An Investigation of Cortical Excitability in Huntington's Disease Using Transcranial Magnetic Stimulation

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B.BNSc. (Hons.)

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Abstract

Huntington's disease (HD) is a neurodegenerative disorder caused by a genetic mutation that is associated with pathological changes in cortico-subcortical pathways. Clinical onset typically occurs in middle adulthood, with an array of neuropathological, cognitive, psychiatric and motor signs evident during the "premanifest" disease stage. Despite increasing knowledge of the progressive structural, microstructural and gross functional brain changes of HD, obtained via magnetic resonance imaging for example, there is inadequate understanding of the pathophysiological changes in neural pathways underlying the disease. An alternative technique, transcranial magnetic stimulation (TMS) involves non-invasive brain stimulation to assess the functional integrity of neurons at a physiological level within targeted circuits. While TMS holds great promise, it has had only limited application in HD to date and mixed findings have resulted from methodological differences between studies.

This thesis sought to address a number of unanswered questions in the literature, relating to the specific pathophysiological changes in excitatory and inhibitory neuronal function occurring in premanifest and symptomatic HD. Moreover, it investigated phenotypic heterogeneity amongst HD gene carriers via examination of the clinical, cognitive and psychiatric correlates of TMS measures and various genetic variants that may modulate disease progression.

Sixteen premanifest, thirteen symptomatic HD participants and seventeen healthy controls were recruited. Participants underwent clinical, cognitive and psychiatric assessment, and provided saliva samples for genotyping. TMS was administered to the left primary motor and dorsolateral prefrontal cortices, and responses were measured through electroencephalography and peripheral electromyography. Various TMS protocols were included in order to comprehensively assess cortical excitability, inhibition and facilitation.

A number of significant findings emerged from these investigations. Firstly, cortical inhibition measures were impaired in premanifest and symptomatic HD, and associated with biological disease burden and development of symptomology. Furthermore, TMS was able to differentiate between pathophysiological changes in specific intracortical inhibitory circuits at different disease stages. Secondly, intracortical inhibition showed significant sex differences, with less inhibition across all female participants (but no interaction with HD-related cortical inhibitory deficits). Thirdly, in combination with the HD mutation, additional genetic variants significantly modulated individual responses to TMS and the age at HD onset. One of these gene variants (rs11789969), coding for a neurotransmitter receptor within the inhibitory pathways affected by HD, was determined to be in the top 10% most deleterious variants genome-wide, and was therefore likely to have a direct functional impact on the gene product.

Taken together, the findings of this thesis provide novel insights into pathophysiology in HD including new knowledge of the sequence of functional neurological changes that occur prior to, and shortly after, clinical onset. Based on the results, it is argued that intracortical inhibitory deficits, mediated by the inhibitory neurotransmitter GABA, may be a primary pathogenic outcome in HD. Building upon this line of research, it is suggested that future studies undertake longitudinal TMS investigations of motor and non-motor cortices with larger premanifest and symptomatic HD samples. This approach will assist in identifying TMS measures that may have utility as sensitive endophenotypic biomarkers in future clinical trials.

General declaration

Monash University

Declaration for thesis based or partially based on conjointly published or unpublished work

In accordance with Monash University Doctorate Regulation 17.2 Doctor of Philosophy and Research Master's regulations the following declarations are made:

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes three original papers published in peer reviewed journals and one paper current accepted and '*in press*'. The core theme of the thesis is the investigation of cortical excitability in Huntington's disease using transcranial magnetic stimulation. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the candidate, working within the School of Psychological Sciences and Monash Institute of Cognitive and Clinical Neurosciences, under the supervision of Prof Nellie Georgiou-Karistianis, Prof Paul Fitzgerald and Dr Tarrant Cummins. The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.

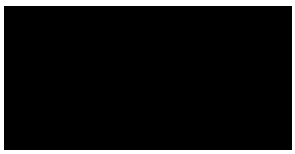
In the case of chapters 1, 3, 4 and 5 my contribution to the work involved the following:

Thesis chapter	Publication title	Publication status	Nature and extent of candidate's contribution
1	**Transcranial magnetic stimulation as a tool for understanding neurophysiology in Huntington's disease: A review**	Published	75%
3	**Cortical inhibitory deficits in premanifest and early Huntington's disease**	Published	70%
4	**Cortical inhibitory deficits in Huntington's disease are not influenced by gender**	Accepted, In press	75%
5	**A *GABBR2* gene variant modifies pathophysiology in Huntington's disease**	Published	70%

I have not renumbered sections of submitted or published papers in order to generate a consistent presentation within the thesis.

Student signature:  **Date:** 12/04/2016

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the student and co-authors' contributions to this work.

Main Supervisor signature:  **Date:** 12/04/2016

Publications during enrolment

1. **Philpott, A.L.,** Fitzgerald, P.B., Cummins, T.D.R., Georgiou-Karistianis, N. (2013). Transcranial magnetic stimulation as a tool for understanding neurophysiology in Huntington's disease: A review. *Neuroscience & Biobehavioral Reviews*, *37*(8)*,* 1420-1433. doi: 10.1016/j.neubiorev.2013.05.009. Impact factor 8.802.

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2. **Philpott, A.L.,** Fitzgerald, P.B., Cummins, T.D.R., Churchyard, A., Georgiou-Karistianis, N. (2015). Characterising cortical inhibition with transcranial magnetic stimulation

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There are countless other people that have provided me with much valued time, resources, expertise and support throughout the production of this thesis. To Dr Neil Bailey and Dr Nigel Rogasch, I am so grateful for the countless hours you spent with me in technical training, investigating equipment failures, data processing and analysis, and reading drafts. Your willingness to provide your support and expertise was instrumental to my completion of this thesis, and I feel very lucky to have had two people like you with such

invaluable skills to share involved from start to finish. I would also like to thank my fellow members of the ENRU lab, past and present. I sincerely value the numerous opportunities I had to practice my talks on you and always felt confident in counting on the many sets of eyes and ears to provide perceptive and constructive feedback on my work. My thanks also go out to the undergraduate psychology student volunteers who helped with my data collection, and made my days in the testing lab far easier and more pleasant.

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I now turn my attention to my valued family members and friends, who supported me over the last four years, and kept me grounded. Now is the time to thank my parents, Vince and Denise, for bringing me to this point. I cannot thank you enough for your dedication to affording me the best educational opportunities, and your continuous and boundless support, emotionally, financially and intellectually. You have always led by example, as people I aspire to be like across all facets of life, and I feel so fortunate to have had the upbringing and experiences I had. Every success I have is a testament to you both and a reflection of your support and love. To my big sister, Olivia, from a very young age I wanted to be just like you and you never failed to set an example for me in living a life of success, integrity, adventure and camaraderie. Though we have chosen vastly different career paths, I still look up to you and derive inspiration and motivation from you, so thank you.

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jobs and lives together, and provide continuous encouragement and positivity has allowed me to finish this thesis with minimal anguish. I know that I could not have gotten through without your love, support and help, and I promise that the next two years will not be as much of an emotional roller coaster ride as the last two. You make me feel like the luckiest girl in the world, and I hope that I can show you the same degree of encouragement and practical support in your future aspirations as you have given to me.

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This thesis is the final product of an extremely challenging, enlightening, rewarding and empowering journey. Thank you once again to everyone, both named above and unnamed, and I sincerely hope that this represents the first major step for me towards a fruitful and fulfilling career in neuropsychology.

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Abbreviations

AMT	Active motor threshold
APB	Abductor pollicis brevis
CSP	Cortical silent period
D_1/D_2	Dopamine receptor type 1/2
DLPFC	Dorsolateral prefrontal cortex
DTI	Diffusion tensor imaging
EEG	Electroencephalography
EMG	Electromyography
EOG	Electrooculography
(f)MRI	(functional) Magnetic resonance imaging
GABA	Gamma amino-butyric acid
$GABA_A/GABA_B$	GABA receptor type A/B
HD	Huntington's disease
HEOG	Horizontal electrooculography
ICF	Intracortical facilitation
LICI	Long-interval cortical inhibition
MEP	Motor-evoked potential
NART-2	National Adult Reading Test – Second edition
PET	Positron emission tomography
Pre-HD	Premanifest Huntington's disease
RMT	Resting motor threshold

rTMS	Repetitive transcranial magnetic stimulation
SD	Standard deviation
SICI	Short-interval cortical inhibition
SNP	Single-nucleotide polymorphism
Symp-HD	Symptomatic Huntington’s disease
TEP	TMS-evoked potential
TMS	Transcranial magnetic stimulation
UHDRS	Unified Huntington’s Disease Rating Scale

Glossary of terms

Biomarker	A quantifiable and objective measure of the disease process
Brain function	Associated with the activity of neurons and other brain cells
Brain structure	The physical substrate of neural tissue
Clinical sign	A characteristic of a disease measured objectively during examination
Neuropathology	The primary brain changes associated with a disease process
Pathophysiology	The alterations in neuronal activity caused by a disease process
Performance	The nature of completion for an individual on a neurocognitive instrument or neuromotor task
Symptom	A phenomenon experienced by an individual affected by a disease

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Chapter one

Monash University

Declaration for thesis chapter one

Declaration by candidate

In the case of chapter one, the nature and extent of my contribution to the work was the following:

Nature of contribution	**Extent of contribution (%)**
The candidate planned and wrote the literature review, with feedback and guidance from co-authors	75

The following co-authors contributed to the work. If co-authors are students at Monash University, the extent of their contribution in percentage terms must be stated:

Name	**Nature of contribution**	**Extent of contribution (%) for student co-authors only**
Paul Fitzgerald	Discussion of literature, critical revision of manuscript	N/A
Tarrant Cummins	Discussion of literature, critical revision of manuscript	N/A
Nellie Georgiou-Karistianis	Discussion of literature, critical revision of manuscript	N/A

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work.

Student signature: [redacted] **Date:** 12/04/2016

Main Supervisor signature: [redacted] **Date:** 12/04/2016

Thesis overview

Huntington's disease (HD)

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by an expanded trinucleotide (CAG) repeat on the gene coding for the protein huntingtin (MacDonald et al., 1993). The clinical phenotype typically manifests in middle adulthood as a variable combination of motor, cognitive and psychiatric changes that ultimately progresses to death. Individuals at risk of inheriting the gene mutation for HD may undergo predictive genetic testing, but diagnosis of symptomatic HD (symp-HD) is currently defined by the emergence of involuntary choreiform movements (Huntington Study Group, 1996). Prior to clinically-defined onset, individuals are considered 'premanifest' HD (pre-HD), a disease stage associated with a myriad of neuropathological processes (i.e., structural and functional brain changes) 15-20 years before onset, as well as subtle clinical changes approximately 10 years before onset (Georgiou-Karistianis, Gray, et al., 2013; Paulsen, 2010; Stout et al., 2011; Tabrizi et al., 2009).

At the core of the pathogenic changes in HD is degeneration of the striatum, which is a critical structure within the basal ganglia-thalamocortical pathways that drive a range of functions (Vonsattel et al., 1985). However, more recent research has established that neurodegenerative changes in the cerebral cortex also represent primary huntingtin-mediated pathogenic outcomes (Gu et al., 2005; Strand et al., 2007). 'Traditional' neuroimaging techniques that have been utilised since the mutation was discovered, including magnetic resonance imaging (MRI), have provided important knowledge about the structural (e.g., atrophic changes), microstructural (e.g., white matter changes via diffusion tensor imaging) and gross functional (e.g., cerebral blood flow via functional MRI) brain changes in HD. However, alternative methods are required to further understand the specific functional changes in key basal ganglia-thalamocortical pathways, and their relationship to symptomology (i.e., motor, cognitive and psychiatric). Neurophysiological techniques, for example, allow for the investigation of causal links between targeted brain pathways and

objective responses, and may differentiate between primary, secondary or compensatory disease effects in HD. Such techniques are the focus of this thesis because they have been identified as potentially capable of yielding endophenotypic biomarkers in HD (Nguyen, Bradshaw, Stout, Croft, & Georgiou-Karistianis, 2010).

Transcranial magnetic stimulation (TMS)

A relatively novel neurophysiological method is transcranial magnetic stimulation (TMS), a non-invasive brain stimulation technique capable of activating the brain by capitalising on the electrical properties of neurons (Barker, Jalinous, & Freeston, 1985). TMS is based upon the principles of *Ampere's* and *Faraday's* laws; together these state that a time-varying electric current (e.g., flowing through a wire coil) induces a time-varying magnetic field, which in turn induces an electric current in the opposite direction to the original stimulation (Chen & Udupa, 2009). When the electric current is applied to the head, the induced magnetic field penetrates the skull and the subsequent electric current depolarises brain cell membranes (Pascual-Leone, Walsh, & Rothwell, 2000). Since the induced electromagnetic fields are oriented perpendicularly to each other, it is thought that intracortical neurons are more preferentially stimulated by TMS pulses, as opposed to projecting or descending tracts (Day et al., 1989).

Historically, TMS studies have principally focused on investigations of corticospinal pathways via stimulation of the motor cortex (Rossini & Rossi, 2007). The effects of stimulation can be measured indirectly via motor-evoked potentials (MEPs) on electromyography (EMG) recordings from peripheral muscles, or directly with TMS-evoked potentials (TEPs) using electroencephalography (EEG; Di Lazzaro & Ziemann, 2013). TMS effects appear to be subserved primarily by trans-synaptic intracortical pathways, as opposed to direct axonal stimulation, since the stimulation typically results in a cascade of downstream effects that outlast the initial stimulus (Rothwell, 1997). The prevailing view regarding TEPs is that inhibitory neurotransmission via ionotropic receptors regulates early TEPs, whilst metabotropic receptor-mediated neurotransmission is crucial for later TEPs

(Premoli et al., 2014). However, the complete TMS-related effects on the brain involve widespread cortical, subcortical and contralateral regions and several neurotransmitter systems (Ferreri et al., 2011; Strafella, Paus, Barrett, & Dagher, 2001).

TMS is a valuable technique for further investigating neuropathological changes in HD as it can tap into the function of targeted circuitry in relative isolation. This allows for investigation of functional neuroanatomy at a causal level, such that neuronal responses from known stimulation parameters can be objectively measured. In particular, TMS can be used to measure pathophysiological changes (i.e., disease-related alterations in neuronal function) in the basal ganglia-thalamocortical pathways known to be affected in HD. To date however, TMS has had limited application in HD and findings (which will be reviewed below) have been mixed. Thus there is no consensus as to what neurophysiological outcomes are affected in HD, and which TMS measures have potential utility as endophenotypic biomarkers; a problem which this thesis will address.

Thesis structure

This thesis is presented in accordance with the Monash University Doctor of Psychology requirements for 'thesis by publication'. As such, there is some unavoidable repetition across chapters. There are seven chapters; specifically: the introduction, the methods, four experimental chapters and the general discussion. This first introductory chapter begins with a general overview, and will continue with a published review article (Philpott, Fitzgerald, Cummins, & Georgiou-Karistianis, 2013), an update on relevant literature since the publication and the thesis aims and hypotheses. Chapter two provides detailed methodological information about the participants, apparatus and procedure adopted in each of the experimental chapters which follow.

The third, fourth and fifth chapters each contain one experimental paper focusing on particular questions relevant to further understanding the pathophysiology of HD. In addition, these chapters also provide the clinical (i.e., signs of disease severity), cognitive, psychiatric and genetic characteristics that are associated with different neurophysiological features.

These three experimental chapters each commence with a preamble section, which serves as a link and places the study in the context of the overarching aims of the thesis. Specifically, chapter three (i.e., the first experimental chapter) builds on previous research by investigating pathophysiological profiles based on a range of TMS measures from the motor cortex in pre-HD and symp-HD to better understand disease mechanisms (Philpott, Cummins, et al., 2016). This chapter also examines inter-relationships between pathophysiological measures and clinical severity, performance on neurocognitive tasks and psychiatric symptoms in HD participants to increase understanding of the phenotypic heterogeneity. Chapter four extends the outcomes of chapter three by investigating whether sex differences modulate pathophysiology in the HD brain. The aim of chapter four is to bring together various lines of evidence suggesting sex differences in disease progression in HD, effects of sex hormones on corticostriatal circuitry and the impact of HD pathology on corticostriatal pathways. Chapter five adds further insights to supplement the findings of chapter three by investigating whether specific candidate gene variants modify HD pathophysiology and age at clinical onset (Philpott, Fitzgerald, et al., 2016). The final experimental investigation, contained in chapter six, describes an intended exploration of pathophysiology in both motor and prefrontal cortices using the concurrent TMS-EEG technique. The aim of this investigation was to compare previous findings with a non-motor brain region that is implicated in cognitive and behavioural control (i.e., the dorsolateral prefrontal cortex; DLPFC). Unfortunately, the experiment was not successful and the data was not recoverable, due to technical difficulties (to be described in detail within the chapter).

To conclude the thesis, chapter seven presents a general discussion that brings together key findings from each aspect of the study, and compares and contrasts findings with previous studies. This final chapter is primarily concerned with highlighting the major contributions of this thesis to the field, and also considers the broader implications of the findings for future research.

Preamble to published review article

There are a number of published review articles that discuss pathophysiological changes in HD. These articles typically consider the topic from a broad perspective; either discussing HD as one of several neurodegenerative diseases (Berardelli et al., 2008), or TMS as one of several electrophysiological techniques (Berardelli et al., 1999). Importantly these reviews generally conclude that neurophysiological measures, including somatosensory and movement-related potentials and quantitative EEG, may be useful as sensitive markers of disease progression (Nguyen et al., 2010). In contrast to these and other traditional neuroimaging techniques, TMS methods represent a relatively novel, and valuable, approach for investigating pathophysiological changes in HD.

TMS is non-invasive, inexpensive, portable, well-tolerated by participants in general and allows for causal inferences to be made about functional neuroanatomy in focal cortical circuits (Pascual-Leone et al., 2000). However, there are very few articles that specifically review the utility of TMS in HD. To our knowledge, the only previous paper was that of Medina and Túnez (2010), which focused on biochemical and metabolic abnormalities in HD, and primarily discussed TMS in terms of its neuroplastic effects and potential use as an intervention tool. As such, current knowledge of pathophysiology in HD is limited due to heterogeneous findings and few attempts to identify possible reasons for discrepancies between studies. This approach is necessary to inform future research, with a view towards implementing TMS techniques diagnostically or therapeutically.

Therefore, the following review article sought to consolidate the prior evidence discussing potential pathophysiological changes in pre-HD and symp-HD participants for the first time, in order to increase understanding of the sequence of pathological events in cortico-subcortical pathways and tease apart likely disease mechanisms. Furthermore, we sought to investigate which TMS measures might represent sensitive markers of early brain degeneration in HD for use in future clinical trials, as this was unclear from the extant body of literature. Highlighting the importance of this line of research, other review articles with a

similar focus have subsequently been published (e.g., Mayer & Orth, 2014). Through conducting this review, we were able to identify important unanswered questions which could be addressed and experimentally tested in this thesis.

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Review

Transcranial magnetic stimulation as a tool for understanding neurophysiology in Huntington's disease: A review

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ABSTRACT

Structural and functional magnetic resonance imaging modalities have been critical in advancing our understanding of the neuroanatomical and pathophysiological changes that emerge during the premanifest and symptomatic stages of Huntington's disease (HD). However, the relationship between underlying neuropathology and the motor, cognitive and behavioural changes associated with the disorder still remain poorly understood. Less conventional technologies, such as transcranial magnetic stimulation (TMS) and electroencephalography (EEG), provide a unique opportunity to further investigate the causal relationships between targeted neural circuits and objective neurophysiological responses together with overt behaviours. In this review, we discuss previous successful applications of TMS in other neurological disorders and its prospective use in HD. We also address the added value of multimodal TMS techniques, such as TMS–EEG, in investigating the integrity of neural networks in non-motor regions in HD. We conclude that neurophysiological outcome measures are likely to contribute towards characterising further the trajectory of decline across functional domains in HD, enhance understanding of underlying neural mechanisms, and offer new avenues for elucidating sensitive endophenotypic biomarkers of disease progression.

Contents

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1. Overview

Gamma amino-butyric acid (GABA) is the most common inhibitory neurotransmitter in mammalian nervous systems, and is widespread in cortical and subcortical regions (Davies et al., 1990). In the cortex, cortical inhibition occurs primarily via GABAergic inhibitory interneurons, which modulate the output of other cortical neurons (Fitzgerald et al., 2008; Krnjevic, 1997). The basal ganglia (BG) also use GABA as their primary neurotransmitter to regulate cortical activity (Parent and Hazrati, 1995). The principal input site to the BG is the striatum, comprising the caudate nucleus and putamen, while the output is driven by excitatory 'corticostriatal' projections from the thalamus to the cerebral cortex (Cepeda et al., 2007). Alterations to this corticostriatal circuitry may result in widespread functional impairments, known to cause a complex presentation of motor and cognitive deficits in neurodegenerative disorders, such as Huntington's (HD) and Parkinson's (PD) diseases (Albin et al., 1989; Di Martino et al., 2008; Saint-Cyr, 2003). While HD will be the main focus of this review, PD, which is caused by selective loss of dopamine-producing neurons in the substantia nigra, affords an important comparison in terms of BG dysfunction (Joel, 2001).

Magnetic resonance imaging (MRI) and positron emission tomography (PET) technologies have been critical in driving forward our understanding of the underlying neuropathology in such disorders. Recent advances in other cutting-edge neurophysiological modalities, including transcranial magnetic stimulation (TMS), promise to provide unique insights into the complex relationship between symptomatology and underlying pathophysiology in clinical populations. We review the existing neuroimaging and neurocognitive/motor literature and examine how outcomes from such studies have informed our current understanding of the premanifest and symptomatic expression of HD. We then focus our review on TMS and electroencephalography (EEG) methods and their capacity to offer new and exciting opportunities to further enhance knowledge relating to the complex biobehavioural relationships underlying the neuroanatomical and pathophysiological abnormalities, as well as the cognitive, motor and behavioural disturbances that characterise HD.

