NEURAL CORRELATES AND PROGRESSION OF SACCADE IMPAIRMENT IN PREMANIFEST AND MANIFEST HUNTINGTON DISEASE

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To Heather, Vincent, Gabriel, Isabelle, 
and my parents
ACKNOWLEDGEMENTS

As is always the case with such endeavors, a large number of people contributed in immeasurable ways to a successful training experience. None of the research we have accomplished is possible without the participation of the families with HD, and I gratefully acknowledge their commitment to the effort. Very early in my training, I met a woman who had recently been diagnosed with the disease, and she was struggling to understand what it meant. As we spoke she seemed to question whether she could even consider herself the same person she had been before the diagnosis. This was the first time that, from my perspective, the disease left the pages of a textbook and became a real concern to a real person, and I am thankful for that experience. More recently I had a chance acquaintance with a man whose wife had been diagnosed. This was an instance where there had been no previously known family history of the disease, and it was quite a shock. As we began talking, I was so excited to share all the things I had learned about the disease, but I quickly realized the inappropriateness of such a response. While he had first-hand experience with academic research and would have enjoyed such a conversation in many other circumstances, his current experience was not academic. His was an experience of uncertainty, of trying to find and pay for medications, of living one day at a time. To those who have participated in this study, who have shared their stories, I thank you.

Dr. Tatiana Foroud has undoubtedly shaped the course of my career and my life. I have learned many things from her, but two stick out. The first is that it’s never too late to change the Specific Aims of a grant, and the second is that sometime you just have to leave work and go to your kid’s parade. These anecdotes capture the type of dedication that I try to emulate; not a dedication that sacrifices everything else, rather one that is committed to the task at-hand but that requires constant re-prioritization. If I learned nothing else from her (and I did!), this would have made me a better person.

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unenviable task of shepherding me, a genetics student, through a project with considerable neuroscience objectives. Though I don’t yet know what a forensic neuropsychologist charges for services, he assures me that the bill is forthcoming. Dr. Andrew Saykin has taken an interest in the imaging project from its conception and has freely offered his lab’s resources and his own expertise. Dr. P. Michael Conneally is one of the forerunners of HD research at Indiana University, and it has been a privilege to see what his foresight has meant to the institution and the families living with HD. Dr. Joanne Wojcieszek is the ultimate patient advocate and has always been that voice asking me why this work makes any difference to a physician or a patient; this question has been a persistent reminder of why I followed this track in the first place.

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I must also thank my parents for the unwavering support they have given me. Their devotion to my happiness and success is something that I can only appreciate now that I have kids of my own. They spent hours driving to basketball gyms and baseball fields, and thousands of dollars for the privilege of doing so. They encouraged me without crossing the line into compulsion. They taught me respect by showing me the same. They made sacrifices that a child can never appreciate, and they did not expect me to appreciate those sacrifices. They let me find my own way through life, letting me get lost sometimes, but never too lost. They loved me.

Finally, I thank my wife and kids for their unending patience. Heather never expected to be 30 years old with a husband who still didn’t have a real job; she might make it to 40. Vincent knows that something is a bit strange about his dad, but he’s still trying to figure out what exactly it is. He recently asked me, “Why does everyone else’s dad go to work but you go to school?” I often have peers who, when they find out that I have a wife and children, ask how I manage to handle everything. In fact, my family makes it easier. Their smiles, laughs, and hugs make sure that I focus on the most important things in my life, and they provide unfailing support that I could never find elsewhere.
ABSTRACT

Jason Douglas Rupp

NEURAL CORRELATES AND PROGRESSION OF SACCade IMPAIRMENT IN PREMANIFEST AND MANIFEST HUNTINGTON DISEASE

Huntington disease (HD) is an autosomal dominant disorder characterized by progressive decline of motor, cognitive, and behavioral function. Saccades (rapid, gaze-shifting eye movements) are affected before a clinical diagnosis of HD is certain (i.e. during the premanifest period of the disease). Fundamental questions remain regarding the neural substrates of abnormal saccades and the course of premanifest disease. This work addressed these questions using magnetic resonance imaging (MRI) and a longitudinal study of premanifest disease progression.

Gray matter atrophy is a characteristic of HD that can be reliably detected during the premanifest period, but it is not known how such changes influence saccadic behavior. We evaluated antisaccades (AS) and memory guided saccades (MG) in premanifest and manifest HD, then tested for associations between impaired saccadic measures and gray matter atrophy in brain regions involved in these saccadic tasks. The results suggest that slowed vertical AS responses indicate cortical and subcortical atrophy and may be a noninvasive marker of atrophic changes in the brain.
We also investigated the brain changes that underlie AS impairment using an event-related AS design with functional MRI (fMRI). We found that, in premanifest and manifest HD, blood oxygenation level dependent (BOLD) response was abnormally absent in the pre-supplementary motor area and dorsal anterior cingulate cortex following incorrect AS responses. These results are the first to suggest that abnormalities in an error-related response network underlie early disease-related saccadic changes, and they emphasize the important influence of regions outside the striatum and frontal cortex in disease manifestations.

Though saccadic abnormalities have been repeatedly observed cross sectionally, they have not yet been studied longitudinally in premanifest HD. We found different patterns of decline; for some measures the rate of decline increased as individuals approached onset, while for others the rate was constant throughout the premanifest period. These results establish the effectiveness of saccadic measures in tracking premanifest disease progression, and argue for their use in clinical trials.

Together, these studies establish the utility of saccade measures as a marker of HD neurodegeneration and suggest that they would be a valuable component of batteries evaluating the efficacy of neuroprotective therapies.

Tatiana Foroud, PhD, Chair
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<th>Description</th>
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<tbody>
<tr>
<td>ACC</td>
<td>Anterior cingulate cortex</td>
</tr>
<tr>
<td>ANCOVA</td>
<td>Analysis of covariance</td>
</tr>
<tr>
<td>AS</td>
<td>Antisaccade</td>
</tr>
<tr>
<td>BDNF</td>
<td>Brain derived neurotropic factor</td>
</tr>
<tr>
<td>BOLD</td>
<td>Blood oxygenation level dependent</td>
</tr>
<tr>
<td>cACC</td>
<td>Caudal anterior cingulate cortex</td>
</tr>
<tr>
<td>CAG-</td>
<td>Unexpanded number of CAG repeats (≤27 repeats)</td>
</tr>
<tr>
<td>CAG+</td>
<td>Expanded number of CAG repeats (≥39 repeats)</td>
</tr>
<tr>
<td>CES-D</td>
<td>Center for Epidemiologic Studies Depression Scale</td>
</tr>
<tr>
<td>cMFG</td>
<td>Caudal middle frontal gyrus</td>
</tr>
<tr>
<td>CVLT</td>
<td>California Verbal Learning Test</td>
</tr>
<tr>
<td>dACC</td>
<td>Dorsal anterior cingulate cortex</td>
</tr>
<tr>
<td>DLPFC</td>
<td>Dorsolateral prefrontal cortex</td>
</tr>
<tr>
<td>DTI</td>
<td>Diffusion tensor imaging</td>
</tr>
<tr>
<td>ERN</td>
<td>Event related negativity</td>
</tr>
<tr>
<td>ERP</td>
<td>Event related potential</td>
</tr>
<tr>
<td>FEF</td>
<td>Frontal eye fields</td>
</tr>
<tr>
<td>fMRI</td>
<td>Functional magnetic resonance imaging</td>
</tr>
<tr>
<td>FXS</td>
<td>Fragile X syndrome</td>
</tr>
<tr>
<td>FXTAS</td>
<td>Fragile X-associated tremor ataxia syndrome</td>
</tr>
<tr>
<td>GPe</td>
<td>Globus pallidus externa</td>
</tr>
<tr>
<td>GPI</td>
<td>Globus pallidus interna</td>
</tr>
<tr>
<td>HD</td>
<td>Huntington disease</td>
</tr>
<tr>
<td>ICV</td>
<td>Intracranial volume</td>
</tr>
<tr>
<td>IFG</td>
<td>Inferior frontal gyrus</td>
</tr>
<tr>
<td>IPL</td>
<td>Inferior parietal lobule</td>
</tr>
<tr>
<td>IPS</td>
<td>Intraparietal sulcus</td>
</tr>
<tr>
<td>MG</td>
<td>Memory guided saccade</td>
</tr>
<tr>
<td>MG1</td>
<td>Memory guided, simple task (see MGs)</td>
</tr>
<tr>
<td>MG2</td>
<td>Memory guided, complex task (see MGc)</td>
</tr>
<tr>
<td>MGc</td>
<td>Memory guided, complex task</td>
</tr>
<tr>
<td>MGi</td>
<td>Memory guided, intermediate task</td>
</tr>
<tr>
<td>MGs</td>
<td>Memory guided, simple task</td>
</tr>
<tr>
<td>MNI</td>
<td>Minnesota Neurological Institute</td>
</tr>
<tr>
<td>MPRAGE</td>
<td>Magnetization prepared rapid gradient echo</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>MT</td>
<td>Movement time</td>
</tr>
<tr>
<td>MTG</td>
<td>Middle temporal gyrus</td>
</tr>
<tr>
<td>NC</td>
<td>Controls (see CAG-)</td>
</tr>
<tr>
<td>PCC</td>
<td>Posterior cingulate cortex</td>
</tr>
<tr>
<td>PEF</td>
<td>Parietal eye fields</td>
</tr>
<tr>
<td>PFC</td>
<td>Prefrontal cortex</td>
</tr>
<tr>
<td>PolyQ</td>
<td>Polyglutamine tract</td>
</tr>
<tr>
<td>PreHD</td>
<td>Premanifest HD</td>
</tr>
<tr>
<td>Pre-SMA</td>
<td>Pre-supplementary motor area</td>
</tr>
<tr>
<td>PS</td>
<td>Prosaccade</td>
</tr>
<tr>
<td>rACC</td>
<td>Rostral anterior cingulate cortex</td>
</tr>
<tr>
<td>rMFG</td>
<td>Rostral middle frontal gyrus</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of interest</td>
</tr>
<tr>
<td>RT</td>
<td>Reaction time</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
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</tr>
<tr>
<td>SDMT</td>
<td>Symbol Digit Modalities Test</td>
</tr>
<tr>
<td>SEF</td>
<td>Supplementary eye fields</td>
</tr>
<tr>
<td>SFG</td>
<td>Superior frontal gyrus</td>
</tr>
<tr>
<td>SMA</td>
<td>Supplementary motor area</td>
</tr>
<tr>
<td>SNpr</td>
<td>Substantia nigra pars reticulata</td>
</tr>
<tr>
<td>SPL</td>
<td>Superior parietal lobule</td>
</tr>
<tr>
<td>STN</td>
<td>Subthalamic nucleus</td>
</tr>
<tr>
<td>TTO</td>
<td>Time to onset</td>
</tr>
<tr>
<td>UHDRS</td>
<td>Unified Huntington Disease Rating Scale</td>
</tr>
<tr>
<td>WAIS-R</td>
<td>Weschler Adult Intelligence Scale - Revised</td>
</tr>
</tbody>
</table>
I. Introduction

A. Genetics of Huntington disease

Huntington disease (HD) is an autosomal dominant disorder caused by an abnormal expansion of a CAG trinucleotide repeat in exon 1 of the huntingtin gene.¹ Current recommendations (Table 1) suggest that thirty-five or fewer CAG repeats should be considered normal (CAG-), and that more than 35 repeats in one allele can lead to disease² (CAG+). However, some additional nuances should be noted. First, individuals with 27-35 repeats, while not at risk for developing the disease, have an increased risk of further expansion during meiosis and, thus, passing on a disease-causing allele to their children. However, some recent reports indicate that HD may result from alleles with between 29 and 35 repeats,³-⁶ though these cases appear to be rare. Second, there is reduced penetrance of the disease in individuals with 36-39 repeats,⁷ while the disease is fully penetrant in those with more than 39 repeats.

The identification of the gene and responsible mutation makes presymptomatic gene testing possible. Prior to the availability of testing, 40-80% of at-risk individuals expressed intent to use the test once it became available,⁸ though the actual use is only 10% in Indiana⁹ and ranges from 4-24% worldwide.¹⁰ Among those who did seek testing, the most common reasons cited were to relieve the anxiety associated with uncertainty, to plan for the future (including family planning), and to inform their children.¹⁰-¹² Perhaps not surprisingly, both positive (CAG expansion present) and negative (CAG expansion not present) test results lead to reports of distress, though at different times.¹⁰ Distress immediately followed positive tests, but was experienced at

Table 1. Association between CAG repeats and clinical outcomes.

<table>
<thead>
<tr>
<th>Number of CAG Repeats</th>
<th>Clinical Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 27</td>
<td>No disease</td>
</tr>
<tr>
<td>27 – 35</td>
<td>No disease, possibly expanded in offspring</td>
</tr>
<tr>
<td>36 – 39</td>
<td>Possible disease (reduced penetrance)</td>
</tr>
<tr>
<td>&gt; 39</td>
<td>Disease (full penetrance)</td>
</tr>
</tbody>
</table>
around 6 months following negative tests. By one year post-testing, distress in those who had received a positive test had returned to baseline, while a slight decrease in distress was found in those with a negative test. One important caveat is that individual responses will greatly vary, and plans should be put in place early for both pre- and post-testing counseling.  

The genetic phenomenon of anticipation is seen in HD. Anticipation is the finding that disease symptoms occur earlier in subsequent generations. One important observation in understanding the mechanisms of anticipation in HD is that the size of the CAG expansion explains about 70% of the variability in age of disease onset. A second observation that helps to explain anticipation in HD is that the size of the repeat tends to expand in abnormally large alleles. Zühlke et al. found a change in repeat size in 72% of 54 transmissions involving an expanded allele (>40 repeats), while only 0.5% of 431 normal allele transmissions resulted in a change. The changes observed in the expanded allele were classified as either small variations (±3 repeats) or large expansions (>4 repeats). While the percentage of altered transmissions was the same in both the maternal and paternal lines, all 10 large expansions (4-28 repeats) resulted from paternal transmission. While studies in mice suggest that this expansion occurs in post-meiotic cells, a post-mortem human study found evidence of substantial pre- and post-meiotic expansion. Interestingly, post-mitotic expansion of the CAG repeat in striatal neurons has been described in mouse models and may help explain some of the variability seen in HD.

The pathogenic repeat expansion is not unique to HD; at least 18 other nucleotide repeat expansion disorders have been identified. Most involve trinucleotide repeats, though tetra- and pentanucleotide repeat sequences can also be pathologically expanded. As is the case in HD, larger expansions are more unstable and thus produce even larger expansions, resulting in anticipation. The expansion seems to occur through a mechanism that involves stalling and restarting of the replication fork. Though it is not known why, all 9 CAG repeat expansion disorders are neurodegenerative. The pathogenic mechanisms of disease include a loss of function at the protein level and a gain of
function at either the RNA or protein level. HD is an example of protein gain of function and will be discussed later. The other mechanisms are exemplified by the \textit{FMRI1} gene that leads to fragile X syndrome (FXS) or fragile X-associated tremor ataxia syndrome (FXTAS) depending on the number of CGG repeats. A normal allele has 5-55 repeats in the 5’ untranslated region of the gene. If there are more than 200 repeats the gene is transcriptionally silenced via methylation and deacetylation. Fewer transcripts leads to a loss of protein, and the clinical result is FXS.\textsuperscript{21,22} However, if the expansion is in an intermediate range (55-200 repeats), a toxic mRNA is transcribed. This mRNA binds proteins important for post-transcriptional modification and sequesters them in intranuclear inclusions,\textsuperscript{23,24} leading to the clinically distinct disease FXTAS.

While modifier genes do not appear to affect the presence or absence of HD, they have been shown to affect the symptoms and progression of the disease. One such modifier is the normal allele of the \textit{huntingtin} gene. Aziz et al.\textsuperscript{25} found that, in individuals with a relatively small number of repeats in the expanded allele (closer to 40), age of onset was delayed and the severity of motor and cognitive symptoms was reduced when the normal allele was relatively small (closer to 10); when the number of repeats in the expanded allele was large, age of onset was delayed and symptom severity was reduced when the normal allele was relatively large. Eight other genes have also been proposed to influence age of onset (\textit{GRIK2},\textsuperscript{26-29} \textit{APOE},\textsuperscript{30,31} \textit{TCERG1},\textsuperscript{32} \textit{UCHL1},\textsuperscript{29} \textit{TP53},\textsuperscript{33} \textit{DFFB},\textsuperscript{33} \textit{GRIN2B},\textsuperscript{34} \textit{GRIN2A}), though a study in a Venezuelan kindred confirmed only the effect of \textit{GRIN2A}.\textsuperscript{35}

\section*{B. Function of huntingtin protein}

The CAG repeat is translated into a poly-glutamine (polyQ) tract in the huntingtin protein. Unfortunately, the mechanism by which this polyQ tract produces disease is unknown. Most studies point to a toxic gain of function in the mutant protein,\textsuperscript{36-44} though haploinsufficiency may also play a role in the development of symptoms.\textsuperscript{44}
Wild-type huntingtin appears to play many roles influenced by developmental timing, cell type, and intracellular location.\textsuperscript{45} The protein is quite large (3144 amino acids, 348 kD), though it does not share sequence homology with other proteins.\textsuperscript{45} Furthermore, it contains only a few known sequence motifs and no structural domains with known function.\textsuperscript{45} Huntingtin is ubiquitously expressed in neural and non-neural tissues, with highest expression in neurons and the testes.\textsuperscript{46-48} A double-knockout is embryonic lethal in mouse models prior to gastrulation.\textsuperscript{38-40} However, there are no apparent developmental defects in individuals homozygous for the expansion,\textsuperscript{49,50} suggesting that the polyQ tract does not exert its deleterious effects during the earliest stages of development. Following gastrulation, decreased amounts of huntingtin have been shown to adversely affect neurogenesis\textsuperscript{41,51} and maintenance of neuronal identity\textsuperscript{52} in mice, but once again the polyQ tract does not appear to play a role in these functions.

