Tracking disease progression in Huntington’s Disease using electrophysiology of the sensorimotor system

Dissertation

Applying for the degree of doctor of human biology (Dr.biol.hum.)

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Munich

2017
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Day of thesis defense: 12th Jan 2018
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### Abbreviations

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<th>Description</th>
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<tr>
<td>AAO</td>
<td>Age at onset</td>
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<tr>
<td>ALS</td>
<td>Amyotrophic lateral sclerosis</td>
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<tr>
<td>AMT</td>
<td>Active Motor Threshold</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
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<tr>
<td>APB</td>
<td>Abductor Pollicis Brevis</td>
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<tr>
<td>ASO</td>
<td>Antisense Nucleotides</td>
</tr>
<tr>
<td>CAG</td>
<td>Cytosin-Adenin-Guanin Nucleobase Triplet</td>
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<tr>
<td>CPO</td>
<td>Cumulative Probability of Clinical Onset</td>
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<tr>
<td>CRISPR</td>
<td>Clustered Regularly Interspaced Short Palindromic Repeats</td>
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<tr>
<td>DBS</td>
<td>Disease Burden Score</td>
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<tr>
<td>DCL</td>
<td>Diagnostic Confidence Level</td>
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<td>DTI</td>
<td>Diffusion Tensor Imaging</td>
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<tr>
<td>EEG</td>
<td>Electroencephalogram</td>
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<tr>
<td>EMG</td>
<td>Electromyogram</td>
</tr>
<tr>
<td>fMRI</td>
<td>Functional Magnetic Resonance Imaging</td>
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<tr>
<td>ICC</td>
<td>Intraclass Correlation Coefficient</td>
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<tr>
<td>HD</td>
<td>Huntington’s Disease</td>
</tr>
<tr>
<td>HTT-gene</td>
<td>Huntingtin-Gene</td>
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<tr>
<td>Hz</td>
<td>Hertz</td>
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<tr>
<td>kHz</td>
<td>kilo Hertz</td>
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<tr>
<td>LLR</td>
<td>Long-Latency Afferent Inhibition</td>
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<tr>
<td>MEP</td>
<td>Motor Evoked Potential</td>
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<tr>
<td>MR</td>
<td>Magnetic Resonance</td>
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<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<td>------------------------------------------------</td>
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<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
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<tr>
<td>ms</td>
<td>Millisecond</td>
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<tr>
<td>MT</td>
<td>Motor Threshold</td>
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<tr>
<td>mV</td>
<td>Millivolt</td>
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<tr>
<td>µV</td>
<td>Microvolt</td>
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<tr>
<td>preHD</td>
<td>Premanifest Huntington’s Disease</td>
</tr>
<tr>
<td>rmANOVA</td>
<td>Repeated Measures Analysis of Variance</td>
</tr>
<tr>
<td>RMT</td>
<td>Resting Motor Threshold</td>
</tr>
<tr>
<td>rTMS</td>
<td>rapid Transcranial Magnetic Stimulation</td>
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<tr>
<td>SAI</td>
<td>Short-Latency Afferent Inhibition</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>SEP</td>
<td>Sensory Evoked Potential</td>
</tr>
<tr>
<td>TALEN</td>
<td>Transcription Activator-Like Effector Nuclease</td>
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<tr>
<td>TMS</td>
<td>Transcranial Magnetic Stimulation</td>
</tr>
<tr>
<td>UHDRS</td>
<td>Unified Huntington’s Disease Rating Scale</td>
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<tr>
<td>VBM</td>
<td>voxel-based morphometry</td>
</tr>
<tr>
<td>ZFN</td>
<td>Zinc Finger Nucleases</td>
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IV
1. Introduction

1.1. Huntington’s Disease

Huntington’s Disease (HD) is a progressive neurodegenerative autosomal dominant hereditary disease characterized by motor, cognitive and behavioral disturbances.

The disorder is caused by a pathological CAG triplet repeat expansion in the Huntingtin-gene (HTT-gene), located on the short arm of chromosome four (Huntington's Disease Collaborative Research Group, 1993). In unaffected people, the repeat length is 17-29, coding the protein Huntingtin, which is involved in transcription, axonal transport, cytoskeletal structure/function, signal transduction, autophagy, and post-transcriptional gene expression, although the mechanisms are not yet fully understood (Culver, et al., 2012; Imarisio, et al., 2008; Savas, et al., 2010; Savas, et al., 2008). Repeats >39 lead to protein misfolding triggering neurodegeneration resulting in the manifestation of HD with the number of CAG repeats correlating with age of symptom onset (Datson, et al., 2017; Lee, et al., 2017; Rosas, et al., 2004; Sweeney, et al., 2017). Depending on the CAG
repeat length, symptoms generally begin between 35-45 years of age and progressively worsen until death; survival after motor onset is approximately 15-20 years (Roos, 2010).

The most prominent symptom of HD is the motor abnormalities. Another name for this disease is Chorea Huntington with *chorea* deriving from the Greek meaning *dance*. The motor phenotype can include facial pouting, grimacing, and lifting of alternate eyebrows; neck and trunk movements, upper and lower extremity asymmetric flexion or extension of both small and larger muscle groups, and frequent crossing of arms and legs (Kramer, 2002).

Harder to diagnose are the psychiatric symptoms. Depression, anxiety, apathy and irritability, however, are common in HD and can be found in around half the patients (Craufurd, et al., 2001; Gelderblom, et al., 2017). Psychotic symptoms can occur as well, but are rather rare (Ding and Gadit, 2014; Nagel, et al., 2014). Other symptoms include impulsivity, irritability, and aggression (Novak and Tabrizi, 2010).

Also, although the motor symptoms are typically the trigger for seeking medical attention, cognitive symptoms often develop years earlier. Among the first cognitive signs are difficulties in planning and decision making as well as cognitive inflexibility, in later stages declining into dementia (Curtin, et al., 2015; Farrar, et al., 2014; Paulsen and Conybeare, 2005).

To now, there is no cure for HD, but there are treatments available to reduce the severity of the symptoms. Indications for drug selection and drug dosing for treating chorea are lacking in research literature, but a survey found that globally clinicians favor antipsychotic drugs or tetrabenazine (Burgunder et al., 2011). Psychiatric symptoms are treated with the same medications as used in the general population, like selective serotonin reuptake inhibitors for depression or atypical antipsychotic drugs for psychosis and behavioral problems (Epping, et al., 2013; Epping and Paulsen, 2011; Novak and Tabrizi, 2011).

Most promising in the last few years as a means of actually treating the cause of HD instead of just reducing symptoms are the more advanced methods of genome editing (Shannon and Fraint, 2015). Since the genetic cause of HD is clearly defined as a mutation in one single gene, as opposed to e.g. multiple genetic influences in Alzheimer’s disease (Waring and Rosenberg, 2008; Wilson, et al., 2011), HD seems an ideal candidate for that kind of approach. The three genome editing tools that have emerged over the past years are
zinc finger nucleases (ZFNs), transcription activator-like effector nuclease (TALEN), and clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 (Brookhouser, et al., 2017). These technologies share that they consist of two domains, a catalytic domain to initiate double-stranded breaks - in which both strands in the double helix are severed - and a programmable domain which recognizes specific DNA sequences. Malankhanova, et al. (2017) review those and other genome editing techniques in their recent article and describe ways these methods can be used in HD research, e.g. correction of the mutation causing gene, disruption or deletion of the mutant allele or the modification of genes involved in the pathogenesis.

In the past two years, the first Phase 1/2a study using gene silencing techniques to reduce mutant HTT in humans has been using antisense oligonucleotides (ASO) a single strand of chemically modified DNA, designed to stick to the message molecule from the huntingtin gene and thus preventing protein expression. The latest press release (IONIS Pharmaceuticals, Inc., 2017) states the completion of participant recruitment and the initiation of an open-label extension.

1.2. Abnormalities in the sensorimotor network

The motor abnormalities are the most visible symptoms of HD and are therefore often used to define the onset of the manifest disease stage (Roos, 2010). As ongoing changes in brain structure and function can already be traced years before motor symptoms first occur, this indicates that mechanisms compensating for structural loss fail at some point leading to overt symptoms. The same pattern might be true for some cognitive skills, where e.g. atrophy in the parietal cortex can be measured before there are overt effects on memory (Klöppel et al., 2015), but motor signs are by nature more visible and more unique than the occasional misplaced key. This makes pinpointing motor symptom onset easier than onset of other pathologies and subsequently makes the sensorimotor system a good target for evaluating ongoing changes around symptom onset.
Pathology can occur on three levels – structure, function, and behavior. In neurodegenerative disorders, brain structure is the first level in which abnormalities can be seen, e.g. localized atrophy. Usually the brain is able to compensate for a certain amount of cell loss, but when that is not possible anymore, brain function is also affected, e.g. in the form of an altered brain metabolism or diminished reactions to external stimuli as measured in the electroencephalogram (EEG). Pathologies in behavior can then include...
abnormalities in motor tasks or difficulties in decision making (for a visual representation see Figure 1). This mechanism is referred to as hypercompensation. Functional magnetic resonance imaging (fMRI) studies on participants genetically at risk for Alzheimer’s disease (AD) showed atrophy in the hippocampal area (Ohnishi, et al., 2001) whereas performance in a memory task was unaffected (Bookheimer, et al., 2000). At the same time participants showed higher activation in brain areas not affected by atrophy. This was interpreted as recruitment of additional brain areas to ensure the execution of certain functions and behaviors despite the ongoing loss of brain structure (Bondi, et al., 2005; Caroli, et al., 2010; Erk, et al., 2011). Similar results have been reported for HD in regards to reward processing (Malejko, et al., 2014), memory (Kloppel, et al., 2015) but also the supplementary motor area in a finger tapping task (Kloppel, et al., 2009).