2. Huntington's disease

2.1. Genetics and neuropathology

HD is an inherited neurodegenerative disorder caused by pathological expansion of the triplet CAG repeat in the IT15 gene coding for the protein 'huntingtin' (Huntington's Disease Collaborative Research Group, 1993; Vonsattel and DiFiglia, 1998). HD is characterised by a triad of symptoms comprising motor, cognitive and psychiatric disturbances (Huntington's Disease Collaborative Research Group, 1996) and clinical diagnosis is obtained once motor symptoms reach a threshold level of severity via the Unified Huntington's Disease Rating Scale (UHDRS; Huntington's Disease Collaborative Research Group, 1996). The age of clinical onset can be estimated using a simple formula that can predict, with up to 50% certainty, the likely timing of symptom onset based on CAG repeat and current age (Langbehn et al., 2004).

The striatum is the primary site of HD pathology (Albin et al., 1992; De La Monte et al., 1988; Diamond et al., 1992; Grafton et al., 1992), with preferential loss of medium spiny GABAergic neurons occurring first in the caudate and then the putamen (Andrews et al., 1999; Douaud et al., 2009; Sapp et al., 1997; Vonsattel and DiFiglia, 1998). Although BG circuitry is complex, the result of striatal atrophy is essentially under-inhibition of the thalamus and consequently over-excitation of cerebral cortex via glutamatergic excitatory thalamic projections (Aron et al., 2003). HD pathology is associated with abnormal neurotransmitter regulation and synaptic communication, as well as glutamate-mediated excitotoxicity and down-regulation of brain-derived neurotrophic factor (Centonze et al., 2005; Cepeda et al., 2003, 2004; Cha et al., 1998; Cummings et al., 2009; DiProspero et al., 2004; Hodgson et al., 1999; Klapstein et al., 2001; Storey et al., 1992; Zuccato et al., 2001). There is considerable evidence supporting a cascade of biochemical abnormalities resulting in numerous morphological cell changes, both degenerative and compensatory, that the HD brain undergoes prior to notable cell loss.

2.2. Overview of neuroimaging findings and functional neuroanatomy

A wealth of research has established that striatal structural changes in HD can be detected up to 15–20 years prior to clinical diagnosis (Bohanna et al., 2011a; Georgiou-Karistianis et al., 2013a; Jurgens et al., 2008; Mascalchi et al., 2004; Sánchez-Castañeda et al., 2012; Thieben et al., 2002). PREDICT-HD (Paulsen et al., 2008) and TRACK-HD (Tabrizi et al., 2009) are two large-scale multi-site longitudinal studies reporting strong effect sizes for neuroimaging measures in the very early premanifest, or 'pre-HD', stages of HD. As part of PREDICT-HD, Paulsen et al. (2010) demonstrated stepwise volumetric changes in individuals more than 15 years before estimated onset, not only in subcortical regions, but also in cortical grey matter (GM), cerebral white matter (WM) and total brain tissue. Furthermore, in TRACK-HD, Tabrizi et al. (2011, 2012) showed stepwise increased rates of change in caudate and putamen from pre-HD through to early and late stages of functional impairment, and highly significant correlations between rate of change in these structures and disease burden scores. To date, however, there are no studies that have examined correlations between longitudinal change in MRI volumetric measures and longitudinal change in measures of disease progression (for a review see Georgiou-Karistianis et al., 2013b).

Regionally-selective cortical thinning in symptomatic HD ('symp-HD'), and pre-HD has also been well-established (Georgiou-Karistianis et al., 2013b; Gómez-Ansón et al., 2009; Nopoulos et al., 2010; Rosas et al., 2002, 2005; Stoffers et al., 2010; Tabrizi et al., 2009). Variations in patterns of cortical thinning have been linked to specific phenotypes in HD and it is possible that these measures may be useful in understanding the heterogeneity in clinical presentation (Rosas et al., 2008b, 2011). PREDICT-HD has established that morphologic changes in cortical GM occur later in the disease process with WM changes seen early (Aylward et al., 2011; Nopoulos et al., 2010). Furthermore, methods such as diffusion tensor imaging (DTI) have revealed widespread microstructural changes in cortical and subcortical regions, which parallel earlier macrostructural findings. Alterations in mean diffusivity (MD)

and fractional anisotropy (FA), which are evident in both symp-HD and pre-HD, have been shown to correlate with motor and cognitive functioning and CAG repeat length (Bohanna et al., 2011a,b; Della Nave et al., 2010; Magnotta et al., 2009; Mascalchi et al., 2004; Reading et al., 2005; Rosas et al., 2006; Sritharan et al., 2010; Weaver et al., 2009). Moreover, and as part of the Australian-based IMAGE-HD study, Georgiou-Karistianis et al. (2013a) showed that a quadratic discriminant analysis demonstrated the highest discriminative accuracy in a comprehensive approach that included motor and neurocognitive scores, as well as multi-modal volumetric and diffusion measures from BG regions. Building on current knowledge, recent research has focused on neuroimaging modalities capable of assessing *function* at the neural level, particularly in critical corticostriatal circuits.

Widespread haemodynamic changes have provided invaluable insight into functional and compensatory changes across the spectrum of the disease (for a review see Georgiou-Karistianis, 2009; Paulsen, 2009). For example, a network of functional abnormalities has been described in PET studies of glucose metabolism in pre-HD, with particular regions associated with either hypometabolism or hypermetabolism (Feigin et al., 2001, 2007). A recent study using functional MRI (fMRI) by Gray et al. (2013) reported significant and more widespread compensatory increases in prefrontal function in symp-HD, compared with controls, during equivalent task performance. Moreover, resting-state MRI has revealed dysfunction in the dorsolateral prefrontal cortex (DLPFC) in pre-HD (Wolf et al., 2011). Overall, fMRI studies have shown region-specific hyperactivation and impaired functional connectivity between regions implicated in corticostriatal pathways during performance of neurocognitive tasks in both pre-HD and symp-HD, which have correlated with CAG repeat length and UHDRS scores (Georgiou-Karistianis et al., 2007; Gray et al., 2013; Klöppel et al., 2009; Paulsen et al., 2004; Saft et al., 2008; Thiruvady et al., 2007; Unschuld et al., 2012; Wolf et al., 2008, 2012; Zimbelman et al., 2007). Taken together, these findings suggest that striatal and cortical degeneration are primary outcomes of the dysfunctional protein and are likely to have independent contributions to phenotypic expression (Cepeda et al., 2007; Paulsen, 2010; Pillai et al., 2012). Imaging modalities that can examine function, track longitudinal change in corticostriatal pathways, and establish links between pathophysiology and neurocognitive/clinical signs in HD, require further investigation to determine their utility as candidates for endophentoypic biomarkers.

2.3. Neurocognitive and motor deficits

Cross-sectional studies have reported deficits in working memory, attentional set-shifting and visual recognition memory, all of which can be related to impaired corticostriatal information processing (Lawrence et al., 1998b). Corticostriatal circuitry has been implicated in a variety of complex cognitive functions (Albin et al., 1989; Alexander et al., 1986), which have also been shown to decline during the pre-HD period (Farrow et al., 2006, 2007; Golding et al., 2006; Harrington et al., 2012; Kirkwood et al., 1999; Klöppel et al., 2008; Lemiere et al., 2004; Tabrizi et al., 2012). PREDICT-HD and TRACK-HD have elucidated a number of very early subtle longitudinal neurocognitive and motor impairments with stepwise deterioration according to clinical severity (Paulsen et al., 2006, 2008; Stout et al., 2011, 2012; Tabrizi et al., 2009, 2011, 2012). While overall performance on certain neurocognitive tasks are reported to be more sensitive to disease progression than the UHDRS motor score, such impairments are subtle and consequently susceptible to sample size/constitution and practice effects (Stout et al., 2011, 2012).

Psychomotor speed has also been shown to be sensitive to disease progression (Bechtel et al., 2010; Harrington et al., 2012; Lemiere et al., 2004; Rosenberg et al., 1995; Rowe et al., 2010; Rupp et al., 2010; Snowden et al., 2002; Solomon et al., 2008; Stout et al., 2011; Tabrizi et al., 2009, 2012, 2011) and has been found to correlate with neuroimaging measures, such as striatal atrophy, extrastriatal WM and cortical thickness in both pre-HD and symp-HD (Bamford et al., 1995; Bechtel et al., 2010; Paulsen et al., 2008). Several studies have shown a reliable deficit in inhibitory control in pre-HD and symp-HD, thought to be related to degeneration of the indirect inhibitory BG circuitry (Aron et al., 2003; Farrow et al., 2007; Fielding et al., 2006; Lawrence et al., 1998a; Swerdlow et al., 1995). There are also consistent reports of slowed processing speed and reaction times, which emerge in pre-HD and significantly worsen with disease progression, and are associated with BG degeneration (Beste et al., 2006; Duff et al., 2010; Georgiou et al., 1995, 1997; Joel, 2001; Kirkwood et al., 2000a,b; Lehéricy et al., 2006; Saint-Cyr, 2003; Schneider et al., 2010; Ward et al., 2006). Although structural MRI demonstrates robust atrophy over 12 and 24 months in pre-HD (Tabrizi et al., 2011, 2012), very few clinical measures have been found to show significant longitudinal changes across short durations (for a review see Georgiou-Karistianis et al., 2013b). Development of more sensitive measures of disease progression that are related to the underlying pathology, including physiological, cognitive, motor, psychiatric and functional measures, will be necessary to validate treatment effectiveness in HD. Given the established links with underlying neuropathology, psychomotor functioning measures generated from neurophysiological technologies, such as TMS and EEG, are likely to show utility as sensitive endophenotypic biomarkers (Bechtel et al., 2010).

2.4. Current conceptualisation and the search for clinical biomarkers

HD is characterised by considerable phenotypic heterogeneity, which is well-documented in relation to the emergence, rate of progression and relative prominence of individual symptoms (Bohanna et al., 2008; Georgiou-Karistianis, 2009; Paulsen et al., 2008). Part of this heterogeneity may relate to the individual's capacity to respond to neuropathological insult (Wu et al., 2008). The premanifest period is envisaged as a time when the brain undergoes numerous pathological alterations, with concomitant functional compensatory processes that may mask overt clinical signs but which vary between gene carriers (Penney et al., 1990). However, given that morphologic cell changes precede neuronal death, it is feasible that therapeutic interventions could substantially extend the premanifest period (Nopoulos et al., 2010; Paulsen, 2010; Reading et al., 2005).

The last decade of research has seen a focus on delineating the trajectory of changes in HD gene carriers. PREDICT-HD and TRACK-HD are principally concerned with identifying sensitive markers of disease progression that will be used to decide appropriate timing of intervention, when therapies become available (Paulsen et al., 2006; Tabrizi et al., 2009, 2012, 2011). Other smaller-scale single-site studies, such as IMAGE-HD, are working to track changes in smaller groups by also incorporating functional imaging to investigate brain reorganisation and compensation (Georgiou-Karistianis et al., 2013a; Gray et al., 2013). Increased functional brain activity in both pre-HD and symp-HD, for example, is often interpreted as a compensatory process, yet there is considerable debate regarding what such changes in activation actually mean in terms of the coupling of neural activity and blood flow (Georgiou-Karistianis, 2009). At this stage, there are no models that adequately explain the wide range of disease manifestations (Esmaeilzadeh et al., 2011; Rosas et al., 2008a). Several authors have discussed the essential features of a practical and robust biomarker (see Aylward, 2007; Georgiou-Karistianis et al., 2013b; Weir et al., 2011), whereas others have noted that a set of multivariate or combined multimodal

imaging biomarkers may be more appropriate to capture the short- and long-term neurobiological outcomes of interventional therapies (Bohanna et al., 2008; Georgiou-Karistianis et al., 2013a; Rizk-Jackson et al., 2011; Sánchez-Castañeda et al., 2012). This conjecture is especially relevant to HD due to the wide-ranging effects of the disease across anatomical, cognitive and motor domains.

Electrophysiological techniques have certain unique qualities that complement and further build on more traditional neuroimaging modalities, however they have received very limited attention in HD (for a review see Nguyen et al., 2010). Electrophysiological outcome measures can be recorded objectively and are likely to be related to causal mechanisms of disease and diagnostic signs (Painold et al., 2010, 2011). The electrophysiological technique, EEG, is the only non-invasive neuroimaging method that provides a direct measure of neuronal firing. EEG data has excellent temporal resolution and test-retest reliability, enabling the detection of minute abnormalities. Moreover, EEG is non-invasive, widely available and inexpensive (Painold et al., 2010; Van Der Hiele et al., 2007). Functional deterioration, known to occur before marked cell loss, can be indexed with EEG and offers much promise in its potential to further inform our understanding of the relationship between clinically-meaningful measures and neural function, particularly during the pre-HD period. Lastly, with a view to discovering endophenotypic biomarkers for use in clinical trials, neural function is likely to be more amenable to treatment in the short-term than neural substrate.

Preliminary studies have indicated that electrophysiological indices of a range of sensory and motor processes in HD have the potential to be sensitive markers of both neuropathology and progression, particularly given their demonstrated sensitivity to decline in a range of other dementias (Berardelli et al., 1999; Beste et al., 2011; De Tommaso et al., 2003a; De Tommaso et al., 2001; Duncan et al., 2009; Ehle et al., 1984; Hodgson et al., 1999; Jurgens et al., 2011; Lefaucheur et al., 2002; Painold et al., 2010, 2011; Shang et al., 2012; Van Der Hiele et al., 2007). Such findings in pre-HD are particularly useful to verify that electrophysiological abnormalities are caused by neuropathology and provide insight into the later development of cognitive and motor impairments (De Tommaso et al., 2003b). Lefaucheur et al. (2006), for instance, demonstrated longitudinal decline in symp-HD with a range of electrophysiological measures, including blink reflexes and somatosensory evoked potentials, as well as significant associations between such measures and functional decline. More novel neurophysiological technologies like TMS are increasingly applied in research and clinical settings and promise to provide equal benefits with their continued advancement (Ziemann, 2011).

3. Transcranial magnetic stimulation: A novel neurophysiological technique

3.1. Overview and mechanisms of action

TMS is a non-invasive technique of stimulating neurons that is founded upon the principal that a time-varying magnetic field will generate a time-varying electric field (Barker et al., 1985; Edelmuth et al., 2010). The technique involves passing a brief current through a wire coil held to the head, which induces a powerful magnetic field that passes through the scalp (Pascual-Leone et al., 2000). This field creates an electric current lasting for less than one millisecond, which depolarises cell membranes, thereby transsynaptically influencing a group of cortical neurons (Siebner et al., 2009). Because the induced magnetic field is perpendicular to the coil direction, axons of corticocortical neurons are preferentially stimulated, rather than corticospinal neurons or cell bodies (Day et al., 1989; Rösler and Magistris, 2008). Furthermore, the lower firing thresholds of inhibitory interneurons render them more susceptible to stimulation than excitatory interneurons (Ridding and Rothwell, 2007). The effects of a TMS pulse at a particular site depends on several parameters, including stimulation intensity, frequency and duration (Miniussi and Rossini, 2011). The temporal resolution of TMS is considered excellent, while the spatial resolution of approximately 5 mm^3 is poorer than other neuroimaging modalities, such as MRI techniques (McClintock et al., 2011). However, the combination of moderate spatial and high temporal resolution that TMS delivers produces a technique that can provide unique insights into the functional neuroanatomy of the brain.

A single supra-threshold magnetic pulse will synchronise the activity of a group of neurons beneath the coil, given certain parameters, and will be followed by a period of long-lasting inhibition (Siebner et al., 2009). This transient interference with neuronal activity (Maccabee et al., 1990), coined "*the virtual lesion*" (Pascual-Leone et al., 2000; Walsh and Rushworth, 1999), has been used to infer how and when certain brain regions are involved in particular behavioural outputs, thereby allowing causal inferences to be made (Miniussi and Rossini, 2011; Pascual-Leone et al., 2000; Sack and Linden, 2003; Taylor et al., 2008). The excitability of a set of neurons underlying the stimulated region reflects the balance between inhibitory and facilitatory processes (Chen, 2004; Komssi and Kähkönen, 2006). Furthermore, the measurable outcome of a TMS pulse reflects neurons stimulated locally and the connectivity of these neurons with distant cortical, subcortical and contralateral areas (Di Lazzaro et al., 1999; Ferreri et al., 2011; Fuggetta et al., 2005; Reithler et al., 2011), as well as the TMS-induced release of endogenous neurotransmitters (Paus and Barrett, 2004; Stagg et al., 2009; Strafella et al., 2001).

3.2. Protocols for measuring cortical excitability and inhibition

Single-pulse TMS paradigms traditionally involve measuring the electromyographic (EMG) response in a hand muscle following stimulation of the motor cortex, which can excite corticospinal tract neurons (Day et al., 1989; Rossini et al., 1994). Outcomes of interest are the motor-evoked potential (MEP) amplitude and latency. While the amplitude is directly related to stimulus intensity, and thus the number of neurons recruited, the latency reflects a combination of factors associated with conduction time, including the number of synapses involved (Miniussi and Rossini, 2011). A fundamental outcome measure of single-pulse paradigms is the resting motor threshold (RMT), which describes the average stimulation intensity required to generate a measurable motor response (arbitrarily defined as 1 mV) in a target muscle (Rossini et al., 1994). The active motor threshold (AMT) describes the target muscle threshold during sustained voluntary isometric contraction. It is normally lower than the RMT by 5–20% and is proposed to reflect axonal excitability (Schippling et al., 2009). While motor thresholds exhibit considerable inter-individual variability, they appear to remain relatively stable within individuals over time (Orth and Rothwell, 2004). The cortical silent period (CSP) is another single-pulse outcome measure that describes the length of time voluntary muscle activity is prohibited by TMS applied to the region of motor cortex that corresponds to a tonically active muscle (Daskalakis et al., 2003; Ziemann, 2004). An early study of the CSP revealed that processes in spinal motor neurons are responsible for inhibition exhibited in the first 50 ms after TMS and cortical mechanisms arise later in the silent period (Inghilleri et al., 1993).

Paired-pulse techniques, on the other hand, assess changes in excitability when a TMS test stimulus is preceded by a conditioning stimulus, quantified as the change in MEP amplitude relative to that elicited by the test stimulus alone (Di Lazzaro et al., 1998; Hallett, 2000; Hanajima and Ugawa, 2008). Whether the effect is inhibitory or facilitatory depends on the inter-stimulus interval (ISI) and

conditioning stimulus intensity (Chen, 2004; Ilić et al., 2002). Short-interval paired-pulse paradigms commonly used in research with a subthreshold conditioning stimulus and suprathreshold test stimulus are "*short-interval cortical inhibition*" (SICI) and "*intracortical facilitation*" (ICF), whereas the long-interval paired-pulse paradigm termed "*long-interval cortical inhibition*" (LICI) requires longer ISIs and a suprathreshold conditioning stimulus. A pivotal study by Davies et al. (1990) explored the inhibitory systems involved in intracortical inhibition and isolated them pharmacologically in the rat hippocampus. This study demonstrated two separate inhibitory systems and confirmed through administration of GABA-receptor antagonists that both were mediated by GABAergic inhibition at the synapse (Davies et al., 1990). A seminal paper by Kujirai et al. (1993) was the first demonstration of SICI and ICF in the motor cortex of healthy individuals. The key outcomes were twofold: first, that SICI was elicited at ISIs of 1–6 ms, while ICF occurred with ISIs of 10 and 15 ms, and second, that response suppression arose at the level of the cortex and was attributable to alterations in synaptic activity in intracortical inhibitory pathways (Kujirai et al., 1993). These findings have since been replicated by a multitude of studies (Ashby et al., 1999; Hanajima et al., 1998; Nakamura et al., 1997) and further studies have established that LICI is elicited with 50–200 ms ISIs (Di Lazzaro et al., 2002; Nakamura et al., 1997). It is thought that the subthreshold conditioning stimulus causes an inhibitory post-synaptic potential at corticospinal neurons without generating a descending corticospinal volley (Di Lazzaro et al., 1998; Ilić et al., 2002). Kujirai et al. (1993) suggested that the GABAergic interneurons comprising these intracortical inhibitory circuits may be particularly susceptible to excitation by a TMS pulse due to their orientation with respect to the stimulating coil.

Pharmacological studies have provided ample evidence for the central role of GABAergic neurotransmission in these inhibitory circuits (Ziemann et al., 1996). For instance, the $GABA_A$-receptor agonist diazepam was demonstrated to significantly increase SICI and decrease ICF when applied to motor cortex corresponding to a muscle at rest (Ilić et al., 2002). Pharmacological studies, together with knowledge of the timeframes for which SICI and LICI occur, have verified that SICI is mediated by $GABA_A$-receptors and LICI is mediated by $GABA_B$-receptors (Chen, 2004; Florian et al., 2008; McDonnell et al., 2006). This notion is further supported by studies showing inhibition of SICI by LICI (Florian et al., 2008; Sanger et al., 2001) because $GABA_B$-receptors are positioned pre- and post-synaptically, and may inhibit GABA release, whilst $GABA_A$-receptors are confined to post-synaptic membranes (Cash et al., 2010; Chu et al., 2008; Daskalakis and Chen, 2008; Werhahn et al., 1999). SICI is considered a pure measure of cortical inhibition in contrast to the CSP, which reflects a combination of corticocortical and corticospinal processes. The corticocortical aspect of the CSP is proposed, with pharmacological evidence, to be modulated by $GABA_B$-receptors (Farzan et al., 2010; Siebner et al., 1998; Ziemann, 2004). Furthermore, neuroimaging and neurophysiological studies have illustrated that different populations of interneurons are responsible for inhibition and facilitation, with ICF thought to be associated with glutamatergic circuitry (Orth et al., 2003; Strafella and Paus, 2001). SICI and LICI are especially useful measures because they intrinsically control for the inter-individual variability of excitability in the general population by investigating the effect of a conditioning stimulus on a previously established MEP (Orth and Rothwell, 2004).

The effect of the summation of TMS pulses to the brain has led to the development of various repetitive TMS (rTMS) protocols that are now widely used, such as "*theta burst stimulation*" (Fitzgerald, 2011). Repeated TMS pulses applied at a certain frequency and intensity cause lasting changes in neuronal reactivity and thus, are used to assess and modulate cortical plasticity in both healthy and dysfunctional brains (McClintock et al., 2011; Paus et al., 2001a). The enduring effects of rTMS seem to be driven by changes in synaptic efficacy, which produces long-term potentiation (LTP) and long-term depression (LTD; Centonze et al., 2007; Cheeran et al., 2010; Chen et al., 1997; Ridding and Rothwell, 2007). Only in more recent years has TMS been associated with metaplasticity, a term used to describe "higher-order" plasticity processes that modulate subsequent instances of LTP- and LTD-inducing events through the integration of prior neural activations over longer timeframes (i.e., minutes to days as opposed to seconds; Mockett and Hulme, 2008). Importantly, metaplastic changes to plasticity thresholds are not specific to synapses undergoing LTP/LTD but may modify activity across entire networks (Abraham, 2008). Such long-lasting and wide-reaching effects, as those possible from treatment with TMS, are promising for neurodegenerative conditions like HD where quite focal pathology causes diverse functional, cognitive and motor changes.