Because HD is neurodegenerative, the role of normal huntingtin in neurons has received particular attention. It appears to have protective effects in response to a variety of apoptotic stimuli including serum deprivation, mitochondrial toxins, death genes, ischemic injury, and excitotoxicity.\textsuperscript{53-55} This property is conferred by the N-terminal 548 amino acids.\textsuperscript{53} Another role for huntingtin in the neuron is that it appears to simulate the production of brain-derived neurotrophic factor\textsuperscript{56,57} (BDNF). BDNF is an important neurotrophin for striatal cells; it is produced in cortical neurons\textsuperscript{58,59} and trafficked along cortico-striatal afferents to striatal targets.\textsuperscript{60-62} BDNF also reduces excitotoxic effects by controlling glutamate release at the cortico-striatal synapse.\textsuperscript{63-66}

Huntingtin also appears to play a role in vesicular trafficking. It facilitates the transport of BDNF and mitochondria along axonal microtubules.\textsuperscript{67,68} The protein also plays a role in endo- and exocytosis at the synaptic terminal; it associates with clathrin via huntingtin-interacting protein 1\textsuperscript{69-72} (HIP-1). Huntingtin also associates with postsynaptic density protein 95 (PSD-95), thereby reducing NMDA-mediated excitotoxic effects.\textsuperscript{73}

Huntingtin has functional nuclear export and nuclear localization signals. Given that the protein is found both in and around the nucleus, it may play a role in transporting
molecules out of the nucleus. Also, the wild-type polyQ tract binds many transcription factors that also contain a glutamine-rich domain. Further supporting the role of huntingtin in transcriptional regulation is the observation of early gene-expression changes in models of HD.

While it is not yet clear how mutant huntingtin causes neurodegeneration, understanding its myriad normal functions can give clues as to potential sources of pathogenesis. Furthermore, it has been observed that huntingtin is cleaved and that the N-terminal fragment accumulates in insoluble aggregates, though it is not clear if these aggregates play a role in neurodegeneration. In summary, the pathogenesis of HD is not clear, though some combination of aggregate formation, excitotoxicity, oxidative stress, and metabolic dysfunction are likely causes.

C. Clinical characteristics of Huntington disease

HD typically has a delayed onset; the average age of disease onset is 40 years, although onset has occurred as early as age 2 and as late as age 80. The size of the CAG expansion is negatively correlated with the age of disease onset and explains up to 70% of the variability of age of onset. This observation has led to the development of models to estimate the number of years prior to onset and the probability of onset within a given number of years. HD progresses steadily until death, typically 10-20 years after diagnosis, which often occurs subsequent to falls, dysphagia, or aspiration.

Diagnosis of HD is made using the Unified Huntington Disease Rating Scale-99 (UHDRS), an instrument that relies heavily on the motor manifestations of the disease. Dentatorubro-pallidoluysian atrophy, Huntington’s disease-like syndromes 1-3, familial prion disease, Friedrich’s ataxia, spinocerebellar ataxias, chorea-acanthocytosis, and Wilson’s disease and other iron-accumulation disorders are phenotypically indistinguishable from HD and must be considered as part of the differential diagnosis, though a gene test showing the CAG expansion in the huntingtin gene is confirmatory. The UHDRS asks the neurologist,
To what degree are you confident that this person meets the operational definition of the unequivocal presence of an otherwise unexplained extrapyramidal movement disorder (e.g., chorea, dystonia, bradykinesia, rigidity) in a person at risk for HD?

0 = normal (no abnormalities)
1 = non-specific motor abnormalities (less than 50% confidence)
2 = motor abnormalities that may be signs of HD (50-89% confidence)
3 = motor abnormalities that are likely signs of HD (90-98% confidence)
4 = motor abnormalities that are unequivocal signs of HD (≥99% confidence)

Before the unequivocal manifestation of HD (response of 4 to the above question), CAG+ individuals are considered to be in the premanifest period of the disease, during which varying degrees of motor, cognitive, and behavioral abnormalities can be detected.

1. Motor abnormalities in Huntington disease

Though it had been described previously, George Huntington’s extensive description of the disease in 1872 led to an enduring association with his name. Huntington focused on the choreic movements, and the disease was referred to as Huntington’s chorea for several years. Chorea can be explained by the preferential loss of indirect pathway neurons in the striatum (Figure 1). This leads to decreased inhibitory output to globus pallidus externa (GPe), increased inhibitory output from GPe to subthalamic nucleus (STN), and subsequent decreased excitatory output from STN to globus pallidus interna (GPi) and substantia nigra pars reticulata (SNpr). Decreased stimulation of GPi/SNpr leads to a loss of

![Figure 1. Basal ganglia signaling pathways.](image)
inhibitory output to the thalamus, which in turn excites the cortex and produces unwanted motor output.

While chorea continues to play an important role in diagnosis, it is not a reliable marker of disease severity. This is partly explained by the observation that chorea often lessens in the late stages of the disease as dystonia and rigidity become more prominent features of HD. Other motor abnormalities include incoordination and impersistence (e.g. the inability to maintain the force of a voluntary muscle contraction). In the premanifest period, the loss of fine motor skills and delayed reaction times are prevalent.

Ocular motor abnormalities, particularly saccadic abnormalities, are also a common feature of HD and have been noted for a number of years. Saccades are rapid eye movements that shift gaze from one location to another. The UHDRS includes a qualitative evaluation of saccade initiation and velocity wherein the neurologist scores performance on a five-point scale. Studies have shown that individuals with HD require blinks or head movements to facilitate saccade initiation, while normal individuals do not require any facilitation. However, Becker et al. suggested that facilitating movements are not required until the very late stages of severe HD. Since the UHDRS five-point scale relies heavily on the observance of head movements or blinks when initiating saccades, this evaluation is likely to be useful only during the late stages of the disease. Velocity is also evaluated as part of the UHDRS, but efficacy of this measure is also questionable. Many studies have shown that individuals with manifest HD have slower saccades, though the effect is associated with age such that impairment in saccade velocity is most pronounced at younger ages. While two studies using qualitative assessment of saccade velocity found differences in premanifest subjects, subsequent studies using quantitative measures have not found saccadic slowing in either premanifest or manifest HD.

Despite the shortcomings of saccadic testing in the UHDRS, quantitative saccades have proven to be quite beneficial in detecting abnormalities even during the premanifest
period of HD. Furthermore, the development of portable saccadometry devices now provides a reasonable means for quantitatively measuring saccades in a clinical setting. Studies using quantitative saccades have reported increased latency of initiation and variability of latency of voluntary saccades, and difficulty inhibiting saccades toward a novel visual stimulus in both premanifest and manifest HD. These results indicate that measures of latency, variability of latency, and correct responses during voluntary saccade tasks are promising biomarkers during the premanifest period of the disease. However, no studies have described the longitudinal progression of these measures in premanifest HD.

2. Cognitive abnormalities in Huntington disease

Cognitive deficits are a considerable source of morbidity in HD, progressively worsen during the course of the disease, and are more strongly associated with functional ability than motor symptoms. The term subcortical dementia has been used to describe the cognitive effects of the disease, but its use has been argued against in part because the symptoms often do not meet DSM-IV criteria for dementia until very late in the disease in spite of clear cognitive difficulty during earlier stages. A Physician’s Guide to the Management of Huntington’s Disease suggests heightened alertness for the following complaints: disorganization, lack of initiation, perseveration, impulsivity, irritability and temper outbursts, perceptual problems, unawareness, altered attention, language difficulties, learning and memory problems, and difficulty estimating time.

A vast array of neuropsychiatric instruments has been used to study cognitive loss in HD, though some cognitive domains have been consistently identified as affected. For example, CAG+ individuals do not employ effective decision-making strategies in a simulated gambling task or a twenty questions game. One possible explanation is that they are unable to appropriately modify their behavior in response to the understanding gained from previous responses. Similarly, CAG+ individuals have difficulty with tasks that require shifting attention from a learned preparatory set to a new
set. Memory and learning problems are among the most common complaints of patients and family members. Based on studies that distinguish between different facets of memory, it appears that encoding and retrieval are impaired while recognition remains relatively intact. Another important cognitive impairment in HD is related to egocentric spatial judgment, which can negatively impact a person’s ability to read maps, maintain a sense of direction, and vary motor actions in response to spatial alterations. Dysfunctional language can severely impact the ability to communicate in HD. Though a major contributor to dysfunction is related to the motor impairments of the disease, non-motor impairments such as reduced fluency, decreased ability to switch between semantic categories, and lack of comprehension of complex sentences and implied information are also evident.

3. Behavioral abnormalities in Huntington disease

Behavioral changes are quite common in HD, but unlike cognitive decline, behavior does not generally correlate with other measures of disease progression. Perhaps the most extensively studied psychiatric disorder in HD is depression. While some studies have found an increased risk of depression in CAG+ individuals (reviewed by Slaughter et al.), an extensive recent study found that the lifetime prevalence of depression is not increased in CAG+ individuals compared to CAG- individuals who have a parent with HD, although the cross sectional prevalence of depression is increased. Suicidal ideation is common and fluctuates throughout the disease process, with highest prevalence in premanifest and late stage disease. Successful suicide is believed to be 5-10 times higher in CAG+ individuals than in the general population.

Irritability is another common feature of HD. The prevalence has been shown to be increased up to 10 years prior to estimated disease onset, though no association between irritability and years to onset was found. Other behavioral manifestations include apathy, anxiety, and psychosis.
D. Magnetic resonance imaging in Huntington disease

Magnetic resonance imaging (MRI) has provided the ability to study the neurodegenerative effects of HD in vivo. Structural MRI analysis of gray matter has been used the longest, and specific patterns of disease have been consistently observed. Bilateral striatal atrophy has been identified in 95% of HD brains\(^1^6^7\) and is caused in part by the preferential loss of medium spiny neurons.\(^1^6^8;1^6^9\) Loss begins in the caudal dorsal medial caudate and progresses rostrally, ventrally, and laterally to eventually involve the caudate head, putamen, and globus pallidus.\(^1^6^7;1^7^0\) Atrophy can be detected up to 20 years prior to estimated disease onset, and the rate of volume loss increases significantly within 10 years prior to onset.\(^1^7^1\) Striatal atrophy is a good indication of disease severity\(^1^7^2-1^7^5\) and is associated with declining total functional capacity,\(^1^7^6\) memory,\(^1^7^7-1^7^9\) executive function,\(^1^7^7;1^8^0\) and psychomotor speed.\(^1^7^7;1^7^9\) In part because of these findings, Kloppel et al.\(^1^8^1\) suggested that striatal volume could be used to stratify patients in clinical trials in order to create more homogeneous groups.

In addition to striatal volume loss, it is also clear that atrophy can be detected throughout the brain.\(^1^0^1;1^7^0;1^7^1;1^7^4;1^7^5;1^8^2-1^9^6\) Rosas et al.\(^1^9^3\) reported that the most consistent regions of cortical thinning in manifest HD occurred in sensorimotor regions of the frontal lobe. Studies of premanifest HD suggest that atrophy begins in the posterior regions of the brain and progresses anteriorly with advancing disease.\(^1^0^1;1^9^3;1^9^4\) While loss of volume and thickness has been most consistently described, there have been reports of increased gray matter volume,\(^1^9^2\) increased ACC thickness,\(^1^9^4\) and enlarged gyral crowns\(^1^9^1\) in premanifest HD, and increased frontal lobe volume in manifest HD.\(^1^8^4\)

A number of studies have investigated neural abnormalities in HD using functional MRI\(^1^9^7-2^0^8\) (fMRI), and these are summarized in Table 2 (modified from Bohanna et al.\(^1^7^0\)). Findings from these reports include both hypo- and hyperactivation of many different regions, and direction of activity changes in CAG+ groups depends on the specific task. Unfortunately, though not unexpectedly, these studies do not point to abnormalities in one common circuit that lead to the observed cognitive deficits. Another
approach in fMRI studies has been to examine the functional connectivity between regions. Wolf et al.\textsuperscript{209,210} found reduced connectivity between the left lateral prefrontal cortex (PFC), parietal cortex, and putamen in premanifest HD, and Thiruvady et al.\textsuperscript{211} found reduced connectivity between ACC and lateral PFC in manifest HD. These studies point to clear functional changes in the premanifest and manifest HD brain, and that activation differences may be detectable before either task performance decline or atrophy.\textsuperscript{206}
<table>
<thead>
<tr>
<th>Authors</th>
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<th>Hypoactivation in CAG+</th>
<th>Hyperactivation in CAG+</th>
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<td>Occipital cortex</td>
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<td>Parietal cortex</td>
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<td>Temporal cortex</td>
<td>Frontal cortex</td>
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<td>Parietal cortex</td>
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<td></td>
<td>Frontal cortex</td>
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<td>Striatum</td>
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<td>Simon task</td>
<td>Parietal cortex</td>
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<td>al. (2007)</td>
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<td>Insula</td>
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<td>Precuneus/SPL</td>
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Table 2. Summary of fMRI studies in premanifest and manifest HD.

<table>
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<th>Hypoactivation in CAG+</th>
<th>Hyperactivation in CAG+</th>
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<tr>
<td>Gavazzi et al. (2007)</td>
<td>Repetitive finger flexion and extension</td>
<td>Precentral gyrus Cerebellum Insula</td>
<td>Caudate ACC Medial frontal gyrus</td>
<td>Infraparietal sulcus Supramarginal gyrus SMA</td>
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<tr>
<td>Wolf et al. (2009)</td>
<td>Working memory</td>
<td>Frontal cortex Parietal cortex Striatum Cerebellum</td>
<td>DLPFC VLPFC IPL Putamen Cerebellum</td>
<td>None</td>
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<td>Reading et al. (2004)</td>
<td>Interference task</td>
<td>PFC Cingulate cortex Parietal cortex Occipito-temporal cortex</td>
<td>ACC</td>
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<td>Time discrimination</td>
<td>Striatum Pre-SMA/cingulate</td>
<td>Caudate Thalamus</td>
<td>ACC Pre-SMA</td>
</tr>
<tr>
<td>Hennenlotter et al. (2004)</td>
<td>Disgust processing</td>
<td>Insula Putamen</td>
<td>Insula Putamen</td>
<td>None</td>
</tr>
<tr>
<td>Authors</td>
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<tr>
<td>Wolf et al. (2007)</td>
<td>Working memory</td>
<td>Striatum, Cerebellum, Parietal cortex, Frontal cortex</td>
<td>Middle frontal gyrus</td>
<td>IPL, Superior frontal gyrus</td>
</tr>
<tr>
<td>Zimbelman et al. (2007)</td>
<td>Time reproduction</td>
<td>Putamen, Cerebellum, ACC, Frontal cortex, Temporal cortex</td>
<td>Far from onset: ACC, Insula, Close to onset: Putamen, SMA, Insula, IFG</td>
<td>Far from onset: Sensorimotor cortex, Medial frontal gyrus, Precentral gyrus, Superior temporal gyrus, Cerebellum</td>
</tr>
<tr>
<td>Saft et al. (2008)</td>
<td>Auditory processing and habituation</td>
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<td>Close to onset: IPL, ACC, Middle frontal gyrus, Insula</td>
<td>Far from onset: Thalamus, Caudate, Manifest HD: Putamen</td>
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<td>Sequential finger movements</td>
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<td>Caudal SMA, SPL</td>
</tr>
</tbody>
</table>

Table 2. Summary of fMRI studies in premanifest and manifest HD.
E. Saccade signaling pathways in the brain

There are several types of saccades that are used in a research setting (Figure 2). One of the simplest types is a prosaccade (PS), a type of visually-guided saccade that shifts gaze toward a visual stimulus. A PS is often termed reflexive, though the accuracy of the term has been questioned because cognitive processes clearly influence PS initiation. A volitional saccade is a second type of saccade. It is an endogenously generated gaze shift in response to a command. Antisaccades (AS), which require the suppression of a reflexive saccade toward a peripheral visual stimulus and the voluntary generation of a saccade to the mirror opposite location, and memory-guided saccades (MG), which require making a saccade to one or more remembered positions that are no longer

**Figure 2. Saccadic tasks and target locations.** The dotted circle, not shown to participants, indicates the correct location of gaze. A. The AS task requires directing gaze away from the target. B. The MGs task requires fixation on the center target until it is extinguished, then directing gaze toward the remembered location of the peripheral target. C. The MGi task requires saccades toward the peripheral targets as they appear, then replication of the sequence to the remembered target locations. D. The MGc task requires fixation on the center target until it is extinguished, then sequentially directing gaze toward the remembered location of the 3 peripheral targets.
identified by a stimulus, are two types of volitional saccades that are particularly affected in premanifest and early manifest HD.\textsuperscript{119,122,124}

Saccades are generated via activity in the brainstem: oculomotor, trochlear, and abducens nuclei; medullary reticular formation; midbrain reticular formation; interstitial nucleus of Cajal; nucleus prepositus hypoglossi; paramedian pontine reticular formation; rostral interstitial nucleus of the medial longitudinal fasciculus; superior colliculus.\textsuperscript{213} However, many cortical regions influence the activity in these brainstem regions and thus control saccade behavior. Functional imaging in humans and single neuron recordings in non-human primates have helped to identify these fairly well-defined cortical regions.