Ways to examine brain function in the sensorimotor system include electrophysiological measures, like the recording of muscle activation or induced motor evoked potentials (MEP) after stimulating the motor cortex with transcranial magnetic stimulation (TMS) for abnormalities concerning the motor efferent pathway. The sensory afferent pathway can be tested using somatosensory evoked potentials (SEPs), alterations in brain activity when the volley from a peripheral sensory stimulus travels to the somatosensory cortex. Different peaks in the recording can be used to trace this, e.g. the N20 is the peak usually associated with the signal reaching the somatosensory cortex. Its’ name refers to a negative peak (N) occurring 20ms after the peripheral stimulus. Other peaks are named the same way with the direction N for negative and P for positive and the time after the peripheral stimulus in ms.

As some of those abnormalities could be sensitive enough to track disease progression, maybe even predict symptom onset or in a few years offer a method of monitoring treatment effects, the current state of research regarding abnormalities in the sensorimotor system in manifest and premanifest HD (preHD) will be reviewed briefly.

Reliably tracking changes occurring in preHD has only been possible since the gene causing HD was identified and genetic testing was widely available. Studies performed before this testing was possible, included an “at-risk” group when testing the children of patients. This offers a problem when interpreting the results these older studies reveal for “premanifest HD”, as there is an extremely high possibility that also unaffected children were included in this group and those could have watered down or distorted the effects. As
a result, in some research areas like electrophysiology of the sensorimotor system, there is relatively little reliable data on actual, genetically confirmed, premanifest HD (preHD) participants.

Structural abnormalities in early manifest HD include whole brain atrophy (Henley, et al., 2009; Tabrizi, et al., 2009; Wild, et al., 2010), as well as regional grey and white matter abnormalities, from cortical thinning to grey matter loss in the neostriatum and cingulate; and white matter loss in posterior-frontal regions (Tabrizi, et al., 2009). These processes have also been observed longitudinally (Gregory, et al., 2015; Poudel, et al., 2015; Tabrizi, et al., 2011).

Also the link between brain structure, brain function and behavior in regards to the motor network has been examined: Muller, et al. (2016) described that sensorimotor white matter organization and functional connectivity in a motor network were independently associated with motor performance. They explained the lack of tract-specific association of structure and function being due to functional adaptation to structural loss differing across patients.


Some research groups report abnormal functioning in the motor efferent pathways of the upper extremities in the sense that MEP sizes are diminished in manifest HD (Meyer, et al., 1992; Modugno, et al., 2001; Priori, et al., 2000), but there are also conflicting results suggesting no difference to healthy controls, especially when looking at premanifest gene carriers (Caramia, et al., 1988; Eisen, et al., 1989; Nardone, et al., 2007). Although already in the premanifest stage, recruitment of nerve cells could be affected as Schippling, et al. (2009) found a more gradual slope in MEP size when TMS stimulation
intensity was increased. Other functional abnormalities include less inhibition in response to continuous theta burst stimulation (cTBS) in preHD and early manifest HD (Orth, et al., 2010) and no increase in MEP following rapid TMS (rTMS) (Lorenzano, et al., 2006) suggesting no plasticity.

Behavioral abnormalities include movement time, alternate button tapping, variability of latency for a memory guided task, error percentage on a more complex memory guided task as assesses by Rupp, et al. (2010).

1.3. Predicting motor symptom onset

The onset of motor symptoms indicates that existing compensation mechanisms have failed, leading to those overt behavioral abnormalities. Most longitudinal studies describe changes in HD in the prodromal phase, but do not include symptom onset itself as a factor or event for the statistical analysis (Epping, et al., 2016; Gregory, et al., 2015; Klöppel, 2015; Müller-Dahlhaus, 2015; Odish, et al., 2015; Poudel, et al., 2015; Sturrock, et al., 2015).

Some exceptions, in the sense that symptom onset was indeed included, are for one Paulsen, et al. (2001), who compared neuropsychological variables between preHD participants and converters. The rates of change over a two year period differed greatly between the two groups with effect sizes of up to 1.7. These findings suggest that brief cognitive measures - like the Stroop test, the Symbol Digit test or verbal fluency - administered over time may capture early striatal neural loss. Hart, et al. (2013) reported a significant deterioration in executive functioning in converters over the course of ten years, when compared to healthy controls. Solomon, et al. (2008) reported faster rates of deterioration in their converter group as opposed to non-converting preHD participants in a few neurocognitive measures, like the Auditory Reaction Time task or the Symbol Digit test.

Analyzing the imaging data from the Track-HD study (Tabrizi, et al., 2012), they did compare the change in brain structure between a group of participants that did either a) reach a Unified Huntington’s Disease Rating Scale (UHDRS) diagnostic confidence level (DCL) of 4, b) have a net 24 month decline in the Total Functioning Capacity (TFC), or c)
1.4. Objectives of this study

It has been established above that there are ongoing changes as Huntington’s Disease progresses. This is especially true for the manifest stage of the disease, but depending on the domain examined, some abnormalities can be observed years before motor symptom onset occurs.
In our study we aim to track deterioration of sensorimotor processing from the premanifest disease stage to the early manifest stage with a special interest in the time closely surrounding motor symptom onset. Study participants who develop motor symptoms and accordingly change group affiliation from preHD to manifest HD will be called converters.

In all cited studies that included a converter group, this group has been compared to a healthy control group or premanifest gene carriers. It would, however, have been worthwhile to also compare the converters with the early HD group to see whether the reported accelerated decline in brain structure and function is continuing after motor symptom onset or whether this is a unique occurrence close to or around symptom onset.

This is the kind of gap we are trying to close in this analysis. We will compare premanifest gene carriers, converters, early manifest HD gene carriers and healthy controls in their development over the course of two years to see whether motor symptom onset does have a direct influence on sensorimotor processing; and we will also look at changes intraindividually in our converter group whether we can see a difference here in the twelve months surrounding motor symptom onset as compared to a comparable timeframe pre or post symptom onset. We will then try to predict motor symptom onset from our variables to see whether electrophysiological measures could help improve onset estimations.

As symptom onset is defined by the occurrence of motor symptoms, we decided to track changes in the sensorimotor network using electrophysiological variables derived from the TrackOn HD study module “Transcranial Magnetic Stimulation”. Paradigms applied were median nerve somatosensory evoked potentials (SEP), long-latency reflexes (LLR), motor thresholds at rest (RMT) and active (AMT), recruitment curves at rest and active, as well as short-latency afferent inhibition (SAI). Response latencies and amplitudes or curve areas were recorded. Using just the healthy controls’ data (n=112), intraclass correlations were calculated for each of the measures to find the ones appropriately powered to detect group differences, meaning having a low within subject variability, but a high between subject variability (Brown et al., 2017). Measures with an intraclass correlation coefficient (ICC) of .8 were defined as reliable. Variables that met the .8 ICC criterion were the SEP amplitudes (.91) and N20 latency (.90), the latencies for LLR I, LLR II, and MEP (.97, .98, .92, respectively), as well as the MEP curve area when stimulated at 150% RMT (.81). Only those variables will be included in the following data analysis.
2. Materials and Methods

2.1. The TrackOn HD study

All data reported here are part of the longitudinal multi-site TrackOn HD study.

The aim of the multisite TrackOn HD study was to extend the results of the Track HD study, which wanted to find out what combination of measures proved the most sensitive in detecting change over the course of premanifest and early HD and to validate these as potential outcome measures for use in future therapeutic trials (Kloppel, et al., 2015; Orth, et al., 2016; Tabrizi, et al., 2012; Tabrizi, et al., 2011). TrackOn HD set out to look at changes in brain structure – using magnetic resonance imaging (MRI) and magnetic resonance (MR) Spectroscopy, brain function – using fMRI and TMS, changes in behavior – using e.g. memory, attention, and cognitive tasks. A total of 243 study participants (110 presymptomatic HD gene expansion carriers, 21 early symptomatic HD gene carriers, and 112 controls) were enrolled in the study and would return for three study visits with an
approx. 12 month interval between the visits. Recruitment of the gene carriers took place at four sites: a) the National Hospital for Neurology and Neurosurgery, London, UK; b) the Department of Medical Genetics at the University of British Columbia, Vancouver, Canada; c) the Department of Genetics and Cytogenetics at the Hôpital de la Salpêtrière-Université Pierre and Marie Curie, Paris, France; d) Department of Neurology at Leiden University Medical Centre, Leiden, Netherlands. HD gene carriers were either previously enrolled participants in the Track HD study or newly recruited gene carriers with a CAG-repeat length ≥40 and a burden of pathology score (CAG -35.5) x age > 250 (disease burden score, DBS) (Penney, et al., 1997). HD gene carriers were divided into pre and early manifest HD using the Total Motor Score of the Unified Huntington’s Disease Rating Scale (UHDRS), with premanifest participants having a UHDRS motor score <5, indicating no substantial motor signs, and early manifest gene carriers having a UHDRS Diagnostic Confidence Level (DCL) of 4. New diagnoses of manifest HD during the study were defined as reaching a UHDRS DCL of 4 at one of the follow-up visits.

Controls were age-matched and gender-matched at the Track HD baseline visit to individuals in the combined premanifest and early HD groups and were selected from the spouses or partners of individuals with the HD gene expansion or were gene-negative siblings, to ensure consistency of environments with gene carriers.

The study was approved by the local ethics committees (London: National Research Ethics Service, Reference: 07/H0716/47; Paris: Committee for Protection of Persons, Reference: CPP/64-07; Vancouver: Office of Research Services, Reference: H07-01759; Leiden: Commission for Medical Ethics, Reference: P07.142/NV/nv), and written informed consent was obtained from each participant.

### 2.1.1. Inclusion and exclusion criteria

Inclusion and exclusion criteria were defined to be valid for all of the TrackOn HD assessments, which included MRI and blood sample donation. Table 1 shows the inclusion and exclusion criteria for the TrackOn HD study. In general, participants were not excluded based on medication usage, unless this medication was part of an experimental therapeutic drug trial; comorbid medical conditions were noted, but unless they prevented
subject assessment were not considered exclusions. For the TMS assessment, certain medication was, however considered an exclusion criterion, as selective-serotonin-reuptake inhibitors (SSRIs), prescribed mainly for depression, are believed to lower the threshold for seizures. Also, as a precaution against seizures, space-occupying lesions in the brain were considered a contraindication.