3.3. Previous use of TMS and EEG and potential applications for future research

3.3.1. EEG studies and ERP components

EEG provides high-density temporal information about neural activity that is either ongoing, spontaneous or triggered by an external event (Siebner et al., 2009). The event-related potential (ERP) is a prominent EEG method for observing patterns of brain activity associated with particular sensory, motor or cognitive events. An ERP represents the summation of post-synaptic potentials from populations of synchronously active, primarily pyramidal cortical neurons (Friedman and Johnson, 2000). Specific ERP abnormalities may occur independently of any behavioural change or may reflect cognitive deficits, and have shown utility in establishing differential diagnoses (Münte et al., 1997). Previous studies employing EEG, and a range of ERP paradigms, have significantly informed our current understanding of the pathophysiology of HD (for a review see Nguyen et al., 2010). For example, a key study by Beste et al. (2009) examined movement-related potentials in pre-HD and symp-HD and revealed region-specific lateralised abnormalities in activation in each HD group, compared with controls, whereas some behavioural measures did not significantly differ between pre-HD and controls. The results of this study pointed to altered inter-hemispheric inhibition in HD and compensatory mechanisms during pre-HD stages that are likely to modify phenotypic expression. ERP studies have also been critical in establishing links between pathophysiological processes in HD and cognitive functioning. For instance, symp-HD participants were found to show increased sensory memory performance compared with pre-HD and controls, which was attributed to increased glutamatergic transmission due to the concomitant alterations observed in two well-established ERP components, namely mismatch negativity and reorienting negativity (Beste et al., 2008). These methods are ideal for study in HD since we can determine which brain systems are dysfunctional and which are relatively intact across the temporal stages of information processing, and findings such as altered inter-hemispheric inhibition also suggest using TMS technologies.

3.3.2. Utility of concurrent TMS and EEG

The existing body of TMS literature is biased towards the motor system due to the ease of measuring an objective outcome (Fitzgerald et al., 2002; Miniussi and Rossini, 2011; Reithler et al., 2011; Ziemann, 2011). In fact, with the exception of rTMS studies (Hoy and Fitzgerald, 2010), the vast majority of research has investigated the effect of TMS pulses on muscle excitability measured with EMG (Ridding and Rothwell, 2007). This bias has therefore generated a substantial literature in movement disorders, with a

particular focus on RMT/AMT and CSP measures in PD, Tourette's syndrome, Attention-Deficit/Hyperactivity disorder and dystonias of various aetiologies (Chen et al., 2008; Orth, 2009). Given the central role of non-motor cortical regions in many neurological disorders, researchers have recently realised the importance of directly measuring cortical excitability (Farzan et al., 2010).

While EEG is valuable as a single modality, combining EEG with TMS provides increased spatial resolution, which in conjunction with high temporal resolution, enables researchers to examine real-time corticocortical connectivity and cortical responses to the TMS pulse, an effect independent of behavioural performance or cognitive task engagement (Ilmoniemi et al., 1997; Komssi et al., 2002; Komssi and Kähkönen, 2006; Miniussi and Thut, 2010; Taylor et al., 2008). Saturation of the EEG recording electrodes by the TMS pulse previously prevented their concurrent use (Mäki and Ilmoniemi, 2010; Virtanen et al., 1999). However, procedures such as the "*sample-and-hold*" method overcame this limitation by sampling the signal immediately before applying the TMS pulse, and latching the signal at that level until residual voltage from TMS had resolved (Virtanen et al., 1999). Other recording methods have been developed more recently (Ives et al., 2006; Thut et al., 2005) and it is now also possible to remove the auditory and somatosensory effects of TMS captured by electrodes after the EEG recording is completed (Nikouline et al., 1999; Thut et al., 2005; Tiitinen et al., 1999).

TMS–EEG has the capability to expose causal relations following stimulation due to the temporal sequence of evoked activity, whereas fMRI for example relies upon existing network models (Komssi and Kähkönen, 2006; Sack and Linden, 2003; Taylor et al., 2008). TMS–EEG enables the measurement of cortical activity directly and objectively with lower stimulus intensities than those required to elicit MEPs, as well as investigation of "*behaviourally silent*" regions, which is a distinct advantage over some ERP research (Ferreri et al., 2011; Ilmoniemi and Kičić, 2010; Mäki and Ilmoniemi, 2010; Miniussi and Thut, 2010; Ziemann, 2011). Furthermore, both the input and output from TMS–EEG techniques occur in the cortex, and can potentially bypass sensorimotor pathways and subcortical structures (Siebner et al., 2009), which is critical to disambiguate primary and compensatory processes in pre-HD. Current knowledge from neuroimaging and cognitive studies has indicated that non-motor corticostriatal circuits, such as the DLPFC loop, are affected very early in pre-HD and are likely to show pathophysiological abnormalities, both dysfunctional and compensatory (Gray et al., 2013; Lawrence et al., 1998a; Stout et al., 2011; Wolf et al., 2011). Thus future TMS–EEG studies will be critical to build upon existing findings of EEG abnormalities in these cortical regions in pre-HD in order to spatially and temporally visualise evoked responses (De Tommaso et al., 2003a; Van Der Hiele et al., 2007). Studies have demonstrated predictable time-locked EEG responses to both sub- and supra-motor threshold pulses in healthy individuals, which increase linearly with stimulus intensity (Bonato et al., 2006; Casarotto et al., 2010; Ferreri et al., 2011; Kähkönen et al., 2005b; Paus et al., 2001b; Van Der Werf and Paus, 2006). Casarotto et al. (2010), however, advise caution when selecting parameters for applying TMS in non-motor cortices because the orientation of fibres is less predictable than in the primary motor area.

The validity of applying protocols conceived for assessment of the integrity of the corticospinal system to non-motor cortical areas is still being established (McConnell et al., 2001; Robertson et al., 2003; Stewart et al., 2001). A TMS–EEG study in healthy participants demonstrated that the degree of suppression elicited by LICI to motor cortex was not significantly different when applied to DLPFC or parietal cortex (Fitzgerald et al., 2008). Other studies have found a significant relationship between LICI in motor and non-motor cortices, and have additionally shown a correlation between EMG and EEG measurements of LICI in the motor cortex (Daskalakis et al., 2008a; Farzan et al., 2010). Nevertheless, LICI in the DLPFC was shown to differentially suppress cortical oscillations compared to motor cortex, specifically those implicated in 'higher-order' cognitive processes, which the authors concluded may reflect the critical importance of cortical inhibitory systems in guaranteeing maximal cognitive performance (Daskalakis et al., 2008b; Farzan et al., 2009, 2010). The association between cortical inhibition in motor and non-motor cortices is thought to indicate similar mechanisms underpinning their production, mediated by GABAergic systems (Ferreri et al., 2011; Fitzgerald et al., 2009).

3.3.3. *Future directions for TMS research*

With the variety of multimodal imaging technologies available, including TMS–fMRI, TMS–PET and TMS–EEG, we can now begin to tease apart the effects of stimulation upon the brain, both at a cortical and subcortical level (Chen et al., 2008; Esser et al., 2006; Hallett and Berardelli, 2008). TMS to the cortex can modulate the activity of BG nuclei, supporting the notion that TMS can tap corticostriatal pathways (Bestmann et al., 2004; Fregni et al., 2005; Orth, 2009; Speer et al., 2003), and multi-modal TMS techniques can illustrate the subsequent pattern of responses across target neural networks. For example, single-pulse stimulation of primary motor cortex activated and subsequently suppressed neurons in the subthalamic nucleus (Strafella et al., 2004) and rTMS of primary motor and prefrontal cortices has been shown to induce dopamine release from the ipsilateral putamen and caudate nucleus, respectively (Keck et al., 2002; Strafella et al., 2001, 2003). Thus, TMS–EEG techniques are extremely useful methods to further understand the link between neuropathophysiology and performance on tasks known to tap corticostriatal circuitry in HD. There have been attempts to ameliorate deficits in disorders such as PD and dystonias with rTMS (Centonze et al., 2007; Koch et al., 2005; Lefaucheur et al., 2004a,b; Mally and Stone, 1999; Siebner et al., 2000; Wu et al., 2008), and other neurodegenerative disorders like Alzheimer's disease (Bentwich et al., 2011; Cotelli et al., 2006). However, many questions remain unanswered concerning the precise impact of single- and paired-pulse protocols in terms of intra- and inter-hemispheric connectivity and neurotransmitter release, and how this might translate to cognitive and behavioural outcomes (Lefaucheur, 2006; Nahas et al., 2001; Rossi et al., 2000; Thut and Pascual-leone, 2010; Wagner et al., 2007). Certainly TMS–EEG has potential utility for further understanding altered transcallosal mechanisms in HD evident in previous ERP studies, which also show associations with cognitive and motor measures (Beste et al., 2009).

Assessment of SICI is considered especially relevant in movement disorders because corticocortical inhibition has been implicated in the cessation of ongoing movements and inhibition of unwanted prepotent movements (Coxon et al., 2006; Stinear and Byblow, 2004b). An early study of corticocortical inhibition and facilitation in PD reported reduced SICI and normal ICF in participants off medication and no difference between PD and controls in RMT or CSP, which underscores the sensitivity of SICI in identifying subtle deficits (Ridding et al., 1995; Stinear and Byblow, 2004a). Investigation of SICI with TMS–EEG in non-motor brain regions, for example, could provide important insight into the relationship between neurophysiology and the development of the cognitive and behavioural aspects of the HD phenotype. There is some debate in the literature, however, surrounding the extent to which reduced SICI might reflect hypoexcitability of inhibitory processes and hyperexcitability of excitatory processes (Curra et al., 2002; Hanajima and Ugawa, 2008; Stinear and Byblow, 2004a). The value of SICI in assessment of neuropathological conditions is unfortunately weakened by its lack of sensitivity in differentiating between conditions, which might be attributed to a restricted range in outcomes (i.e., 0–100% reduction in MEP) and the amalgamation

of different ISIs when reporting strength of inhibition (Chen et al., 2008; Fisher et al., 2002). With a view to overcome these limitations, Fisher et al. applied a novel "*threshold-tracking*" method, which determines the test stimulus intensity required to produce a designated MEP size. This method revealed two different types of SICI occurring at discrete ISIs, which has been corroborated (Daskalakis et al., 2002; Roshan et al., 2003) and may afford increased specificity for future uses of SICI protocols in clinical neurophysiology.

4. The utility of TMS in Huntington's disease

4.1. Review of past research findings

4.1.1. Motor thresholds

There is a small body of published research describing the use of TMS in HD with mixed results (refer to Table 1 for a summary), likely attributed to small sample sizes, differential choice of stimulation parameters (such as the intensity of pulses) and poor control of phenotypic heterogeneity (such as the degree of choreic symptoms; Nardone et al., 2007). For instance, several researchers have repeatedly found no difference in motor thresholds or MEP amplitude between symp-HD and controls (Crupi et al., 2008; Lorenzano et al., 2006; Nardone et al., 2007; Orth et al., 2010; Priori et al., 1994, 2000; Tegenthoff et al., 1996). Schippling et al. (2009), on the other hand, reported higher RMT and AMT in pre-HD and symp-HD participants and argued that HD is associated with a disturbance in both post-synaptic excitability and axonal thresholds. However, given that motor cortex excitability did not differ between pre-HD and symp-HD, and there was no association between responses to TMS and clinical measures, abnormal excitability may arise from the presence of the mutated protein in the brain, rather than progressive pathophysiological changes (Orth et al., 2010; Schippling et al., 2009). Indeed, emerging research is now beginning to tease apart the responses in pre-HD and symp-HD across the array of TMS paradigms (Orth et al., 2010; Schippling et al., 2009).

Priori et al. (2000) found that MEPs were smaller in symp-HD participants compared with controls and suggested that the motor cortices in HD are not globally excitable. However, others have argued that the abnormal corticospinal excitability is likely to result from some disturbance of the complex modulation of BG output by cortical interneurons (Abbruzzese et al., 1997; Tegenthoff et al., 1996). There is ample evidence to support the BG dysfunction model of HD, given intact corticospinal efferents and normal patterns of central conduction times to upper and lower limbs in HD participants regardless of symptom severity (Hömberg and Lange, 1990; Nardone et al., 2007).

4.1.2. Cortical silent period

Findings regarding the CSP are similarly mixed and have been proposed to reflect the difficulty symp-HD participants have in maintaining a voluntary muscle contraction (refer to Table 1; Schippling et al., 2009). Furthermore, CSP measurements should ideally be adjusted for differences in MEP size, which has scarcely been done (Schippling et al., 2009), and there is considerable variability between studies in the demarcation of the beginning and

Table 1
Single- and paired-pulse TMS studies with HD participants since the CAG repeat genetic test became available.

Authors	Symp-HD (*n*)	Pre-HD (*n*)	Control (*n*)	TMS measures	Main findings
Priori et al. (1994)	13	–	11	RMT/AMT	Prolonged CSP in symp-HD
				CSP	Positive correlation between CSP and severity of chorea in symp-HD
Tegenthoff et al. (1996)	13	–	21	RMT	Prolonged CSP in symp-HD
				CSP	Prolonged inhibition after LICI in symp-HD
				LICI (resting)	
Abbruzzese et al. (1997)	9	2	12	RMT	Reduced SICI and increased ICF in symp-HD compared to controls
				SICI, ICF (resting)	Correlations for SICI and ICF with UHDRS dyskinesia score in the combined HD group
					Inverse correlation between SICI and age of onset in symp-HD
Priori et al. (2000)	16	–	28	AMT	Smaller MEP amplitude at each stimulus intensity in symp-HD
				SICI, LICI (active)	No group differences in motor thresholds or inhibition
Modugno et al. (2001)	17	–	15	RMT	Smaller MEP amplitude in symp-HD
				CSP	Prolonged CSP in symp-HD
Lefaucheur et al. (2002)	36	–	–	CSP	CSP was abnormal in 24 participants: 1 was absent, 11 were prolonged, 12 were shortened
Lefaucheur et al. (2006)	20	–	–	CSP	CSP duration shortened within participants across a 2-year period
					Positive correlation between slope of CSP decline and UHDRS TFC score in symp-HD
Lorenzano et al. (2006)	11	–	11	RMT/AMT	MEP amplitude increased during repetitive TMS administration in controls but not in symp-HD
				CSP	CSP duration increased during repetitive TMS administration in both groups equally
Nardone et al. (2007)	12	–	15	RMT/AMT	Reduced ICF in symp-HD at three different inter-stimulus intervals
				CSP	Correlation between ICF and UHDRS motor and TFC score in symp-HD
				SICI, ICF (resting)	
Crupi et al. (2008)	8	–	8	RMT	MEP amplitude increased following paired associative stimulation in controls but not in symp-HD
Schippling et al. (2009)	8	8	22	RMT/AMT	Higher RMT and AMT in the combined HD group compared to controls
				CSP	Recruitment slopes were flatter in symp-HD and pre-HD compared to controls
				SICI (resting)	Thresholds for eliciting SICI were higher in the combined HD group than in controls
Orth et al. (2010)	8	7	14	RMT/AMT	Greater decreases in MEP amplitude during repetitive TMS administration in controls than in either HD group
					No group differences between symp-HD and pre-HD

Abbreviations: Symp-HD: symptomatic HD gene carriers; Pre-HD: premanifest HD gene carriers; RMT: resting motor threshold; AMT: active motor threshold; CSP: cortical silent period; LICI: long-interval cortical inhibition; SICI: short-interval cortical inhibition; ICF: intracortical facilitation; UHDRS: Unified Huntington's Disease Rating Scale; MEP: motor-evoked potential; TFC: total functional capacity.

end of the silent period (Daskalakis et al., 2003). Various studies have reported reduced (Eisen et al., 1989), unchanged (Lorenzano et al., 2006; Nardone et al., 2007; Schippling et al., 2009) or prolonged (Modugno et al., 2001; Priori et al., 1994; Tegenthoff et al., 1996) CSPs in HD participants compared with controls, and there is some evidence that CSP length might correlate with clinical indices (Priori et al., 1994). Nevertheless, while a cross-sectional study failed to find any consistent alteration of CSPs in symp-HD (Lefaucheur et al., 2002), assessment of the same participants two years later revealed a significant reduction over time, which also correlated with functional capacity (Lefaucheur et al., 2006). Conversely, it has been shown that rTMS to the motor cortex increased the CSP in both symp-HD and controls, suggesting that the excitability of inhibitory interneurons is normal in HD, while the excitability of facilitatory interneurons was reduced (Lorenzano et al., 2006). Priori et al. (1994) contend that, based on the primate model of BG functioning, the increased excitation of cortical inhibitory interneurons that results from striatal degeneration in HD should prolong the CSP.

4.1.3. Cortical inhibition

To our knowledge, only five published studies have investigated SICI in HD (refer to Table 1). The most recent by Schippling et al. (2009) reported that SICI thresholds were significantly higher in pre-HD and symp-HD participants compared with controls. The authors concluded that, because recruitment curves did not differ between HD groups and controls, the effect was likely to be a result of changes in axonal thresholds, rather than post-synaptic excitability (Schippling et al., 2009). The earliest of these studies also reported reduced SICI and greater ICF in pre-HD and symp-HD participants compared with controls, which correlated with dyskinesia scores and age of clinical onset (Abbruzzese et al., 1997). Although normal SICI in symp-HD has been reported by Hanajima et al. (1999), this study was confounded by the inclusion of participants with chorea of various aetiologies. Similarly, Priori et al. (2000) also found SICI and LICI to be comparable between symp-HD and controls, however, participants were investigated while maintaining a voluntary muscle contraction to remove any interference from subthreshold involuntary activity, which raises the initial excitability level of the corticospinal pathway and may explain their negative findings. Lastly, Nardone et al. (2007) reported normal SICI in symp-HD, but showed that reduced ICF correlated with UHDRS scores. Lorenzano et al. (2006) have likewise found evidence that it is facilitatory but not inhibitory interneurons that are affected in HD and there is corresponding evidence that LTP-like plasticity in glutamatergic excitatory synapses is altered in symp-HD (Crupi et al., 2008). Critically, however, the effect of stimulation on MEP amplitude reflects a complex interaction of both facilitatory and inhibitory processes; thus, ICF is not a pure measure of cortical facilitation, but likely captures cortical inhibitory activity (Nardone et al., 2007). Overall, the research to date highlights the fact that SICI and CSP are modulated by different inhibitory systems and that abnormal excitability parameters in HD are likely to be attributed to functional more so than structural alterations.

4.1.4. Repetitive TMS

Preliminary studies have begun to experiment with rTMS in HD with modest success. Brusa et al. (2005) have demonstrated a reduction in abnormal involuntary movements in symp-HD participants with low-frequency subthreshold rTMS applied to the supplementary motor area, which lasted for approximately 30 min after treatment. A recent review commented on the future utility of rTMS in treating the symptoms of HD, as well as potentially slowing the disease progression, and attributed the mechanisms of action to its antioxidant and neuroplasticity-inducing effects (Medina and Tunez, 2010). In line with this, rTMS is currently known to deliver lasting improvement from symptoms in several neurological conditions, including tinnitus (Burger et al., 2011; Khedr et al., 2009), schizophrenia (Freitas et al., 2009) and chronic stroke (Yozbatiran et al., 2009), and could offer utility as a possible treatment option in HD. Emerging research employing an exposure-based learning task, that reflects LTP-inducing protocols, has indicated that pre-HD individuals may have an enhanced capacity for perceptual learning, which has been shown to correlate with genetic load (Beste et al., 2012). It has thus been proposed that increased glutamatergic activity not only accelerates excitotoxic neurodegeneration but also increases neural plasticity; this suggests that TMS-based therapies could be particularly effective in individuals with the HD gene.

4.2. Issues of complexity and future directions: The use of multimodal TMS in Huntington's disease

There is considerable phenotypic heterogeneity in both pre-HD and symp-HD. Lawrence et al. (1998c) noted that the HD mutation seems to have a continuous rather than a discrete mode of action, and that future studies need to focus on understanding what additional factors describe and/or modulate the genotype-phenotype relationship. Furthermore, studies have employed different exclusion criteria for HD groups, with some exclusions based on cognitive impairment that might constitute signs of dementia and/or depression, but which might also be a typical part of HD progression (Papp et al., 2011).

Other issues include the fact that pre-HD participants are a self-selected group; they comprise a minority of individuals at risk of inheriting the defect that have decided to undergo genetic testing and may represent a subset of HD carriers who systematically differ from individuals that choose not to be tested (Biglan et al., 2009; Papp et al., 2011; Paulsen et al., 2001). Furthermore, it is possible that knowledge of the approaching disease may affect responses to experimental procedures (Johnson et al., 2007). Pre-HD individuals are significantly younger than symp-HD, which renders matching of control groups problematic (Wolf et al., 2009). While most studies address age discrepancies by covarying for age, others have chosen to match groups by age to eliminate possible confounds from generational subtleties (Dumas et al., 2012; Golding et al., 2006). Finally, the typical trajectory of age-related decline in neuroanatomical, neurophysiological and cognitive domains known to be affected in HD have been inadequately characterised in the literature, which can influence the abnormality observed in HD (Aylward et al., 2011; Chen et al., 2008; Sasaki et al., 2011; Swerdlow et al., 1995; Ward et al., 2006). These complexities deserve consideration when comparing results across studies.