PS generation is preceded or accompanied by activity in several cortical regions (Figure 3), including visual cortex, parietal cortex, and frontal and supplementary eye fields (FEF, SEF) (reviewed in McDowell et al.\textsuperscript{214}). The visual stimulus is sent to primary (V1) and secondary (V2/V3, middle occipital gyrus) visual cortex where its location is mapped in visual space. Contrary to all other regions, visual cortex may activate more strongly when making PS than when making volitional saccades.\textsuperscript{215} Visual cortex then projects directly to the superior colliculus\textsuperscript{216,217} and to the parietal cortex via the dorsal stream.\textsuperscript{218} Widespread and varied activation has been noted throughout the parietal cortex,\textsuperscript{215,219-222} but the most consistently activated regions are located in the superior parietal lobule (SPL). The parietal cortex is important for visuo-spatial processes.\textsuperscript{223-226} The parietal cortex has direct projections to the superior colliculus\textsuperscript{227,228} and is reciprocally connected with regions in the frontal lobe,\textsuperscript{229,230} particularly the FEF and SEF. FEF are located in Brodmann area 6, immediately anterior to the motor strip\textsuperscript{231,232} and are important in generating saccades.\textsuperscript{233,234} SEF are located on the dorsomedial surface of the frontal lobe, just anterior to the supplementary motor area\textsuperscript{235,236} (SMA). While activated during PS, SEF appear to be more important for tasks involving remembered ocular motor sequences or predictable stimuli.\textsuperscript{237-242} Both FEF and SEF project to the brainstem saccade generators,\textsuperscript{243-246} and direct stimulus of either region is sufficient to produce a saccade.\textsuperscript{247-249}
Volitional saccades activate the same regions as PS, though typically to a greater degree (with the previously noted exception of the visual cortex). Parietal cortex plays a number of important roles in AS performance. The inferior parietal lobule (IPL) appears to inhibit saccades toward the peripheral stimulus, while an area along the intraparietal sulcus (IPS) is responsible for vector inversion (remapping the location of the stimulus to its mirror opposite location). FEF activity is detected prior to the initiation of an AS, suggesting an increase of preparatory inhibition and prospective saccadic coding. Similar to FEF, SEF activation is detected prior to saccade initiation. Evidence suggests that SEF activation may slow the PS response, thereby allowing the AS to be initiated first.

As with AS tasks, MG tasks elicit activation similar to PS though to a greater degree. In a simple version of the task (MGs, Figure 2B), the parietal cortex, and IPS in particular, is activated during the delay period after the stimulus but prior to the response, consistent
with its role in visuo-spatial attention. FEF activation is also persistent during the delay period and may represent maintenance of the location of the cue.\textsuperscript{257,261-266}

In a more complex MG task (MGc, Figure 2D), both the location and sequence of the visual stimuli must be remembered. Given the role supplementary motor areas in motor sequence learning,\textsuperscript{267-269} it is not surprising that SEF plays a prominent role in the planning, learning, and execution of MGc tasks.\textsuperscript{220,270} IPS is also activated under these conditions and may represent an online visuo-spatial recoding of the stimulus locations in order to account for new eye position after making a saccade to a previous target.\textsuperscript{220,271-273} Greater activation compared to PS is also found in FEF,\textsuperscript{274,275} supporting its important role in volitional saccade generation.

In addition to activating regions that overlap with PS, volitional saccades also activate dorsolateral prefrontal cortex\textsuperscript{214,221,222,250,255,276-278} (DLPFC) and anterior cingulate cortex (ACC). These regions play an important role in both saccadic and non-saccadic cognitive control: DLPFC is involved in attention, planning, spatial orientation, and inhibition;\textsuperscript{279,280} and ACC is involved in conflict and error monitoring.\textsuperscript{280-282}

During an AS task, DLPFC activation precedes the saccadic response prior to correct trials,\textsuperscript{255,277,283,284} and lesions in DLPFC lead to more AS errors, but do not affect PS.\textsuperscript{233,285,286} In MG tasks, DLPFC appears to play a role in inhibition\textsuperscript{266,276,283,287} and maintenance of spatial orientation.\textsuperscript{263,288-291} Relevant to saccadic control, DLPFC sends projections to FEF\textsuperscript{292} and superior colliculus.\textsuperscript{293,294}

ACC is activated during both AS and MG tasks. Prior to saccadic response in an AS task, ACC activity is associated with subsequent correct responses,\textsuperscript{283} consistent with its role in conflict monitoring; however, post-response ACC activation is associated with incorrect responses,\textsuperscript{283,295} consistent with an error monitoring role. This error monitoring role appears to be the major contribution of ACC during MG tasks.\textsuperscript{220} While connectivity in the context of saccades has not been studied in ACC, regions of the ACC activated by
saccadic tasks are connected with parietal cortex, motor and pre-motor cortex, and dorsal prefrontal cortex.\textsuperscript{296}

**F. Biomarkers of Huntington disease**

Unfortunately, there are no current pharmacologic or therapeutic interventions shown to delay or slow the onset or progression of HD. Therefore, it is essential that sensitive and specific biomarkers in the prediagnostic period be identified that could be used to evaluate future therapeutic interventions. Biomarkers are objective measurements that are evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to therapeutic interventions.\textsuperscript{297} They must be sensitive and specific for the process or response being evaluated, and they must also show reliability, validity, responsiveness, precision, interpretability, acceptability, and feasibility.\textsuperscript{298} It has been shown that the proposed saccadic measurements are good candidates for HD biomarkers.\textsuperscript{119,122} We have recently shown that saccadic measures satisfy biomarker criteria such as high between-session reliability, strong genetic influence, and limited abnormalities in a CAG+ population.\textsuperscript{299}

Several studies have sought to identify potential prediagnostic biomarkers, and it is well-established in cross sectional studies that prediagnostic CAG+ individuals experience deficits in tests of attention,\textsuperscript{300} executive function,\textsuperscript{99,301,302} memory,\textsuperscript{131,300,301,303,304} psychomotor speed,\textsuperscript{98,99,118,131,300,305} and ocular movements.\textsuperscript{98,99,111,119,120,122,306} However, biomarkers must be characterized longitudinally in order to be effective measures of therapeutic intervention. In a large cross sectional sample of 438 prediagnostic individuals, Paulsen and colleagues\textsuperscript{100} reported that the detectable changes begin one to two decades prior to the estimated age of onset, and that this initial period is followed by more rapid change in the years just prior to diagnosis. While this study was very well-powered (a weakness of many prediagnostic biomarker studies), care must be taken when making longitudinal interpretations of cross sectional data because cross sectional studies cannot control for effects of learning, training, etc.
Only a few longitudinal studies have explored rates of decline in prediagnostic CAG+ individuals. Some have reported differential rates of progression between premanifest CAG+ and CAG- controls in measures of attention, psychomotor speed, and memory;\textsuperscript{130,307-310} however, others have not been able to replicate these results.\textsuperscript{311-314} These discrepant results may be due to the modest sample sizes of most studies and to the challenge presented by the extensive heterogeneity of the disease phenotype.

G. Statement of Purpose

HD is, in many ways, ideally suited for studying neurodegenerative disease; its Mendelian inheritance is relatively straightforward, a simple gene test can unambiguously predict future onset of the disease, and its late onset allows for a thorough study of the prodromal phase. Studying the disease is not, however, without challenges. The ubiquitous expression of huntingtin protein belies the focal neural pathology, and the variable symptomology greatly complicates research intended to characterize the defining characteristics of premanifest disease. The goal of these studies was to gain a better understanding of the mechanisms of saccadic impairment in HD and of the potential for using saccades to follow premanifest HD. This was accomplished through the following aims:

1. Identify the neural correlates of saccade impairment in premanifest and manifest HD.

   A. Using structural MRI and a focused region of interest (ROI) approach, identify specific regions of brain atrophy associated with saccade impairment.

   B. Using fMRI, determine the functional brain changes that underlie AS impairment.

2. Determine the pattern of progression of neuropsychological and ocular motor decline within the premanifest period of HD.
A. Identify measures for which the rate of decline increases as premanifest individuals approach onset.

B. Identify measures for which the rate of decline is faster in CAG+ than CAG-individuals.
II. Vertical antisaccade latency tracks gray matter atrophy in premanifest and early manifest Huntington disease

A. Introduction

Huntington disease (HD) is an autosomal dominant disorder caused by an expanded number of CAG repeats in the huntingtin gene.\(^1\) The diagnosis of HD is currently based on the Unified Huntington Disease Rating Scale (UHDRS), although abnormalities can be detected before a clinical diagnosis of HD becomes certain (i.e. during the premanifest period of the disease). These abnormalities are potential biomarkers that offer insights into premanifest disease progression, and indicate the brain systems that are first to be affected.

Quantitative measurements of saccadic eye movements are one such potential set of biomarkers of disease progression. A saccade is a rapid eye movement that shifts gaze from one location to another. An extensive and systematic study of manifest HD\(^{107}\) found that the most profound changes were in the ability to initiate voluntary saccades and to maintain fixation. Subsequent studies in premanifest and manifest HD have found abnormalities in antisaccade (AS) and memory guided (MG) measures of latency, variability of latency, and error rates.\(^{98;99;104;111;119-124;306}\) There is also evidence that the rate of impairment increases in some MG tasks for measures of the variability of latency and error rate.\(^{315}\) Interestingly, despite early evidence that abnormalities of vertical saccades were possibly more prominent than those of horizontal saccades,\(^{107}\) recent studies have focused on horizontal saccades.

Striatal atrophy is a well-known and long-established sign of HD progression\(^{170;187}\) and can be detected using structural MRI up to 10 years prior to disease onset.\(^{101;171}\) However, atrophy is not limited to the striatum, and has also been detected in the thalamus and multiple cortical regions.\(^{101;174;175;182-186;188-192;195;196;316}\) It has been suggested that cortical atrophy begins in posterior regions and progresses anteriorly.\(^{101;193;194}\)
The association between gray matter changes and saccade performance in HD has not been described. This study examines the relationship between regional loss of cerebral gray matter and saccadic parameters that differentiate between CAG- and CAG+ groups. By understanding these relationships, clinically assessable markers can be derived that are closely associated with the loss of both striatal and cortical tissue in the premanifest period.

B. Methods

1. Participants

Participants were recruited primarily from individuals who had taken part in previous studies at Indiana University. The inclusion criteria were: 1) a parent diagnosed with HD; 2) age between 18 and 65; 3) no diagnosis of HD, or if diagnosed, having received the diagnosis within the past 2 years. 121 participants completed the saccade protocol, and a subset of 31 participants was also imaged. All those who were imaged self-reported right handedness. No participants reported a concurrent neurologic illness, major psychiatric diagnosis (e.g. schizophrenia, bipolar disorder), or current alcohol or drug abuse. Participants were asked not to disclose their CAG status, if known, to study staff. This study was approved by the local institutional review board (IUPUI IRB Study Nos. 0109 and 0707) and all participants provided written informed consent.

2. Clinical Evaluation and Study Group Assignment

Molecular testing was used to determine the number of CAG repeats in the huntingtin gene. Participants with 2 alleles having fewer than 28 repeats were considered CAG unexpanded (CAG-; n=47; 12 in imaging subset), while those having at least 1 allele with more than 38 CAG repeats were considered CAG expanded (CAG+; n=74; 19 in imaging subset).
An experienced movement disorder neurologist (J.W., X.B.) administered the motor portion of the Unified Huntington Disease Rating Scale-99\(^7\) (UHDRS). The neurologists were aware that the participants were at-risk for HD, but were blinded to the results of all other study assessments, including huntingtin gene testing. On the basis of the motor examination only, the neurologist assigned an overall confidence rating (UHDRS diagnosis confidence level) that represented the likelihood of motor abnormalities attributable to HD. The ratings are defined as: (0) normal (no abnormalities); (1) nonspecific motor abnormalities (less than 50% confidence); (2) motor abnormalities that may be signs of HD (50% to 89% confidence); (3) motor abnormalities that are likely signs of HD (90% to 98% confidence); and (4) motor abnormalities that are unequivocal signs of HD (≥ 99% confidence). Those CAG+ subjects with a confidence rating from 0-3 were considered premanifest (preHD; n=49; 12 in imaging subset), while those receiving a 4 were considered to have manifest HD (HD; n=25; 7 in imaging subset). Estimated onset was defined as the age at which a person had a 50% probability of having manifest disease, and the estimated time to onset (TTO) was calculated for each preHD participant.\(^8\):\(^{100}\) PreHD subjects were further classified as far from estimated onset (Far; TTO>13 years; n=25) and near to estimated onset (Near; TTO<13 years; n=24). Because of the small sample, dichotomization of the preHD group was not used in the imaging data analysis.

3. Eye Movement Recording and Analysis

Participants were seated in front of a 22 inch computer LCD monitor in a standard ophthalmology exam chair. Visual targets (3 mm red spot) were displayed on a monitor placed 23.5 inches from the participant. As part of the pre-testing procedure, calibration and validation were completed. Four saccadic tasks were administered (Figure 2): AS; MG, simple (MGs); MG, intermediate (MGi); and MG, complex (MGc). The vertical and horizontal positions of the participants’ pupils were recorded binocularly with two ultra-miniature high-speed (250 Hz) video cameras attached to a headband. Four sensors monitored head movements; eye positions were adjusted for small head movements (EyelinkII, SR Research Ltd, spatial resolution < 0.1 degree). Before each task, the
examiner instructed the participant verbally and then provided a brief view of the task to ensure that the participant understood the instructions. Each of the tasks consisted of 24 trials. After the participant completed the testing procedure, an interactive computerized analysis of the right eye position was performed.

Measures of latency, variability of latency, and percentage of errors (including missed flashes for MGc) were tested for group differences (CAG-, Far, Near, HD) using analysis of covariance (ANCOVA) in SAS v9.13. A significant ANCOVA test \((p \leq 0.05)\) was followed by one-tailed t-tests between CAG- and Far groups, Far and Near groups, and Near and HD groups. Age, gender, and education were included in the model as covariates when they had a significant effect \((p \leq 0.05)\). A linear trend analysis was also performed with Far, Near, and HD groups to test for evidence of linear decline in CAG+ subjects, suggesting progressive impairment in the premanifest and early manifest stages of disease. Those measures with a significant linear trend \((p \leq 0.05)\) were used in the correlation analysis with structural measures.

4. Image Acquisition and Analysis

A subset of participants (31 total: 12 CAG-, 12 preHD, 7 HD) were imaged in a Siemens (Erlangen, Germany) 3T Magnetom Trio-Tim scanner with a 12-channel head-coil array. A whole-brain, high resolution \((1.0 \times 1.0 \times 1.2 \text{ mm voxels})\) structural image volume was acquired using a 3D magnetization prepared rapid gradient echo (MPRAGE) sequence.

An automated parcellation and segmentation procedure in FreeSurfer V4 was used to extract cortical thickness and volume measures. Analyses focused on FreeSurfer segmented and parcellated structures that overlap with regions known to mediate saccade function: dorsolateral prefrontal cortex (DLPFC) in rostral middle frontal gyrus (rMFG), frontal eye fields (FEF) in caudal MFG (cMFG), supplementary eye fields (SEF) in superior frontal gyrus (SFG), inferior parietal lobule (IPL), and the caudate nucleus. \(104;214;253;262;322-327\) The rostral and caudal anterior cingulate cortex (rACC, cACC) were also included because of their monitoring role in volitional
saccades. ANCOVA was used to test for group differences (CAG-, preHD, HD) in thickness and volume. A significant ANCOVA test ($p \leq 0.05$) was followed by two-tailed t-tests for all groupwise comparisons. Age, gender, and intracranial volume (ICV) were included in the model as covariates, when they had a significant effect ($p \leq 0.05$).

5. Structural-Saccadic Relationships

Due to the modest size of the imaged sample, a Spearman nonparametric correlation model was used to test for an association between saccade impairment and brain atrophy in saccade-related regions. Only saccadic measures with a significant linear trend (see above) and structural regions with a significant group difference were used. This assured that the saccadic measures used are those that show progression, and that the relationships between cortical volume and saccadic measures occur in regions where cerebral degeneration can be measured. Gender, age, and ICV were included in the model as partial variables.