Table 1: Inclusion and exclusion criteria for the TrackOn HD study. MRI: Magnetic Resonance Imaging; HD: Huntington’s Disease; CAG: Cytosin-Adenin-Guanin Nucleobase Triplet; UHDRS: Unified Huntington’s Disease Rating Scale.

<table>
<thead>
<tr>
<th>Inclusion Criteria</th>
<th>Exclusion Criteria</th>
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<tr>
<td>- 18-65 years old</td>
<td>- major psychiatric disorder at time of enrollment</td>
</tr>
<tr>
<td>- able to tolerate MRI assessment</td>
<td>- known history of epilepsy</td>
</tr>
<tr>
<td>- for HD gene carriers:</td>
<td>- Concomitant significant neurological disorder</td>
</tr>
<tr>
<td>o positive genetic test with CAG repeat length ≥40</td>
<td>- Concomitant significant medical illness</td>
</tr>
<tr>
<td>o Disease Burden Score &gt;250 or</td>
<td>- Unsuitability for MRI, e.g. claustraphobia, metal implants</td>
</tr>
<tr>
<td>o UHDRS motor score &gt;5</td>
<td>- History of significant head injury</td>
</tr>
<tr>
<td>- for control participants:</td>
<td>- Predictable non-compliance by drug and/or alcohol abuse</td>
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<tr>
<td>o partner/spouse of a HD subject or</td>
<td>- Significant hand injuries that preclude either writing or rapid computerized responding</td>
</tr>
<tr>
<td>o HD normal repeat length sibling or</td>
<td>- Participant in Predict-HD</td>
</tr>
<tr>
<td>o HD normal repeat length control volunteer</td>
<td>- currently participating in a clinical drug trial</td>
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<td></td>
<td>- unwillingness to donate blood</td>
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</table>
2.1. Participants

For this analysis we wanted to concentrate on HD gene carriers who developed motor symptoms during the three TrackOn visits, i.e. developed an UHDRS DCL of 4. In TrackOn there were a total of 23 participants who met this criterion and completed all three study visits. This group will be referred to as converters. 13 of them developed symptoms between visits 1 and 2, whereas the other 10 developed symptoms between visits 2 and 3 (Fig. 2).

**Figure 2: Number of motor symptom onsets over 24 months.** In the TrackOn HD baseline visit (V1) 96 HD gene carriers were categorized as premanifest HD. By the second study visits (V2) 13 of those had developed motor symptoms (dashed arrow) and another 10 participants by the third study visit (V3), while others remained symptom free and categorized as preHD (solid arrow).
As control groups, we used data from 73 preHD participants, who did not develop symptoms over the data collection period of TrackOn; 17 early HD participants, who had been categorized as early manifest since the first visit they attended; and 76 healthy control participants. Only data from participants with the two necessary visits (i.e. Visits 1 and 3) was used.

As the Leiden study site did not perform the TMS assessment, but only the SEPs, subject numbers for the analysis of the LLR, motor threshold, and MEP data were smaller. Only data from London, Paris and Vancouver were analyzed here. This subsample of the one described above included 10 converters, 51 controls, 63 preHD participants and 11 earlyHD participants.

For the second part, trying to predict symptom onset from electrophysiological, as well as clinical data, we used data from all preHD participants completing the SEP assessment at three study visits, including the converter group, a total of 96 participants.

### 2.2. Data collection

Participants were seated in a comfortable chair and asked to relax as much as possible. All measures were collected from the dominant hemisphere and hand, assessed with the Edinburgh Handedness Questionnaire (Oldfield, 1971).

#### 2.2.1. Somatosensory evoked Potentials (SEPs)

Somatosensory evoked potentials (SEPs) were recorded following median nerve stimulation (pulse width 200 μs, square wave pulse, cathode distal, anode proximal) with surface electrodes using routine techniques (Fischer and Orth, 2011; Yamada, et al., 1991). Cortical SEPs were recorded with a silver/silver-chloride disk electrode over the somatosensory cortex (2 cm posterior of C3 in the international EEG 10-20 system) referenced against Fz.
Briefly, stimulation at 3Hz was delivered at 150% of the motor threshold, defined as the minimum intensity required to evoke a visible twitch in the target muscle. Recordings from 300 stimuli were collected. At three sites (London, Paris, Vancouver), surface electromyograms (EMG) were recorded from the right abductor pollicis brevis (APB) muscle using silver/silver-chloride disc surface electrodes (1 cm diameter) in a belly tendon montage. The signal was amplified and analogue filtered (30 Hz to 1 kHz) with a Digitimer D150 amplifier (Digitimer Ltd., Welwyn Garden City, UK) in London and Paris, or Powerlab 4/30 EMG System (AD Instruments, Colorado Springs, CO) in Vancouver. Leiden used Medelec Synergy version 11.0 (Oxford Instruments, Abingdon, United Kingdom). Data were digitized (sampling rate 4 kHz) for offline analysis using Signal software (Cambridge Electronic Devices, Cambridge, UK) in London and Paris, or LabChart (AD Instruments, Colorado Springs, CO) in Vancouver.

In order to analyze SEP data, an average trace for each stimulation intensity separately was produced to extract the SEP components.

The P14 component was defined as the first positive peak in a time window of 10-20ms post-stimulus; the N20 component as the first negative peak in a time window of 15-25ms post-stimulus; the P25 as the first positive peak in a time window of 20-30ms post-stimulus, and the N33 as the first negative peak in a time window of 25-40ms post-stimulus. Amplitudes were measured as peak-to-peak amplitudes, e.g. the N20 amplitude was measured between the N20 and P25 peaks. Latency was determined as peak latency from the 150% of motor threshold trace.

2.2.2. Long-Latency Reflexes

LLRs were collected using standard procedures (Deuschl, et al., 1989). 300 stimuli were delivered over the median nerve at the wrist (pulse width 200 μs, square wave pulse, cathode distal, anode proximal) as individuals maintained an APB contraction of 20-30% of their maximal voluntary contraction (MVC). To activate the APB, individuals were instructed to abduct their thumbs against a force transducer while monitoring visual feedback to ensure consistency. EMG was collected as described above.
Average traces were used to determine the latencies of both LLR I and LLR II. LLR I was defined as the first deflection from baseline between 35-45ms post-stimulus, while LLR II was identified in a time window of 45-55ms.

2.2.3. Motor thresholds and Motor Evoked Potentials

TMS assessment was not done at the Leiden study site, so only data from London, Paris and Vancouver will be reported on the threshold data.

Surface electromyograms (EMG) were recorded from the dominant APB muscle using silver/silver-chloride disc surface electrodes (1 cm diameter) in a belly tendon montage. The EMG signal was amplified and analogue filtered (30Hz to 1kHz) with a Digitimer D150 amplifier (Digitimer Ltd., Welwyn Garden City, UK) in London and Paris, or Powerlab 4/30 EMG System (AD Instruments, Colorado Springs, CO) in Vancouver. Data were digitized (sampling rate 4 kHz) for offline analysis using Signal software (Cambridge Electronic Devices, Cambridge, UK) in London and Paris, or LabChart (AD Instruments, Colorado Springs, CO) in Vancouver.

Determination of motor thresholds also followed routine protocol (Schippling, et al., 2009). First, the best location for the TMS coil on the scalp for stimulating the contralateral APB was located and marked with a felt pen. Using a High Power Magstim 200 stimulator (Magstim Co., Whitland, Dyfed, UK), magnetic stimuli were given with a figure-of-eight coil (outer winding diameter 9cm). This stimulator induces a current in the brain with an anterior-posterior flow when the coil is placed tangentially to the scalp with the handle positioned at an angle of 45° pointing backwards.

Resting motor threshold (RMT) was defined as the minimum intensity (in % of maximum stimulator output) needed to evoke a MEP of 50µV amplitude in the relaxed APB in at least five out of ten consecutive trials. The stimulation intensity was set to elicit a clearly visible MEP and then lowered in steps of 1% stimulator output until there were less than five 50µV MEPs in ten trials. The stimulation intensity was slowly raised again until the criterion was met. This intensity was taken as the RMT.

Active motor threshold (AMT) followed a similar protocol, with the differences that the criterion was set to an MEP of >200µV in five out of ten consecutive trials in the tonically active APB (~20% of MVC).
Threshold determination was followed by recording of recruitment curves, where stimulation was given at 110, 130, and 150% RMT with a relaxed APB or at 125, 150, and 175%AMT with the tonically active APB. Ten trials were recorded for each intensity. The resulting motor evoked potentials (MEP) were recorded from the APB were recorded and averaged. MEP latency was defined as the time from the stimulus artefact to onset of the MEP wave, the area under the curve was taken as MEP size (McDonnell, et al., 2004).

As the MEP in the 150%RMT condition was the only one that met the .8 ICC criterion, only that will be used for this data analysis.

2.3. Data analysis

Assessments were completed using a fully electronic data capture and content management system that provided secure access to the system for sites worldwide. All subject phenotypic data was pseudonymised and securely stored in the European Huntington’s Disease Network (EHDN) Clinical Trial Management System (CTMS, Ulm). Stringent quality control and assurance measures were implemented, e.g. continuous online and monthly on-site data monitoring or twice-yearly progress reports detecting possible data errors and providing feedback to the investigators.

2.3.1. Differences between study sites

As mentioned before in the methods section, hardware and software used differed between study sites. Furthermore, although a detailed study protocol was distributed to all sites to follow and study personal were trained in the paradigms used, different protocol executions could not be completely excluded. To test for difference between the study sites and whether, subsequently, we have to control for study site in the following analysis we decided to perform a one-way analysis of variance (ANOVA) with the between-subjects factor “Study Site” with our SEP amplitudes and latencies, the SEP stimulation intensities used, the LLR and MEP latency data and the TMS motor thresholds and MEP size at
150%RMT using our healthy controls’ data. Analyses including latency variables were controlled for arm length.

### 2.3.2. Group differences over 24 months

To test for differences in somatosensory processing between our four subject groups, we used a repeated measures analysis of variance (rmANOVA) with the between-subjects factor “Group” (Control v preHD v early HD v converter) and the within-subjects factor “Visit” (Visit 1 v 3). In addition to those, for the motor thresholds, we also used a within-subjects factor “Threshold” (RMT vs AMT) to avoid multiple testing. We decided to only use those two visits at this point in the analysis to avoid losing testing power looking at four experimental groups.