There are also inherent difficulties in applying TMS to motor cortices and using EMG as the outcome measure in movement disorders. For example, Cantello (2002) asserted that previous studies have generally failed to make a distinction between the underlying pathophysiology of movement disorders and associated signs, such as altered muscle tone and difficulties initiating and executing movements. It is therefore conceivable that TMS responses may vary according to specific symptomatology, rather than being directly caused by neuropathology. A key focus for future research should certainly be the delineation of relationships between clinical signs of HD and TMS outcomes. Furthermore, the movement disorder can cause differential motor artefacts between patient and control groups and the use of a variety of image analyses software can make comparison across studies difficult. Previous studies also differ in their choice of studying participants at rest or while maintaining a slight muscle contraction in order to avoid the confounding effect of incomplete relaxation in some participants. In their review of the use of TMS in various neurological disorders, Curra et al. (2002) highlighted the considerable inter-individual

differences evidenced between HD participants compared to other clinical groups, and suggested that future research concentrate on response variability as opposed to reporting deficits at the group level.

Despite such issues, TMS–EEG is an especially useful method to apply in HD; the direct measurement of cortical potentials in response to stimulation permits the investigation of small populations of neurons, which in comparison to other neuroimaging methodologies like MRI and DTI, has the potential to reveal subtle disturbances in very focal brain regions. This advantage is beneficial in terms of endophenotypic biomarker discovery and also for informing current understanding of neuropathology and inter-individual phenotypic variability (Pascual-Leone et al., 2011). However, there is a paucity of research describing the differential effects of TMS to regions outside the motor cortex (McConnell et al., 2001; Robertson et al., 2003). There is some evidence of different responses of the prefrontal and motor cortices to equivalent TMS pulses, suggested to result from differential influences upon neurotransmitter systems (Kähkönen et al., 2004; Sack and Linden, 2003). There is also the potential that SICI/LICI could be tested in non-motor cortices at lower intensities, thus minimising risk to participants (Komssi et al., 2004).

There are additional design complexities uniquely associated with TMS–EEG that should also be considered as part of future research. For instance, it is possible that TMS pulses applied to participants wearing an EEG cap differ from TMS used in isolation because the coil is further from the scalp (Fitzgerald, 2010; McConnell et al., 2001; Rossi et al., 2009). Also, localisation of non-motor areas is less accurate than primary motor areas due to the absence of an overt response, although DLPFC has been localised under electrodes F3/F4 of the EEG cap with minimal inter-individual variability. Moreover, it has yet to be shown whether TMS application to non-motor cortices with a similar coil orientation, to that used to stimulate motor regions, actually activates similar neural populations (Kähkönen et al., 2005a).

5. Conclusions

The vast body of research in HD to date has greatly contributed towards our current understanding of the earliest changes associated with the genetic mutation. However, the wealth of anatomical and biochemical features associated with HD have not been well mapped onto the functional and behavioural outcomes of the disease. In fact the disconnection between structural brain changes and cognitive function has led to speculation that compensatory processes may be at play early in the disease process. Future TMS and/or TMS–EEG studies are likely to offer new and exciting opportunities to further enhance knowledge relating to the complex biobehavioural relationships underlying neuroanatomical and pathophysiological abnormalities, and motor, cognitive and behavioural disturbances in HD. Not only do such techniques allow for non-invasive investigation of critical cortico-subcortical circuits, their outcomes constitute a key midpoint in the pathway between genotype and phenotype. Thus, neurophysiological responses in HD might be envisaged as the missing link for further enhancing our understanding of such causal relationships between the gene mutation and symptomatology. The design complexities that arise in research of this nature do not preclude its value, but offer an opportunity to further understand the pathophysiological mechanisms not only of HD but also other neurodegenerative and neuropsychiatric conditions. Regarding their utility as endophenotypic biomarkers, the proximity of neurophysiological outcomes to the gene product generates larger statistical effect sizes and permits smaller clinical samples, which further attests to their inherent value as objective, sensitive and cost-effective measures. Future research should investigate TMS paradigms as part of large-scale longitudinal multi-site studies so as to map their trajectories in terms of neuroanatomical, motor, cognitive and psychiatric decline. Powerful studies of this type are likely to offer increased insight into the underlying neural mechanisms that occur early in the disease and offer new avenues for elucidating which electrophysiological paradigms might provide increased sensitivity to disease progression and also have symptom relevance.

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Additional relevant literature

Structure and function of the basal ganglia and corticostriatal pathways

The basal ganglia comprise a number of subcortical structures at the base of the forebrain that are highly interconnected with various brain regions and associated with a range of functions (Albin, Young, & Penney, 1989). These structures form a series of parallel loops generating a reciprocal network of communication between discrete regions of the basal ganglia, thalamus and cortex via several neurotransmitters (Alexander, DeLong, & Strick, 1986; see Figure 1). There is a substantial body of literature regarding the topology of basal ganglia-thalamocortical projections, the majority of which stems from animal studies (Haber & Knutson, 2010). However, the emergence of sophisticated neuroimaging techniques, such as DTI and event-related fMRI, has provided further insights into the functional neuroanatomy of these circuits in the human brain.

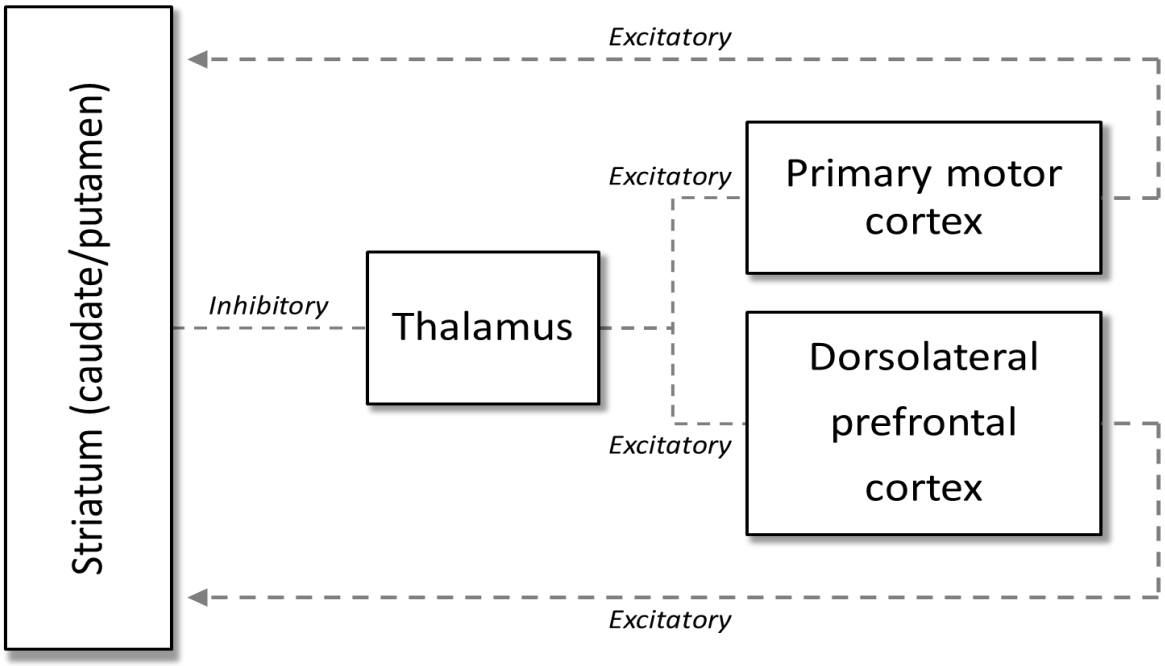


Figure 1. Schematic representation of two parallel corticostriatal circuits.

Figure adapted from Alexander et al. (1986). The net output between structures is labelled as either 'excitatory' (e.g., glutamate-mediated) or 'inhibitory' (e.g., GABA-mediated).

Primate models of basal ganglia interconnectivity have been able to propose two distinct pathways, namely the direct and indirect, through which structures within the basal ganglia exert their effect (DeLong & Wichmann, 2009). The direct pathway comprises a circuit between the cortex, striatum, substantia nigra pars reticulata, globus pallidus interna and thalamus. The indirect pathway encompasses an additional loop between the striatum, globus pallidus externa and subthalamic nucleus, and is a net inhibitory pathway (Hallett & Obeso, 2015). The striatum comprises predominantly GABAergic interneurons and output neurons (Wichmann & DeLong, 1996). The substantia nigra pars compacta, on the other hand, produces the neuromodulator dopamine and provides dopaminergic innervation of the striatum, with dopamine (D_1 and D_2) receptors located throughout both direct and indirect pathways. D_1 and D_2 receptors are expressed in GABAergic neurons in the striatum and differentially influence release of GABA, essentially 'gating' corticostriatal transmission (Bernath & Zigmond, 1989). However, these dopamine receptors are also found in widespread brain regions, including in the frontal cortex, diencephalon, limbic areas and cerebellum (Beaulieu & Gainetdinov, 2011).

Several polymorphic variants have been described for each of the dopamine receptors, which exhibit unique anatomical, pharmacological and physiological features (Beaulieu & Gainetdinov, 2011). Some dopamine receptors are located both presynaptically on dopaminergic neurons and postsynaptically (e.g., D_2), while others (e.g., D_1) are only found postsynaptically on dopamine-receiving cells, such as GABAergic striatal neurons. GABA receptors ($GABA_A$ and $GABA_B$) also show polymorphisms, which contribute to neurophysiological differences between individuals and are implicated in a number of neurological conditions (e.g., Korpi & Sinkkonen, 2006). Moreover, interactions between GABAergic and dopaminergic neurons are essential to the healthy functioning of the basal ganglia (André, Cepeda, & Levine, 2010). Indeed, pathophysiological activity in key basal ganglia-thalamocortical networks has been implicated in movement disorders, such as Parkinson's disease and dystonia (DeLong & Wichmann, 2009). However, knowledge of the

role of these circuits has evolved over time with further human research to now include goal-directed behaviours, learning and emotion processing, in addition to motor and sensory functions (Balleine, Liljeholm, & Ostlund, 2009). Critically, the majority of striatal outputs project via the basal ganglia and thalamus to the frontal cortices, which highlights the importance of considering cognitive and psychiatric disturbances in HD studies (Nopoulos et al., 2010).

HD pathogenesis

The hallmark HD symptom of chorea is believed to arise from over-excitation of the cortex due to abnormal functioning of the indirect basal ganglia pathway, whilst later symptoms of rigidity and hypokinesia appear to be caused by further striatal degeneration disrupting the direct pathway (Berardelli et al., 1999). Age at onset in HD is diverse but the prevailing determinant is the size of the CAG expansion, with most affected individuals possessing 40-55 repeats (Langbehn, Brinkman, Falush, Paulsen, & Hayden, 2004). A post-mortem study revealed that neuronal loss in the striatum correlated with CAG repeat, even after controlling for disease duration and age at death (Furtado et al., 1996). Moreover, CAG is also associated with measures such as rates of whole-brain atrophy and D_2 receptor binding (Antonini et al., 1996; Henley et al., 2009). A study of the 'Venezuelan kindreds', the best characterised sample of HD carriers in the world, suggested that approximately 40% of the variance in age at onset that remained after accounting for CAG is explained by additional genetic variation (Wexler, 2004). Thus, the burden of the mutated protein explains some of the variability in the rate of HD progression but other genetic and environmental influences are also at play. Although sexual dimorphisms in the basal ganglia, thalamocortical tracts and cortex are well-established, sex differences do not feature prominently in the HD literature (Beyer, Pilgrim, & Reisert, 1991; Savic, 2014). However, there is a growing body of evidence from animal models suggesting sex differences in terms of motor performances, with greater impairments in male rats (Fielding et al., 2012).

The huntingtin protein is highly conserved in species ranging from drosophila to mammals, suggesting that it is likely to be involved in a core aspect of cell functioning (Jia-Yi Li, Plomann, & Brundin, 2003). It is expressed in neural and non-neural tissues, and is localised in nerve cell bodies and endings within the brain (Trottier et al., 1995). Huntingtin's pathological impact is essentially confined to the central nervous system, although it is not preferentially localised in the striatum (Sapp et al., 1997). However, the striatum receives the greatest dopaminergic innervation in the brain and dopamine promotes the formation of mutated huntingtin aggregates (Cyr, Sotnikova, Gainetdinov, & Caron, 2006). Moreover, animal studies have revealed a greater frequency of $GABA_A$ receptor-mediated activity in striatal neurons, which has numerous downstream effects, including on glutamatergic and $GABA_B$ receptor-mediated activities (Cepeda et al., 2004; Raymond et al., 2011). Post-mortem and *in vivo* neuroimaging studies suggest a complex interplay of pathological processes very early in HD, which follow discrete time courses in different cell types (Bohanna, Georgiou-Karistianis, Hannan, & Egan, 2008). As such, there are numerous candidates currently undergoing rigorous investigation as potential biomarkers of pathogenic processes and disease progression in HD (see Figure 2), particularly during the premanifest period (Ross et al., 2014).

Although a full review of corticostriatal circuitry is beyond the scope of this thesis, certain loops are of particular relevance to pathological disturbances in HD. For example, the loop involving the DLPFC is a focus of recent research due to concomitant evidence of selective atrophy of the DLPFC in pre-HD, DLPFC dysfunction in event-related fMRI studies and progressive impairment in cognitive functions associated with the DLPFC in pre-HD and symp-HD (Paulsen et al., 2004; Rosas et al., 2005; Wolf, Vasic, Schönfeldt-Lecuona, Landwehrmeyer, & Ecker, 2007). Altered function of corticostriatal pathways, via recruitment of additional brain regions and changes in functional coupling, has been repeatedly reported in pre-HD, and usually interpreted in terms of compensatory mechanisms (Beste et al., 2007; Georgiou-Karistianis, 2009; Klöppel et al., 2009; Paulsen, 2009; Poudel, Egan, et al., 2014).

Accordingly, evidence from animal models of HD has suggested that greater efficiency in neural networks, equated to 'cognitive reserve' paradigms, is associated with later disease onset and slower progression (Borroni, Premi, Bozzali, & Padovani, 2012; Hannan & Nithianantharajah, 2006). A vast body of literature from animal models and human participants indicates that cognitive reserve is built up across the lifespan through different brain activities, such as education, and is driven by neurogenesis and neuroplasticity (Nithianantharajah & Hannan, 2009, 2011). Evidence from pre-HD individuals of abnormal neuroplastic adaptation in corticostriatal projections supports the notion of inter-individual differences in response to pathogenic changes (Beste, Wascher, Dinse, & Saft, 2012; Centonze, Bernardi, & Koch, 2007; Nithianantharajah, Barkus, Vijiaratnam, Clement, & Hannan, 2009; Pascual-Leone et al., 2011). In sum, evidence from research into HD and other neurodegenerative disorders shows that clinical phenotypes may be modified by differential effects of environmental factors upon neuropathological mechanisms.

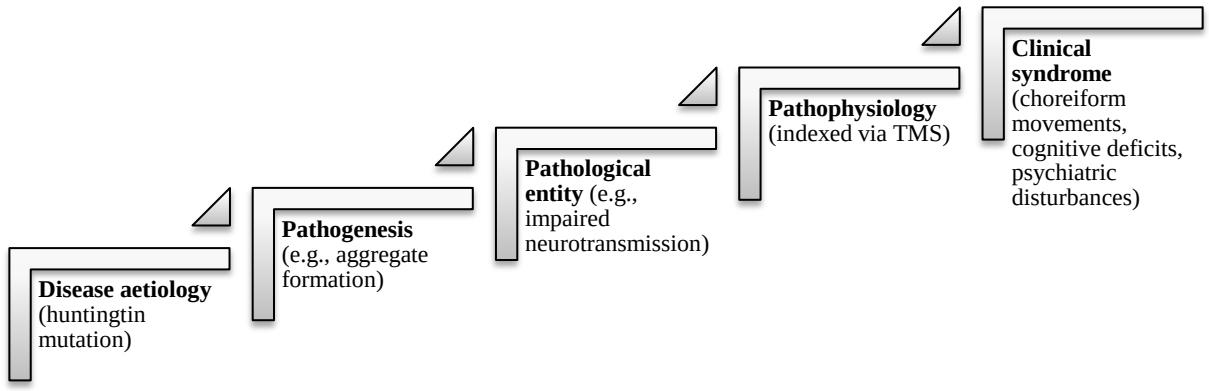


Figure 2. Schematic representation of the disease process in HD.

Figure adapted from Lewis et al. (2005).

Understanding the complexities of TMS data

As outlined in our review, TMS-EMG has generated important new knowledge about motor cortex excitability and connectivity over the past three decades. Understanding the repeatability and sensitivity of such data is critical, particularly given that the stimulation is 'non-ecological' (i.e., does not reflect natural processes) and mediated by several parameters and that the technology continues to evolve (Rothwell, 2011). Various peripheral muscles have been studied with TMS-EMG, but a common target is the abductor pollicis brevis (APB) muscle in the hand. This muscle is usually targeted because it is superficially located on the cortical gyrus in most individuals, and shows minimal inter-trial and inter-individual variability (Jung & Ziemann, 2006). In terms of reliability, Casarotto et al. (2010) used EEG to show that evoked potentials were 97% accurate in detecting whether a change in stimulation parameters had occurred. Moreover, inter-trial variability in MEP amplitudes appears largely attributable to intrinsic time-varying neuronal excitability, which may be due to inputs from inhibitory cells or peripheral sensory receptors (Darling, Wolf, & Butler, 2006). Cortical excitability measures do not appear to show predictable diurnal variability (Strutton, Catley, & Davey, 2003) and are generally unaffected by physiological aging, despite cortical atrophy (Casarotto et al., 2011). Furthermore, TMS studies do not typically control for participant sex because the consensus from past research is that it does not have a significant or large impact on MEP characteristics (Chipchase et al., 2012). Importantly, research into reliability, variability and specificity of TMS data is ongoing and has been discussed in depth in the existing literature.

An early study by Devanne et al. (1997) was instrumental for advancing understanding of the input-output relationship between stimulus intensity and MEP amplitude, showing that the relationship was best explained by a sigmoidal function. The shape of the distribution appeared to be caused by phenomena other than activity of single motor neurons (because the discharge probability of individual cells is linear), including increasing synchronisation of discharges and an ultimate balance between excitatory and inhibitory responses. It was

recently established that repeated MEP recordings within individuals were statistically independent measurements, thereby validating the use of ANOVA (Roy Choudhury et al., 2011). In fact, a mixed-model ANOVA design with Participant and Stimulation intensity as factors explained 86% of the variability in excitability data, which might imply a lack of 'memory' in the motor system.

The wide-ranging effects of TMS on the brain have been investigated in studies using a variety of imaging techniques with concurrent TMS, including fMRI and PET. TMS generates focal haemodynamic changes, which spread to distant interconnected areas (Fox et al., 1997). However, these methods preclude inferences regarding the type of brain cells involved, whether excitatory or inhibitory neurons, or non-neuronal brain cells (Bohning et al., 1999). Nevertheless, combining TMS with other neuroimaging techniques ensures that the physiological effects of TMS may be measured objectively and with increased spatio-temporal sensitivity. In particular, TMS-EEG represents a relatively novel approach. In early TMS-EEG studies, it was revealed that motor cortex stimulation resulted in activation of ipsilateral premotor and parietal regions, as well as the contralateral motor cortex (Komssi et al., 2002). Furthermore, TMS-EEG has been used to demonstrate that stimulation can cause entrainment of brain oscillations that mirror oscillatory signatures characteristic of particular cognitive operations (Thut et al., 2011), which is important for establishing ecological validity.

Combined techniques can more effectively target focal brain regions and pathways, providing increased understanding of pathophysiological effects. For example, TMS-EEG methods are extremely valuable for contributing to our knowledge of cortico-subcortical networks (Pascual-Leone et al., 2011). The last decade has seen significant progress in refining the TMS-EEG technique, with a view to overcoming inherent complexities and establishing validity (Daskalakis, Farzan, Barr, Maller, et al., 2008; Lioumis, Kičić, Savolainen, Mäkelä, & Kähkönen, 2009). TMS-EEG has been employed to investigate the brain mechanisms engaged by changes in functional connectivity and compensatory processes in abnormal neurological states, such as stroke (Shafi, Westover, Fox, & Pascual-

Leone, 2012). Moreover, the potential utility of specific TMS-EEG outcome measures as biomarkers in neurodegenerative diseases is now starting to be recognised. For example, TEP amplitudes correlated with cognitive decline in Alzheimer's disease, and were able to discriminate participants with Alzheimer's disease from those with mild cognitive impairment and healthy controls (Julkunen et al., 2011). Approaches such as these are encouraging, offering new avenues of research for similar applications in HD.

Investigating HD pathophysiology using TMS

As outlined in our published review article, there is a wealth of evidence supporting structural brain changes, as well as emerging evidence suggesting functional changes as possible compensatory mechanisms, in pre-HD (Philpott et al., 2013). However, traditional neuroimaging techniques (e.g., fMRI) are limited in their ability to establish causal connectivity between brain activity and behaviour, or investigate the compensatory cortical plasticity that may occur in response to pathological changes (Pascual-Leone et al., 2000). Therefore, further research employing TMS techniques may help to elucidate the specific mechanisms underlying compensatory processes in pre-HD participants, as they measure objective and focal responses to stimulation, rather than lesioned brain regions or observed behaviours.

Pharmacological TMS studies, in particular, have highlighted the dynamic role of certain neurotransmitter systems in cortico-subcortical pathways in individuals with neurological diseases (Ziemann, Tergau, Bruns, Baudewig, & Paulus, 1997). For instance, a reduced CSP has been demonstrated in participants with Parkinson's disease, but it was lengthened by treatment with the dopamine precursor L-dopa (Priori, Berardelli, Inghilleri, Accornero, & Manfredi, 1994). Moreover, genetic variation of specific molecules involved in TMS-activated pathways has been implicated in mediating responses to stimulation and corticospinal neuroplasticity (Cheeran, Ritter, Rothwell, & Siebner, 2009). It is clear from the body of literature that TMS is a useful tool to complement traditional neuroimaging

techniques for investigating neuroplasticity, exploring genetic modifiers of neuronal function and further understanding individual differences in response to pathogenic changes in HD.