C. Results

In the large primary sample, the 4 study groups (CAG-, Far, Near, HD) did not significantly differ in education, gender, race, or handedness ($p \geq 0.6$; Table 3), although the Far group was significantly younger than the other three groups ($p \leq 0.0005$). In the subset of participants that underwent imaging, there were no significant group (CAG-, preHD, HD) differences ($p \geq 0.1$).
**Table 3. Participant demographics.** Data for imaged participants are listed in parentheses.

<table>
<thead>
<tr>
<th></th>
<th>PreHD</th>
<th></th>
<th></th>
<th>Near</th>
<th></th>
<th>HD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Participants</td>
<td>CAG- 47 (12)</td>
<td>Far 25 (12)</td>
<td>Imaged Subset 24 (12)</td>
<td>Near 24 (7)</td>
<td></td>
<td>HD 25 (7)</td>
</tr>
<tr>
<td>Age* (years)</td>
<td>47.1 ± 11.2</td>
<td>36.0 ± 11.1</td>
<td>47.6 ± 11.7</td>
<td>48.9 ± 11.7</td>
<td>45.5 ± 15.0</td>
<td></td>
</tr>
<tr>
<td>(46.1 ± 11.4)</td>
<td>(43.9 ± 14.4)</td>
<td>(14.0 ± 15.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Education (years)</td>
<td>15.2 ± 2.3</td>
<td>15.3 ± 2.3</td>
<td>16.1 ± 3.2</td>
<td>15.3 ± 2.6</td>
<td>14.0 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>(14.8 ± 2.1)</td>
<td>(16.6 ± 4.1)</td>
<td></td>
<td>(14.0 ± 1.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male:Female</td>
<td>12:35 (5:7)</td>
<td>8:17 (5:7)</td>
<td>10:14 (10:14)</td>
<td></td>
<td>8:17 (2:5)</td>
<td></td>
</tr>
<tr>
<td>Race (% Caucasian)</td>
<td>100 (100)</td>
<td>100 (100)</td>
<td>100 (100)</td>
<td></td>
<td>100 (100)</td>
<td></td>
</tr>
<tr>
<td>Handedness (% right)</td>
<td>89.4 (100)</td>
<td>88.0 (100)</td>
<td>87.5 (100)</td>
<td>88.0 (100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAG Repeats in Larger Allele</td>
<td>19.9 ± 3.0</td>
<td>42.4 ± 2.5</td>
<td>42.9 ± 2.9</td>
<td>43.7 ± 4.0</td>
<td>45.4 ± 5.5</td>
<td></td>
</tr>
<tr>
<td>(20.2 ± 3.3)</td>
<td>(42.5 ± 2.2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estimated Time to Onset (years)</td>
<td>18.1 ± 3.4</td>
<td>9.0 ± 2.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(13.1 ± 6.4)</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

* The Far group was significantly younger than all other groups for the larger non-imaging sample (p≤0.0005)
1. Saccade Abnormalities and Linear Decline

There was a significant difference ($p \leq 0.05$) in the performance of the Far and Near groups (Table 4) for: AS) percentage of horizontal errors, and latency of correct horizontal and vertical AS; MGs) percentage of horizontal errors; and MGc) percentage of errors. Additionally, there was a significant difference between the Near and HD groups for the measures: AS) percentage of horizontal and vertical errors; MGs) variability of latency of correct vertical saccades; MGi) percentage of errors; MGc) percentage of errors and missed flashes. There were no saccadic measures for which a significant difference between the CAG- and Far groups was detected.

A significant linear decline ($p \leq 0.05$) across CAG+ groups (Far, Near, HD) was found for the measures (Table 4): AS) percentage of horizontal and vertical errors, and horizontal and vertical latency; MGs) latency of horizontal saccades; MGi) percentage of errors; MGc) percentage of errors and missed flashes.

2. Atrophic Brain Changes

There was a significant group (CAG-, preHD, HD) effect on thickness and volume for many cortical and subcortical regions (Table 5), consistent with previous studies. There was a significant loss of thickness in preHD compared with CAG- subjects in the frontal lobe (bilateral SFG, left rMFG and cMFG) and parietal lobe (bilateral IPL). There was also a loss of volume bilaterally in the caudate. When comparing the HD with preHD subjects, there was a loss of thickness in the parietal lobe (right IPL) and of volume bilaterally in the caudate. There was a significant difference between CAG- and HD for all above mentioned regions. There was no significant loss of thickness in the rACC or cACC.
Table 4. Group differences in saccade performance.

<table>
<thead>
<tr>
<th>Antisaccades</th>
<th>Unadjusted Mean ± SD</th>
<th>Group Differences (p value)</th>
<th>Linear Trend in CAG+ (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CAG-</td>
<td>Far</td>
<td>Near</td>
</tr>
<tr>
<td>% Errors</td>
<td>Hrz 20.3 ± 18.9</td>
<td>16.3 ± 22.4</td>
<td>29.4 ± 22.8</td>
</tr>
<tr>
<td></td>
<td>Vrt 30.6 ± 19.3</td>
<td>25.8 ± 23.0</td>
<td>32.2 ± 25.5</td>
</tr>
<tr>
<td>Latency (ms)</td>
<td>Hrz 288.9 ± 47.7</td>
<td>283.4 ± 49.9</td>
<td>309.5 ± 44.8</td>
</tr>
<tr>
<td></td>
<td>Vrt 304.6 ± 47.2</td>
<td>300.2 ± 51.2</td>
<td>344.3 ± 75.5</td>
</tr>
<tr>
<td>Variability of Latency (ms)</td>
<td>Hrz 50.5 ± 23.8</td>
<td>60.0 ± 32.5</td>
<td>62.1 ± 28.5</td>
</tr>
<tr>
<td></td>
<td>Vrt 55.4 ± 25.7</td>
<td>73.1 ± 48.8</td>
<td>73.1 ± 58.5</td>
</tr>
<tr>
<td>Memory Guided Saccades (simple)</td>
<td>% Errors</td>
<td>Hrz 24.8 ± 15.0</td>
<td>25.5 ± 20.6</td>
</tr>
<tr>
<td></td>
<td>Vrt 17.6 ± 17.3</td>
<td>27.5 ± 16.0</td>
<td>29.4 ± 20.7</td>
</tr>
<tr>
<td>Latency (ms)</td>
<td>Hrz 299.9 ± 64.1</td>
<td>309.7 ± 60.8</td>
<td>326.5 ± 59.4</td>
</tr>
<tr>
<td></td>
<td>Vrt 319.2 ± 68.0</td>
<td>306.3 ± 63.3</td>
<td>339.5 ± 67.3</td>
</tr>
<tr>
<td>Variability of Latency (ms)</td>
<td>Hrz 82.6 ± 40.6</td>
<td>104.1 ± 59.1</td>
<td>106.9 ± 47.8</td>
</tr>
<tr>
<td></td>
<td>Vrt 94.8 ± 46.6</td>
<td>91.9 ± 67.3</td>
<td>110.1 ± 55.3</td>
</tr>
</tbody>
</table>

a - CAG- vs. Far; b - Far vs. Near; c - Near vs. HD; * - non-significant test; † - significant ANCOVA with no significant post hoc tests. Significance of p≤0.05 used for all tests. Hrz- horizontal, Vrt - vertical.
Table 4. Group differences in saccade performance.

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted Mean ± SD</th>
<th>Group Differences (p value)</th>
<th>Linear Trend in CAG+ (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Memory Guided Saccades (intermediate)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Errors</td>
<td>14.8 ± 14.9</td>
<td>24.5 ± 21.0</td>
<td>32.9 ± 28.7</td>
</tr>
<tr>
<td>Latency (ms)</td>
<td>359.9 ± 88.0</td>
<td>362.3 ± 113.9</td>
<td>363.3 ± 121.5</td>
</tr>
<tr>
<td>Variability of Latency (ms)</td>
<td>151.4 ± 48.5</td>
<td>147.9 ± 41.3</td>
<td>151.4 ± 78.5</td>
</tr>
<tr>
<td><strong>Memory Guided Saccades (complex)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Errors</td>
<td>18.3 ± 14.0</td>
<td>24.2 ± 20.2</td>
<td>43.7 ± 25.7</td>
</tr>
<tr>
<td>% Missed Flashes</td>
<td>7.9 ± 10.9</td>
<td>11.4 ± 15.7</td>
<td>14.2 ± 18.8</td>
</tr>
</tbody>
</table>

a - CAG- vs. Far; b - Far vs. Near; c - Near vs. HD; * - non-significant test; † - significant ANCOVA with no significant post hoc tests. Significance of p≤0.05 used for all tests. Hrz- horizontal, Vrt - vertical.
Table 5. Gray matter atrophy in saccade-related brain regions.

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CAG-</td>
</tr>
<tr>
<td>Frontal Lobe</td>
<td></td>
</tr>
<tr>
<td>Superior Frontal Gyrus</td>
<td>Left¹</td>
</tr>
<tr>
<td></td>
<td>Right¹</td>
</tr>
<tr>
<td>Rostral Middle Frontal Gyrus</td>
<td>Left¹,²</td>
</tr>
<tr>
<td></td>
<td>Right¹</td>
</tr>
<tr>
<td>Caudal Middle Frontal Gyrus</td>
<td>Left¹</td>
</tr>
<tr>
<td></td>
<td>Right¹</td>
</tr>
<tr>
<td>Parietal Lobe</td>
<td></td>
</tr>
<tr>
<td>Inferior Parietal Lobule</td>
<td>Left¹</td>
</tr>
<tr>
<td></td>
<td>Right¹</td>
</tr>
<tr>
<td>Cingulate Cortex</td>
<td></td>
</tr>
<tr>
<td>Rostral Anterior Cingulate</td>
<td>Left</td>
</tr>
<tr>
<td></td>
<td>Right¹</td>
</tr>
<tr>
<td>Caudal Anterior Cingulate</td>
<td>Left²,³</td>
</tr>
<tr>
<td></td>
<td>Right</td>
</tr>
<tr>
<td>Subcortical</td>
<td></td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td>Left¹,²,³</td>
</tr>
<tr>
<td></td>
<td>Right¹,²,³</td>
</tr>
</tbody>
</table>

Cortical structural measure is gray matter thickness in mm while subcortical measure for the caudate nucleus is volume in mm³. Post hoc testing indicates a significant difference (p≤0.05) between a) CAG- and preHD, b) CAG- and HD, and c) preHD and HD. Superscripts indicate significant effects (p≤0.05) of covariates: 1) age, 2) gender, 3) ICV.
Table 6. Correlation coefficients (p values) of significant associations between measures of brain atrophy and saccade impairment.

<table>
<thead>
<tr>
<th></th>
<th>AS</th>
<th>MGi</th>
<th>MGc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Errors (vertical)</td>
<td>Latency (vertical)</td>
<td>% Errors</td>
</tr>
<tr>
<td><strong>Frontal Lobe</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SFG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>0.53 (0.04)</td>
<td>-0.62 (0.05)</td>
<td></td>
</tr>
<tr>
<td><strong>Parietal Lobe</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IPL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>-0.74 (0.01)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Subcortical</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>-0.88 (0.0008)</td>
<td>-0.49 (0.05)</td>
<td>-0.58 (0.02)</td>
</tr>
<tr>
<td>Right</td>
<td>-0.91 (0.0003)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AS: antisaccade; IPL: inferior parietal lobule; MGc: memory guided, complex; MGi: memory guided, intermediate; SFG: superior frontal gyrus.
Figure 4. Plots of vertical AS latency and structural measures. Filled circles represent CAG+ individuals, and unfilled triangles represent CAG-. Plots are of vertical AS latency vs. left SFG thickness (A) and right caudate volume (B).

A.

B.
A significant negative association (Table 6) was found between vertical AS latency and thickness in the left SFG (Figure 4A) and left IPL and bilateral caudate volume ($p \leq 0.05$, Figure 4B). The percentage of errors of MGi and MGc were negatively associated with left caudate volume, and the percentage of missed flashes (MGc) was negatively associated with bilateral caudate volume. The percentage of vertical AS errors was positively associated with left SFC thickness. There were a number of saccadic and structural measures tested that had no significant associations: percentage of horizontal AS errors, horizontal AS and MGs latency, right SFG and IPL, and left rMFG and cMFG.

D. Discussion

This study is the first to describe the gray matter correlates of saccade impairment in HD. We found that horizontal and vertical eye movements dissociated Far, Near, and HD groups. On the basis of saccade measures alone, it was not clear that either horizontal or vertical movements were better suited for studying the disease. However, only vertical AS latency was related to both cortical and subcortical gray matter loss in several areas, suggesting that vertical AS are more informative of disease-related atrophy than either horizontal AS or MG measures.

1. Eye movement findings

Our results confirm findings from previous studies that AS latency and error rate are affected in preHD. Horizontal and vertical latency are slowed in the premanifest period (Far vs. Near). While we did not find a significant difference between the Near and HD groups, we did find a significant linear trend across the 3 CAG+ groups. This could be explained by a ceiling effect wherein latency does not continue to slow indefinitely in individuals with manifest disease. We also found group differences in the percentage of AS errors in both directions. The significant increases were found between
the Near and HD groups in both horizontal and vertical directions, and between Far and Near in horizontal AS only. This demonstrates that the AS task is quite useful for measuring premanifest disease progression, and that both latency and percentage of errors are informative.

The results from the MG tasks suggest that the more challenging MGi and MGc tasks are more sensitive than MGs in measuring decline during the premanifest period. There was significant impairment in the HD group in the percentage of errors (MGi and MGc) and the percentage of missed flashes (MGc). Furthermore, the Near group made more errors than the Far group during the MGc task. The linear trend among CAG+ groups was significant for all three measures. The percentage of correct saccades in the MGc task appears more sensitive than other MG measures in detecting a gradual progression in premanifest and early manifest HD, although the very low percentage of correct responses in manifest HD (33.3%) suggests that a ceiling effect may be reached when studying progression beyond the earliest stages of manifest disease. It is also important to note that none of the measures were able to detect differences between the CAG- and Far from onset groups. This may be explained in part by the observation that the Far group was significantly younger than the CAG- group.

*Association Between Atrophy and Saccade Impairment*

The neural correlates of these saccade impairments are largely unknown in HD. In healthy individuals FEF and SEF are activated to a greater extent during volitional saccades than during reflexive saccades. These regions send projections to the superior colliculus directly and via the caudate. Reflexive saccades are thought to be triggered in the parietal lobe via direct projections to the superior colliculus, though there is considerable evidence that the parietal lobe plays an important role in AS and MG saccades as well. Importantly, the frontal and parietal lobes are interconnected so that an absolute distinction between their functions is unlikely. The one study examining neural correlates of saccades in HD used diffusion tensor imaging to examine the relationship between white matter integrity
and voluntary saccades. They found that the variability of voluntary saccade latency increased as the percentage of fibers connecting the FEF and the caudate decreased, suggesting that fiber loss in this important connection in the saccadic pathway could be the source of increased variability of latency in preHD.

In this study we examined the gray matter correlates of saccade function. We limited our analysis to regions of interest in saccade function, including FEF, SEF, IPL, DLPFC, and caudate (see Methods). The most striking associations between cerebral degeneration and saccadic measures were with the latency of vertical AS. This was the only saccadic measure that was sensitive to both cortical and subcortical atrophy, and it explained more of the variation in size in left SFG, left IPL, and bilateral caudate than any of the other saccadic measures. Subsequent analysis of the associations between the structural measures (left SFG, left IPL, bilateral caudate) indicated that this finding is not entirely explained by correlation between the structural measures; left IPL thickness was significantly associated with the other structures ($p\leq0.01$), but left SFG thickness was not associated with caudate volume ($p\geq0.2$). On the other hand, MG task performance is only affected by subcortical atrophy. The caudate is a major site of cortical input to the basal ganglia, and appears to play a role in both the initiation and inhibition of saccades. This is consistent with its role as a relay between the cortex and ocular motor output. Our findings suggest that increased vertical AS latency is related to changes in the caudate, SEF, and IPL, and that vertical AS latency may be one of the more sensitive markers of cortical and subcortical volume loss in premanifest and early manifest HD.

One major strength of this study was that all imaging and saccadic data were collected at the same study visit, thus avoiding any time-dependent discrepancies between the saccadic and structural measures. Also, all participants in the study had a parent diagnosed with HD which increased the degree of matching between the groups for environmental and other non-measurable influences. On the other hand, this study was limited by the relatively small number of individuals who were imaged. This precluded whole brain analysis of the neural correlates of saccade function and reduced our power to detect significant associations. We are also limited in our ability to conclude that there
are no group differences in saccades between CAG- and individuals far from onset due to the age differences between these groups.