This analysis was performed for all amplitude, latency, and threshold data collected controlling for study site and arm length, respectively.

A main effect “Visit” would indicate changes over time independent of subject group, a main effect “Group” would indicate that the participant groups differ in both observed visits, and an interaction between the two factors would indicate that the development over the course of 24 months differs depending on which group a subject belongs to.

### 2.3.3. Intraindividual changes around symptom onset

In this analysis we wanted to focus on a group of HD gene carriers, who developed motor symptoms over the 24 month testing period. As some of the converters developed motor symptoms between Visits 1 and 2, while others did so between Visits 2 and 3, this enables us to compare the development between those pre and post symptom onset visits with the third study visit. These intrasubject comparisons could give information whether changes in somatosensory processing occur in a linear manner, regardless of symptom onset, or whether there is a rapid change around the time of symptom onset.
We only used the SEP amplitudes for this analysis, as for all other variables we are missing data from the Leiden study site. Comparing the two converter groups with each other on variables taken from the LLR or MEP assessments would have resulted in group sizes of 6 vs 4, which makes reliable statistical testing impossible.

First we applied a rmANOVA with the within-subjects factor “Visit” (Visit 1 v 2 v 3) and the between-subjects factor “Group” (Onset 1-2 v Onset 2-3). A main effect “Visit” would indicate changes over time independent of subject group, a main effect “Group” would indicate that the participant groups differ in all the observed visits, and an interaction between the two factors would indicate that the development over the course of 24 months differs depending on which group a subject belongs to. This interaction effect would be most interesting, since it would indicate that development of somatosensory processing is different even in two groups close around symptom onset.

In a second step, we calculated the slope between the pre and post symptom onset visits and used a paired-samples t-test to compare it to the slope between the remaining study visit and the one following or preceding it, depending on when symptoms occurred.

Figure 3 gives a visual representation of that process.
We decided to pool all converters into one group for this part, regardless of the time of symptom onset, as we did not seek to discriminate between processes preceding or following symptom onset, but rather were interested in the intrasubject development around onset and a timeframe close by. A significant result here would indicate that in the twelve months around symptom onset changes in the somatosensory system happen in another speed or extent than in a comparable timeframe close to symptom onset. A non-significant result would indicate rather linear changes over the course of the disease.

2.3.4. Predicting motor symptom onset

The aim of this analysis was to find out whether there was an electrophysiological variable sensitive enough to be able to predict symptom onset. We estimated new diagnosis risk in preHD participants using Cox proportional hazard models.

As the dependent variable we defined the binary variable “Onset” (yes vs no), with yes indicating that a participant developed motor symptoms at some point during the 24-month TrackOn data collection period, defined as reaching a UHDRS diagnostic confidence score (DCL) of 4.

We estimated hazard ratios for electrophysiological predictors. Since we wanted to take the intraindividual development into account, we used the slopes of the amplitudes between the visits and entered them as time-dependent predictor, i.e. depending on whether the event (symptom onset) occurred between Visit 1 and 2 or Visits 2 and 3, we used the respective slope as a predictor. For those preHD participants that did not develop symptoms, we used the average slope across all time points. Since we expect amplitudes to decrease over time, we multiplied our slopes with -1 for easier readability, so that a stronger negative slope actually represents a higher hazard ratio. We also controlled for age.
and CAG length, as they propose the strongest risk for disease onset. We combined those two, as for our 20 events (i.e. symptom onsets), only two predictors per analysis are recommended (Peduzzi, et al., 1995).

Time was defined by longitudinal visit number, and we used the exact method for tied survival times.

Since we would otherwise be missing a lot of data, as the Leiden site did only complete the SEP assessment and not LLRs, motor thresholds or MEP, we only calculated the hazard ratios for SEP variables.

Other possible methods for predicting symptom onset we considered were a logistic regression, or the Kaplan-Meier estimation with the log-rank-test (George, et al., 2014).

The logistic regression tries to predict group affiliation, i.e. preHD or converter, from given predictor variables. In our case, as there are a very small number of symptom onsets in relation to the whole preHD sample, the prediction of group might be highly significant, just due to the fact that affiliation to the preHD group has an extremely high probability.

A reason to decide against Kaplan-Meier and the log-rank-test was that it only allows the comparison between categorical covariates. This is not the case for SEP amplitudes or DBS, CPO or expected years to onset. It would require e.g. a median split to meet this requirement, which would mean a simplification of data and loss of information. Because of that and the high dependency on the pattern of censored data in this method, we decided against it.

An assumption of the Cox regression is that time duration is a continuous variable (Cox, 1972). That is not the case for two follow-up time points. When a subject is newly diagnosed at a visit, we don’t literally think that the subject reached the diagnosable point exactly on that day they were seen. Rather, it seems sensible to assume that the subject reached that point at an unknown time between the current and previous examination. Thus, it would not be appropriate to measure follow-up time in days and assign new diagnosis to the exact date of follow-up. Measuring follow-up by visit number rather than exact calendar time leads to tied failure times for all participants developing symptoms between two given visits. If a Cox model is fit using the “exact” method to handle ties, all
possible permutations of the order of failure among the tied times are evaluated and this information is incorporated into the estimates and attendant p values, etc.

In their longitudinal analysis of Track HD data Tabrizi, et al. (2013) do use the Cox regression with three follow up time points, following the same rationale.

The Cox regression was the method of choice for our problem, as it was also the only one able to include time dependent variables (Bellera, et al., 2010; Sargent, 1997) and, as we can see from the intraindividual comparison of N20 amplitude slopes, those seem to be influenced by the close motor symptom onset.

All statistical analysis was performed using SPSS 20 (IBM, Armonk, New York). Mauchly tests for sphericity were performed and Greenhouse Geisser corrections were applied where necessary. The alpha-level was set to .05. All descriptive statistics are reported as mean (SD) unless otherwise indicated.
3. Results

3.1. Participants

See Table 2 for the demographic variables of our whole study sample completing the SEP assessment. An univariate analysis of variance could not find significant differences between all the groups regarding age and arm length (p = .317 to .961); just looking at the HD groups, there were no significant differences in CAG repeat length (p = .432); but in DBS (F_{2,110} = 13.225; p < .001); cumulative probability of symptom onset (CPO) (Langbehn, et al., 2004) (F_{2,110} = 23.451; p < .001); and expected years to onset (F_{2,110} = 23.451; p < .001). These differences had to be expected, as those variables mirror disease progression.

The subsample of the one described above, completing the LLR and MEP assessments, included 10 converters, 51 controls, 63 preHD participants and 11 earlyHD participants (see Table 3 for the demographic variables). Here, an univariate ANOVA revealed significant differences in expected years to onset (F_{2,81} = 7.276; p = .001), DBS (F_{2,81} = 8.995; p < .001) and CPO (F_{2,810} = 13.982; p < .001) between the HD groups. No
difference in CAG repeat length between the HD groups was found (p = .436). Testing for differences between all the groups, there was a significant effect for age ($F_{3,131} = 7.276; p = .009$), with post-hoc t-tests showing that the preHD participants were significantly younger than the healthy controls ($t_{112} = 3.336; p = .001$), no other comparison showed a significant result ($p = .207$ to .902). No differences were found regarding armlength ($p = .587$).

Table 2: Demographic variables as recorded in Visit 1 for the SEP analysis. Shown are averages and standard deviations if not indicated otherwise. Age and age to onset reported in years; armlength reported in cm; HD: Huntington’s Disease; preHD: premanifest Huntington’s Disease gene carrier; UHDRS: unified Huntington’s Rating Scale; DBS: Disease Burden Score; CPO: cumulative probability of symptom onset; n.a.: not applicable

<table>
<thead>
<tr>
<th></th>
<th>converters (n=23)</th>
<th>preHD (n=73)</th>
<th>early HD (n=17)</th>
<th>Controls (n=76)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>46.11 (9.22)</td>
<td>41.92 (9.30)</td>
<td>46.07 (8.15)</td>
<td>48.37 (10.07)</td>
</tr>
<tr>
<td>Sex m/f</td>
<td>13/10</td>
<td>36/37</td>
<td>7/10</td>
<td>28/48</td>
</tr>
<tr>
<td>Armlength</td>
<td>74.87 (4.12)</td>
<td>74.37 (5.86)</td>
<td>73.95 (6.19)</td>
<td>74.64 (4.61)</td>
</tr>
<tr>
<td>CAG repeat length</td>
<td>42.96 (2.74)</td>
<td>42.96 (2.21)</td>
<td>43.65 (2.45)</td>
<td>n.a.</td>
</tr>
<tr>
<td>DBS</td>
<td>324.91 (66.02)</td>
<td>290.16 (46.40)</td>
<td>358.49 (57.63)</td>
<td>n.a.</td>
</tr>
<tr>
<td>CPO</td>
<td>.34 (.19)</td>
<td>.19 (.14)</td>
<td>.44 (.14)</td>
<td>n.a.</td>
</tr>
<tr>
<td>Expected years to onset</td>
<td>9.38 (3.99)</td>
<td>11.66 (3.59)</td>
<td>7.28 (2.05)</td>
<td>n.a.</td>
</tr>
</tbody>
</table>
For the second part, trying to predict symptom onset we used data from all preHD participants completing the SEP assessment at three study visits, including the converter group, a total of 96 participants. In Visit 1 this sample consisted of 49 men and 47 women, who were on average 42.72 years old (9.38). Mean CAG repeat length was 42.89 (2.35), mean DBS 298.58 (54.19), and mean CPO .22 (.16). Table 2 gives an overview of the demographics for the included converters and included preHD participants separately. The two groups differed in respect to their DBS (F$_{1,94}$ = 7.911; p = .006), their CPO (F$_{1,94}$ = 17.358; p < .001) and their expected years to onset (F$_{1,94}$ = 6.726; p = .011), as revealed by a one-way ANOVA. They did not differ in age or CAG repeat length.