In vitro and *in vivo* research has revealed that high-frequency cortical activity is primarily subserved by $GABA_A$ receptor-mediated interneurons (McCormick, 1992). These interneurons, which can be investigated functionally using TMS (e.g., SICI paradigms), are credited with controlling the phasing of action potentials and ultimately the synchronicity of cortical networks (Hasenstaub et al., 2005). These same interneurons may be significantly affected by the pathogenic process in HD. A seminal study by Gu et al. (2005), using a mouse model of HD, revealed that mutant huntingtin accumulated in cortical interneurons. These cells showed dysfunction early in the disease, which caused reduced GABAergic neurotransmission and thereby contributed to pathogenesis in pyramidal cells. This study in fact purported that huntingtin-induced pyramidal cell dysfunction by itself was insufficient to cause the pattern of cortical pathology observed in HD, suggesting that GABAergic interneuron dysfunction represents a primary pathogenic process. In keeping with these findings, numerous studies using animal models and human participants have demonstrated dysfunction of cortical interneurons and disturbed synaptic transmission early in the disease, which precedes cellular atrophy (H. Li, Wyman, Yu, Li, & Li, 2003; Walker, Miller, Fritsch, Barton, & Rebec, 2008). Moreover, the function of inhibitory interneurons, indexed via TMS, is affected in other movement disorders, such as focal hand dystonia (Simonetta-Moreau et al., 2006). This suggests that interneuron dysfunction is also likely to contribute to the phenotypic expression of HD, at least in terms of motor symptomology.

Outcome measures from a range of TMS protocols are differentially affected across a number of clinical disorders, including Tourette's syndrome and schizophrenia (Orth, 2009; Rogasch, Daskalakis, & Fitzgerald, 2014). However, as highlighted in our review article (Philpott et al., 2013), findings are often inconclusive due to mixed results, a large component of which can be attributed to methodological differences, as well as poor control of potentially confounding variables including sex. The majority of studies select stimulus

intensities for TMS protocols according to RMT/AMT. Although it has been suggested that neurophysiological measures should be investigated independently of each other, this approach has not been widely adopted (Kimiskidis et al., 2005). With regards to paired-pulse protocols, the degree of inhibition and facilitation is dependent upon the conditioning stimulus intensity (Schäfer, Biesecker, Schulze-Bonhage, & Ferbert, 1997). However, studies have used various criteria and evidence sources to select stimulus intensities and other variable parameters, which complicates the comparison of findings across studies. Moreover, equipment and technological advances over time create additional difficulties for interpreting discrepant findings. Thus, despite three decades of TMS research and many important advances, we still lack critical knowledge about the relationship between local and network TMS effects and neurological or degenerative changes. To date, specific TMS measures have not shown sensitivity in terms of differentiating between neuropsychiatric conditions, which is important to establish if using TMS in a diagnostic capacity (Farzan et al., 2010a).

The DLPFC is a well-established target for stimulation using TMS (Rusjan et al., 2010) and is central to HD neuropathology (Wolf et al., 2007). In particular, the large-scale longitudinal IMAGE-HD study showed that the functional integrity of prefrontal circuits was reflective of cognitive and psychiatric disturbances in symp-HD, and that pre-HD participants also exhibited compensatory changes in prefrontal activations (Gray et al., 2013). As such, TMS measures following DLPFC stimulation warrant investigation as potential endophenotypic biomarkers sensitive to disease progression in HD. To our knowledge however, such measures, which require TMS-EEG techniques to obtain, have not yet been examined in HD. Indeed, determining the most appropriate method for localising the DLPFC has been a focus of recent research. For example, Fitzgerald et al. (2009) compared the two most widespread methods, which involve measuring 5 cm anterior to the motor ‘hotspot’ or the more expensive and time-consuming method of an MRI-based neuronavigational procedure. It was revealed that the optimal site for stimulating DLPFC was considerably

more anterior than that determined using the 5 cm method. An equally simple method that was generally appropriate across individuals involved using electrode position F3 of the standard 10-20 EEG co-ordinate system, and it has subsequently been adopted in many studies (Rusjan et al., 2010). Investigations such as this have enabled researchers to reliably tap into previously unmeasurable 'behaviourally silent' brain regions, in order to further develop the technique and assess a broad range of functions in healthy and clinical populations (Sandrini, Umiltà, & Rusconi, 2011).

Update on literature

Recent neuroimaging and clinical findings in HD

Since the publication of our literature review, there have been a number of articles that have provided important new knowledge about HD. For example, longitudinal 36-month data from TRACK-HD revealed significantly increased rates of decline in participants 'close to onset', compared with controls, for several quantitative motor and neurocognitive measures that were not significantly different after 24 months (Tabrizi et al., 2013). Moreover, pre-HD participants who progressed to a symptomatic state during the course of study differed significantly on a number of neurocognitive measures compared with those who did not progress. Given that similar changes were not observed in participants 'far from onset' despite striatal and white matter loss, these findings support the notion that clinical progression occurs faster as individuals approach onset. Since the structural brain changes do not strictly align with the longitudinal functional and clinical changes (see also: Wolf et al., 2012; Wolf et al., 2013), further work is necessary to bridge this gap and better understand the pathological factors driving phenotypic expression. Indeed, one report has emerged that suggests that disease progression may be quicker in symp-HD females, compared with males, in terms of UHDRS motor, functional and independence scores (Zielonka et al., 2013).

Recent publications from PREDICT-HD have also provided additional insights (Bonner-Jackson et al., 2013; Paulsen et al., 2014). For example, investigators used novel

statistical shape analysis to measure atrophy of specific subcortical structures in pre-HD (Younes et al., 2014). This method revealed regionally selective atrophy of the caudate and globus pallidus, which varied as a function of years to estimated onset. Similarly, a different group used a tractography-based approach, capable of mapping basal ganglia and thalamus connectivity directly onto the cortex (Marrakchi-Kacem et al., 2013). Key findings were that associative temporal, parietal and frontal corticostriatal connections showed greater degeneration than limbic pathways in symp-HD, with up to 76% connectivity reduction compared with controls. Moreover, the structural correlates of specific clinical signs in symp-HD have been described using voxel-based DTI; for example, an association between mean diffusivity in the right prefrontal cortex and self-paced finger-tapping performance was reported (Delmaire et al., 2013). These and other findings afford better understanding of basal ganglia atrophy and how this corresponds with dysfunction of cortico-subcortical circuitry and HD symptomology.

Additional evidence has emerged of dysfunctional neural networks in the context of normal cognitive performance in pre-HD individuals. For example, one study reported that both pre-HD and symp-HD participants exhibited reduced functional coupling of medial prefrontal and left premotor cortices, compared with controls (Unschuld et al., 2013). This reduced connectivity was related to putaminal atrophy and varied according to the complexity of executive functioning that was required, even though executive function *per se* was only impaired in symp-HD. Resting state fMRI has also demonstrated widespread dysfunction of the default-mode network in symp-HD, which correlated with frontal executive changes but not with atrophic changes (Quarantelli et al., 2013). Furthermore, reduced connectivity of the medial visual network with frontal, parietal and cingulate regions has been observed in both pre-HD and symp-HD (Dumas et al., 2013). Given that results from Dumas et al. (2013) were also independent of atrophic changes, it seems clear that fMRI abnormalities in pre-HD reflect altered neuronal function, which precedes cell loss and may represent pathological or compensatory processes.

Indeed, further work employing task-related fMRI has enriched the literature supporting a complex pattern of compensatory processes underlying motor and neurocognitive functioning in pre-HD. For instance, Scheller et al. (2013) investigated cognitive and executive aspects of motor functioning in pre-HD and found evidence of compensatory mechanisms enabling complex movements with high cognitive demands. The investigators reported that the core brain regions responsible for maintaining motor performances were bilateral premotor cortices, with supplementary motor cortices recruited depending on the complexity. Moreover, the interaction between regions differed according to proximity to clinical onset, such that the coupling of specific regions increased as time to onset reduced. Identifying the specific brain regions involved in compensatory responses during pre-HD is important for identifying potential targets for interventional and neuroprotective therapies, including TMS, that might be able to enhance neuroplasticity and slow disease progression.

Correspondingly, a series of papers from the Australian-based IMAGE-HD study have emerged recently, with a focus on tracking longitudinal changes in activation and functional connectivity in pre-HD individuals. Comparing 18-month longitudinal data from multiple imaging modalities, Domínguez et al. (2013) reported that longitudinal caudate volume change was the only sensitive measure for discriminating HD groups according to time to diagnosis. Longitudinal change in DTI measures of the caudate was also sensitive, but only in differentiating symp-HD from controls. Moreover, changes in volume and diffusion metrics were inter-related and correlated with clinical measures, suggesting that these neuropathological alterations have measurable functional implications. Extending these findings, it was revealed that radial diffusivity in white matter tracts was associated with cognitive and motor functioning in pre-HD, and white matter connectivity in symp-HD correlated with UHDRS ratings of clinical severity (Poudel, Stout, et al., 2014). Taken together, it is clear from recent research that pre-HD is characterised by early aberrant structural connectivity due to axonal dysfunction, which deteriorates with disease progression and underpins many of the clinical symptoms of HD (Poudel, Egan, et al., 2014).

Whilst an array of image acquisitions were included as part of IMAGE-HD, a major component was the investigation of functional changes during pre-HD stages in light of the known structural changes. One functional operation of interest in the IMAGE-HD study was spatial working memory as assessed by an “n-back” task. Cross-sectional data suggested functional brain reorganisation in cortical and subcortical regions in both pre-HD and symp-HD, which showed further change with increased working memory demands and progression into early symp-HD (Georgiou-Karistianis, Stout, et al., 2013). Moreover, these findings revealed abnormalities on average up to 15 years prior to estimated onset, supporting previous results from other large-scale studies (Paulsen et al., 2008; Tabrizi et al., 2009). Longitudinally, 30-month data showed increased activation over time in multiple brain regions during working memory performance in pre-HD but not symp-HD, which varied according to disease burden score and years to estimated onset despite no change in performance (Poudel et al., 2013). Furthermore, functional connectivity between the left caudate and DLPFC lessened over time in pre-HD but not symp-HD, signifying an early neurodegenerative role for the DLPFC. Lastly, IMAGE-HD results were comparable from 18- to 30-month follow-up, indicating that changes in activation and functional connectivity in certain brain regions follow a mostly progressive and linear trajectory (Georgiou-Karistianis, Poudel, et al., 2013; Poudel et al., 2013).

Neurophysiology of TMS effects

Knowledge around the neurophysiological underpinnings of TMS effects has continued to evolve in recent years. In particular, a novel model coined the ‘canonical microcircuit model of neocortex’ has the potential to explain the known anatomical and physiological features of the corticospinal system (Di Lazzaro & Ziemann, 2013). Essentially, this model stipulates that a TMS pulse produces strong excitation of superficial cells, which leads to recruitment of ‘fully synchronised clusters’ of excitatory and inhibitory neurons. Inhibitory neurons are key to the modulation of induced responses, in that they are capable of entraining the firing of excitatory networks and controlling the frequency and magnitude of

discharges. In terms of paired-pulse TMS, Di Lazzaro and Ziemann (2013) noted, and others have reiterated (Kojima et al., 2013), that inhibitory protocols like SICI have been well characterised within the canonical microcircuit model. However, the mechanisms of ICF have been comparatively more difficult to elucidate; they are presumed to depend on distinct neural substrates but remain somewhat elusive (Kossev, Siggelkow, Dengler, & Rollnik, 2003; Ziemann, Rothwell, & Ridding, 1996).

The DLPFC has become the most commonly selected non-motor target for TMS (Radhu et al., 2015). It is well recognised for its role in executive function, cognitive control and working memory, however it is also implicated in motor control. For example, Hasan et al. (2013) recently investigated the exact timing, and excitatory and inhibitory features of motor-DLPFC interactions using TMS. This study indicated that during movement selection there were task- and muscle-specific functional connectivity interactions between the motor cortex and DLPFC, which were temporally and spatially selective. These findings represent early efforts to break down the functional complexities of the DLPFC, which can be further investigated with future TMS-EEG studies, in order to expand the extrapyramidal use of TMS across a range of disorders.

State-dependent stimulation effects are a well-established phenomenon, reflecting the fact that neuronal responses to TMS depend on the activation state of underlying neurons at the exact moment of stimulation (Berger, Minarik, Liuzzi, Hummel, & Sauseng, 2014). In fact, a novel study using TMS-EMG and TMS-EEG demonstrated that MEP amplitude was affected by cortical beta-band oscillatory processes in the motor cortex, which might serve to gate incoming motor commands (Keil et al., 2014). Whilst methods outlining the use of a TMS ‘adaptation’ procedure have been suggested to limit state-dependent effects, these are not commonly employed (Dayan, Censor, Buch, Sandrini, & Cohen, 2013). Despite variability in evoked TMS responses within and between individuals, further work has verified the stability of an individual’s excitability profile over time. For instance, Du et al. (2013) administered paired-pulse TMS to healthy participants on two occasions, approximately

three weeks apart. It was revealed that individual profiles of facilitation and inhibition emerged, which were highly variable between participants but stable over time within individuals. Unfortunately, this inter-individual variability complicates the use of TMS in clinical populations, in terms of identifying disease-driven abnormalities in comparison with control groups. Lastly, Groppa et al. (2013) employed TMS-DTI to investigate the influence of less superficial regions, such as the thalamus, on cortical oscillatory coupling. These researchers demonstrated that suprathreshold pulses caused increased inter-hemispheric coherence in the alpha band for up to 200 milliseconds (msec) following stimulation. Given that alpha activity represents the predominant 'idling' brain rhythm, these findings support the notion that TMS influences existing physiological activity, rather than generating artificial oscillations.

TMS-EEG validation studies

A number of papers have emerged in recent years examining different methods for recording and analysing TMS-EEG data, establishing the reliability and validity of TMS-EEG methodologies, and further probing the underlying mechanisms involved. In their review, Rogasch and Fitzgerald (2013) noted that various motor paradigms had been validated using TMS-EEG, which additionally provided information on signal propagation and cortical connectivity. It has also been established that LICI of early and late TEPs was differentially modulated according to conditioning and test stimulus intensities, pointing to discrete mechanisms of action (Rogasch, Daskalakis, & Fitzgerald, 2013). The authors postulated that early TEPs were related to suppression of excitatory activity, whereas the N100 component reflected the well-established $GABA_B$-mediated inhibition known from TMS-EMG studies; a postulation that has been substantiated by other recent research (Premoli et al., 2014). As such, data from this type of research can now be reliably compared to results obtained from TMS-EMG. Considerable work that characterises the nature and extent of TMS-induced EEG artefacts has also emerged, allowing for understanding of the accuracy and limitations of TEP data. For instance, Rogasch et al. (2013) identified a primary artefact,

which resolved within approximately 12 msec. A secondary artefact, which occurred following stimulation over lateral scalp areas, and was presumed to be related to muscle activation, resolved after approximately 25-40 msec (Rogasch, Thomson, et al., 2013). These findings have been replicated by other groups, and data processing techniques using principal or independent component analysis have been recommended in order to suppress the influence of these artefacts (Ter Braack, De Jonge, & Van Putten, 2013).

In order to minimise the burden on participants, recent studies have been conducted to refine the optimal TMS protocols for determining corticospinal excitability. For example, Cuypers et al. (2014) reported that the RMT intensity did have a significant effect on the estimation process, such that participants with higher thresholds required fewer pulses to attain a reliable value for corticospinal excitability. This study also asked healthy participants to self-rate levels of attention, arousal and fatigue before and after TMS, in an attempt to control for individual physiological changes across the session. Despite increased ratings of fatigue and decreased attention and arousal from participants over time, these perceived changes had no significant impact on experimental estimates of corticospinal excitability (Cuypers et al., 2014), which is important knowledge for future TMS studies.

Utility of TMS in HD

A review was recently published on the use of TMS for understanding the degenerating brain in normal aging, as well as in neurological conditions, such as Alzheimer's, Parkinson's and Huntington's diseases (Ljubisavljevic, Ismail, & Filipović, 2013). Based on TMS research, together with studies employing other neuroimaging technologies, this review purported that disturbances to excitatory activity in pre-HD, such as the RMT, seemed to parallel the compensatory changes resulting from striatal degeneration. An alternative hypothesis was that such neurophysiological changes might reflect an excitotoxicity phase that preceded the more well-established neurodegenerative processes. Regardless, TMS research in HD represents an important avenue for future research, in order to establish sensitive biomarkers of progression related to underlying pathophysiology,

especially during early pre-HD stages when structural changes become notable (Berardelli & Suppa, 2013). Indeed, further studies may be able to ascertain a ‘signature’ of very early pathophysiological changes in HD, which could be utilised to implement interventions prior to the initiation of irreversible neurodegenerative processes (Ljubisavljevic et al., 2013).

With regards to non-human studies, recent work has emerged examining the physiological and therapeutic effects of extremely low frequency electromagnetic fields, using a rat model of 3-nitropropionic acid (3NP)-induced HD (Tasset et al., 2012). Animals were administered 3NP, which mimics HD by causing excitotoxicity, oxidative stress and neuronal death, prior to magnetic stimulation. Magnetic stimulation was administered through two coils placed dorsally and ventrally to the head, for four hours daily over 21 days. Findings revealed that stimulation had a neuroprotective effect, in that; animals performed better on behavioural tests than those who underwent mock stimulation, and exhibited increased levels of dopamine and neurotrophic factors, increased neuronal density, and reduced oxidative and cell damage (Tasset et al., 2012). A supplementary study demonstrated that this type of stimulation increased levels of the Nrf2 transcription factor, which induced an antioxidant pathway and reversed the toxic effects of 3NP (Tasset et al., 2013). These findings confirmed that low frequency electromagnetic stimulation, considered analogous to rTMS in humans, has considerable potential to be an efficacious therapy for neurodegenerative diseases, such as HD, by normalising various aspects of disease pathology. However, consideration must be given to the numerous differences between animal and human studies before extensive conclusions can be drawn.

TMS has also been utilised in a novel way to provide further information regarding the function of the indirect inhibitory pathway of the basal ganglia in humans. A selective stopping task, completed concurrently with TMS-fMRI, was used to show that striatal and pallidal structures were critical to effective proactive motor suppression (Majid, Cai, Corey-Bloom, & Aron, 2013). Specifically, the degree of suppression was associated with activation in particular areas of the basal ganglia and better performance was correlated with greater

striatal activation. Moreover, participants with pre-HD were impaired on the task in terms of behavioural selectivity and proactive motor suppression. Such functional evidence of specific basal ganglia pathways in humans is both valuable and scarce, as it has been previously difficult to obtain due to the limited spatial resolution of traditional neuroimaging technologies. The current study therefore represents a tangible demonstration of the potential utility of TMS for investigating critical cortico-subcortical circuits, and exploring sensitive endophenotypic biomarkers of disease progression in HD.

Rationale, aims and hypotheses

Novel technologies, such as TMS-EMG and TMS-EEG, provide a unique opportunity to measure underlying pathophysiology of the human brain and offer important new knowledge regarding neuropathology in clinical populations. Moreover, these technologies allow for the investigation of 'causal' relationships between the targeted neural circuits that are stimulated and the objective neurophysiological responses that are elicited. This serves to complement and build upon previous findings using traditional neuroimaging techniques, such as MRI. To our knowledge, TMS-EEG has not been previously studied in HD, an important gap that this thesis will address. It has been argued, based on other neurophysiological evidence, that HD is associated with a disturbed balance of function in inhibitory and facilitatory cortico-subcortical pathways, likely to manifest in the expression of motor, cognitive and psychiatric symptoms (Cepeda, Wu, André, Cummings, & Levine, 2007). Such disturbances are likely to generate poorly integrated, less synchronised responses to stimulation, leading to a possible reduction across a number of different TMS measures. However, there is no conclusive evidence to support this conjecture, as heterogeneous findings from previous studies using TMS in HD have resulted from differing methodologies and equipment, limited inclusion of pre-HD individuals and investigation of motor circuits only.

Therefore, the primary aim of this thesis is to characterise the excitability profile of motor and non-motor circuits (such as the DLPFC) in pre-HD and symp-HD individuals using

a number of TMS protocols. Additionally, it seeks to investigate the relationship between underlying neurophysiology and clinical severity, neurocognitive performance, psychiatric symptoms and genetic variants, in order to better understand how pathophysiology in HD maps onto the heterogeneous manifestation of symptoms. Increasing our knowledge of such relationships will assist with selecting participants for future neuroprotective and clinical interventions, and in tailoring therapies according to inter-individual differences.

Hypotheses were developed according to prior research using TMS in HD, and in other neurological disorders, together with theoretical models of basal ganglia pathways, cortical excitability and mechanisms of action for TMS. A number of hypotheses were generated in accordance with each of four aims, as outlined below.

Aim 1: Investigate neuronal function following primary motor cortex TMS, as measured indirectly with peripheral EMG. This is achieved by fully characterising a profile of EMG outcome measures in response to a range of TMS protocols that assess corticospinal and intracortical excitability (both facilitation and inhibition). Additionally, investigate whether clinical severity, neurocognitive performance and psychiatric symptoms are related to cortical excitability profiles. This aim is addressed in chapter three, the first experimental chapter.

Hypothesis 1a: Corticospinal excitability, CSP duration, SICI, ICF and LICI will be significantly decreased in symp-HD individuals compared with pre-HD, which in turn will be reduced relative to controls (i.e., symp-HD < pre-HD < controls). As corticospinal excitability is measured via RMT and AMT, it is expected that reduced excitability will manifest as increased thresholds in symp-HD compared with pre-HD, which in turn will be increased relative to controls.

Hypothesis 1b: Decreased corticospinal excitability/CSP duration/SICI/ICF/LICI will be associated with increased clinical severity, poorer neurocognitive performance and increased psychiatric symptoms and in both pre-HD and symp-HD groups.

Aim 2: Investigate whether sex differences influence pathophysiology, specifically one of the cortical inhibition measures investigated in chapter three, in pre-HD and symp-HD participants. This aim is addressed in chapter four.

Hypothesis 2: There will be a significant interaction effect between Group (i.e., symp-HD, pre-HD, controls) and Sex for a TMS measure of cortical inhibition, such that males will show a different pattern of pathophysiological changes, compared with females.

Aim 3: Investigate whether variants in corticostriatal receptor genes influence cortical excitability in pre-HD and symp-HD, and age at onset in symp-HD participants. This aim is addressed in chapter five.

Hypothesis 3: Cortical excitability measures in pre-HD and symp-HD, and age at onset in symp-HD individuals, will differ according to the particular GABA and dopamine receptor gene variants present.

Aim 4: Using EEG to investigate neuronal function following TMS delivered over the primary motor cortex and DLPFC across groups. This is achieved by investigating one of the measures of cortical inhibition (described in chapter three) with TMS-EEG in both cortices. This aim is discussed in chapter six.