This study is the first to describe the gray matter correlates of saccade impairment in HD. The results suggest that cortical and subcortical atrophy contribute to slowed vertical AS responses, while MG performance is only influenced by subcortical volume loss. While there were few behavioral differences between vertical and horizontal AS, the strong associations between gray matter loss and vertical AS latency suggest that future studies would be well-served to measure vertical AS, which may be an early clinical indication of disease manifestation.
III. Abnormal error-related antisaccade activation in premanifest and early manifest Huntington disease

A. Introduction

Huntington disease (HD) is an autosomal dominant disorder caused by an expanded number of CAG repeats (CAG+) in the huntingtin gene.\(^1\) The disease is characterized by progressive worsening of motor, cognitive, and behavioral control. Diagnosis is based on the Unified Huntington Disease Rating Scale\(^87\) (UHDRS), which emphasizes motor abnormalities. However, many studies indicate that cognitive,\(^99;131;300-303\) psychomotor,\(^98;99;118;131;300;305\) and psychiatric signs\(^154;157;339-344\) can be detected during the premanifest (presymptomatic) period, before a diagnosis of HD is certain.

Abnormalities in saccades, rapid eye movements that shift gaze from one location to another, are widely observed in HD.\(^98;99;104;111;119-124;306\) However, a distinction between types of saccades is important. A prosaccade (PS) is a type of visually-guided saccade that shifts gaze toward a visual stimulus. A PS is often termed reflexive, though the accuracy of the term has been questioned\(^212\) because cognitive processes clearly influence PS initiation. On the other hand, a volitional saccade is an endogenously generated movement in response to a command. It is these volitional saccades that are particularly affected in premanifest and early HD.\(^111;119;120;122\) An antisaccade (AS) is a type of volitional saccade that requires the suppression of a reflexive saccade toward a peripheral visual stimulus, and the voluntary generation of a saccade to the mirror opposite location of the stimulus. As compared to those who do not have the disease-causing expansion (CAG-), both premanifest and manifest HD subjects make more AS errors and have longer and more variable latencies of AS initiation.\(^119;122;124\)

PS are generated by activity in known brain regions, including the visual cortex, parietal cortex, frontal and supplementary eye fields (FEF, SEF), striatum, and superior colliculus (reviewed recently by McDowell and colleagues\(^214\)). Given that AS require a conscious executive component, their successful execution depends on multiple processes such as
planning, reflex suppression, and error monitoring. Based on functional magnetic resonance imaging (fMRI) studies, AS generation activates the same regions as does PS generation, albeit to a greater extent,\textsuperscript{214} with the possible exception of the visual cortex.\textsuperscript{215;277} In addition to regions activated by PS, AS also activate dorsolateral prefrontal cortex\textsuperscript{214;221;222;250;255;276-278} (DLPFC) and anterior cingulate cortex\textsuperscript{221;295;322;328;331;345} (ACC). Lesions in the DLPFC lead to more AS errors, but do not affect PS.\textsuperscript{213;285;286} The ACC plays a role in conflict monitoring generally,\textsuperscript{280-282} and increased activity in the period preceding an AS is associated with better performance.\textsuperscript{283} On the other hand, increased ACC activity is associated with errant reflexive saccades during the response phase of an AS task,\textsuperscript{283;295} suggesting an error monitoring role for the ACC as well.

Separating the preparatory period leading to an AS (i.e. knowing the instruction to make an AS while awaiting the cue to execute it) and the response period (i.e. the presentation of the peripheral stimulus and the saccadic response) is one strategy to help disentangle planning and error. Using such an approach, Brown et al.\textsuperscript{324} identified the FEF, SEF, DLPFC, ACC, and intraparietal sulcus (IPS) as active during AS preparation, while FEF, SEF, and IPS regions were involved in the response period. Others have similarly identified the pre-supplementary motor area (pre-SMA), FEF, and SEF as important regions in maintaining the preparatory set necessary for correct AS performance.\textsuperscript{258;346}

A number of studies have investigated neural abnormalities in HD using fMRI.\textsuperscript{197;199-206;208-211;347} Findings from these reports include both hypo- and hyperactivation of many different regions. However, the neural abnormalities underlying impaired AS in HD have not been investigated. We used an event-related AS paradigm to investigate whether performance of an AS task in a sample of individuals at-risk for HD (at least one parent with diagnosed HD) was affected by: 1) abnormal brain activity while preparing for an AS response, or 2) abnormal activity while executing an AS.
B. Methods

1. Participants

Participants were recruited primarily from individuals who had taken part in previous studies. The inclusion criteria were: 1) parent diagnosed with HD; 2) age between 18 and 65; 3) no diagnosis of HD, or if diagnosed, having received the diagnosis within the past 2 years; and 4) self-reported right-handedness. No participants reported a concurrent neurologic illness, major psychiatric diagnosis (e.g. schizophrenia, bipolar disorder), or current alcohol or drug abuse at any visit. Participants were asked not to disclose their CAG status, if known, to study staff. This study was approved by the local institutional review board (IUPUI IRB Study No. 0109). All participants provided written informed consent.

2. Clinical Evaluation and Study Group Assignment

Molecular testing of the huntingtin gene was performed to determine the number of CAG repeats. Individuals in the CAG unexpanded (CAG-) group had 2 alleles with fewer than 28 CAG repeats (n=12). Individuals with at least 1 allele of more than 38 CAG repeats were considered CAG expanded (CAG+; n=19). One CAG+ participant was not included in the analysis due to excessive motion during imaging, resulting in a final sample size of 18 CAG+ individuals.

An experienced movement disorder neurologist (J.W.) administered the motor portion of the UHDRS. The neurologist was aware that the participants were at-risk for HD, but was blind to the results of all other study assessments, including huntingtin gene testing. On the basis of the motor examination only, the neurologist assigned an overall confidence rating (UHDRS diagnosis confidence level) that represented the likelihood of motor abnormalities attributable to HD. The ratings were defined as: (0) normal (no abnormalities); (1) nonspecific motor abnormalities (less than 50% confidence); (2) motor abnormalities that may be signs of HD (50% to 89% confidence); (3) motor
abnormalities that are likely signs of HD (90% to 98% confidence); and (4) motor abnormalities that are unequivocal signs of HD (≥ 99% confidence). Those CAG+ participants with a confidence rating from 0-2 were considered premanifest (preHD, n=10), while those receiving a 3 or 4 were considered to have manifest HD (n=8).

Neuropsychological performance was evaluated using measures from four tests: 1) Wechsler Adult Intelligence Scale-Revised (WAIS-R): Digit Symbol subtest; 2) Symbol Digit Modalities Test (SDMT); 3) Stroop Color-Word Interference Task: Word Reading, Color Naming, Interference; and 4) H-scan system: Movement Time (MT) and Alternate Button Tapping. Analysis of covariance (ANCOVA) was used to test for group differences, with age, gender, and education included as covariates. Covariates were removed from the model when not significant to preserve statistical power.

3. Antisaccade Paradigm

A mixed event-related design was used to study brain activation elicited by PS and AS (Figure 5). Similar to Brown et al., participants were initially given a color-coded instruction to perform a PS or an AS (Figure 5, panel 1). The instruction was then extinguished with the simultaneous appearance of a peripheral stimulus. Subjects were instructed to look at the stimulus (PS trials) or in the mirror-opposite location of the stimulus (AS trials; Figure 5, panel 2). The peripheral stimulus was then extinguished and a centrally-located white circle appeared, upon which participants fixated their gaze while awaiting the next instruction (Figure 5, panel 3). Sixteen PS and sixteen AS trials were presented in each 5:20 minute functional imaging scan in a pseudorandom order using E-prime (www.pstnet.com/eprime.cfm). All but two participants completed four functional imaging scans; one terminated the protocol after the third scan due to loss of sensation in his arms, and eye movement data were not collected during one scan for another.
4. Eye Movement Recording and Analysis

An R-LRO 6.1 eye-tracking system designed for fMRI (Applied Science Laboratories, Bedford, MA) was used to track eye movements during imaging at a sampling rate frequency of 60 Hz. Eye movements were analyzed offline using a semi-automated, in-house software program written in Matlab (http://www.mathworks.com) as described previously. For each trial, the saccade was determined to be either correct or incorrect. We also identified self-corrected AS errors, defined as an initial saccade made toward the stimulus that was corrected by making a saccade away from the stimulus. No external feedback was given to the participants regarding accuracy. The percentages of incorrect trials and of self-corrected AS errors were then determined, and ANCOVA was used to test for between-group differences (CAG-, preHD, manifest HD) with age, gender, and

Figure 5. The fMRI task protocol. A central circle turns green (G) for a PS (A) and red (R) for an AS (B) (panel 1). The circle is extinguished as a horizontal peripheral square (stimulus) appears. For a PS, participants look at the square; for an AS, participants look directly opposite the square (panel 2). The central circle then reappears (panel 3), upon which participants fixate while awaiting the next instruction. Repetition time = 2000 ms. Dotted circle = correct eye position (shown here for illustration purposes, but not visible to participants during testing).
education included as covariates when appropriate. A significant ANCOVA \((p \leq 0.05)\) was followed by one-tailed \(t\)-tests of all pairwise comparisons.

5. Image Acquisition and Analysis

Subjects were imaged using a Siemens (Erlangen, Germany) 3T Magnetom Trio-Tim scanner with a 12-channel head-coil. A whole-brain, structural image volume \((1.0 \times 1.0 \times 1.2 \text{ mm voxels})\) was acquired first using a 3D magnetization prepared rapid gradient echo (MPRAGE) sequence to enable anatomic registration of the functional volumes.\(^{352}\) Functional imaging was performed with a blood oxygenation level dependent (BOLD) contrast sensitive gradient echo, echo-planar imaging sequence (repetition time 2000 ms, echo time 29 ms, flip angle 76˚, field of view 220 × 220 mm, 35 interleaved axial slices, \(2.5 \times 2.5 \times 3.0 \text{ mm voxels}\)) incorporating a 3D prospective acquisition correction algorithm, which adjusts the acquisition in real time to account for head movement.

Given that atrophy in the caudate and putamen have been consistently described,\(^{173;178;182;183;186;192;353;354}\) an automated segmentation procedure in FreeSurfer \(^{V4}^{321}\) was used to extract caudate and putamen volumes from each individual’s structural image. We then used ANCOVA with age, gender, and intracranial volume (ICV) as covariates to test for group differences. Post hoc analysis was carried out on all measures with a significant group effect using a two-tailed \(t\)-test for all pairwise comparisons.

Image analysis was performed using SPM5 (Wellcome Trust Centre for Neuroimaging, University College London, UK, http://www.fil.ion.ucl.ac.uk/spm/). Functional image volumes were corrected for slice acquisition timing differences and rigid-body realigned to the initial volume of the first functional imaging scan, which was also a reference volume for MPRAGE co-registration. The MPRAGE volume segmentation into tissue classes generated nonlinear spatial transformation parameters enabling a conversion of functional image volumes to a common coordinate system (Montreal Neurological Institute; MNI). The resulting functional image volumes were resampled to 2 mm
isotropic voxels and smoothed by a 6 mm full-width at half-maximum isotropic Gaussian kernel.\textsuperscript{355}

Brain responses to eye movements in each participant were modeled in a general linear model using SPM’s canonical hemodynamic response function. Six movement parameters (three translations and three rotations) obtained during realignment were included as regressors to account for residual movement-induced effects. Serial correlations in the fMRI time series were accounted for using an autoregressive model implementing classical (restricted maximum likelihood) parameter estimation. A high-pass filter with a cut-off of 1/128 Hz was applied to each voxel’s time series to remove low frequency noise.

A first level model yielded contrast images for each participant that represented the mean BOLD response to three eye movement conditions: [correct AS > correct PS], [correct AS > incorrect AS], [incorrect AS > correct AS]. Incorrect AS trials included both self-corrected and uncorrected AS errors. BOLD activity associated with PS error trials was not modeled due to the very small number of errors made by all participants on the PS trials. Event onsets were defined for: 1) the preparation phase, which included the times of the instructional stimulus presentations; and 2) the response phase, which included the times of the peripheral stimulus presentations. This second approach was feasible since all participant reaction times were less than 750 msec. A second level, random effects analysis within the CAG-group was then used to identify activated regions that achieved a corrected cluster level significance ($p_{\text{cluster}}<0.05$) under a voxel-wise height threshold of $p_{\text{voxel}}<0.001$. The correction for multiple comparisons was performed within a whole-brain search volume common across all CAG-participants, with implicit rejection of cerebrospinal fluid voxels and exclusion of predominantly white matter voxels (probability of white matter from SPM segmentation>0.70). Functional regions of interest (ROI) were defined for each significant cluster in the CAG-group under each eye movement condition described above. These ROIs were then used as the criterion to define “normal” activation in an unaffected, healthy sample. Mean activity within each
ROI was extracted in all participants using the MarsBaR toolbox (http://marsbar.sourceforge.net/).

To compare the mean activation among the groups, it is important to account for the frequency of incorrect responses. Thus, a multiple linear regression model implemented in SAS version 9.13 was used to examine the relationship between mean activation and group, percentage of incorrect AS, and the interaction between group and percentage of incorrect AS. The preHD group was treated as the reference group in the model so that comparisons could be made between the CAG- and preHD groups and between the preHD and manifest HD groups. Age, gender, and education were included as covariates in the model when they had a significant effect on the model ($p \leq 0.05$).

C. Results

The demographics, neuropsychological test performance, and caudate and putamen volumes of the sample are shown in Table 7. The groups did not differ significantly in age, education, or gender, and there was no significant difference in the number of CAG repeats in the larger allele for the two CAG+ groups. There was a significant difference ($p \leq 0.03$) between the groups in measures of psychomotor speed (movement time and alternate button tapping). Post hoc testing revealed a difference between the CAG- and manifest HD groups ($p = 0.005$) for movement time and between the manifest HD group and both the CAG- and preHD groups ($p \leq 0.01$) for alternate button tapping. As expected, and consistent with previous studies, the groups also differed in caudate and putamen volumes bilaterally, and all three pairwise group comparisons were highly significant ($p \leq 0.001$).
Table 7. Participant demographics.

<table>
<thead>
<tr>
<th></th>
<th>CAG- (n=12)</th>
<th>PreHD (n=10)</th>
<th>Manifest HD (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>46.4 ± 11.4</td>
<td>44.2 ± 15.2</td>
<td>42.8 ± 13.4</td>
</tr>
<tr>
<td>Education (years)</td>
<td>14.8 ± 2.1</td>
<td>15.7 ± 3.2</td>
<td>15.8 ± 4.4</td>
</tr>
<tr>
<td>Gender (M:F)</td>
<td>5:7</td>
<td>4:6</td>
<td>3:5</td>
</tr>
<tr>
<td># of CAG repeats in</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>larger allele</td>
<td>20.2 ± 3.3</td>
<td>42.2 ± 2.1</td>
<td>45.5 ± 5.1</td>
</tr>
<tr>
<td>Movement Time (s)</td>
<td>0.14 ± 0.03</td>
<td>0.16 ± 0.05</td>
<td>0.19 ± 0.05</td>
</tr>
<tr>
<td>Alternate Button</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tapping (s/30 round</td>
<td>17.3 ± 3.0</td>
<td>18.1 ± 3.0</td>
<td>21.9 ± 4.1</td>
</tr>
<tr>
<td>trips)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroop Color Naming</td>
<td>76.9 ± 16.7</td>
<td>85.3 ± 10.3</td>
<td>70.5 ± 14.6</td>
</tr>
<tr>
<td>(correct/45 s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroop Word Reading</td>
<td>97.0 ± 23.9</td>
<td>103.9 ± 15.4</td>
<td>94.5 ± 14.2</td>
</tr>
<tr>
<td>(correct/45 s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroop Interference</td>
<td>47.2 ± 10.7</td>
<td>48.9 ± 9.2</td>
<td>43 ± 8.9</td>
</tr>
<tr>
<td>(correct/45 s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Symbol Digit Modalities</td>
<td>54.2 ± 8.8</td>
<td>48.8 ± 13.3</td>
<td>41.8 ± 9.0</td>
</tr>
<tr>
<td>Test (correct/90 s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WAIS-R Digit Symbol</td>
<td>59.0 ± 14.9</td>
<td>60.4 ± 10.3</td>
<td>50.0 ± 14.9</td>
</tr>
<tr>
<td>(correct/90 s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caudate (mm³)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>3437 ± 400</td>
<td>3151 ± 251</td>
<td>2384 ± 463</td>
</tr>
<tr>
<td>Right</td>
<td>3523 ± 439</td>
<td>3215 ± 343</td>
<td>2541 ± 368</td>
</tr>
<tr>
<td>Putamen (mm³)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>5448 ± 885</td>
<td>4925 ± 785</td>
<td>3644 ± 720</td>
</tr>
<tr>
<td>Right</td>
<td>5116 ± 701</td>
<td>4679 ± 893</td>
<td>3359 ± 616</td>
</tr>
</tbody>
</table>

Post hoc testing indicates a significant difference (p≤0.05) between a) CAG- and preHD, b) CAG- and manifest HD, and c) preHD and manifest HD.
1. Antisaccade task performance

ANCOVA was used to test for group differences in the percentages of incorrect PS and AS. A significant group effect was found for both the percentages of incorrect PS and AS ($p \leq 0.02$) (Figure 6). For the PS task, post hoc testing revealed that the CAG- group made significantly fewer errors than both CAG+ groups ($p \leq 0.01$), although the error rates were low overall. For the AS task, the manifest HD group made significantly more errors than the CAG- and preHD groups ($p \leq 0.05$), and there was a trend toward a difference between the CAG- and preHD groups ($p=0.08$). More than 85% of AS errors were self-corrected in all groups, with no statistical difference between the groups ($p=0.6$).