Table 3: Demographic variables as recorded in Visit 1 for the long latency reflex and motor threshold analysis. Shown are averages and standard deviations if not indicated otherwise. Age and age to onset reported in years; armlength reported in cm; HD: Huntington’s Disease; preHD: premanifest Huntington’s Disease gene carrier; UHDRS: unified Huntington’s Rating Scale; DBS: Disease Burden Score; CPO: cumulative probability of symptom onset; n.a.: not applicable

<table>
<thead>
<tr>
<th></th>
<th>converters (n=10)</th>
<th>preHD (n=63)</th>
<th>early HD (n=11)</th>
<th>Controls (n=51)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>45.27 (7.91)</td>
<td>41.17 (9.65)</td>
<td>45.61 (8.13)</td>
<td>47.50 (10.56)</td>
</tr>
<tr>
<td>Sex m/f</td>
<td>7/3</td>
<td>32/31</td>
<td>5/6</td>
<td>21/30</td>
</tr>
<tr>
<td>Armlength</td>
<td>76.00 (3.86)</td>
<td>74.39 (6.62)</td>
<td>72.23 (7.04)</td>
<td>73.94 (4.29)</td>
</tr>
<tr>
<td>CAG repeat length</td>
<td>42.70 (2.71)</td>
<td>43.13 (2.16)</td>
<td>43.88 (2.53)</td>
<td>n.a.</td>
</tr>
<tr>
<td>DBS</td>
<td>308.65 (70.50)</td>
<td>297.01 (42.60)</td>
<td>364.86 (67.93)</td>
<td>n.a.</td>
</tr>
<tr>
<td>CPO</td>
<td>.27 (.14)</td>
<td>.20 (.14)</td>
<td>.45 (.12)</td>
<td>n.a.</td>
</tr>
<tr>
<td>Expected years to onset</td>
<td>10.27 (4.21)</td>
<td>11.18 (3.27)</td>
<td>7.04 (2.44)</td>
<td>n.a.</td>
</tr>
</tbody>
</table>
3.2. Differences between study sites

To ensure comparability between data from the three different study sites, we performed a one-way analysis of variance with the between-subjects factor “study site”. For this analysis we used the healthy controls’ electrophysiological data averaged across the two time points. Table 4 displays the averages and standard deviations for the central SEP components, the latency data and the MEP size at 150%RMT by study site.

Stimulation intensity is missing for Vancouver, as their stimulator only had an output in mA, whereas the other sites worked with mV. LLR, motor threshold, and MEP data are missing for Leiden, as they did not perform that part of the protocol.

The ANOVA with between-subjects factor study site showed significant differences in almost all of the SEP amplitude data from stimulation at 150% motor threshold (MT) (p = .0001 to .136). Post-hoc t-tests revealed that at 150%MT, the London and Leiden study sites differed significantly in P14 (p = .019), N20 (p = .029), and N33 (p = .015) amplitudes; London and Paris differed in P14 (p = .03), N20 (p = .02) and N33 (p = .012) amplitude. At 150%MT, there was no difference in amplitude between the Leiden and Paris sites and no differences between Vancouver and the other sites.

Looking at the stimulation intensities used at the different study sites, there could be an explanation for the site differences in SEP amplitudes. Here, the ANOVA showed significant differences between the sites for stimulation at 150%MT (p = .015). Only data from London, Paris and Leiden could be used for this test. In post-hoc t-tests London differed significantly from Leiden and Paris (p = .01). Leiden and Paris did not differ from each other (p = .084). Since we are lacking the intensities from Vancouver, we cannot use intensity as a cofactor for future analysis, but will include “study site”.

The ANOVA did not reveal any site differences regarding SEP latencies (p = .124 to .267), nor regarding the TMS motor thresholds at rest or active (p = .195 to .675), the LLRs (p = .065 to .110) or the MEP size (p = .321).
Table 4: Averages and standard deviations for the controls’ data by study site. Stimulation intensities in mV, amplitudes in µV; latencies in ms; thresholds in %maximum stimulator output; MEP area in mVms. MT: motor threshold; RMT: resting motor threshold; AMT: active motor threshold; MEP: motor evoked potential; n.a.: not applicable.

<table>
<thead>
<tr>
<th></th>
<th>Leiden (n=23)</th>
<th>London (n=20)</th>
<th>Paris (n=19)</th>
<th>Vancouver (n=14)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stim Intensity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>150% motor</td>
<td>12.55 (4.33)</td>
<td>6.49 (1.68)</td>
<td>9.93 (5.10)</td>
<td>n.a.</td>
<td>.015</td>
</tr>
<tr>
<td><strong>SEP Amplitudes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>150% MT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P14</td>
<td>1.65 (.78)</td>
<td>.71 (.30)</td>
<td>1.84 (.55)</td>
<td>1.43 (.88)</td>
<td>.001</td>
</tr>
<tr>
<td>N20</td>
<td>3.17 (1.73)</td>
<td>1.66 (.69)</td>
<td>1.65 (1.52)</td>
<td>2.45 (1.27)</td>
<td>.001</td>
</tr>
<tr>
<td>P25</td>
<td>2.07 (1.28)</td>
<td>1.02 (.51)</td>
<td>1.56 (1.07)</td>
<td>1.34 (1.57)</td>
<td>.136</td>
</tr>
<tr>
<td>N33</td>
<td>1.92 (1.16)</td>
<td>.98 (.47)</td>
<td>3.24 (1.50)</td>
<td>1.98 (1.73)</td>
<td>.005</td>
</tr>
<tr>
<td><strong>Latencies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P14</td>
<td>15.95 (.66)</td>
<td>15.46 (1.26)</td>
<td>15.01 (.57)</td>
<td>14.98 (1.74)</td>
<td>.267</td>
</tr>
<tr>
<td>N20</td>
<td>20.56 (1.15)</td>
<td>19.40 (.88)</td>
<td>19.66 (1.09)</td>
<td>20.46 (.79)</td>
<td>.124</td>
</tr>
<tr>
<td>P25</td>
<td>26.30 (1.26)</td>
<td>25.90 (1.97)</td>
<td>24.75 (1.13)</td>
<td>25.31 (1.62)</td>
<td>.173</td>
</tr>
<tr>
<td>N33</td>
<td>33.79 (1.92)</td>
<td>32.22 (2.23)</td>
<td>32.14 (1.54)</td>
<td>33.11 (1.68)</td>
<td>.218</td>
</tr>
<tr>
<td>LLR I</td>
<td>n.a.</td>
<td>36.90 (3.67)</td>
<td>39.27 (3.67)</td>
<td>38.50 (1.17)</td>
<td>.110</td>
</tr>
<tr>
<td>LLR II</td>
<td>n.a.</td>
<td>47.78 (3.78)</td>
<td>50.57 (3.59)</td>
<td>49.78 (1.20)</td>
<td>.065</td>
</tr>
<tr>
<td>MEP</td>
<td>n.a.</td>
<td>21.98 (1.78)</td>
<td>22.94 (1.84)</td>
<td>20.50 (.81)</td>
<td>.321</td>
</tr>
<tr>
<td><strong>Motor thresholds</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RMT</td>
<td>n.a.</td>
<td>47.70 (10.17)</td>
<td>43.50 (6.78)</td>
<td>46.26 (8.32)</td>
<td>.675</td>
</tr>
<tr>
<td>AMT</td>
<td>n.a.</td>
<td>40.30 (7.84)</td>
<td>34.50 (6.44)</td>
<td>38.84 (7.38)</td>
<td>.195</td>
</tr>
<tr>
<td><strong>MEP area</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>150%RMT</td>
<td>n.a.</td>
<td>9.42 (7.50)</td>
<td>8.77 (6.11)</td>
<td>5.63 (3.98)</td>
<td>.660</td>
</tr>
</tbody>
</table>
3.3. Group differences over 24 months

3.3.1. Somatosensory Evoked Potentials

See Figure 4 for the mean SEP traces by group from Visits 1 and 3.

To look for deterioration over time in Huntington’s disease we applied rmANOVAs with the within-subjects factor “Visit” (V1 v V3) and the between-subjects factor “Group” (converters v healthy controls v preHD v early HD), controlling for study site. Table 5 shows the component amplitudes by group for stimulation at 150%MT.

Table 5: Averages and standard deviations of the cortical SEP components’ amplitudes at 150% motor threshold stimulation in μV. preHD: premanifest Huntington’s Disease gene carrier; HD: Huntington’s Disease; V1: Visit 1; V3: Visit 3.

<table>
<thead>
<tr>
<th>Component</th>
<th>Group</th>
<th>V1</th>
<th>V3</th>
<th>V1</th>
<th>V3</th>
<th>V1</th>
<th>V3</th>
<th>V1</th>
<th>V3</th>
</tr>
</thead>
<tbody>
<tr>
<td>P14</td>
<td>converters (n=23)</td>
<td>1.13</td>
<td>1.11</td>
<td>1.77</td>
<td>1.01</td>
<td>1.08</td>
<td>1.01</td>
<td>1.76</td>
<td>1.42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(.64) (.09)</td>
<td>(.91) (.68)</td>
<td>(1.09) (1.06)</td>
<td>(1.09) (.94)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N20</td>
<td>preHD (n=73)</td>
<td>1.31</td>
<td>.91</td>
<td>2.21</td>
<td>1.92</td>
<td>1.47</td>
<td>1.35</td>
<td>1.55</td>
<td>1.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1.09) (.72)</td>
<td>(1.71) (1.40)</td>
<td>(1.32) (1.41)</td>
<td>(1.25) (1.25)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P25</td>
<td>early HD (n=17)</td>
<td>.76</td>
<td>.43</td>
<td>1.13</td>
<td>.89</td>
<td>.74</td>
<td>1.12</td>
<td>1.02</td>
<td>1.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(.57) (.56)</td>
<td>(1.04) (1.23)</td>
<td>(.63) (.77)</td>
<td>(.54) (.51)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N33</td>
<td>healthy controls (n=76)</td>
<td>1.24</td>
<td>1.55</td>
<td>2.48</td>
<td>2.53</td>
<td>1.38</td>
<td>1.44</td>
<td>1.61</td>
<td>1.66</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(.71) (1.00)</td>
<td>(1.40) (1.48)</td>
<td>(1.00) (.97)</td>
<td>(1.29) (1.36)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 4: Somatosensory evoked potentials traces by group in Visits 1 and 3. X-axis displays time in ms post peripheral stimulus.
For the N20 we found a significant main effect “Group” (F\(_{3,147} = 8.704;\ p > .001;\ \eta^2_p = .261\)) and a significant interaction “Group” x “Visit” (F\(_{3,147} = 3.270;\ p > .0001;\ \eta^2_p = .117\)). Post-hoc t-tests showed that controls had shallower slopes than preHD (t\(_{106} = -3.025;\ p = .001\), early HD (t\(_{80} = 2.334;\ p = .026\)) or the converters (t\(_{85} = -3.585;\ p < .001\)). The converter group had a steeper slope than early HD (t\(_{33} = 2.143;\ p = .040\)) and showed a tendency towards a steeper slope than preHD (p = .070).