Hypothesis 4: LICI of TEPs in both cortices will be significantly decreased in symp-HD individuals compared with pre-HD, and in pre-HD compared with controls (i.e., symp-HD > pre-HD > controls).

Chapter two

This chapter provides detailed information regarding the methodology of this study. It begins with a description of the recruitment process and participants involved, then continues with an explanation of the materials and apparatus, including the neurophysiological, neurocognitive, psychiatric and genetic measures. The chapter concludes with an outline of the procedure that was followed in conducting the study and an explanation of the statistical techniques that were applied for data analysis. Several figures and tables are included for further illustration of the methodologies involved. There is some unavoidable duplication between this chapter and the following chapters (due to the nature of a thesis by publication), but details provided in experimental papers have been omitted where appropriate.

Participants

Recruitment

This study was approved by the Monash University Human Research Ethics Committee (Project number: CF12/3045 – 2012001533). Written informed consent was obtained from each participant in accordance with the Declaration of Helsinki (World Medical Association, 2013).

Pre-HD and symp-HD individuals were recruited through the 'ENRU-STOUT' participant database at Monash University (managed by the Georgiou-Karistianis Experimental Neuropsychology Research Unit and the Stout Group), which is comprised of individuals that had previously participated in research and consented to be contacted about future opportunities. Control participants were recruited through a variety of channels, including the ENRU-STOUT database, family members of individuals with HD and word-of-mouth techniques. Participants travelled from metropolitan Melbourne, rural Victoria and interstate to be involved.

The total sample consisted of 46 participants, comprising 16 pre-HD, 13 symp-HD and 17 healthy controls. The pre-HD group comprised six males and ten females with a mean age of 42 years (median = 41, standard deviation (SD) = 8, range = 26-54). Symp-HD individuals comprised eight males and five females with a mean age of 56 years (median = 59, SD = 9, range = 43-69). Controls were matched to pre-HD individuals on sex and age and included six males and eleven females with a mean age of 42 years (median = 48, SD = 12, range = 26-57).

Clinical information

Only pre-HD and symp-HD individuals underwent genetic testing to confirm their HD gene status. All HD individuals were clinically assessed by a collaborating neurologist (Dr Andrew Churchyard) and underwent a UHDRS motor assessment (Huntington Study Group, 1996). Similar to Tabrizi et al. (2009), inclusion in the pre-HD group in our study required a UHDRS total motor score less than five. The years until onset of motor symptoms warranting clinical diagnosis was estimated for pre-HD individuals using the predictive parametric survival model proposed by Langbehn et al. (2004); negative values were corrected to zero. Estimates of years to onset had a mean of 19 years (median = 14, SD = 12, range = 0-43). Age at onset for symp-HD individuals ranged from 40 to 63 years (mean = 52, median = 52, SD = 8) and the duration of illness ranged from 1 to 12 years (mean = 5, median = 4, SD =3). Disease burden scores were calculated for pre-HD and symp-HD individuals using the formula of Penney et al. (1997), based on current age and CAG repeat length. Pre-HD individuals had a mean CAG repeat length of 42 (median = 42, SD = 2, range = 38-44), a mean UHDRS motor score of 0 (median = 0, SD = 1, range = 0-2) and a mean disease burden score of 250 (median = 268, SD = 89, range = 97-408). The symp-HD group had a mean CAG of 43 (median = 43, SD = 2, range = 39-47), a mean UHDRS of 17 (median = 18, SD = 8, range = 5-30) and a mean disease burden of 408 (median = 404, SD = 91, range = 220-523).

Screening criteria

Participants underwent a rigorous screening process prior to recruitment. Individuals were excluded if they were; left-hand dominant, aged younger than 20 or older than 70 years, pregnant or non-Caucasian. Only Caucasian individuals with European ancestry were included in order to avoid population stratification artefacts in the genetic analyses (The International HapMap 3 Consortium, 2010). Participants were also excluded if they had any neurological or severe diagnosed psychiatric conditions (other than HD; e.g., psychotic disorder), had a pacemaker or metallic implant, or had a history of central nervous system disease or events (e.g., traumatic brain injury).

Full-scale IQ (estimated using the NART-2, see Materials section below) did not differ across the three groups (median = 113, range = 80-128; $F_{2,42} = 1.58$, $p > .05$). All participants were of normal intellectual ability on this measure, with the exception of two individuals with self-reported literacy problems (but no formal diagnoses). Similarly, the number of years of formal education did not differ between groups in the total sample (median = 15, range = 7-25; $F_{2,42} = 1.45$, $p > .05$). Pre-HD participants had an average IQ of 109 (SD = 11) and education of 15 years (SD = 4), symp-HD participants had an average IQ of 110 (SD = 13) and education of 12 years (SD = 3), and control participants had an average IQ of 116 (SD = 9) and education of 16 years (SD = 3).

Medication

Medications taken by participants included prescriptions for; diabetes and respiratory, vascular/heart, gastro-oesophageal and urinary conditions (pre-HD: n=3; symp-HD: n=4; control: n=2), nicotine addiction and hormone replacement therapies (pre-HD: n=2), and oral contraceptives (pre-HD: n=1; control: n=3). Female participants were not asked about their menstrual status or phase. Some pre-HD and symp-HD individuals were also medicated with antidepressants of the selective serotonin or serotonin-norepinephrine reuptake inhibitor varieties (pre-HD: n=1; symp-HD: n=6), risperidone (symp-HD: n=2), haloperidol (symp-HD: n=1) or clonazepam (symp-HD: n=1). Four symp-HD individuals were not taking any

prescribed medications. Over the counter and natural remedies were not noted or restricted. Typical daily caffeine consumption was recorded and did not differ significantly between groups, with an overall mean of 2 caffeinated drinks per day (median = 2, SD = 2, range = 0-6; $F_{2,42} = 0.17$, $p > .05$). Four participants were regular cigarette smokers (pre-HD: n=2; symp-HD: n=2).

Materials

Neurophysiological techniques

TMS-EMG set up. Biphasic TMS pulses, with the induced current in the brain flowing first in a posterior-anterior direction and then anterior-posterior, were administered unilaterally. A MagVenture MagPro X100 stimulator was used with a MagOption unit, connected to a hand-held figure-of-eight coil with a 70 mm wing diameter. Surface EMG recordings were taken from the APB muscle of the right hand by placing two disposable self-adhesive silver-silver chloride (Ag/AgCl) disc electrodes in a tendon-belly arrangement (see Figure 3). A ground electrode common to both recording electrodes was placed over the styloid process of the ulna.

Participants were instructed to relax their hand for most procedures, except the AMT and the CSP protocols, and EMG activity was continuously monitored on a computer screen. For the AMT and CSP measures, participants maintained a constant voluntary isometric contraction of the muscle at approximately 10 Newton using a grip force transducer with visual display feedback.

TMS was applied to the area of the left motor cortex corresponding to the APB muscle. The optimal position for stimulation was obtained by shifting the coil in 1 cm movements over the scalp region typically corresponding to the motor cortex and gradually increasing the stimulation intensity to find the position that generated the largest EMG response. The coil was held with the handle pointing backwards and laterally, angled at approximately 45° to the mid-sagittal line, which is most favourable for eliciting MEPs over the motor cortex

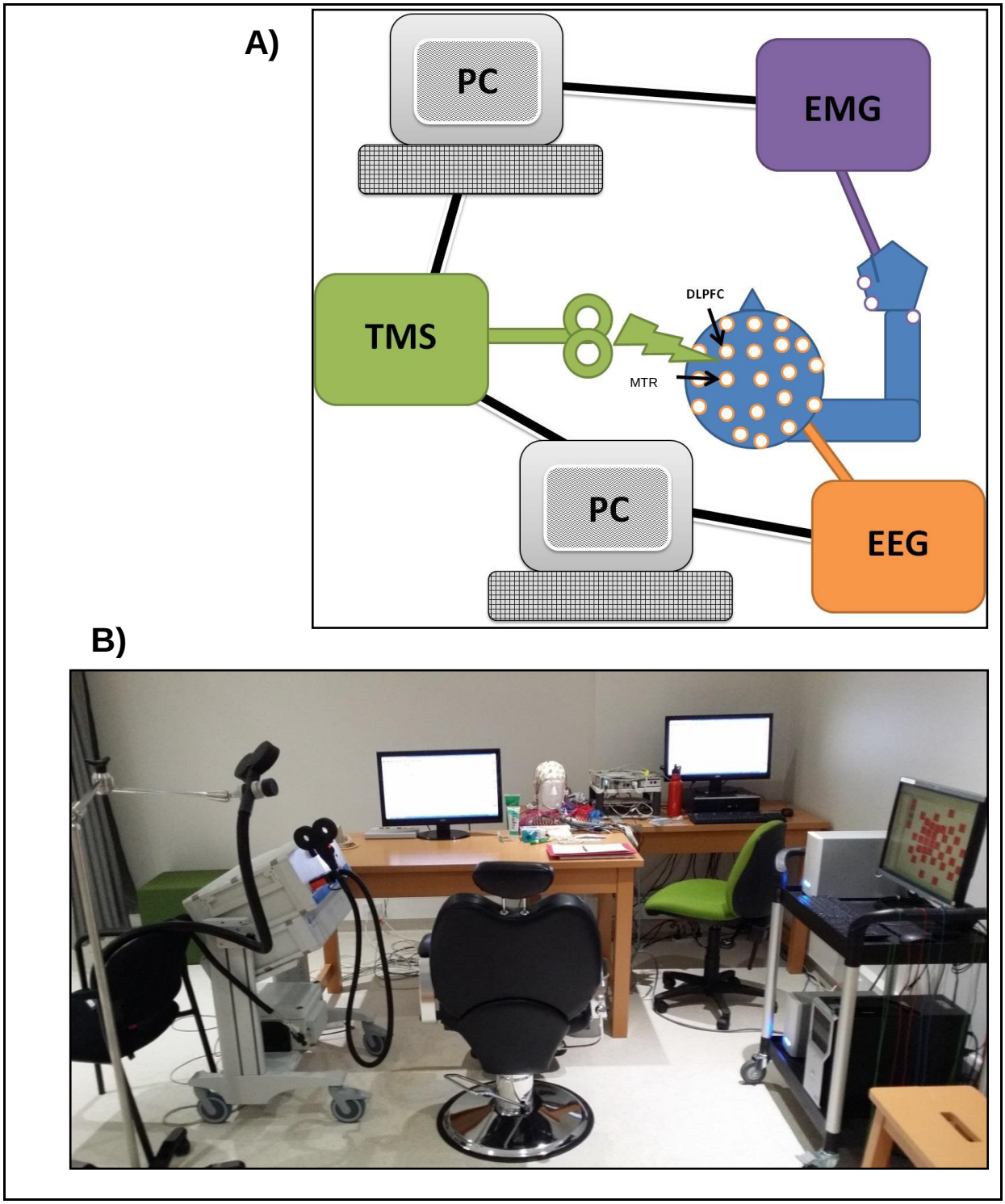


Figure 3. Illustration of the experimental apparatus.

A) Schematic of the equipment set up with TMS, EMG, EEG and computers for recording data (PC). Sites of stimulation were the left primary motor cortex (MTR) and DLPFC. Electrodes are represented by unfilled circles (illustrative only, see Figure 4 for exact electrode placement with EEG).

B) Photograph of equipment set up. The TMS machine is on the left connected to the hand-held coil, and with the cooled coil in the stand. Separate computers can be seen for EEG (on right) and EMG recordings (middle and left screens). The comfortable chair with a headrest for participants is in the middle.

(Kammer, Beck, Thielscher, Laubis-Herrmann, & Topka, 2001). The optimal spot was marked on the scalp with a pen traced along the edge of the coil, to ensure consistent positioning of the coil across trials. The intensity of the TMS pulses was set at the beginning of each procedure based on the individual's RMT/AMT. The RMT was defined as the minimum stimulation intensity required to evoke a peak-to-peak MEP of more than 50 μV in at least five of ten consecutive trials (Rossini et al., 1994). The AMT was defined as the minimum stimulation intensity required to produce a MEP of approximately 200 μV in at least five of ten trials during voluntary APB muscle contraction (Orth & Rothwell, 2004).

Cortical silent period. The CSP was investigated in the active muscle at two stimulus intensities (120% and 140% AMT), with 12 pulses at each intensity. These were presented in a pseudorandomised train with a 10 sec inter-pulse interval, to ensure that the TMS-induced cortical activity had resolved before the next stimulus was applied. The average duration of the silent period was calculated on the EMG trace at each stimulus intensity. This was measured offline by the same rater for all participants using LabChart 7 (ADInstruments).

Recruitment curve. This was determined through 12 pulses administered at four different stimulation intensities (110, 120, 130 and 140% RMT), presented in a pseudorandomised train with a 10 sec inter-pulse interval. The average MEP amplitude was calculated at each intensity. The slope of the recruitment curve was calculated as the increase in average MEP amplitudes between 110% and 140% RMT, divided by the 110% amplitude.

SICI and ICF. These were investigated in the resting muscle, with a subthreshold conditioning stimulus (80% RMT) preceding the suprathreshold test stimulus (120% RMT). Twenty paired-pulse stimuli were administered of each, with inter-stimulus intervals of 3 and 10 msec, respectively (Kujirai et al., 1993), and inter-pulse intervals of 10 sec. These were presented in a single train of 60 stimuli, pseudorandomly interspersed with 20 single-pulse stimuli (120% RMT), administered to determine the size of the MEP elicited by the test

stimulus alone. The average degree of inhibition and facilitation was expressed as a function of the average MEP amplitude generated by the paired-pulse stimuli compared to that generated by the test stimuli.

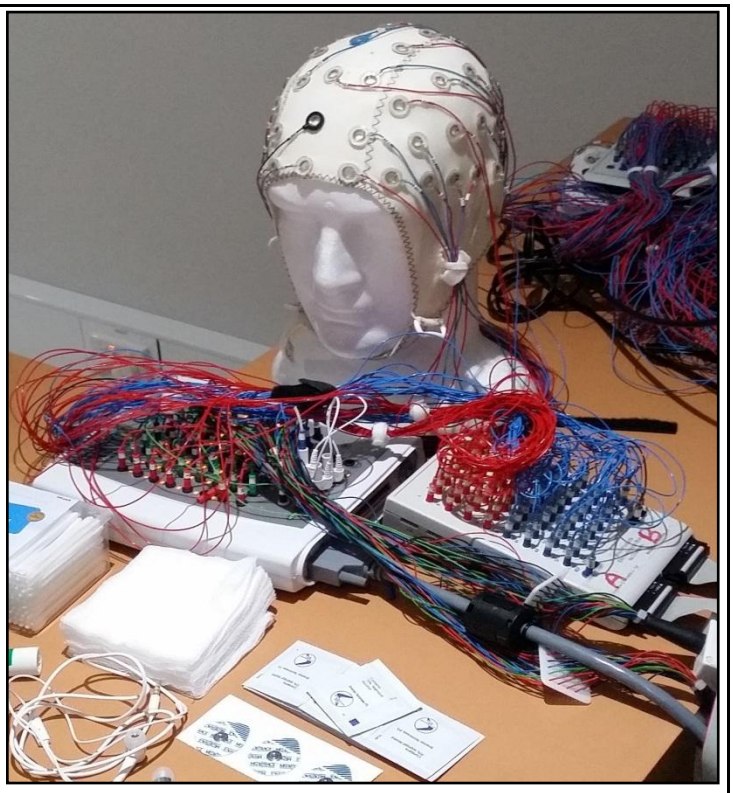
TMS-EEG set up. For the TMS-EEG procedures, stimulation was administered via a figure-of-eight cooled coil with a 75 mm wing diameter to prevent over-heating during longer stimulation trains. The coil was aligned with the site of stimulation and held in a stand for these protocols, with the participant's head supported. The cortical signal was recorded using a commercially-available 64-channel EEG cap with standard 10-20 positions, fitted with thin sintered Ag/AgCl electrodes. Thirty-four relevant channels were prepared and connected, according to a selective 32-channel configuration with increased density around stimulation sites (see Figure 4). Electrode lead wires were arranged perpendicularly to the TMS coil handle to reduce interference from electromotive force artefacts (Sekiguchi, Takeuchi, Kadota, Kohno, & Nakajima, 2011) and the capacitor recharge was delayed by 1000 msec (Rogasch, Thomson, et al., 2013). Participants were provided with earphones playing white noise to reduce the auditory responses on the EEG recordings resulting from the TMS coil click (Nikouline, Ruohonen, & Ilmoniemi, 1999). To monitor eye movement artefacts, four individual Ag/AgCl electrodes were placed around the eyes for electrooculography (EOG) recordings; on the outer side of each eye, and above and below the left eye (see HEOG, VEOU and VEOL, respectively, on Figure 4). Electrodes were referenced to an electrode placed on the vertex positioned posterior to the CZ electrode on the EEG cap, with the exception of the horizontal EOG electrodes (HEOG), which were referenced to each other. Additional recordings were also taken from the left and right mastoid processes for alternate referencing (see M1 and M2 on Figure 4). Electrode impedance levels for EEG and EOG were kept below 5 kΩ throughout the experiment. EEG signals were acquired using Curry 7 Neuroimaging Suite (Compumedics Neuroscan). Signals were recorded DC with a 20 kHz sampling rate; they were amplified (x1000), low-pass filtered at 2,000 Hz and digitized at 10 kHz.

Figure 4. Illustration of the EEG apparatus.

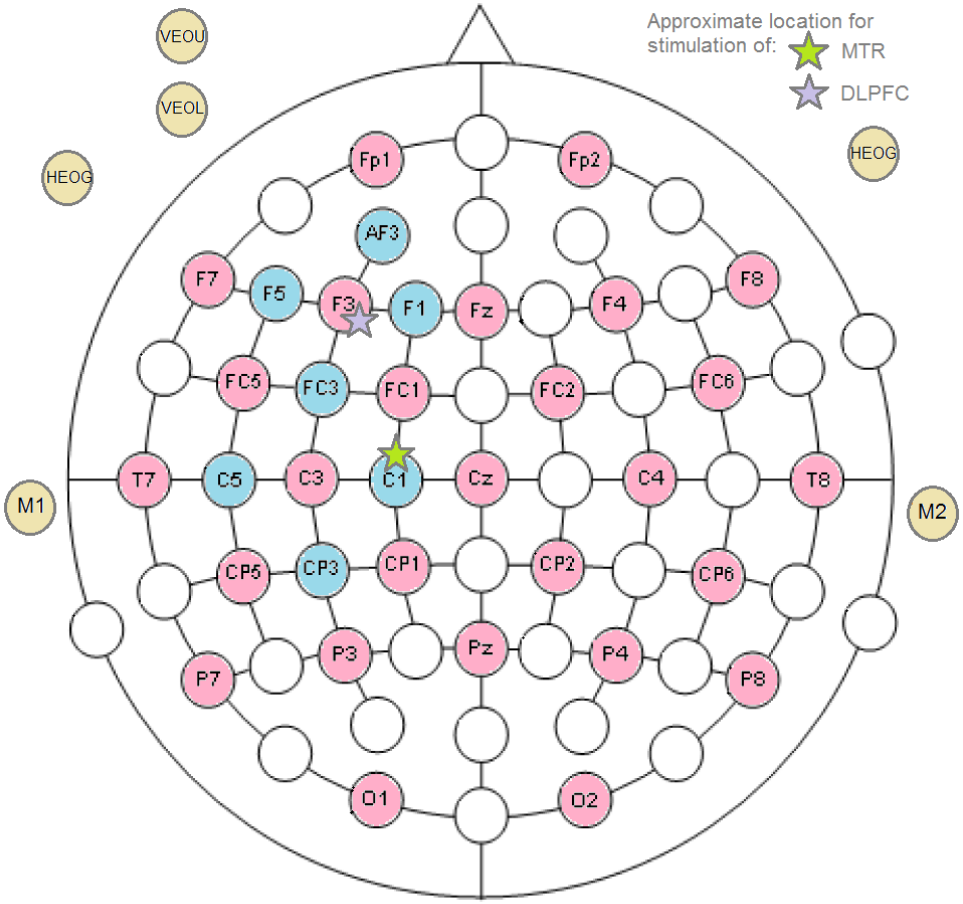
A) Photograph of the EEG cap and head box.

B) Electrode arrangement based on the 10-20 system for EEG and EOG with approximate sites of stimulation via TMS; MTR = primary motor cortex; DLPFC = dorsolateral prefrontal cortex; VEOU = vertical EOG upper; VEOL = vertical EOG lower; HEOG = horizontal EOG; M_1/M_2 = mastoid 1/2).

A)



B)



RMT was recalculated after the EEG cap was fitted given that higher stimulation intensities are required because the coil is further from the scalp. EEG and EMG were recorded simultaneously on separate computers when LICI was administered to the motor cortex. LICI stimulation was then administered to the left DLPFC, which was localised under electrode F3 of the EEG cap, and only EEG was recorded. The method of DLPFC localisation was chosen over other methods that are used in the absence of MRI coregistration for an optimal balance between inter-individual consistency and participant tolerability (Fitzgerald et al., 2009).

LICI. This was investigated at rest by pairing a suprathreshold conditioning stimulus with a suprathreshold test stimulus (both at 120% RMT). An inter-stimulus interval of 100 msec was used (Valls-Solé, Pascual-Leone, Wassermann, & Hallett, 1992) with an inter-pulse interval of 5 sec, which does not result in long-term depression of the MEP amplitude with repeated stimulation (Farzan et al., 2009; Sanger, Garg, & Chen, 2001). A pseudorandomised train of 75 paired-pulse stimuli and 75 single-pulse stimuli were administered to the left motor cortex in a single block. Participants were then given a trial of prefrontal stimulation before commencing the second LICI train because it can be uncomfortable for some individuals, due to the potential activation of superficial nerves innervating facial muscles (Rogasch, Thomson, et al., 2014). If participants agreed to proceed, they were administered an identical pseudorandomised train of 150 stimuli. The degree of inhibition was calculated from EMG and EEG recordings using the same formula as for SICI.