![Figure 6. Performance on the PS and AS tasks.](image)

For the PS task, the CAG- group made significantly fewer errors than the preHD and manifest HD group. For the AS task, the manifest HD group made significantly more errors than the CAG- and preHD groups.

2. BOLD responses during AS task (Table 8, Figure 7).

a. Preparation, [correct AS > correct PS]. Eleven functional ROIs emerged as significant within the CAG- group (Figure 7A): Left and right DLPFC; left and right FEF; right anterior insula/frontal operculum; pre-SMA/dorsal ACC (dACC); left and right parietal eye field (PEF); left and right middle occipital gyrus; and right calcarine cortex.

When testing extracted mean activations in a multiple linear regression model, there was a significant main effect of group and an interaction between group and percentage of incorrect AS in the left middle occipital gyrus ($p=0.03$). Further testing revealed a significant difference between the slopes and intercepts of the regression lines in the
preHD and manifest HD groups (Table 8, Figure 8A). There was also a significant effect of the percentage of incorrect AS in the left DLPFC (Figure 8B), pre-SMA/dACC, left and right PEF, and left middle occipital gyrus ($p \leq 0.03$). In all cases, the BOLD response decreased as the percentage of incorrect AS responses increased.

b. Response, [correct AS > correct PS]. One functional ROI emerged as significant within the CAG- group in the right PEF (Figure 7B). There were no significant effects of group, percentage of incorrect AS, or their interaction in this ROI ($p \geq 0.3$, Table 8).

c. Preparation and response, [correct AS > incorrect AS]. No functional ROIs were defined as there were no regions that met our criteria in the CAG- group during either preparation or response.

d. Preparation, [incorrect AS > correct AS]. Two functional ROIs were significant within CAG- group (Figure 7C): Left and right calcarine cortices. ANCOVA revealed a significant effect of group in the left calcarine cortex ($p=0.03$). Post hoc testing showed a significant difference between the CAG- and preHD groups ($p = 0.02$, Table 8).

e. Response, [incorrect AS > correct AS]. Six functional ROIs emerged as significant within the CAG- group (Figure 7D): 1) Left inferior frontal gyrus (IFG); 2) pre-SMA; 3) dACC; 4) posterior cingulate cortex (PCC); 5) right inferior parietal lobule (IPL); and 6) left middle temporal gyrus (MTG).

A significant interaction between group and percentage of incorrect AS ($p \leq 0.03$) was found for all functional ROIs except the PCC, with the same general pattern across these ROIs (Table 8). In all cases the slope of the regression line was significantly different between the CAG- and preHD groups ($p \leq 0.02$), but not significantly different between the preHD and manifest HD groups ($p \geq 0.3$). Specifically, the BOLD response decreased as the percentage of incorrect AS increased in the CAG- group, but not in either of the CAG+ groups (Figures 8C: pre-SMA, and 4D: dACC).
Table 8. ROI locations and sizes as defined by CAG- controls, and non-zero linear regression parameter estimates and $p$ values (with preHD as the reference group) from the modeling of ROI-extracted mean activations.

<table>
<thead>
<tr>
<th>Contrast/ROI</th>
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</tr>
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<tbody>
<tr>
<td></td>
<td>Group</td>
</tr>
<tr>
<td></td>
<td>Size (mm$^3$)</td>
</tr>
<tr>
<td>Correct AS &gt; Correct PS Preparation</td>
<td></td>
</tr>
<tr>
<td><strong>Left DLPFC</strong></td>
<td>464</td>
</tr>
<tr>
<td><strong>Right DLPFC</strong></td>
<td>1480</td>
</tr>
<tr>
<td><strong>Left FEF</strong></td>
<td>3184</td>
</tr>
<tr>
<td><strong>Right FEF</strong></td>
<td>3696</td>
</tr>
<tr>
<td><strong>Right Anterior Insula/Frontal Operculum</strong></td>
<td>640</td>
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<tr>
<td><strong>Pre-SMA/dACC</strong></td>
<td>6776</td>
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Covariates that remained in the model due to a significant effect ($p<0.05$) are indicated by superscripts: * education, † age. dACC: dorsal anterior cingulate cortex; DLPFC: dorsolateral prefrontal cortex; FEF: frontal eye field; IPL: inferior parietal lobule; MTG: middle temporal gyrus; PCC: posterior cingulate cortex; PEF: parietal eye field; pre-SMA: pre-supplementary motor area.
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<table>
<thead>
<tr>
<th>Contrast/ROI</th>
<th>Parameter Estimates (p values)</th>
<th>Group</th>
<th>Percentage of Incorrect AS</th>
<th>Group x Percentage of Incorrect AS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Group</td>
<td>Percentage of Incorrect AS</td>
<td>Group x Percentage of Incorrect AS</td>
</tr>
<tr>
<td></td>
<td>Size (mm$^3$)</td>
<td>Peak MNI Location (x,y,z)</td>
<td>PreHD vs CAG-</td>
<td>PreHD vs Manifest HD</td>
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<tr>
<td>Left PEF*</td>
<td>1584</td>
<td>-20, -58, 52</td>
<td>-0.03 (0.001)</td>
<td></td>
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<tr>
<td>Right PEF*</td>
<td>4384</td>
<td>18, -66, 52</td>
<td>-0.04 (0.0008)</td>
<td></td>
</tr>
<tr>
<td>Left Middle Occipital Gyrus*†</td>
<td>480</td>
<td>-26, -80, 20</td>
<td>-1.66 (0.02)</td>
<td>-0.05 (0.0004)</td>
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<tr>
<td>Right Middle Occipital Gyrus</td>
<td>1024</td>
<td>30, -80, 20</td>
<td></td>
<td></td>
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<tr>
<td>Right Calcarine Gyrus</td>
<td>1240</td>
<td>12, -80, 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stimulus-response Right PEF</td>
<td>1136</td>
<td>8, -60, 54</td>
<td></td>
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<td></td>
<td>Size (mm$^3$)</td>
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<td>Incorrect AS &gt; Correct AS</td>
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<tr>
<td>Right Calcarine Gyrus</td>
<td>1304</td>
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<tr>
<td>Stimulus-response</td>
<td></td>
</tr>
<tr>
<td>Left Inferior Frontal Gyrus</td>
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<tr>
<td>Pre-SMA</td>
<td>1704</td>
</tr>
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<td>Group</td>
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<tr>
<td></td>
<td>Size (mm³)</td>
</tr>
<tr>
<td>dACC</td>
<td>1224</td>
</tr>
<tr>
<td>PCC</td>
<td>664</td>
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<tr>
<td>Right IPL</td>
<td>456</td>
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<tr>
<td>Left MTG</td>
<td>720</td>
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</tbody>
</table>

Covariates that remained in the model due to a significant effect (p<0.05) are indicated by superscripts: * education, † age. dACC: dorsal anterior cingulate cortex; DLPFC: dorsolateral prefrontal cortex; FEF: frontal eye field; IPL: inferior parietal lobule; MTG: middle temporal gyrus; PCC: posterior cingulate cortex; PEF: parietal eye field; pre-SMA: pre-supplementary motor area.
Figure 7. Functional ROIs defined in CAG- participants for each saccade comparison. (A) Significant activation during preparation for the [correct AS > correct PS] comparison in the bilateral DLPFC, FEF, PEF, and middle occipital gyri; the right insula and calcarine cortex; and the pre-SMA/dACC. (B) Significant response-related activation for [correct AS > correct PS] in the right PEF. (C) Significant activation during preparation for the [incorrect AS > correct AS] comparison in the bilateral calcarine cortices. (D) Significant activation during responses for [incorrect AS > correct AS] in the left IFG, pre-SMA, dACC, PCC, right IPL, and left MTG. AS = antisaccade; dACC = dorsal anterior cingulate cortex; DLPFC = dorsolateral prefrontal cortex; FEF = frontal eye fields; IFG = inferior frontal gyrus; IPL = inferior parietal lobule; L = left; MTG = middle temporal gyrus; PCC = posterior cingulate cortex; PEF = parietal eye fields; pre-SMA = pre-supplementary motor area.
Figure 8. BOLD response [incorrect AS > correct AS] as a function of the percentage of incorrect AS. In the left middle occipital gyrus (MOG) (A), activation is dependent on the percentage of incorrect AS in the CAG- and preHD groups but not in the manifest HD group, and the manifest HD group has lower overall activation. In the left DLPFC (B), activation decreases as the percentage of incorrect AS increases in all 3 groups. In the pre-SMA (C) and dACC (D) activation is dependent on the percentage of incorrect AS in the CAG- group but not in either CAG+ group; the CAG- group has greater overall activation. Furthermore, there are no differences in activation between the preHD and manifest HD groups. dACC = dorsal anterior cingulate cortex; DLPFC = dorsolateral prefrontal cortex; pre-SMA = pre-supplementary motor area.
D. Discussion

1. Identified brain regions

This is the first study to explore the BOLD fMRI response during an AS task in CAG+ individuals. The brain regions activated by AS (with a baseline of PS) within the CAG-group closely resemble those from previous studies of other populations\textsuperscript{322-324} (Figure 7A). In particular, our healthy control CAG- participants activated the DLPFC, FEF, PEF, insula, ACC, pre-SMA. The increased activation in the visual cortex was consistent with some studies,\textsuperscript{255} though other studies have found the opposite pattern.\textsuperscript{215,277} During the response phase, activation was limited to the right PEF.

Activation related to AS errors (with a baseline of correct AS) was limited to visual cortex during preparation, but was more widespread during the response phase as evidenced by activation in the IFG, pre-SMA, ACC, PCC, IPL, and MTG. Previous studies have typically identified the ACC as being activated by errant saccades,\textsuperscript{283,295} and Polli et al.\textsuperscript{295} also found increased activation in the IFG, pre-SMA, and anterior insula. Similar error-monitoring activity in the ACC and in a more dorsal pre-SMA area has been described in non-saccadic tasks as well,\textsuperscript{281,356-363} suggesting that these areas are critical to the monitoring of errant behavior more generally.

2. Abnormal Activation in CAG+ Groups

Abnormalities in AS performance are sensitive markers of the premanifest period of HD.\textsuperscript{110,119,364} Given these previous findings, it would be reasonable to hypothesize that activation abnormalities would be found either in the preparation or response phase of an AS task (compared to a PS baseline). Our analysis found some group differences in the left middle occipital gyrus while subjects prepared to make AS, but further examination makes this finding of questionable significance: In particular, although the regression line was significantly less negative in the manifest HD, no one in this group had an AS error rate of less than 20%. Since the BOLD response was dependent on the percentage of
incorrect AS in the other 2 groups, it is possible that the differences in this group can be explained solely by the restriction of range. Instead, the cortical activity that did distinguish between these groups was related to AS errors in the response phase (with a baseline of correctly executed AS). Specifically, activity was inversely related to the percentage of incorrect AS in the CAG- controls, but not in the preHD or manifest HD patients. In the case of the preHD, restriction of range cannot explain this difference, as the distribution of error rates was similar across both the preHD and the CAG- groups.

Activity in the ACC and pre-SMA (two of the five areas in which group × error interaction emerged) is likely to be particularly important. Both of these regions are repeatedly noted as sites of error-related activation, even in studies that do not involve ocular motor responses. Furthermore, an event related potential (ERP) study of error processing showed decreased error-related negativity (ERN) during a Flanker task in patients with manifest HD. That is, brain responses to provoked behavioral errors were less prominent in HD subjects than in healthy controls—a result that mirrors our findings with AS errors.

Detection of errant behavior, or of events that violate expectations, is a necessary function for executive control, and the failure to process such errors will lead to poor adaptive behavior. The dopaminergic mesocorticolimbic brain circuit has received much attention for its hypothesized roles in reward related processing. However, a key element in this processing is the learned anticipation of outcomes based on experience, and the detection of events that do not conform to these learned expectations. For example, seminal work by Schultz and his collaborators has shown that dopaminergic midbrain neurons increase their firing to unanticipated events, and decrease their firing when an anticipated (cued) reward fails to arrive. Holroyd and Coles elaborate on such findings and hypothesize that medial frontal (ACC, pre-SMA) areas are signaled by this midbrain activity, provoking their engagement in error (deviant event) processing. Moreover, midbrain dopaminergic neurons are smaller and have a loss of tyrosine hydroxylase mRNA in HD. Thus, one potential explanation of our findings is an early loss of midbrain signaling in preHD patients.
Although both the ACC and pre-SMA are consistently implicated in error-related activation, there are also questions about the exact nature of each region’s role in saccadic pathways. Compelling evidence from a rare patient with a small focal lesion to the left supplementary eye field (SEF), posterior to the pre-SMA, suggests SEF involvement in resolving conflict both from internally generated saccadic plans and during rule switching, but not in saccade generation per se. Similarly, functional imaging data show that activity in caudal SMA is related to sudden changes in planned saccades, while SEF activity is related to successfully implemented plan changes. In primates, Schall et al. localized different populations of neurons in the ACC, pre-SMA, and adjacent SEF, wherein one population of neurons accounted for conflict-related activation, one for reinforcement-related activation, and one for error-related activation; thus, one region may play a role in multiple functions.

The ACC also has connections to important regions in saccadic pathways. In a meta-analysis of cingulate cortex, Beckmann et al. characterized a cluster in the dACC (their “cluster 4”) that overlaps with the region activated in our study by AS error trials, and which appears to mediate conflict resolution and error detection. Through tractography, Beckmann et al. showed this dACC region has white matter projections to prefrontal and premotor areas, as well as to the dorsal striatal regions that are an early site of degeneration in HD. Picard and Strick similarly identified an overlapping rostral cingulate motor zone in their meta-analysis as governing conflict and action selection.

Reinforcing our findings in HD, other disorders with presumed frontal and dopaminergic involvement also show functional brain abnormalities linked to errors in AS. In particular, similar error-related activation in the ACC was significantly reduced in a sample of schizophrenic patients at peak coordinates [8, 13, 25]/[-13, 17, 25] that were quite close to our own [0, 16, 24]. Conversely, Thakkar et al. reported that in autism spectrum disorders there is dACC hyperactivation in response to correct AS, which was in turn related to rigid and repetitive behavior.
While pre-SMA and cingulate cortex have received the most attention for their roles in error-related activation, there is evidence that the IPL and IFG have related roles. A number of studies identify the IPL as playing an important role in the inhibition of an unwanted response, while others have found that the IPL is activated in response to error commission. Interestingly, most of these studies found evidence for asymmetric activation of the right IPL. Findings in the IFG have been reported even more rarely than in the IPL, but Hodgson et al. showed that lesions in ventrolateral frontal cortex (including IFG) predict impaired AS performance. There is also evidence of error-related activation in the IFG. These previous findings and the similarity of the activation patterns between the IPL and IFG and the pre-SMA and dACC suggest that these regions may play a role in the error-related network along with pre-SMA and cingulate cortex, although more study is necessary.

This study was limited by the inability to dichotomize the preHD group into groups estimated to be closer to or farther from onset. Similarly, we were unable to use estimated time to onset in our analysis because of the limited number of participants in the preHD group. As we focused our group analysis on those regions with significant activation in the CAG- healthy control group, we cannot make conclusions regarding the recruitment of other brain regions in an attempt to compensate for pathology in regions normally involved in task performance. However, our method did permit detecting deviant activation in CAG+ individuals in regions usually involved in task performance (though only decreased activation was found). Unlike prior studies, and contrary to expectations, there was only a marginal difference in the percentage of incorrect AS between the CAG- and preHD groups ($p = 0.08$). However, it is likely that a smaller sample size and adaptation of the task to a more difficult mixed event-related design contributed to this finding.

In summary, this is the first study to examine the underlying functional neuroanatomy associated with AS performance in HD. While future studies, including longitudinal ones, are necessary to determine the temporal appearance of abnormalities within the context of disease progression, our data suggest that impaired AS performance may be related to
abnormal cortical activity during the processing of saccadic errors. Importantly, deficits in this error-related activity appear to occur early in the disease process (i.e., in premanifest individuals with normal AS performance), pointing to a prodromal decline in an important supervisory executive network.
IV. Progression in prediagnostic Huntington disease

A. Introduction

Huntington disease (HD) is an autosomal dominant disorder characterized by progressive decline of motor, cognitive, and behavioral function. The disease-causing mutation is a trinucleotide (CAG) repeat expansion in the 5' translated region of the huntingtin gene.\(^1\) The average age of onset is 40 years, although onset occurs as early as age 2 and as late as age 80.\(^{81,82}\)

Disease onset is insidious, often with a long prediagnostic period prior to the clinical diagnosis. Typically, diagnosis is made based on the presence of unequivocal motor signs consistent with HD. Unfortunately, there are no current pharmacologic or therapeutic interventions shown to delay or slow the onset or progression of HD. Therefore, it is essential that sensitive and specific biomarkers in the prediagnostic period be identified that could be used to evaluate future therapeutic interventions.