For the P14, the rmANOVA showed a significant interaction between “Group” x “Visit” (F\(_{3,102} = 2.90;\ p = .041;\ \eta^2_p = .116\)). Post-hoc tests comparing the slopes showed that preHD had a steeper slope than controls (t\(_{100} = 3.247;\ p = .003\)) as did early HD (t\(_{27} = 3.133;\ p = .004\)).

No significant results were found for the P25 or N33 component.

### 3.3.2. Latency variables

Average latencies and the respective SDs for the different SEP components can be taken from Table 6.

Looking at the LLR I in Visit1, the converter group had a latency of 40.47ms (1.86), the healthy controls had a latency of 38.31ms (3.04), the preHD group 37.94ms (2.56), and the earlyHD group 38.90ms (3.06). In Visit 3 the LLR I latencies were 40.72ms (1.91), 37.98ms (3.16), 37.91ms (2.28), and 38.16ms (2.78) for the converters, control, pre and early HD group, respectively.

For the LLR II, in Visit 1 the latencies were 48.75ms (1.22), 49.94ms (2.81), 52.21ms (2.12), and 50.42ms (2.36) for the converter, control, pre and early HD group, respectively.
### Table 6: Averages and standard deviations of the cortical SEP components’ latencies at 150% motor threshold stimulation in ms.

preHD: premanifest Huntington’s Disease gene carrier; HD: Huntington’s Disease; V1: Visit 1; V3: Visit 3.

<table>
<thead>
<tr>
<th>Converter (n=23)</th>
<th>P14</th>
<th>N20</th>
<th>P25</th>
<th>N33</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V1</td>
<td>V3</td>
<td>V1</td>
<td>V3</td>
</tr>
<tr>
<td></td>
<td>16.82</td>
<td>16.64</td>
<td>20.94</td>
<td>21.11</td>
</tr>
<tr>
<td></td>
<td>(.99)</td>
<td>(1.74)</td>
<td>(1.15)</td>
<td>(1.18)</td>
</tr>
<tr>
<td>PreHD (n=73)</td>
<td>P14</td>
<td>N20</td>
<td>P25</td>
<td>N33</td>
</tr>
<tr>
<td></td>
<td>V1</td>
<td>V3</td>
<td>V1</td>
<td>V3</td>
</tr>
<tr>
<td></td>
<td>15.18</td>
<td>15.50</td>
<td>19.87</td>
<td>19.82</td>
</tr>
<tr>
<td></td>
<td>(1.27)</td>
<td>(1.20)</td>
<td>(1.20)</td>
<td>(1.40)</td>
</tr>
<tr>
<td>Early HD (n=17)</td>
<td>P14</td>
<td>N20</td>
<td>P25</td>
<td>N33</td>
</tr>
<tr>
<td></td>
<td>V1</td>
<td>V3</td>
<td>V1</td>
<td>V3</td>
</tr>
<tr>
<td></td>
<td>16.05</td>
<td>15.30</td>
<td>19.99</td>
<td>19.87</td>
</tr>
<tr>
<td></td>
<td>(1.43)</td>
<td>(1.14)</td>
<td>(1.51)</td>
<td>(.99)</td>
</tr>
<tr>
<td>Healthy controls (n=76)</td>
<td>P14</td>
<td>N20</td>
<td>P25</td>
<td>N33</td>
</tr>
<tr>
<td></td>
<td>V1</td>
<td>V3</td>
<td>V1</td>
<td>V3</td>
</tr>
<tr>
<td></td>
<td>15.19</td>
<td>15.67</td>
<td>19.69</td>
<td>19.87</td>
</tr>
<tr>
<td></td>
<td>(1.02)</td>
<td>(1.31)</td>
<td>(.93)</td>
<td>(1.20)</td>
</tr>
</tbody>
</table>

In Visit 3 the latencies were 49.43ms (1.39), 49.96ms (2.35), 51.68ms (2.38), and 49.16ms (2.01).

For the mean MEP latency, Visit 1 presented itself as follows: 23.03ms (1.39) for the converter group, 23.12ms (1.51) for the healthy controls, 23.55ms (1.29) for the preHD group, and 22.48ms (1.26) for the early HD group. In the same order, latencies for Visit 3 are 21.98ms (1.17), 21.16ms (5.19), 22.21ms (.84), and 21.92ms (1.66).

rmANOVAs were calculated for each latency variable separately. Latencies were similar in all groups.
3.3. Motor thresholds and Motor Evoked Potential

Group averages and standard deviations can be taken from Table 7.

A rmANOVA including the within-subjects factors “Visit” (V1 vs V3) and “Intensity” (RMT vs AMT), as well as the between-subjects factor “Group” (peri vs pre vs early vs control) revealed a significant main effect “Threshold” ($F_{1, 42} = 488.542; p < .001; \eta_p^2 = .921$). A post-hoc paired-sample t-Test showed that RMT was significantly larger than AMT over all groups ($t_{49} = 10.917; p < .001$).

The average MEP size at 150%RMT in mVms were 6.97 (4.75) in the converter group, 10.02 (6.93) in the healthy control group, 7.61 (5.76) in the preHD group, and 8.46 (3.37) for the early HD group. In Visit 3 this changed to 4.36 (2.46), 8.17 (6.49), 6.49 (4.45), and 8.21 (5.87) for the converter, control, pre and early HD group, respectively.

A rmANOVA did not find any significant group differences or interactions.

Table 7: Averages and standard deviations of the TMS motor thresholds at rest and active in %maximum stimulator output. RMT: resting motor threshold; AMT: active motor threshold. V1: Visit 1; V3: Visit 3; preHD: premanifest Huntington’s Disease gene carrier; HD: Huntington’s Disease.

<table>
<thead>
<tr>
<th></th>
<th>RMT</th>
<th>AMT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V1</td>
<td>V3</td>
</tr>
<tr>
<td>converter (n=10)</td>
<td>41.00 (8.00)</td>
<td>41.78 (7.41)</td>
</tr>
<tr>
<td>preHD (n=63)</td>
<td>42.82 (7.26)</td>
<td>43.73 (5.12)</td>
</tr>
<tr>
<td>early HD (n=11)</td>
<td>44.63 (9.74)</td>
<td>46.88 (9.05)</td>
</tr>
<tr>
<td>healthy controls (n=51)</td>
<td>46.22 (8.56)</td>
<td>46.94 (6.67)</td>
</tr>
</tbody>
</table>
3.4. Intraindividual changes around symptom onset

We then concentrated on the converter group, whether we could see an influence of symptom onset on central somatosensory processing, i.e. the N20 amplitude. Here, we tested the participants developing symptoms between visits 1 and 2 against those developing symptoms between visits 2 and 3. A rmANOVA with the within-subjects factor “Visit” and the between-subjects factor “Group” showed a significant main effect “Visit” (F_{2,36} = 18.223; p > .001; \eta^2_p = .503) at 150%MT stimulation with Visit 1 differing from Visits 2 (p = .040) and 3 (p > .001), and Visits 2 and 3 from each other (p > .001). There was no main effect “Group”, nor any significant interaction, meaning that in both groups, there was a comparable significant reduction of amplitude over the observation period. Figure 5 depicts the N20 amplitude at the three study visits for participants developing symptoms between Visits 1 and 2 and participants developing symptoms between Visits 2 and 3.

![Figure 5: N20 amplitude group means and standard errors in converters for Visits 1, 2 and 3 in µV. Converter group with motor symptom onset between Visits 1 and 2 displayed in blue (circles), converter group with motor symptom onset between Visits 2 and 3 displayed in green (squares).](image)
Looking at intraindividual changes close to symptom onset, participants showed a mean slope of -.59 (SD .63) between the pre and post symptom onset study visits. The mean slope using the remaining visit was -.17 (SD .15). The paired-samples t-test showed a significant difference between the two ($t_{19} = -2.66; p = .016$), indicating a steeper slope around symptom onset than in a similar time frame before or after symptom onset.

### 3.5. Predicting symptom onset

Participants with preHD who progressed to DCL 4 showed no significant differences compared with those who did not reach that threshold (Table 8). In our Cox hazard models, neither N20, nor P14 slopes served as significant predictors, both with and without correction for the combined CAG repeat length and age risk.

#### Table 8: Electrophysiological predictors of disease onset in premanifest Huntington’s Disease.

Data are from the comparison of participants with premanifest HD who progressed to a diagnostic confidence score of 4 compared with those who did not reach this threshold. CI: Confidence Interval; *Wald $\chi^2$ value (1df): test statistic for the significance of the predictor in a survival model, with and without adjustment for age x CAG repeat length; $^1$: For a 1 unit increase in the predictor (in the direction expected a priori to represent greater risk). Ratios are in terms of actual units of measurement for each variable. $^2$: Cag repeat length x age was predictive of disease onset ($p = .030$, 3 df).