Neurocognitive and psychiatric measures

A set of pen-and-paper and computerised neurocognitive and psychiatric measures were selected based on their ability to discriminate between pre-HD and symp-HD groups and controls, and their sensitivity to frontostriatal brain dysfunction in previous large, single-site and longitudinal multi-site studies (Georgiou-Karistianis, Stout, et al., 2013; Gray et al.,

2013; Stout et al., 2012; Stout et al., 2011; Tabrizi et al., 2009; Tabrizi et al., 2012; Tabrizi et al., 2011; Tabrizi et al., 2013).

National Adult Reading Test – Second edition (NART-2). The NART-2 (Nelson & Willison, 1991) is a word-reading task involving non-phonetic, low frequency English words. It provides a brief measure of retrospective premorbid verbal intelligence, and is relatively robust to changes in cognition associated with neurological deficits. It has 50 items of increasing difficulty and takes approximately 5 min to complete. Raw scores range from 0 - 50 and can be converted to an estimated full-scale IQ based on normative data (Strauss, Sherman, & Spreen, 2006).

Trail Making Test. This pen-and-paper task has two parts that assess processing speed, complex attention and executive function (Reitan & Wolfson, 1985). It takes approximately 5 min to complete and completion time is recorded in seconds. Part A requires participants to connect numbered circles in numerical order, whereas part B requires participants to alternate between numbers and letters in numerical and alphabetical order, respectively. Any errors are corrected during the task such that they only contribute to increased performance time. The key outcome measure is the difference score between parts A and B, which reflects the complex attention and executive function aspects of the task, whilst reducing the impact of psychomotor deficits (O'Rourke et al., 2011).

Symbol Digit Modality Task. This is a speeded task that requires participants to transcribe symbols paired with the numbers 1 - 9 in a key at the top of the page within a 90 sec period (Smith, 1982). It is a test of visuomotor integration, involving visual scanning, tracking and motor speed, as well as working memory. The total number of items completed correctly within the time limit is summed, with scores ranging from 0 - 110.

Finger Tapping Task. This is a computerised task assessing processing speed, motor timing and sequencing, that is known to engage a number of brain regions, including non-motor cortices (Hinton et al., 2007; Jäncke, Loose, Lutz, Specht, & Shah, 2000; Paulsen et al., 2004). It takes approximately 10 min to complete and has two parts. The first part,

'speeded tapping', requires the participant to use their non-dominant index finger to press the mouse button repeatedly as fast as possible. The task has five 10 sec trials and the primary outcome measure is the average inter-tap interval, with poorer performances represented by higher scores. The second part of the task is 'paced tapping' and involves the participant tapping the mouse buttons with alternating thumbs at the same rate as a tone, and then continuing to tap at the same rate for 42 taps after the tone stops. There are two blocks at different rates (3.00 Hz and 1.82 Hz) with five trials of each. The primary outcome measure is the inter-tap variance, which designates the precision of motor timing. Lower scores on this component are indicative of poorer performance. All participants completed speeded tapping first, followed by slow-paced tapping and then fast-paced tapping.

Beck Depression Inventory – Second edition. This is a self-report measure of the mood, somatic and cognitive symptoms of depression experienced by the individual over the past two weeks (Beck, Steer, & Brown, 1996). It has 21 items and takes approximately 5 min to complete. Participants rate each item on a four-point scale (0 - 3) reflecting the presence and severity of a symptom. For example, *Sadness: I do not feel sad, I feel sad much of the time, I am sad all the time, I am so sad or unhappy that I can't stand it*. Scores on individual items are summed to determine depression severity, with higher total scores indicating more severe symptoms. Total scores range from 0 - 63, with scores ≥ 20 considered indicative that a participant may be at risk of a depressive disorder. Participants meeting this criterion (pre-HD: n=1; symp-HD: n=3) were contacted by one of the researchers (clinician A.C.) to discuss ways of addressing their reported symptoms.

Beck Anxiety Inventory. This is a 21-item self-report measure, with a similar structure to the Beck Depression Inventory (Beck & Steer, 1990). It enquires about symptoms of anxiety and takes approximately 5 min to complete. For example, *Nervous: Not at all, Mildly (It did not bother me much), Moderately (It was very unpleasant, but I could stand it), Severely (I could barely stand it)*. Scores on individual items are summed to determine anxiety severity, with higher total scores indicating more severe anxiety symptoms. Total

scores range from 0 - 63, with scores ≥ 17 indicative that an individual may be at risk of an anxiety disorder. As with the Beck Depression Inventory, any participants meeting this criterion (pre-HD: n=1; symp-HD: n=1) were contacted by one of the researchers.

Frontal Systems Behaviour Scale. This is a 46-item self-rating scale of behaviours associated with frontal pathology in the brain that takes approximately 10 min to complete (Grace & Mallory, 2001). The scale yields a total score and scores for subscales measuring apathy (14 items), disinhibition (15 items) and executive dysfunction (17 items). Each item is rated on a five-point Likert scale, with higher scores indicating greater levels of pathology.

Genetic analyses

Genes investigated were GABA ($GABA_A$ and $GABA_B$) and dopamine (D_1, D_2 and D_4) receptor variants. Saliva samples were obtained from all participants using the Oragene-DNA self-collection kit, which allowed specimen stability at room temperature until all samples were collected. Dissolved DNA was plated in deep well plates with 500 µL of solution at a concentration of 25 ng/mL. Samples consisting of 200-250 ng dried DNA from this solution were sent to the Australian Genome Research Facility for genotyping. Single-nucleotide polymorphism (SNP) fine mapping of genes was conducted for *GABRA2*, *GABBR1*, *GABBR2*, *DRD1*, *DRD2* and *DRD4*. Haplotype-tagging SNPs were chosen using the HapMap project database of European Caucasians (http://hapmap.ncbi.nlm.nih.gov/), resulting in the selection of 161 SNPs. Eighteen SNPs failed the Australian Genome Research Facility's quality control protocol and one SNP had a successful completion rate less than 90% rates across all samples. Thus, 142 SNPs from six genes remained.

Experimental procedure

Potential participants were screened over the telephone using a questionnaire that determined their eligibility to participate and safety to undergo TMS procedures, modelled on the version recommended by the Safety of TMS Consensus Group (see Table 1; Rossi, Hallett, Rossini, & Pascual-Leone, 2009).

Table 1. Telephone questionnaire administered to screen participants for exclusion criteria and safety to undergo transcranial magnetic stimulation

1. What is your date of birth?
2. Are you left-handed or right-handed?
3. What is your ethnicity?
4. (If Caucasian): Are your parents and all your grandparents also Caucasian?
5. Are you taking any medications? If so, which ones and at what dosages?
6. Do you have epilepsy, or have you ever had a convulsion or a seizure?
7. Do you have, or have you ever had, a stroke or any other brain-related/neurological or psychiatric condition?
8. (If female): Are you pregnant or is there any chance you might be?
9. Do you have a cardiac pacemaker or intracardiac lines or metal in your body?
10. Do you have an implanted neurostimulator?
11. Do you have cochlear implants?
12. Have you ever had a severe head injury where you lost consciousness?
13. Do you have a medication infusion device?
14. Do you have any metal in your brain or skull (not including titanium), such as splinters, fragments or clips?
15. Have you ever had a surgical procedure on your spinal cord?
16. Do you have spinal or ventricular derivations?
17. Have you ever had a fainting spell or syncope?
18. Do you suffer from severe or frequent headaches?
19. Have you ever undergone transcranial magnetic stimulation in the past? If yes, were there any problems or anything unusual?
20. Have you ever had an MRI scan in the past? If yes, were there any problems or anything unusual?
21. Do you have any hearing problems or ringing in your ears?
22. Do you have any visual problems or wear glasses?

Neurocognitive and psychiatric measures were administered in an initial one-hour session conducted either in the participant's home or in a quiet room at Monash University prior to the TMS session. Tests were administered in the same order for each participant. Individuals that travelled from rural Victoria or interstate completed this session on the same day as the TMS session. Saliva samples were also collected at this time.

The second and final session was conducted at the Monash Biomedical Imaging facility (Clayton, Melbourne). This session took approximately three hours with breaks and comprised all TMS-EMG and TMS-EEG procedures. TMS protocols were administered in the same order for each participant. Participants were seated in a comfortable armchair with a headrest throughout the TMS procedures, with their eyes open and hand resting on a pillow placed over their lap. Participants continued their normal medication regimen and were asked to refrain from consuming caffeine on the day of testing (and any intake at odds with this was noted).

Design and analysis

The present study employed a non-randomised cross-sectional design. Participants were allocated to groups based on their gene status (i.e., number of CAG triplet repeats in the huntingtin gene) and symptomology (i.e., UHDRS score). Some participants were unable to complete the EEG component of the study due to a high RMT, which meant that the TMS machine was out of range for the suprathreshold LICI stimuli. Poor TMS tolerability, technical faults and unwillingness to complete particular aspects of the study led to an additional small number of participants with random missing data, who were excluded in an analysis-by-analysis manner. For these reasons, the sample differs slightly across each of the following chapters.

Analyses were performed using SPSS Statistics 22 with two-tailed tests. The Shapiro-Wilks test was used for assessing assumptions of normality. Tukey's 'ladder of powers' was used for transforming data that was in violation of the assumption of normality. The nominal threshold for significance was set at $p < .05$, with the exception of the genetic analyses

where a correction for multiple comparisons was applied. The presence of univariate and multivariate outliers was explored subsequent to primary analyses, and further statistical analyses were conducted excluding such individuals to investigate their effect on the results. Further data processing and analysis is described in more detail in the following chapters.

Chapter three

Monash University

Declaration for thesis chapter three

Declaration by candidate

In the case of chapter three, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
The candidate planned the study and reviewed relevant literature, attained ethics approval and met ongoing reporting requirements, recruited participants, collected, analysed and interpreted data, and planned and wrote the manuscript, with guidance and feedback from co-authors	70

The following co-authors contributed to the work. If co-authors are students at Monash University, the extent of their contribution in percentage terms must be stated:

Name	Nature of contribution	Extent of contribution (%) for student co-authors only
Tarrant Cummins	Conception and design of study, discussion and interpretation of findings, critical revision of manuscript	N/A
Neil Bailey	Collection and analysis of data, discussion and interpretation of findings, critical revision of manuscript	N/A
Andrew Churchyard	Provision of HD individuals, discussion of study design and findings	N/A
Paul Fitzgerald	Conception and design of study, discussion and interpretation of findings, critical revision of manuscript	N/A
Nellie Georgiou-Karistianis	Conception and design of study, discussion and interpretation of findings, critical revision of manuscript	N/A

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work.

Student signature: **Date:** 12/04/2016

Main Supervisor signature: [redacted] **Date:** 12/04/2016

Preamble

The primary aim of the following paper was to establish a comprehensive profile of cortical excitability, inhibition and facilitation in the largest sample of pre-HD and symp-HD individuals to date. It presents findings from TMS-EMG techniques using a number of single-pulse and paired-pulse protocols. This paper is presented first because of the heterogeneity of findings in previous published studies using TMS with HD participants. We attempted to address such heterogeneity through our inclusion of HD participants across a broad range of disease stages by: controlling for potentially confounding variables, standardising voluntary muscle activity using a grip force transducer, investigating the CSP at two different stimulus intensities and investigating intracortical inhibition together with ICF at two different inter-stimulus intervals,. Previous studies had drawn conclusions about pathophysiology in HD based on a select few TMS measures, which makes the interpretation of the heterogeneous literature highly complex, because TMS measures tap different underlying mechanisms. We therefore focused on measures that would assist with differentiating possible pathophysiological mechanisms, such as synaptic or axonal effects and inhibition mediated by specific GABA receptors. For this reason, we did not report the recruitment curve data in this paper as it is difficult to interpret and the motor thresholds also reflect overall corticospinal excitability.

We also sought to investigate the clinical correlates of neurophysiological deficits in pre-HD and symp-HD by considering measures of pathological burden, motor symptoms, neurocognitive performance and psychiatric disturbance. This approach was relatively novel given that the majority of previous work had chosen to investigate a limited number of clinical correlates, predominantly in symp-HD participants, typically including CAG repeat length, total motor score and functional capacity ratings from the UHDRS. In contrast, we included a number of measures of processing speed, attention, executive function, mood and behaviour, with previously demonstrated sensitivity in HD. Increasing the understanding of such relationships is important for identifying which TMS measures might represent candidate

endophenotypic biomarkers for use in future clinical trials, and may also provide increased prognostic accuracy and assist with identifying pre-HD individuals to undergo neuroprotective therapies.

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Research report

Cortical inhibitory deficits in premanifest and early Huntington's disease

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HIGHLIGHTS

- Understanding pathophysiology in HD may provide new insights on disease mechanisms.
- We studied the largest premanifest and symptomatic HD sample with TMS to date.
- Cortical inhibitory deficits were observed in both premanifest and symptomatic HD.
- Reduced GABAergic inhibition in HD was correlated with the triad of symptomology.
- TMS is a useful tool for investigating pathophysiology in HD.

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Pathophysiological mechanism

ABSTRACT

Although progress has been made towards understanding the gross cortical and subcortical pathology of Huntington's disease (HD), there remains little understanding of the progressive pathophysiological changes that occur in the brain circuits underlying the disease. Transcranial magnetic stimulation (TMS) enables investigation of the functional integrity of cortico-subcortical pathways, yet it has not been widely applied in HD research to date. This study sought to characterise profiles of cortical excitability, including inhibition and facilitation, in groups of premanifest and symptomatic HD participants via the use of TMS. We also investigated the clinical, neurocognitive and psychiatric correlates of cortical excitability to better understand the development of phenotypic heterogeneity. The sample comprised 16 premanifest HD, 12 early symptomatic HD and 17 healthy control participants. Single- and paired-pulse TMS protocols were administered to the left primary motor cortex, with surface electromyography recorded from the abductor pollicis brevis muscle. Short-interval cortical inhibition was significantly reduced in symptomatic HD, compared with premanifest HD and controls, and was significantly correlated with pathological burden and neurocognitive performance. There was also reduced long-interval cortical inhibition in both premanifest and symptomatic HD, compared with controls, which was associated with pathological burden and psychiatric disturbances. Motor thresholds, cortical silent periods and intracortical facilitation did not differ across groups. Our results provide important new insights into pathophysiological changes in cortico-subcortical circuits across disease stages in HD. We propose that neurophysiological measures obtained via TMS have potential utility as endophenotypic biomarkers in HD, given their association with both pathological burden and clinical phenotype.

1. Introduction

1.1. Huntington's disease

Huntington's disease (HD) is an inherited neurodegenerative disorder caused by an expansion of CAG repeats in the huntingtin gene. The pathogenic process primarily involves the basal ganglia

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and cerebral cortex [1]. In particular, there is early dysfunction and loss of GABAergic striatal neurons, causing a constellation of motor, cognitive and psychiatric signs. Clinical diagnosis of HD (symp-HD) by observation of involuntary choreiform movements typically occurs in middle adulthood. Gene-positive premanifest HD (pre-HD) individuals exhibit a range of neuropathological changes 15–20 years prior to clinical onset [2], whereas neurocognitive, psychiatric and subtle motor signs are evident <10 years prior [3]. Despite the wealth of knowledge about the gross pathology of HD at different disease stages, there remains little information regarding the pathophysiological properties of neurons within cortico-subcortical circuits underlying the disease. Such information may provide critical new insights on how neurons progress to death in HD.

1.2. Transcranial magnetic stimulation

GABA is a common inhibitory neurotransmitter with widespread functions mediated by $GABA_A$ and $GABA_B$ receptors in cortical and subcortical regions [4]. Transcranial magnetic stimulation (TMS) is able to assess the functional integrity of cortico-subcortical circuits noninvasively, by generating a magnetic field that penetrates the skull and depolarises neurons. Following motor cortex stimulation, amplitudes of motor-evoked potentials (MEPs) from TMS can be recorded through electromyography (EMG) in a peripheral muscle, and manipulated via changes in stimulus intensity and other parameters [5]. The hyperpolarisation of postsynaptic neurons via $GABA_A$ receptors can be assessed with the well-established short-interval cortical inhibition (SICI) paradigm, whereas both long-interval cortical inhibition (LICI) and cortical silent period (CSP) protocols probe $GABA_B$ receptor-mediated circuits [6]. It is widely recognised that TMS is useful for understanding pathophysiology in a range of neurological disorders and in establishing functional biomarkers, yet its application in HD to date has been limited [7].

1.3. Pathophysiology in HD

Previous structural and functional magnetic resonance imaging (MRI) studies have shown cortical thinning in HD, as well as regionally-selective cortical changes, especially in motor cortices [8]. TMS enables investigation of both the pathophysiological results of cortical thinning and the functional impact of regional cortical changes. TMS–EMG has been applied in HD using a number of approaches, including exploring cortical excitability with single- and paired-pulse protocols [9–14]. However, none of the previous studies included a comprehensive protocol of TMS measures in order to establish and compare the profile of corticospinal excitability, including cortical inhibition and intracortical facilitation (ICF), in pre-HD and symp-HD participants. For example, investigating both SICI and LICI, in the resting muscle, might enable the isolation of specific GABAergic deficits without pre-existing synaptic activity. This is an important feature if we are to further delineate the pathogenic mechanisms in terms of axonal and synaptic effects, and extend our knowledge of cortico-subcortical changes.

It has been argued that HD is associated with a disturbed balance of inhibitory and facilitatory connections, which may manifest phenotypically in the heterogeneous expression of symptoms [15]. This disturbed balance may impair the brain's capacity to produce an integrated response following TMS, thereby leading to reduced responses across multiple neural pathways and/or phenotypic measures. However, while animal models support early disturbances in cortical interneuronal activity [16], there is no conclusive evidence from human participants to support this conjecture. Mixed TMS findings for humans are likely to have resulted from a multitude of factors, including differences in sample constitution, coil types, current direction, stimulus intensities and muscle activation. Moreover, comparison between studies is problematic because the various TMS measures employed tap into different mechanisms, which makes interpretation of the literature complex. This is particularly true when studies make inferences based on a select few measures, which may not reflect the full range of pathophysiological deficits in HD. The two TMS studies that directly compared pre-HD and symp-HD participants did not elicit group differences and postulated that neurophysiological abnormalities were caused by the presence of the gene mutation in the brain during development, as opposed to degenerative effects [14,17]. In contrast, numerous studies have demonstrated neurophysiological decline in HD across a range of non-TMS measures, including evoked synaptic responses, which also correlate with increasing clinical severity with disease progression [18,19]. Thus, the question of what pathophysiological abnormalities typically develop in HD, and whether these occur in parallel with the development of types of symptomology, remain unanswered. More comprehensive investigation of pathophysiology in pre-HD and symp-HD participants, that encompass a broad range of disease stages, together with increased neurocognitive and psychiatric phenotypic characterisation, is therefore warranted.

1.4. Aims and hypotheses

The aim of the present study was to examine and compare pathophysiological changes in pre-HD and symp-HD. Seven relevant TMS measures were investigated; specifically, the resting and active motor thresholds (RMT and AMT, respectively), the CSP (at two stimulus intensities), SICI, ICF and LICI. Our aim was achieved, firstly, by characterising a profile of excitability, inhibition and facilitation in samples of pre-HD and symp-HD participants, and secondly, by investigating the clinical, neurocognitive and psychiatric correlates of TMS measures. To our knowledge, this is the first study to utilise a comprehensive TMS procedure in pre-HD as well as symp-HD groups, and to investigate relationships between TMS measures and the triad of symptoms that characterise HD (as indexed by a number of neurocognitive and psychiatric instruments). In particular, we seek to replicate past TMS findings in symp-HD and to build upon the seminal work of Schippling et al. [14], which investigated the RMT and AMT, the CSP and SICI in 16 pre-HD and symp-HD participants. We build upon this work by including ICF, LICI and a range of neurocognitive/psychiatric measures. Moreover, we also used a grip force transducer, investigated a target muscle with lower inter-trial variability [20] and recruited a gender-balanced sample to refine the methodology of the Schippling et al. study. It was hypothesised that corticospinal excitability, cortical inhibition and ICF would be reduced in symp-HD compared with pre-HD, which in turn would be reduced relative to healthy controls. It was also hypothesised that reductions of TMS measures would be associated with greater clinical severity, poorer neurocognitive performance and increased psychiatric symptoms.

2. Material and methods

2.1. Participants

The sample comprised 45 participants, consisting of 16 pre-HD (age range = 26–54 years), 12 symp-HD (43–69 years) and 17 healthy controls (26–57 years). Controls were matched for age and gender to the pre-HD group. One-way ANOVA revealed that the symp-HD group was significantly older than both the pre-HD and control groups. There were no significant differences in gender across groups. Moreover, there were no significant differences in IQ scores (National Adult Reading Test—2nd edition) [21], years of

formal education or typical caffeine consumption across any of the three groups.

Only pre-HD and symp-HD participants underwent gene testing prior to enrolment in the study. The CAG expanded repeat lengths were significantly longer in the symp-HD group (range = 41–47) compared with pre-HD participants (38–44). All HD participants were clinically assessed by a clinician (A.C.) prior to enrolment and underwent a Unified Huntington's Disease Rating Scale (UHDRS) motor assessment. Similar to Tabrizi et al. [2], inclusion in the pre-HD group in our study required a UHDRS total motor score of <5, with scores ranging from 0 to 2. Estimated years to clinical onset for pre-HD participants ranged from 0 to 44, according to the widely-used published formula based on participant age and CAG repeat [22]. Participants in the symp-HD group had UHDRS motor scores ranging from 5 to 30, which was significantly higher than that for the pre-HD group. Duration of diagnosed HD (ascertained by the clinician A.C.) ranged from 1 to 12 years and age at onset from 40 to 63 years. Calculation of disease burden scores [23] revealed significantly higher pathological burden in the symp-HD group compared with pre-HD.

Participants underwent a rigorous screening process prior to recruitment, including a comprehensive review of medical history [24]. All participants were right-handed and free from brain injury, neurological and/or severe diagnosed psychiatric conditions (e.g., bipolar, psychosis) other than HD. Participants remained on their normal medication regimen, which included selective serotonin or serotonin-norepinephrine reuptake inhibitors (pre-HD: $n = 1$; symp-HD: $n = 6$), risperidone (symp-HD: $n = 2$) and haloperidol (symp-HD: $n = 1$). Demographic, clinical, neurocognitive and psychiatric information for all participants is presented in Table 1.

The study was approved by the Monash University Human Research Ethics Committee and written informed consent was obtained in accordance with the Declaration of Helsinki.