Several studies have sought to identify potential prediagnostic biomarkers. Some cross sectional studies reported prediagnostic CAG expanded individuals (CAG+) exhibited deficits in tests of attention,\(^{300}\) executive function,\(^{301,302}\) memory,\(^{131,300;301;303;304}\) psychomotor speed,\(^{131,300}\) and ocular movements;\(^{111;119;120;122;306}\) however, other studies of these same domains have not confirmed these results.\(^{128;312;379-383}\) In a large cross sectional sample of 438 prediagnostic individuals, Paulsen and colleagues\(^{100}\) reported the commencement of detectable changes begins one to two decades prior to the estimated age of onset, and this initial period is followed by more rapid change in the years just prior to diagnosis. Few studies have explored longitudinal rates of decline in prediagnostic CAG+ individuals. There have been reports of differential rates of progression between prediagnostic CAG+ and nonexpanded (CAG-) controls in measures of attention, psychomotor speed, and memory;\(^{130;307-310}\) however, others have not been able to replicate these results.\(^{311-314}\) These discrepant results may be due to the modest sample sizes of most studies.
The goal of this study was to examine longitudinal rates of change in a sample of at-risk individuals for a series of neurocognitive, psychomotor, and oculomotor measures. We use estimated time to onset in two ways: 1) as a continuous variable to evaluate change within a group of CAG+ individuals, and 2) as a means of dichotomizing a group of CAG+ individuals (into those Near and Far from onset) in order to compare the rate of decline in each with the rate in CAG- individuals. We hypothesize that the rate of progression is not uniform within the prediagnostic period and that it increases as CAG+ individuals approach onset. In addition, the rate of change is faster in CAG+ than in CAG- individuals.

B. Methods

1. Participants

Participants were recruited primarily through the National Research Roster for Huntington Disease Patients and Families (HD Roster). The inclusion criteria were: 1) a parent diagnosed with HD; 2) between the ages of 18 and 65; and 3) a non-diagnostic motor exam at the first study visit (Unified Huntington Disease Rating Scale-9987 (UHDRS) diagnostic confidence level less than 4). All participants completed two study visits, approximately 2.5 years apart. The testing protocol was identical at both visits. Medical history, current medications, and history of alcohol and recreational drug use were collected at both visits. Any participants reporting a concurrent neurologic illness, major psychiatric diagnosis (e.g. schizophrenia, bipolar disorder), or current alcohol or drug abuse were excluded from the analyses. Participants were asked not to disclose their CAG status, if known, to study staff. This study was approved by the local institutional review board (IUPUI IRB Study No. 0109-12). All participants provided written informed consent.
Molecular testing of the huntingtin gene was performed to determine the number of CAG repeats. Normal controls (NC; n=68) were defined as those having 2 unexpanded alleles (<28 CAG repeats). Individuals with at least 1 expanded allele (>38 CAG repeats) were considered CAG expanded (CAG+; n=39). Subjects whose larger allele contained 28 to 38 CAG repeats, inclusive, were considered inconclusive and were not used in the analyses (n=10).

Two movement disorder neurologists (J.W., X.B.) administered the motor exam portion of the UHDRS. Both were aware that the participants were at-risk for HD, but were blinded to the results of all other study assessments, including the results of huntingtin gene testing. The motor examination was performed for each participant at both study visits. On the basis of the motor examination only, they assigned an overall confidence rating which represented the likelihood that any observed abnormalities represented HD. The ratings were defined as: (0) normal (no abnormalities); (1) nonspecific motor abnormalities (less than 50% confidence); (2) motor abnormalities that may be signs of HD (50% to 89% confidence); (3) motor abnormalities that are likely signs of HD (90% to 98% confidence); and (4) motor abnormalities that are unequivocal signs of HD (≥99% confidence). CAG+ subjects with a confidence rating from 0-3 at their second visit were considered prediagnostic (n=34). Five subjects who were prediagnostic at their first visit became diagnostic (confidence rating of 4) at their second visit. Estimated onset was defined as the age at which a person had a 50% probability of having manifest disease, and the estimated time to onset (TTO) was calculated for each participant at each study visit. The distribution of TTO at the first study visit was reviewed and one subject was removed due to a very large TTO (>3.5 SD from the mean) so that 38 CAG+ subjects were included in the analyses.
3. Study Assessment

The study battery included an assessment of neurocognitive performance, psychomotor speed, and saccadic eye movements. All testing was conducted in a private examination room by trained study staff.

Neurocognitive performance and psychomotor speed were evaluated using measures from six tests: 1) Wechsler Adult Intelligence Scale-Revised\textsuperscript{348} (WAIS-R): Arithmetic, Picture Arrangement, and Digit Symbol subtests; 2) Stroop Color-Word Interference Task\textsuperscript{350} Word Reading, Color Naming, Interference; 3) Trail Making Test\textsuperscript{384} Parts A and B; 4) WAIS-III\textsuperscript{385} Letter-Number Sequencing Test; 5) California Verbal Learning Test\textsuperscript{137} (CVLT): Total Learning, Semantic Clustering, Short Delay Recall, Long Delay Recall, Recognition Discriminability; 6) H-scan system\textsuperscript{351} reaction time (RT) (Auditory RT, Visual RT, Decision RT) and motor speed (Movement Time (MT), Decision MT, Alternate Button Tapping). We also assessed depressive symptomatology using the Center for Epidemiologic Studies Depression Scale (CES-D).

Saccadic eye movement testing was performed as described previously\textsuperscript{119}. Briefly, the participant was seated 1 meter from a large white screen in front of a bar with vertical and horizontal target lights (light-emitting diodes, LED). Three saccadic tasks were administered: anti-saccade (AS), memory guided, simple version (MG1), and memory guided, complex version (MG2). The vertical and horizontal positions of the participant’s pupils were recorded binocularly with two ultra-miniature high-speed (250 Hz) video cameras attached to a headband and digitized at 250 Hz for later analysis (Eyelink II, SR Research Ltd, spatial resolution < 0.1 degree). Before each task, the examiner instructed the participant verbally to ensure that the participant understood the instructions. Each of the tasks consisted of 25 trials. After the participant completed the testing procedure, an interactive computerized analysis\textsuperscript{122} of the right eye position was performed. Current analyses focused on the AS and MG measures (saccadic latency, the standard deviation of saccade latency, and percentage of errors) previously reported to demonstrate abnormalities in prediagnostic HD\textsuperscript{119}.
4. Statistical Analysis

We tested for group differences in depression at each visit using a Fisher’s exact test. All analyses evaluated the change in the performance of neurocognitive, psychomotor, and oculomotor tasks between the two study visits in the NC and prediagnostic CAG+ participants.

To evaluate longitudinal change during the prediagnostic period, we analyzed neurocognitive, psychomotor, and oculomotor performance using a repeated measures, mixed linear model (SAS v9.13). The model included three terms: 1) a main effect of TTO, indicating a linear relationship between TTO and performance; 2) a main effect of visit, indicating a change in performance between the study visits, and perhaps indicating either training/learning or disease progression between the visits; and 3) an interaction between TTO and visit, indicating an effect of TTO on the between-visits change in performance. The model also included age, sex, and education as covariates, when there was a significant effect ($p \leq 0.05$). This analysis included only prediagnostic CAG+ participants.

We also evaluated how the rate of change in prediagnostic CAG+ subjects compared with that in CAG- subjects. To do this, a median split in the TTO distribution was used to define two prediagnostic groups: 1) Far from onset (Far), defined as those participants whose TTO at the first study visit was greater than 11 years ($n=19$); and 2) Near to onset (Near), defined as those participants whose TTO was less than 11 years ($n=19$).

The rate of change in the prediagnostic (CAG+) and NC (CAG-) groups for each study measure was calculated for each participant as follows:

\[
\text{rate} = \frac{\text{visit 2 measure} - \text{visit 1 measure}}{\text{months between visits}}.
\]

These rates were analyzed using analysis of covariance (ANCOVA) to test for group effects (3 groups: NC, Far, Near) with sex, age, and education as covariates. For variables
with a significant group effect \((p\leq0.05)\), post hoc analysis was performed using two-sided t-tests of all pairwise comparisons.

For all analyses we employed a nominal significance value \((p\leq0.05)\). We recognize that we are testing multiple outcomes; however, our approach was to review results to identify trends or domains consistently affected in prediagnostic individuals.

C. Results

The 106 participants included in the analysis completed two visits approximately 2.5 years apart \((28.6 \pm 5.2 \text{ months})\). The three groups (NC, Far, Near) did not differ significantly \((p\geq0.6)\) for sex, race, handedness, education, or months between study visits, nor was there a significant difference \((p=0.1)\) between the Near and Far groups for the number of CAG repeats (Table 9). The groups did, however, differ significantly for age \((p=0.02)\), with the Far group being significantly younger than the other two groups (Table 9). Due to technical difficulties, saccade tasks were completed in only a subset of the participants \((n=74)\). The prevalence of depression was assessed in our patients using the CES-D. At the first study visit, there was no significant difference between the NC,

Table 9. Participant demographics.

<table>
<thead>
<tr>
<th>Number of participants</th>
<th>NC (68)</th>
<th>Far (19)</th>
<th>Near (19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at first visit* (years)</td>
<td>45.2±8.7</td>
<td>39.5±9.2</td>
<td>47.7±10.8</td>
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<tr>
<td>Months between visits*</td>
<td>28.2±4.1</td>
<td>29.5±7.0</td>
<td>29.1±6.6</td>
</tr>
<tr>
<td>Education* (years)</td>
<td>15.6±2.8</td>
<td>15.5±1.9</td>
<td>15.6±3.4</td>
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<tr>
<td>Male:Female</td>
<td>19:49</td>
<td>6:13</td>
<td>7:12</td>
</tr>
<tr>
<td>Race (% Caucasian)</td>
<td>98.5%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Handedness (% right)</td>
<td>91.2%</td>
<td>89.5%</td>
<td>89.5%</td>
</tr>
<tr>
<td>CAG repeats*</td>
<td>41.7±2.7</td>
<td>43.4±4.0</td>
<td></td>
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<tr>
<td>Estimated time to onset computed from first visit* (years)</td>
<td>18.2±4.7</td>
<td>8.1±1.7</td>
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</table>

*Mean ± SD
Far, and Near groups ($p=0.4$). At the second visit, the prevalence was significantly higher in the Far group ($p=0.01$).

### 1. Repeated Measures Analysis in CAG+ Individuals

Repeated measures analysis was used to examine the main effects of TTO and visit, and the interaction between the two on each of the performance outcomes. Representative plots of the data are shown in Figure 9. Each subject’s performance at the first and second visits is connected by a line. For alternate button tapping (Figure 9A), a significant main effect of TTO indicated that subjects require more time to complete 30 round trips as they approach onset. A similar trend is not seen for variability of latency of MG1 (Figure 9C) or for percentage of errors of MG2 (Figure 9E), indicating no significant effect of TTO. The interaction between TTO and visit can be seen by examining the changes from visit 1 to visit 2. For alternate button tapping (Figure 9A) the changes for subjects with a larger estimated TTO tend to be relatively flat, and become steeper as onset approaches. For variability of latency of MG1 and the percentage of errors of MG2 (Figure 9, panels C and E), the changes for subjects with a larger estimated TTO suggest improvement or learning from the first to second visit, while the changes for subjects with a smaller estimated TTO suggest a failure to learn from the first visit or reduction in performance that cannot be compensated for with learning effects. To facilitate visualization of the interaction, the slope of the line in Figure 9A, C and E has been plotted as a single point for each subject in Figure 9, panels B, D, and F, respectively.

The results of the repeated measures analysis are shown in Table 10. A significant main effect of TTO ($p \leq 0.04$) was found for subtests of the H Scan (audio and visual RT, MT, decision MT, and alternate button tapping), WAIS (picture arrangement, digit symbol, and letter number sequencing), CVLT (long delay recall), Stroop (color naming, word reading, and interference), Trail Making (Part A), and the AS task (percentage of errors). In all cases, performance was worse in subjects with a smaller TTO than in those with a larger TTO. A significant main effect of visit ($p \leq 0.05$) was found for subtests of the H
Scan (alternate button tapping), WAIS (picture arrangement), the MG1 task (percentage of errors, latency, and variability of latency), and the MG2 task (percentage of errors). Of these measures, only picture arrangement demonstrated an overall improvement at the second visit, indicating a learning effect. Performance on the other tests with a significant visit effect was worse at the second visit. A significant interaction between TTO and visit \( (p \leq 0.02) \) was found for subtests of the H Scan (MT and alternate button tapping), the MG1 task (variability of latency), and the MG2 task (percentage of errors). For all four measures, the rate of decline was more rapid as subjects approached onset.

2. ANCOVA in CAG+ and CAG- Individuals

ANCOVA was used to test for differences in the rate of change between NC, Far, and Near groups. Table 11 shows the raw group means and the \( p \) values adjusted for covariates. Significant group differences \( (p \leq 0.03) \) were found for 3 measures from the H-Scan (audio and visual RT, and alternate button tapping). For all three measures, post hoc testing demonstrated that the rate of change was significantly greater \( (p \leq 0.007) \) for the Near group as compared with the NC. Furthermore, a significantly faster rate of change was found in the Near group as compared with the Far group \( (p=0.007) \) for alternate button tapping. For the saccadic tasks, all measures from the MG1 task (percentage of errors, latency, and variability of latency) and the percentage of errors from the MG2 task also yielded significant group effects \( (p \leq 0.03) \). Subsequent post-hoc testing found that the rate of change was significantly faster in the Near group as compared with the NC group \( (p \leq 0.008) \) for all but the variability of latency of MG1, though a trend was also found for this measure \( (p=0.055) \). Furthermore, the rate of change was faster in the Near group as compared with the Far group \( (p=0.04) \) for all but the percentage of errors of MG1. Additionally, the Far group declined faster than the NC for the percentage of errors of MG1 \( (p=0.02) \). No other study measure showed a significant group effect for the rate of change.
Figure 9. Performance of CAG+ subjects. Progression is shown both by connecting a subject’s performance at each study visit with a line vs. TTO at each visit (A,C,E) and as the change in performance/TTO year vs. TTO at the first study visit (B,D,F). A,B: Alternate Button Tapping; C,D: Variability of latency of MG1; E,F: Percentage of Errors of MG2.

A.

B.
Figure 9. Performance of CAG+ subjects. Progression is shown both by connecting a subject’s performance at each study visit with a line vs. TTO at each visit (A,C,E) and as the change in performance/TTO year vs. TTO at the first study visit (B,D,F). A,B: Alternate Button Tapping; C,D: Variability of latency of MG1; E,F: Percentage of Errors of MG2.
Figure 9. Performance of CAG+ subjects. Progression is shown both by connecting a subject’s performance at each study visit with a line vs. TTO at each visit (A,C,E) and as the change in performance/TTO year vs. TTO at the first study visit (B,D,F). A,B: Alternate Button Tapping; C,D: Variability of latency of MG1; E,F: Percentage of Errors of MG2.

E.

F.
Table 10. Results of repeated measure mixed model.

<table>
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<th>TTO*Visit</th>
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<td>Audio Reaction Time</td>
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<td><strong>Trail Making Test</strong></td>
<td></td>
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<tr>
<td>Part A</td>
<td>0.02</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>Part B</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td><strong>Anti-Saccade</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Percentage of Errors</td>
<td>0.0001</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Latency</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Variability of Latency</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

¹ NS = not statistically significant (p>0.05)
<table>
<thead>
<tr>
<th></th>
<th>TTO</th>
<th>Visit</th>
<th>TTO*Visit</th>
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<tbody>
<tr>
<td><strong>Memory Guided 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage of Errors</td>
<td>NS</td>
<td>0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Latency</td>
<td>NS</td>
<td>0.03</td>
<td>NS</td>
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<tr>
<td>Variability of Latency</td>
<td>NS</td>
<td>0.005</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Memory Guided 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of Errors</td>
<td>NS</td>
<td>0.0003</td>
<td>0.0008</td>
</tr>
<tr>
<td>% of Missed Flashes</td>
<td>NS</td>
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<td>NS</td>
</tr>
</tbody>
</table>

1 NS = not statistically significant (p > 0.05)
Table 11. Results of ANCOVA with three groups.

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>p-values</th>
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<tr>
<td></td>
<td>NC</td>
<td>Far</td>
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<tr>
<td><strong>H Scan</strong></td>
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<tr>
<td>Audio Reaction Time</td>
<td>-0.00038 ± 0.00077</td>
<td>-0.00022 ± 0.00062</td>
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<tr>
<td>Visual Reaction Time</td>
<td>-0.00016 ± 0.00064</td>
<td>5.8E-05 ± 0.00067</td>
</tr>
<tr>
<td>Alternate Button Tapping</td>
<td>-0.011 ± 0.076</td>
<td>-0.0042 ± 0.063</td>
</tr>
<tr>
<td><strong>Memory Guided 1</strong></td>
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</tr>
<tr>
<td>Percentage of Errors</td>
<td>-0.20 ± 0.40</td>
<td>0.34 ± 0.78</td>
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<tr>
<td>Latency</td>
<td>-0.015 ± 1.94</td>
<td>0.26 ± 2.41</td>
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<tr>
<td>Variability of Latency</td>
<td>0.47 ± 2.55</td>
<td>-0.13 ± 2.38</td>
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<tr>
<td><strong>Memory Guided 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of Errors</td>
<td>-0.036 ± 1.16</td>
<td>-0.25 ± 1.16</td>
</tr>
</tbody>
</table>
D. Discussion

The identification of potential biomarkers of disease progression in prediagnostic HD is a largely unmet requisite for performing neuroprotective drug trials in CAG+ individuals. We have used two complementary approaches to examine longitudinal changes in prediagnostic CAG+ subjects, and to compare these changes between CAG- and CAG+ subjects.