<table>
<thead>
<tr>
<th></th>
<th>Predictive value before age x CAG length adjustment*</th>
<th>Predictive value after age x CAG length adjustment*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hazard ratio (95% CI) $^1$   p value</td>
<td>Hazard ratio (95% CI) $^2$   p value</td>
</tr>
<tr>
<td>Amplitude N20 slope</td>
<td>2.229 (.858 to 5.789) .10</td>
<td>1.988 (.779 to .5.070) .150</td>
</tr>
<tr>
<td>Amplitude P14 slope</td>
<td>1.159 (.843 to 1.594) .363</td>
<td>1.172 (.841 to 1.634) .349</td>
</tr>
</tbody>
</table>
The N20 amplitude slope showed a tendency towards a significant prediction and with a hazards ratio of approximately 2, irrespective of whether controlling for CAG repeat length and age, meaning that by each unit the slope increases, i.e. the SEP decreases (as we multiplied the slopes by -1), the diagnose is about twice as likely.
4. Discussion

4.1. Summary of the main results

In this longitudinal analysis electrophysiological data was used to investigate changes in the functioning of the sensorimotor system over time and to examine whether those variables can predict the event of motor symptom onset in HD. We compared 73 premanifest and 17 manifest HD gene carriers with 23 gene carriers developing motor symptoms over the course of this study and 76 healthy control participants. As electrophysiological variables we used median nerve somatosensory evoked potentials (SEPs), long-latency reflexes (LLRs), motor thresholds and motor-evoked potentials (MEPs), as those showed stable results in our reliability analysis, conducted prior.

The following are the main results of this analysis which will be discussed more thoroughly below:

1. Neuronal recruitment in the sensory part of the network proved more sensitive to disease progression than in the motor part, as some SEP components’
amplitudes did show differences in accordance to group affiliation, whereas MEPs and motor thresholds did not.

2. The latencies of electrophysiological variables seem to be unaffected at least in this very early part of the disease, indicating that the neurons’ myelin sheath seem mostly intact and the axons are still unaffected by the disease progression.

3. The slope of the N20 SEP component does differ intraindividually depending on motor symptom onset.

4. SEP slopes were, however, no significant predictors for new diagnosis risk.

4.2. Group differences over 24 months

The first aim of this study was to evaluate whether closeness to motor symptom onset influences brain functioning in the sensorimotor network in HD. In rmANOVAs we tested preHD participants, early HD participants, converters and healthy controls for differences in different electrophysiological variables over the course of 24 months.

Those rmANOVAs showed a) a steeper slope between Visits 1 and 3 for HD gene carriers as opposed to controls for the P14 and N20, and b) that resting motor thresholds (RMT) for stimulation with TMS are higher than active motor thresholds (AMT), irrespective of group affiliation. Participant groups were similar in regards to their latencies and motor evoked potentials (MEP).

The steeper slopes in all HD gene carrier groups as opposed to healthy controls indicate an ongoing destructive process in HD gene carriers, irrespective of the stage of the disease. Lefaucheur, et al. (2006) reported a significant decrease of N20 amplitude in manifest HD over 24 months, when testing their slope against 0. This showed that there was a significant decrease in amplitude, but did not take a possible decrease due to e.g. aging processes into account (Hume, et al., 1982). In comparing the slope to a healthy control group, we were able to show that there is not only an ongoing decrease in amplitude, but also that it seems to be due to disease specific factors.
In our sample, also different rates of change depending on the stage of disease became apparent. For the N20, the converter group had a bigger decrease in amplitude than the early HD group and, by tendency, than the preHD group. As our three HD groups are not that far apart in their disease progression, as can be seen in the expected years to onset or CPO, it is worthwhile to note that the development of the N20 amplitude seems to be sensitive for motor symptom onset in a very short time frame.

Furthermore, we found that control and HD gene carriers did not differ in regards to their TMS motor thresholds or the size of the motor evoked potential (MEP) at 150% resting motor threshold (RMT). At threshold intensities, TMS to the motor cortex activates axons of cortical neurons that synaptically excite pyramidal tract neurons. These conduct impulses to the spinal cord where they synaptically activate alpha motoneurons in the ventral horn. So, thresholds depend on the excitability of axon membranes at the site of stimulation and the membrane potential of postsynaptic neurons in the motor cortex and spinal cord (Schippling, et al., 2009). Above threshold, recruitment of MEPs depends strongly on the distribution of both axonal and postsynaptic excitability in the corticospinal system. If there is little difference in the excitability between the most and the least excitable neuron, a small change in stimulus intensity can recruit a bigger number of neurons and thus result in a bigger MEP, than when the excitability varies broadly across the stimulated neuron population.

As we did not see significant differences between the groups either regarding their motor thresholds or their MEP size, this suggests in contrast to Schippling, et al. (2009) findings, that in our case the distribution of axonal thresholds seemed to be similar across all groups. Neither Schippling, et al. (2009), nor Orth, et al. (2010), could find associations of their reported motor abnormalities with the severity of the patients’ motor signs, leading them to the conclusion that motoneurons may not necessarily change as HD advances from the premanifest to the early manifest stage and that reduced motor cortex excitability or plasticity might be more likely to be an intrinsic consequence of abnormal genotype rather than a consequence of symptoms.

Unaffected latencies for the sensory afferent, as well as the motor efferent pathways indicate normal myelin sheathing of the axons of the neurons involved. Apart from individual-related factor like age, sex, temperature, or a person’s height, the most relevant factor influencing nerve conduction velocity is demyelination (Busch and Schulte-Mattler,
In other neurodegenerative diseases like amyotrophic lateral sclerosis (ALS) distal motor latencies and slowing of conduction velocity worsens as the severity of muscle weakness increases. Both symptoms are consistent with the axonal degeneration occurring in ALS patients (Joyce and Carter, 2013). Slowing of SEP latencies has also been described in Guillain-Barré syndrome, a peripheral neuropathy involving the degeneration of myelin sheathing (Parry and Steinberg, 2007), and diabetes mellitus (Kanazawa, et al., 2013).

In Huntington's disease there are conflicting reports regarding latency variables of the sensory and motor pathways. For SEP latencies, there are reports on normal (Noth, 1985; Oepen, et al., 1982) and pathological latencies (Abbruzzese, et al., 1990; Ehle, et al., 1984; Josiassen, et al., 1982; Takahashi, et al., 1972). LLR latencies were found to be unaffected in HD (Deuschl, et al., 1989). Meyer, et al. (1992) described a higher variance in MEP latency between trials when compared to the variability in healthy participants. They rejected demyelination in the descending motor tracts, influence of the non-systematic fiber degeneration in the spinal cord, and anterograde degeneration of the cortico-spinal tract as possible explanations for their findings, but postulated that abnormalities result from a functional rather than a structural change, which alters the excitability at the motor cortical or spinal level.

Taking together this first part of our results, it seems that the sensory part of the sensorimotor network is more sensitive to mirroring disease progression, as previously reported motor abnormalities seem rather unaffected by disease progression in our sample. Furthermore, the myelin sheathing seems to be intact whereas the axonal excitability, i.e. the synapses, in the sensory system might be more vulnerable.

4.3. Intraindividual changes around symptom onset

For the closer inspection of processing close around symptom onset, we decided to use only the SEP data, as we are missing data from the Leiden study site for the other variables and this would have meant a loss in power when splitting the already small converter group according to symptom onset and rather unreliable results.
When applying a rmANOVA to the N20 amplitude we found a significant decrease in N20 amplitude over 24 months in both groups. There was no main effect for the between-subjects factor “Group”, nor a significant interaction. We attribute that to the fact that the subject number was too small and that of the three visits compared, two were very much alike in the two different groups and only Visit 2 showed a real difference.

However, when comparing the slope of the study visits around symptom onset with the slope between the other visits, there was a significant difference in the sense that a steeper slope or greater change occurs in the 12 months surrounding motor symptom onset than a comparable time frame pre or post symptom onset.

Lefaucheur, et al. (2006) also looked at SEP slopes longitudinally over 24 months in manifest HD participants. They report an average slope of -0.43 for the development of the N20, which, when tested against 0, indicates a significant decrease of amplitude. Our converter group had average slopes of -0.59 and -0.17 for the visits surrounding symptom onset and a comparable time frame, respectively. Our overall average falls roughly in Lefaucheur’s dimension.

Our results indicate that the previously reported steeper decline does indeed occur close before symptom onset, as measured in SEPs in this case, but that this “acceleration” of deterioration might be time-limited and doesn’t seem to continue after symptom onset.

What might have contaminated our data, though, is that we used the combined slope of pre and post symptom onset for the comparison. This might have diluted a steeper slope post symptom onset.

4.4. Predicting symptom onset

Disease progression can be described using group comparisons, slopes of progression or the prediction of an event, e.g. motor symptom onset. In comparing gene carrier groups at different disease stages, we found that the rate of change in SEP amplitude differs depending on disease stage and that converters showed the steepest slopes. We could also find those steeper slopes when comparing rates of change intraindiviually. These first results made the SEP amplitudes a promising candidate for
predicting symptom onset as they proved sensitive enough to show inter- and intraindividual differences.

The time-dependent N20 slope showed a tendency towards predicting a new diagnose of manifest HD. A slope of -1 would result in twice the risk to develop motor symptoms. As the average slope in our converter group around symptom onset was just half that size, the average amplitude about 1.8µV and both with a very high variability, this trend seems to be the result of a few participants whose SEP amplitudes were higher to begin with.

A problem in this prediction might have been the definition of symptom onset. We recognize that all participants may have shown disease progression over 24-months, but the aim was to use objectively-defined thresholds as defined by traditional clinical measures to distinguish between faster and slower progression. In our case, we defined a UHDRS DCL of 4 as progression to manifest HD. Tabrizi, et al. (2012) also considered any net 24-month decline in TFC, an increase in TMS of 5 or more as progression, but in their follow-up paper (Tabrizi, et al., 2013) came to the conclusion that only DCL was relatively stable over time. For example, 60% participants who had an increase in TMS of 5 or more at 12 months and 36% of those with an increase at 24 months no longer met the TMS criteria for “progression” at 36-months. In contrast, 9 out of 11 preHD participants with DCL 4 at either 12 or 24 months, continued to have DCL 4 at 36 months.

Until now, there are only a moderate number of studies trying to predict symptom onset, which tends to concentrate on imaging or functional imaging data. Tabrizi, et al. (2013) found, longitudinal change of grey matter volume and intertap-interval as candidates for predictors, when controlling for CAG repeat length and age-related risk. Aylward, et al. (2012) identified the putamen as a predictor and Herben-Dekker, et al. (2014) got similar results with low average or abnormal putaminal metabolism.

To our knowledge, there is no research on using electrophysiological variables as predictors for symptom onset in HD. We could show here, that SEP might be sensitive enough to track changes in brain function over time and that the rate of change differs between different stages of disease inter- and intraindividually. At the same time, however, variability in SEP amplitude as well as in change over time is very high, making predictions on a group level unreliable and for an individual patient practically impossible.
4.5. Limitations

4.5.1. Sample size

As in most studies involving a clinical group, our sample size for the converter group is rather small. We did have a larger overall subject pool that we used as control groups. Reasons for the small number of converters in particular and HD study participants in general are discussed below.

For one, Huntington’s is a rare disease, with only 5.7 in 100,000 persons being affected by it in Europe (Pringsheim, et al., 2012). One can imagine that since not everybody affected is interested in participating in research projects, this only leaves an even smaller number of participants available. The characteristic that symptom onset in Huntington’s mostly occurs in mid-adulthood provides a special difficulty in recruiting premanifest gene carriers. Not every child of an affected HD patient actually wants a genetic confirmation of their status, whatever it might be. Also, this premanifest group is less likely to seek out a specialist for HD on a regular basis, making it hard to spread information about upcoming clinical trials or research projects.

Furthermore, considering the TrackOn assessment started with a total of 110 participants in our preHD group in the four study sites, of those 96 completed all three visits, and in the course of twenty-four months only 23 of these participants developed motor symptoms, this gives an idea of how large a sample would have to be recruited to raise the number of converting participants. Of course some of the preHD participants had a short CAG-repeat length and were relatively young, so were considered far from onset and having a low risk to develop symptoms in the next years. Naturally one could argue to only include HD gene carriers close to symptom onset in such study, as they have a higher risk, but there is the question of practicability. Would one just include those HD gene carriers considered close to onset at study recruitment, so a very short time window? Our subject numbers speak against that approach. Or would one wait until preHD participants visiting one’s clinic are considered close to onset? This would result in a very lengthy recruitment process and thus is not an economical solution either.

Lastly, as in any other longitudinal study we did have dropouts. Reasons given to drop out of the whole study included a busy work schedule, a move to another city, and personal reasons. In the electrophysiology assessment, we for one had a case of allergic
reaction to the tape for attaching the recording EMG electrodes and changes in medication that excluded participants from this study module. The 23 converters included here completed all three study visits; all other groups used in the rmANOVA analysis completed at least Visits 1 and 3. The whole study was demanding for the participants as assessments did last a whole day and some had to travel far to the study sites forcing them to take leave from work and maybe even stay at a hotel for two nights. Of course the participants were reimbursed for their travel expenses, but it still took them a lot of effort to participate.

4.5.2. Differences between study sites

Resulting from the problem of passable sample size, this study included participants from four countries being examined at four study sites. We created an almost step-by-step protocol with suggestions on how to brief the participant for each paradigm and held a training meeting before the study commenced. This way we sought to minimalize the influence of the different examiners. If applied correctly electrophysiological paradigms are quite objective as they do not require a subjective opinion or rating from the examiner.

What we only had limited influence on was the equipment used in the different sites. As mentioned in the Methods section, the sites differed in hardware and software used. Since all of the manufacturers are known providers for medical research tools, we do not expect vast differences in data processing, but one cannot exclude that a mixture of both the technical and the human factor are responsible for the significant differences we found between the sites. Including site as a cofactor was a way to handle this problem. It would have been preferable to avoid differences in the first place, but for getting decent sample sizes in clinical groups participants recruitment at multiple study sites is almost inevitable and purchasing new study equipment when comparable hard- or software is available on site is not economic.
4.6. Future Directions

Disease pathology can occur on three levels: structure, function and behavior. In Huntington’s Disease (HD) all of these domains are affected to some extent at some point during disease progression.

In this data analysis, we tried to assess whether electrophysiological variables are sensitive enough to mirror disease progression or even predict motor symptom onset.

We were able to show that, at least SEPs can be used to track disease progression, but not predict symptom onset. Next steps following in this path might be for one, not only recording the SEPs from one scalp electrode, but maybe use an electrode cap or include the peripheral parts of the sensory afferent tract to examine abnormalities in the SEPs more carefully.

Another approach could be the combination of assessment techniques, especially using imaging or functional imaging data. This combination will enable enhanced information on spatial localization of brain structures as well as precise time course of neural activity in HD. This will not only accelerate our understanding of mechanisms associated with disease onset and progression but could also identify sensitive biomarkers to test efficacy of therapeutic intervention.

Realizing this combined recording might, however, prove difficult for the recording of SEP because of the peripheral nerve stimulator’s construction. But for illustrating the relationship between brain structure and function, also the successive recording of both domains is helpful for correlations or regression models. As imaging data was part of TrackOn HD, the next step in this particular case would be to see whether our reported changes in the SEP amplitude can be replicated in imaging variables and whether there is a significant association between those and the SEP development.

What is possible, on the other hand, is the recording of fMRI while stimulating the brain with TMS. Although we did not find any group differences in regard to motor thresholds or motor evoked potentials (MEPs), results in this field seem to be rather controversial (Orth, et al., 2010; Schippling, et al., 2009). So, fMRI or diffusion tensor imaging (DTI) might help shed some light on the areas stimulated, the connectivity between areas or the order of networks activated. Even the combined recording of TMS
and EEG could be an alternative here. Of course, it doesn’t allow the spatial resolution like imaging techniques, but this combination of methods offers the better temporal resolution and it might be less unpleasant for study participants.

Also, more large-scale multisite longitudinal studies are needed to further understand the development of the sensorimotor system in HD. The 24-month TrackOn HD study already was able to deliver some insights, in this particular data analysis on how somatosensory processing is altered depending on disease. But more study visits, either spaced more closely together or following the participants for a longer time might lead to a better understanding for processes closely surrounding symptom onset or increasing the sample size for preHD participants converting to the early HD stage.

Reliability of data is important especially for longitudinal studies, where e.g. a clinical group is compared to healthy controls and changes are expected in the clinical group, whereas data should remain relatively stable in the controls. After data collection, we calculated intraclass correlation coefficients (ICCs) with our healthy controls’ electrophysiological data, to see which of the variables are actually reliable in a longitudinal comparison. Naturally, this procedure might lead to a loss of data, if some variables prove to be non-reliable, but only that way sound conclusions can be drawn.
4.7. Conclusion

We used electrophysiological data to track longitudinal changes in brain function of the sensory afferent and motor efferent pathways in Huntington’s Disease (HD) and identify variables sensitive enough to a) mirror disease progression, and b) predict motor symptom onset.

We found sensitivity for change in somatosensory rather than motor variables. This indicates that sensory afferent pathways might be more affected by ongoing changes in brain structure and more precise to track development than motor efferent pathways.

In short, we were able to replicate findings of reduced SEP amplitudes and deterioration over time in HD gene carriers, with the steepness of the slope differing inter- and intra-individually depending on closeness to motor symptom onset. Also this would have made the SEPs a good candidate for predicting motor symptom onset, variability in amplitude and slope might be too high for reliable predictions on an individual level.

Our non-significant results for the motor efferent pathway from the TMS data offer a new argument that motoneurons may not necessarily change as HD advances from the premanifest to the early manifest stage and that reported reduced motor cortex excitability or plasticity might be more likely to be an intrinsic consequence of abnormal genotype rather than a consequence of symptoms. Normal latencies indicate no significant demyelination processes of the neurons’ axons in these stages of the disease.
5. Summary

Huntington’s Disease (HD) is an autosomal dominant hereditary progressive neurodegenerative disorder with pathologies occurring on structural, functional and behavioral levels in the course of the disease.

Since visible symptoms only begin to occur in mid-adulthood, being able to predict motor symptom onset would be valuable for clinical use, to predict for individual patients when diagnosable symptoms will occur, as well as for research, e.g. clinical trials where diagnosis of HD is an outcome measure. Most of the work on this topic has been done using structural and functional brain imaging data and here, the putamen seems to provide the most promising results. But also the caudate, striatum and grey-matter volume are candidates.

Being interested in whether closeness to motor symptom onset had an influence on somatosensory processing, we identified 23 participants who, in the 24-month observation period of the multisite TrackOn HD study, progressed from the premanifest to the early
manifest disease stage (converters) and compared their data to 73 premanifest participants who did not convert, 17 early manifest HD participants and 76 healthy controls.

As progression to the manifest stage of Huntington’s is most commonly defined by the onset of motor symptoms, our aim was to track changes in the sensorimotor network close around and identify predictors for motor symptom onset using electrophysiology.

In the four study sites in London, Leiden, Vancouver and Paris we performed paradigms examining the sensory afferent as well as the motor efferent pathways of the sensorimotor system, like somatosensory evoked potentials (SEPs) of the median nerve, long-latency reflexes (LLRs), as well as motor thresholds at rest and active and motor evoked potentials (MEPs) after stimulating the motor cortex with transcranial magnetic stimulation (TMS).

We found that over the 24-month observation period, all HD gene carrier groups had a stronger deterioration in SEP amplitude than healthy controls. Within the HD gene carriers, the converter group showed a steeper deterioration than the early manifest group and tended towards having a steeper decline than premanifest group. Groups were similar in regards to their latencies, motor thresholds and MEPs.

When comparing slopes intraindividually in the converter group, we were able to show that SEP deterioration was steeper around symptom onset than it was in a comparable timeframe before or after onset.

Even though these results indicate that SEPs are sensitive for tracking disease progression inter- and intraindividually, we were unable to predict motor symptom onset from the N20 slope.

Summarizing our results, we found sensitivity for disease related change in the sensorimotor network in somatosensory rather than motor variables. This indicates that sensory afferent pathways might be more precise to track development than motor efferent pathways. But variability in amplitude as well as slope might be too high to reliably predict motor symptom onset.
6. Literature


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6. Literature


Curriculum Vitae

Der Lebenslauf wurde aus Gründen des Datenschutzes entfernt.