Initial analyses using a repeated measures model with only prediagnostic CAG+ subjects confirmed that performance on a number of neurocognitive, psychomotor, and oculomotor tests declines during the prediagnostic period. The results from this study indicate that psychomotor measures (H Scan subtests, digit symbol) are particularly sensitive, and that certain neurocognitive and oculomotor measures are also sensitive to declining performance in the prediagnostic period.

The central hypothesis of the study was that the rate of decline in functioning increases as subjects approach estimated disease onset. This hypothesis was addressed through the interaction term: TTO x visit. Four subtests (MT, alternate button tapping, variability of latency of MG1, and percentage of errors of MG2) were able to detect a significant change in the rate of decline as subjects approach onset. In all cases, the rate of decline increased as the subjects approached their estimated age of onset. Our results also emphasize the importance of longitudinal studies. For variability of latency of MG1 and percentage of errors of MG2, no cross sectional effect of TTO was detectable. However, it is clear that longitudinal performance changes as subjects approach onset for these two measures. Subjects with a larger estimated TTO tend to perform slightly better at their second visit, indicating a training or learning effect; but those with a smaller estimated TTO perform worse at their second visit, suggesting that they no longer benefit from having done the task previously. It is also noteworthy that all of the measures with a significant TTO x visit interaction have a motor component, suggesting that motor measures (with or without a cognitive component) may be the most sensitive to detect rate differences during the prediagnostic period.
We also tested the hypothesis that the rate of decline is different between CAG- and CAG+ subjects. The CAG+ subjects were dichotomized into either a Far from or Near to onset group. Of the seven subtests with a significant ANCOVA test ($p \leq 0.05$), post hoc tests revealed a difference between the NC and Near groups for six of the subtests. For the seventh subtest (variability of latency of MG1), a trend was also found ($p=0.055$). Only the percentage of errors of MG1 was sufficiently sensitive to detect a difference between the NC and Far groups. Interestingly, this measure did not detect a significant difference in the rate of decline during the prediagnostic period using either method, suggesting that the rate of change is different between CAG- and CAG+ subjects but that the rate is constant throughout the prediagnostic period.

ANCOVA confirmed the results from the repeated measures analysis that supported a changing rate of decline during the prediagnostic period for alternate button tapping, variability of latency of MG1, and percentage of errors of MG2. The only discrepancies between the two methods were with MT and latency of MG1. Further examination of these data suggests that a difference in the rate of decline in MT is subtle and that dichotomization of the sample increased variability so that differences could not be detected. On the other hand, it appears that the significant difference found in latency of MG1 is likely due to a few subjects, and a larger sample may be required to have sufficient power to test this hypothesis.

These results provide a functional correlate to longitudinal anatomical findings. Aylward et al. \textsuperscript{171} reported significantly smaller striatal volume cross sectionally in subjects up to 20 years before onset; however, the rate of striatal atrophy was significantly increased only 10 years prior to onset. Many previous cross sectional studies detected performance differences during the prediagnostic period;\textsuperscript{100,111,119,120,122,131,300-304,306} however, fewer longitudinal studies have been performed and differences in the rate of change during the prediagnostic period have not been consistently reported.\textsuperscript{130,307-314} Our data would appear to suggest that the differences in rates are subtle but can be detected with particular measures. As seen from the ANCOVA, the most significant differences in rate of change are between the NC and Near groups, indicating that the most rapid decline occurs close
to onset. Furthermore, by directly plotting the rate of change for each subject against TTO at the first visit (Figure 9), it appears that for alternate button tapping and the percentage of errors of MG2 the rate of decline increases only as subjects are within approximately 10 years of their estimated age of onset. However, for the variability of latency of MG1 the rate of decline appears to increase earlier in the prediagnostic period. Further work is required to see if this pattern is consistent, but it may indicate that saccadic measures are more sensitive in detecting differences in rate of change early in the prediagnostic period; however, they may not be more sensitive than other measures when examining the entire prediagnostic period.

While in many instances CAG+ individuals further from their estimated onset do not appear to decline more rapidly than CAG-, this does not imply an absence of pathology. Others have noted the likelihood of compensatory mechanisms sufficient to mask the behavioral effects of underlying pathology early in the prediagnostic period. This study does not use methods to investigate underlying neural integrity (e.g. MRI, fMRI, EEG, etc.) and thus cannot test whether there is an absence of pathology in our subjects or neuronal compensation that masks pathological changes.

This study had several strengths and weaknesses. One strength was that all study participants had a parent with HD and thus were at-risk for HD. This generates groups that have greater matching for unmeasurable factors as compared to a study in which the CAG- group is not at-risk for HD. In addition, all subjects completed a uniform study visit that evaluated a number of domains reported to be affected early in disease progression. The study also had several weaknesses. The size of the sample is similar to that of previous studies but is still relatively modest to detect small differences in rates of change. Furthermore, the sample of CAG+ individuals tended to have a smaller number of expanded repeats with fewer subjects having greater than 50 repeats. As a result, we have limited power to test whether those with a larger number of CAG repeats (i.e. >50 CAG) have more rapid rates of decline as compared with those having the more typical number of repeats (39-50 CAG repeats). We collected data regarding depression using the CES-D and found that depressive symptomology was significantly higher in the Far
group than the other two groups at the second visit. One possible explanation may be that as subjects approach onset (Near group), the depressive symptoms worsen and they seek medical attention to alleviate its effects. Unfortunately, we were not able to assess this explanation because we did not ask subjects if they had sought out medical care for depression. Finally, both a strength and a weakness of the study was that the CAG+ individuals were distributed throughout the prediagnostic continuum as estimated by their TTO. This allowed the use of TTO as a continuous variable and evaluated a linear relationship between TTO and performance; however, it also likely resulted in extensive heterogeneity within each group when the CAG+ participants were divided into Near and Far groups.

We are currently collecting data for a third time point in these subjects. We will evaluate whether these new data provide improved model fitting to better estimate the rate of disease progression across study variables in a sample of subjects who are either prediagnostic or in the early stages of clinically diagnosable disease. We anticipate that these data will further improve our ability to identify sensitive and specific biomarkers in the early stages of disease progression.
V. Summary

Many studies have examined the changes that take place in premanifest HD, and it is clear that motor, ocular motor, cognitive, and behavioral abnormalities can be reliably detected. Furthermore, imaging studies have shown striatal and extra-striatal gray matter changes and white matter abnormalities during the premanifest period of the disease. Taken together, this is overwhelming evidence that neurodegeneration begins many years prior to diagnosis of HD.

The current standard of therapy for HD is to manage the symptoms of the disease, though this strategy has limited success. Neuroprotective intervention promises to slow the rate of progression and delay or even prevent disease onset. It would ideally occur during the premanifest period before quality of life is adversely and irreparably affected. A significant challenge to evaluating neuroprotective therapies in premanifest HD is that the only acceptable endpoint for regulatory agencies is disease onset; Hersch and Rosas suggest that a study using this endpoint would require up to 3000 subjects and 6 years of follow-up to detect a 40% decline in the frequency of onset. One strategy to avoid such costly and unreasonable studies is to evaluate a therapy in both manifest HD using regulatory agency-acceptable outcomes and in premanifest HD using biomarkers of progression. The hope is that demonstration of efficacy in manifest HD combined with premanifest biomarker improvement would be sufficient to gain regulatory approval, though this method has not yet been tried.

The work presented herein further establishes saccades as effective measures of premanifest disease progression. In response to previous work showing the cross sectional sensitivity of saccadic measures, we decided to further characterize the nature of saccades in HD by investigating the neural correlates of saccade impairment and the longitudinal progression of saccadic measures in premanifest HD. This work has shown that saccadic impairment is associated with cortical and subcortical atrophy, that identifiable functional brain changes underlie saccade impairment, and that saccade
performance decline can be detected over a relatively short period of time in premanifest HD.

Because of the early and extensive atrophy that occurs in the striatum, many attempts have been made to interpret cognitive and motor abnormalities in the context of striatal atrophy. Indeed, it has been hypothesized that degeneration in the putamen is associated with motor deficits, while degeneration in the caudate is associated with cognitive deficits.\textsuperscript{170} This is supported by imaging studies reporting that putamen atrophy was associated with atrophy in cortical regions that make up the motor cortico-striatal loop, and caudate atrophy with those that make up the cognitive part of the loop.\textsuperscript{193} While it is impossible to completely separate the striatum from cortical regions given the density of neuronal connections between the two, our findings suggest that cortical and striatal atrophy contribute to saccade dysfunction in a non-redundant manner. The AS task in particular appears well suited for evaluating cortical changes; AS performance is associated with gray matter loss in SFG and IPL, and there is a strikingly absent BOLD response in ACC and pre-SMA following AS errors. It is likely that saccades are not unique in this regard and that cortical changes also contribute to other signs and symptoms of the disease.

Further studies are needed in order to better understand the connection between brain pathology and saccade performance. In particular, whole-brain rather than ROI-based approaches provide an opportunity to discover truly unexpected findings. While an ROI approach is helpful in that it reduces the number of potentially spurious and biologically implausible findings, it also constrains the conclusions that can be drawn. For example, based on our study design we were able to discuss abnormalities in our \textit{a priori} ROIs, but could not make any conclusions regarding functional compensation outside of these ROIs as others have done.\textsuperscript{202,393} These studies should also be carried out longitudinally, as evidence of co-decline will strengthen the likelihood of causative relationships between atrophy and saccade decline. Another imaging method with great potential is the use of diffusion tensor imaging (DTI). This method relies on the diffusion characteristics of water to examine microstructural changes, including the integrity of fibers connecting
discrete regions. The use of DTI in studying HD is growing, and one study has shown a negative association between fiber tract integrity connecting FEF and caudate and the variability of latency of voluntary saccades. Being able to link gray matter atrophy, functional abnormalities, and white matter tract integrity should provide important insights to the nature of saccade, cognitive, and motor dysfunction in HD.

Previous studies identified saccades as potential biomarkers of premanifest disease progression, and our imaging studies have provided biological insight into the underlying neural mechanisms of impairment. A longitudinal study was conducted to further explore the potential of saccadic measures as biomarkers. This study confirmed impairment in premanifest HD and provided the first look at the longitudinal behavior of saccades in this population. Not surprisingly, we found different patterns of decline. Some measures declined constantly in premanifest HD, though more rapidly in CAG+ than in CAG-.

These measures would be useful in clinical trials to evaluate the effectiveness of a therapy to slow disease progression. Another pattern of decline was one in which the rate of decline increased just prior to disease onset. One possible explanation is that some additional disease process is triggered, leading to more rapid decline. Another explanation is that, prior to this increase, compensatory mechanisms attenuated decline, but that a critical threshold was reached, beyond which compensation was no longer effective. Measures with this pattern of decline could be used to evaluate the ability of an intervention to prevent a disease-augmenting trigger or to facilitate compensation. Importantly, these changes could be detected over a relatively short period of time. However, still more work needs to be done to longitudinally characterize saccades. More time points are needed to confirm the findings described above. Furthermore, examination over a shorter time period would be helpful to determine the minimum length of a clinical trial in which saccades could be useful.

While studies have consistently confirmed the potential of saccades to track premanifest disease, technological advancements have also taken place that make the wide-spread use of quantitative saccades possible. Scleral search coils are still considered the gold standard for quantitatively measuring eye movements, but they are slightly invasive and
can lead to discomfort, increased intraocular pressure, and temporary reduction in visual acuity.\textsuperscript{394} We used a non-invasive, high-frequency video-recording system, but widespread use of such a system is limited by both its cost and, more importantly, its relative immobility. Fortunately, the newly developed systems mentioned above are non-invasive, and their ease-of-use and portability make adoption in a clinical setting much more likely.

In addition to saccades, other measures will likely be included in a battery to follow disease progression. Structural MRI, particularly of the striatum, would be a good fit given the extensive characterization that is already in place. Qualitative assessment of the striatum seems unlikely to be sufficiently sensitive, so quantitative measurements must be made. While such measurements previously required extensive human effort, the methods that we employed use computer processing capabilities to reduce human time involvement. Furthermore, these methods remove inter-rater variability by using a standard algorithm to define structures. A third measure that would almost certainly be included in such a battery would be speeded or self-paced finger tapping. Both of these have been repeatedly shown to be quite sensitive in the premanifest period,\textsuperscript{98-100} and there is fMRI evidence of neural substrates for this impairment\textsuperscript{198} (Table 2).

The relative temporal appearance of disease-related changes is an important question, but one that is not often addressed explicitly in published studies partly because the sample sizes for such an assessment would need to be quite large. However, the results that have been published allow for some speculation on the matter. It appears that striatal atrophy is among the earliest detectable changes; it has been found up to 20 years prior to estimated disease onset\textsuperscript{171} (Figure 10). Motor impairment, including speeded and self-paced finger tapping,\textsuperscript{100} and incorrect and slowed saccades (unpublished data) appears to be present 10-15 years prior to onset. Cognitive impairment, while detectable in premanifest disease, appears to onset around 10 years prior to onset.\textsuperscript{100} Psychiatric manifestations of the disease, while prevalent, do not follow a prescribed time course, nor do they progress with other disease-related impairment.\textsuperscript{86} The fMRI findings described above are interesting in that none of the premanifest CAG+ subjects had normal activation in
response to AS errors even though they were predicted to be as much as 18 years prior to disease onset. While these functional changes may be some of the earliest that can be detected, more studies are needed to confirm these initial findings.

There are many reliable measures of impairment and disease progression during premanifest HD, but it is unlikely that one measure will be sufficient to track progression throughout the disease continuum. For example, we have seen that the percentage of errors during MGc is sensitive early in premanifest disease but that individuals with manifest disease often complete only a few correct trials, suggesting a limiting floor effect of the task. On the other hand, cognitive measures may not be as sensitive during the earliest periods, but they may have utility further into the manifest disease range. Because these measures will be used to evaluate therapeutic efficacy in clinical trials, longitudinal characterization will inform a selection of measures that are able to capture a wide range of disease progression.

While prevention remains the ultimate goal of therapy in HD, there are significant obstacles that must be overcome. First, prevention will require correcting the pathogenic nature of mutant huntingtin, yet there are many fundamental questions that remain regarding the pathogenesis of the disease. Second, the disease is clearly progressive and begins many years before a clinical diagnosis can be made. Given the protein’s ubiquitous expression throughout both development and the body, it is not unreasonable to expect that as yet unidentified pathology exists from birth or even earlier. It is in this

Table 10. Temporal appearance of changes in premanifest HD. Striatal atrophy is one of the earliest detectable changes in premanifest HD, with motor and cognitive impairment following. Psychiatric manifestations vary in their temporal appearance. Preliminary fMRI studies indicate early activation abnormalities in response to errors, though more study is necessary.
sense that Hersch and Rosas argue that neuroprotective therapy in HD is inherently not preventative.

Neuroprotective therapy that delays disease onset seems to be a more attainable goal, at least in the short term. Intervention would begin during the premanifest period of the disease. Given our current understanding of premanifest disease progression, the above-mentioned evaluative battery consisting of quantitative saccades, finger tapping, and MRI would inform physicians regarding the earliest signs of the disease. Therapy would begin with these earliest signs of the disease, probably 10-15 years prior to predicted onset, in order to preserve quality of life. Although it is tempting to intervene even earlier, there are important considerations that temper the “earlier is better” approach. For example, it is impossible to establish therapeutic efficacy before the presence of any signs or symptoms of the disease, underscoring the importance of identifying early markers of disease progression. In fact, efficacy is probably the most important of these considerations because it is central to other discussions of therapeutic and financial cost-benefit analyses. Furthermore, I am hopeful that development and implementation of effective neuroprotective therapies will also lead to a better understanding of the disease, which in turn will increase our ability to identify earlier disease processes, thus providing the opportunity to intervene even earlier.

While not prevention, I believe that the development of these neuroprotective interventions could have effects extending beyond simply delaying onset. More people may seek presymptomatic gene testing if a disease-altering therapy is available, though predicting responses to gene testing is notoriously imprecise in HD. Assuming that presymptomatic testing does increase, the associated counseling and family planning combined with delayed onset could have important consequences on the future prevalence of the disease.
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