2.2. Neurocognitive and psychiatric measures

A battery of neurocognitive tests was selected based on their sensitivity in previous large-scale single-site and longitudinal multi-site studies [25–27]. The tests assessed psychomotor processing and executive attention (Trail Making Test, TMT) [28], psychomotor processing and working memory (Symbol Digit Modalities Test, SDMT) [29], and motor timing abilities (Finger Tapping Task, FTT, with speeded tapping and self-paced tapping at 1.82 and 3 Hz) [30]. Although these tasks rely on motor performance to an extent, they also require significant cognitive control processes, thus they engage broader corticostriatal circuits [31]. Participants also completed behavioural questionnaires, which included assessments of psychiatric disturbances (Beck Depression Inventory—2nd edition, BDI-II, and Beck Anxiety Inventory, BAI) [32,33] and behaviours associated with frontostriatal brain dysfunction (Frontal Systems Behaviour Scale, FrSBe) [34]. Neurocognitive and psychiatric outcomes were completion time (TMT), total score (SDMT, BDI-II, BAI, FrSBe), response time (FTT-speeded; mean inter-tap interval) and response accuracy (FTT-paced; inverse of inter-tap interval standard deviation × 100). One-way ANCOVAs (controlling for age) revealed that neurocognitive performance was poorer and psychiatric symptoms were greater in the symp-HD group on all measures, compared with pre-HD and controls; the pre-HD group did not differ from controls on any measure (see Table 1).

2.3. TMS administration

Biphasic TMS pulses were administered to left primary motor cortex via a hand-held 70 mm figure-of-eight coil, using a MagVenture MagPro X100 stimulator (Farum, Denmark). The coil was held tangential to the scalp, with the handle angled backwards and 45° away from the midline. EMG activity was recorded from the right abductor pollicis brevis (APB) muscle, using self-adhesive surface electrodes in a tendon-belly montage. The site of optimal motor response in the APB muscle was located by stimulation at modestly suprathreshold intensity and moving the coil until the position was located that produced the largest MEP. This position was marked on the scalp and used throughout the TMS procedures. EMG was recorded through commercially available software (LabChart 7, ADInstruments, Bella Vista, New South Wales), and signals were amplified (×1000), band-pass filtered (low-pass = 1000 Hz, high-pass = 10 Hz) and digitised (10 kHz).

The RMT was defined as the minimum stimulation intensity required to evoke a peak-to-peak MEP of more than 50 μV in at least five of ten consecutive trials [35]. The AMT was defined as the minimum stimulation intensity required to produce a MEP of approximately 200 μV in at least five of ten trials during voluntary APB muscle contraction [36]. The AMT was measured during a sustained low intensity contraction of approximately 10 N using a grip force transducer (ADInstruments). The CSP was investigated in the active muscle at two stimulus intensities (120 and 140% AMT), with 12 pulses at each intensity presented in a single pseudorandomised train with a 10 s inter-pulse interval (IPI).

A paired-pulse TMS paradigm investigating SICI and ICF consisted of the administration of a subthreshold (80% RMT) conditioning stimulus followed by a suprathreshold (120% RMT) test stimulus in the resting muscle. This paradigm involved a pseudo-

Table 1
Demographic, clinical, neurocognitive and psychiatric data for each group (presented as mean ± standard deviation).

Measure	Control	Pre-HD	Symp-HD	p
n	17	16	12	–
Age	42 ± 12	42 ± 8	55 ± 9	<.01[a]
Gender (M/F)	6/11	6/10	7/5	>.05
Years of education	16 ± 3	15 ± 4	13 ± 3	>.05
IQ	116 ± 9	109 ± 12	110 ± 13	>.05
Caffeine	2.0 ± 1.4	2.3 ± 1.8	2.3 ± 1.9	>.05
CAG repeat length	–	41.6 ± 2.1	43.3 ± 1.6	.03[b]
Disease burden score	–	250 ± 89	423 ± 74	<.01[b]
UHDRS motor	–	0.2 ± 0.5	17.6 ± 7.7	<.01[b]
Years to onset	–	19 ± 12	–	–
Duration of illness	–	–	4.8 ± 3.5	–
Age at clinical onset	–	–	51 ± 7	–
TMT: A	22 ± 7	24 ± 10	53 ± 27	<.01[a]
TMT: B	48 ± 12	65 ± 33	150 ± 93	<.01[a]
TMT: B–A	26 ± 11	41 ± 26	99 ± 70	<.01[a]
SDMT	58 ± 11	54 ± 10	31 ± 15	<.01[c]
FTT: speeded	216 ± 25	221 ± 33	403 ± 140	<.01[a]
FTT: slow-paced	25 ± 7	24 ± 7	12 ± 6	<.01[c]
FTT: fast-paced	29 ± 8	25 ± 11	13 ± 8	<.01[c]
BDI-II	2.7 ± 3.0	5.1 ± 6.5	9.3 ± 7.9	.03[d]
BAI	2.0 ± 2.2	4.0 ± 4.7	6.8 ± 6.0	<.01[a]
FrSBe: total	76 ± 15	80 ± 21	104 ± 23	<.01[a]
FrSBe: apathy	23 ± 5	26 ± 10	33 ± 10	.01[a]
FrSBe: disinhibition	24 ± 5	24 ± 5	31 ± 9	<.01[a]
FrSBe: executive	29 ± 7	31 ± 7	41 ± 9	<.01[a]

Pre-HD = premanifest Huntington's disease; Symp-HD = symptomatic Huntington's disease; IQ = estimate of premorbid intellectual ability using the National Adult Reading Test—2nd edition; Caffeine = typical daily consumption of caffeinated drinks; UHDRS = Unified Huntington's Disease Rating Scale total motor score; Duration of illness = years since clinical diagnosis; TMT = trail making test (parts A and B, time taken in seconds); SDMT = symbol digit modalities test (number of items correct); FTT = finger tapping test (speeded = mean inter-tap interval, msec; slow/fast-paced = inverse of inter-tap interval standard deviation × 100); BDI-II = Beck depression inventory—second edition; BAI = Beck anxiety inventory; FrSBe = frontal systems behaviour scale (total and subscale scores).
AN(C)OVA/χ^2/t-test results from significance testing of group differences:
[a] Symp-HD > Pre-HD, Control.
[b] Symp-HD > Pre-HD.
[c] Symp-HD < Pre-HD, Control.
[d] Symp-HD > Control.

randomised train of 60 trials with a 10 s IPI, comprising 20 of each of the following three conditions: paired-pulse with a 3 ms inter-stimulus interval (ISI, measuring SICI) [37], paired-pulse with a 10 ms ISI (measuring ICF) and single-pulse TMS at 120% RMT. A paired-pulse TMS paradigm investigating LICI was administered separately and involved a pseudorandomised train of 150 trials with a 5 s IPI. This paradigm comprised 75 single pulses at 120% RMT and 75 paired-pulses with both conditioning and test stimuli at 120% RMT and a 100 ms ISI [5].

2.4. Procedure

Neurocognitive and psychiatric measures were administered prior to TMS. TMS procedures were conducted at the Monash Biomedical Imaging facility (Clayton, Victoria). Protocols were administered in the same order for each participant. Participants were seated in a comfortable armchair with a headrest throughout TMS, with their eyes open and right hand resting on a pillow. Participants were asked to refrain from consuming caffeine on the day of testing. No adverse events were reported following TMS.

2.5. Data analysis

EMG data was examined offline using LabChart 7. Trials were excluded if baseline muscle activity was greater than 40 μV prior to TMS or grip force strength was outside 10 ± 3 N for the CSP measure. The duration of the CSP was calculated as the time between MEP onset and resumption of voluntary activity, and average peak-to-peak amplitudes of MEPs were determined for the other TMS measures. The degree of inhibition and facilitation was calculated using a formula that compared the average MEP amplitude generated by paired-pulse stimuli to the MEP from test stimuli alone (whereby negative values signify inhibition and positive signify facilitation).

Analyses were performed in SPSS Statistics 22. Distributions of variables were assessed for normality using the Shapiro–Wilks test and log-transformed where appropriate. Age was used as a covariate in analyses of group differences. All TMS measure group differences were investigated with one-way ANCOVAs with the exception of the CSP measure, which was examined with a mixed-model ANCOVA (repeated measure: stimulus intensity). Given the exploratory nature of these investigations, Fisher's LSD was applied in post-hoc tests. The associations between TMS measures, clinical severity, neurocognitive performance and psychiatric symptoms were assessed with Pearson's and partial correlations and hierarchical multiple regression analyses. UHDRS motor score was entered as a covariate in secondary partial correlation analyses in order to assess the influence of motor performance differences on the relationships between TMS measures and neurocognitive/psychiatric measures. The nominal threshold for significance throughout the analyses was $p < .05$.

3. Results

3.1. TMS between-group comparisons

Estimated marginal means and standard errors for responses to the different TMS protocols across the three groups are presented in Table 2.

Levene's test indicated homogeneous variances across groups on all TMS measures. For paired-pulse TMS, there was a significant main effect of Group for SICI ($F_{2,38} = 4.44$, $p = .02$, $\eta^2 = .19$). Post-hoc analyses revealed significantly reduced inhibition in symp-HD, compared with pre-HD and controls (both $p = .01$). Additionally, there was a significant main effect of Group for LICI ($F_{2,35} = 4.11$, $p = .03$, $\eta^2 = .19$). Post-hoc analyses revealed significantly reduced inhibition in pre-HD and symp-HD participants, compared with controls (both $p = .02$). Thresholds were similar across all three groups (RMT: $F_{2,40} = 0.96$, AMT: $F_{2,38} = 0.52$, both $p > .05$). There was no significant main effect of Group for ICF ($F_{2,38} = 0.09$, $p > .05$) or the CSP ($F_{2,35} = 0.66$, $p > .05$). There was a main effect of Intensity on the CSP ($F_{1,35} = 11.85$, $p < .01$), with longer CSP duration at the higher stimulus intensity, but no Group-Intensity interaction effect ($F_{2,35} = 1.21$, $p > .05$). Excluding the symp-HD participants on risperidone or haloperidol did not change the pattern of results.

Table 2
Estimated marginal means and standard errors on TMS measures for each group, after adjusting for differences in age (presented as estimated marginal mean ± standard error).

Measure	Control	Pre-HD	Symp-HD	p
n	17	16	12	–
Resting motor threshold	53 ± 2	49 ± 3	54 ± 3	.39
Active motor threshold	45 ± 2	43 ± 2	43 ± 3	.60
Cortical silent period (120%)	52 ± 9	55 ± 8	38 ± 11	.53
Cortical silent period (140%)	78 ± 9	90 ± 7	77 ± 11	.53
Short-interval cortical inhibition	−0.9 ± 0.2	-0.9 ± 0.2	−0.3 ± 0.2	.02[a]
Intracortical facilitation	0.5 ± 0.1	0.6 ± 0.1	0.5 ± 0.2	.92
Long-interval cortical inhibition	−2.9 ± 0.3	−1.9 ± 0.3	−1.6 ± 0.4	.03[b]

Pre-HD = premanifest Huntington's disease; Symp-HD = symptomatic Huntington's disease; Resting/active motor thresholds expressed as a percentage of maximum stimulator output; Duration of cortical silent period expressed in milliseconds, with stimulation intensity at 120/140% active motor threshold; Short- and long-interval cortical inhibition and intracortical facilitation measures were log-transformed to satisfy the assumptions of normality for statistical analysis.
ANCOVA results from significance testing of group differences with post-hoc LSD:
[a] Symp-HD > Pre-HD, Control.
[b] Pre-HD, Symp-HD > Control.

3.2. Clinical, neurocognitive and psychiatric correlations

Pearson's correlations were conducted to examine the clinical correlates of SICI and LICI in the pre-HD and symp-HD samples separately because these TMS measures differed across groups. The threshold for significance was not adjusted due to the exploratory nature of these analyses. With regards to clinical severity, SICI did not correlate with clinical measures in the pre-HD participants but was significantly associated with CAG ($r = .72$, $p = .01$) and disease burden score ($r = .64$, $p = .03$) in symp-HD. LICI was significantly correlated with CAG in the pre-HD participants ($r = .64$, $p = .01$) but was not associated with clinical measures in symp-HD. Of the neurocognitive measures, SICI significantly correlated with slow-paced ($r = -.55$, $p = .04$) and fast-paced FTT ($r = -.58$, $p = .03$) in the pre-HD participants but was not associated with neurocognitive performance in symp-HD. Additionally, LICI was not significantly correlated with neurocognitive measures in either group. There was also no evidence of associations between SICI and psychiatric symptoms in either group. In contrast, LICI was significantly correlated with FrSBe total ($r = -.54$, $p = .04$), apathy ($r = -.51$, $p = .05$) and disinhibition scores ($r = -.54$, $p = .04$) in the pre-HD participants, and with FrSBe executive scores in symp-HD ($r = -.68$, $p = .03$). However, when UHDRS motor score was entered as a covariate in partial correlational analyses, the associations between SICI and FTT in pre-HD were no longer significant. Similarly, associations between LICI and FrSBe scores were no longer significant in either group in the secondary partial correlational analyses.

Separate hierarchical multiple regression analyses were performed to assess whether each of SICI and LICI predicted age at onset in symp-HD beyond the influence of CAG repeat length. Age at onset was initially log-transformed due to its known curvilinear relationship with CAG repeat length [38]. CAG was entered at step 1, explaining 38 and 46% of the variance in age at onset for the SICI and LICI models, respectively. Prediction of age at onset was not significantly improved in the two-step model with the addition

of SICI (R square change = .07, $F_{1,7}$ = 0.96, p > .05) or LICI (R square change = .19, $F_{1,6}$ = 3.34, p > .05).

4. Discussion

4.1. Key findings

This study builds on previous TMS research and importantly includes the largest sample of pre-HD and symp-HD participants to date. In particular, we sought to investigate the CSP, at two different stimulus intensities using a grip force transducer, and examined TMS measures of both SICI and LICI in order to differentiate between cortical inhibitory circuits. Moreover, we also contributed to the literature by investigating whether TMS measures were associated with clinical severity, neurocognitive performance and psychiatric symptoms. There were a number of key findings, which were broadly in accordance with our hypotheses. Firstly, SICI was reduced in symp-HD but not pre-HD participants. SICI was significantly correlated with clinical severity and neurocognitive performance in both pre-HD and symp-HD groups. Secondly, there was reduced LICI in both pre-HD and symp-HD, which was related to clinical severity and psychiatric symptoms in both groups. The profile of TMS–EMG responses suggests that motor cortex excitability changes in HD predominantly involve intracortical inhibitory pathways rather than corticospinal or facilitatory. Our results provide further evidence of functional impairment of cortical neurons during early stages of HD.

Our findings suggest that SICI is relatively normal in HD until individuals develop overt motor symptoms, in line with one previous report [12]. In contrast, other studies have reported no difference in SICI between early HD and controls; although different target muscles and stimulus intensities were used [9]. A higher threshold for eliciting SICI has been described in a combined pre-HD and symp-HD group, which, together with differences in motor threshold between HD and controls, was concluded to reflect deficits in axonal membrane excitability in HD [14]. However, this result is difficult to compare with present findings because a theoretical threshold was determined from a different target muscle, and the actual degree of inhibition did not differ between groups. Moreover, we did not find differences in RMT/AMT across our three groups in the APB muscle, which suggests that synaptic effects in HD should not be ruled out.

The possibility of synaptic effects is a valid inference considering our novel results also indicated reduced LICI in pre-HD and symp-HD, which has not been previously described, as this measure is not typically included with other TMS protocols. In contrast to our study, Priori et al. [11] investigated symp-HD participants using a circular coil during active contraction of a different target muscle and reported normal LICI, as well as normal SICI; however, coil types and muscle activation are known to have a significant impact on MEP amplitudes [39], which may have confounded their results. Taken together, our findings of cortical inhibitory deficits, for both the short- and long-interval paradigms in the resting muscle, suggest that synaptic transmission could be disturbed in HD.

4.2. Clinical, neurocognitive and psychiatric correlates

Notably, previous studies have not found TMS measures to correlate with the triad of deficits in HD as we have shown; however, these results should be interpreted with caution given our exploratory approach. Indeed, many previous studies only investigated UHDRS motor and functional ratings [9,12]. The negative correlations between TMS and psychiatric measures warrant further investigation and may reflect a weakness of relying on self-report measures in participants with cognitive disturbances. However, the existence of significant correlations in both pre-HD and symp-HD indicates they may be driven by genuine relationships, possibly related to reactive psychiatric changes. Indications from secondary analyses that the SICI associations (e.g., with FTT) were largely driven by motor deficits must also be considered. Nevertheless, there is ample evidence from previous studies that the clinical phenotype in HD is at least partly driven by pathophysiological changes [15,40], consistent with present results. Such evidence is encouraging for the future development of disease-modifying treatments, which could include brain stimulation.

4.3. Non-significant findings

In line with recent studies, we found unchanged silent periods in pre-HD and symp-HD [9,14,41]. In contrast, earlier studies using higher stimulus intensities predominantly reported prolonged silent periods [10,13,42]. The stimulus intensities we used for eliciting the CSP, which produced relatively short periods, may have been too low to capture the range of pathophysiological responses resulting from changes in cortical excitability. However, our use of a grip force transducer to standardise muscle activity (and exclude trials with excessive activity) may explain the differing results. Reports of increased [12] or decreased ICF [9] are also at odds with our findings. Whilst Nardone et al. [9] reported reduced ICF at ISIs ranging from 7–20 ms, it is possible the present study failed to elicit facilitation in participants with a single ISI. In accordance with numerous studies, we found no difference in RMT or AMT, which suggests that overall corticospinal excitability is likely to be normal in HD [9–13].

Comparison between TMS studies is particularly difficult due to important methodological differences, including the muscle activity, the coil type and the intensity of conditioning and test stimuli. Nevertheless, the present study constitutes the largest pre-HD and symp-HD sample investigated to date with TMS and it administered a number of measures assessing corticospinal excitability, including cortical inhibition and facilitation, thus allowing inferences regarding the cortico-subcortical pathways that may be affected at different disease stages. Moreover, our cross-sectional study encompassed a wide range of participants in terms of disease progression, with pre-HD individuals up to 44 years prior to estimated onset and symp-HD individuals with a range of UHDRS motor scores, who were well-characterised phenotypically regarding neurocognitive and psychiatric assessments.

4.4. Pathophysiological mechanisms

The cortical inhibitory deficits observed in HD may be caused by a number of factors, possibly including altered GABA transmission. There is abundant evidence supporting changes in interneuronal activity, trafficking of receptors and synaptic transmission early in HD, primarily from animal models [16,43]. The trajectories of such changes are time-dependent, region-specific and bi-directional, but likely precede the loss of striatal and pyramidal cells [44]. Indeed, corticostriatal pathways become disconnected early in HD [45], which might cause striatal degeneration through deprivation of trophic support [46]. An alternative, though not mutually exclusive, hypothesis is that reduced net inhibition reflects increased excitatory drive. This corresponds with models of basal ganglia function and has been demonstrated in HD mouse models [47]. Lastly, it is possible that the cortical inhibitory deficits reflect a state of perpetual movement preparation in HD [48]. Whilst this possibility should be considered in future studies, it is unlikely to account for our group differences given that UHDRS motor score was not significantly associated with SICI or LICI and we excluded trials with EMG activity prior to TMS. Taken together, our findings highlight the importance of further investigating the mechanisms underpinning

pathophysiological changes in HD and their specific contributions to phenotypic manifestation.

SICI and LICI rely on distinct inhibitory systems involving $GABA_A$ and $GABA_B$ receptors, respectively [49]. We propose that the $GABA_B$-mediated pathway is impaired early in disease progression because LICI was the only measure that was significantly affected in pre-HD. This could be due to the long timeframe for LICI or the complex second messenger pathway [50]. Indeed, cannabinoid receptors, another class of G-protein coupled receptors, are also selectively vulnerable early in HD [51]. Our findings thus fit with evidence from animal studies, which has suggested presynaptic and postsynaptic changes within the corticostriatal system in HD [52]. The range of possible $GABA_B$ alterations and bi-directional effects might also explain the inconsistent findings for the CSP in past research. Increased knowledge of cortical inhibitory changes across disease stages, which can be differentiated using TMS, is therefore important for determining the earliest pathophysiological changes in HD.

4.5. Limitations and future directions

There were a number of limitations in the current study. For example, it might be underpowered to detect group differences on TMS measures with a cross-sectional design; thus, larger sample sizes are suggested for future studies. Moreover, a longitudinal investigation, with a wider range of stimulus intensities and intervals, should be conducted given that the disease progresses non-linearly. The impact of cortical atrophy on stimulation effects, particularly in symp-HD, must be considered and could be addressed with MRI-based neuronavigation techniques. Lastly, we cannot exclude a confounding effect of psychoactive medication on neurophysiological responses, despite excluding these medicated HD participants in secondary analyses.

5. Conclusion

In conclusion, the present findings provide important additional insights into the pathophysiology of HD. For the first time, we found GABA-mediated cortical inhibitory deficits in both pre-HD and symp-HD participants using paired-pulse TMS paradigms, which were related to clinical severity, neurocognitive performance and psychiatric disturbances. Our findings reinforce the notion that a neurophysiological approach assists with teasing apart complex biobehavioural relationships between the gene mutation and symptomology in HD. Such information is potentially beneficial for developing interventions that target pathophysiology in HD. The question of whether the cortical inhibitory deficits change with disease progression will need to be investigated through longitudinal studies. This will enable the identification of TMS measures that may have utility as sensitive and robust endophenotypic biomarkers in future clinical trials with HD.

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Chapter four

Monash University

Declaration for thesis chapter four

Declaration by candidate

In the case of chapter four, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
The candidate planned the study and reviewed relevant literature, attained ethics approval and met ongoing reporting requirements, recruited participants, collected, analysed and interpreted data, and planned and wrote the manuscript, with guidance and feedback from co-authors	75

The following co-authors contributed to the work. If co-authors are students at Monash University, the extent of their contribution in percentage terms must be stated:

Name	Nature of contribution	Extent of contribution (%) for student co-authors only
Tarrant Cummins	Conception and design of study, discussion and interpretation of findings, critical revision of manuscript	N/A
Neil Bailey	Collection and analysis of data, discussion and interpretation of findings, critical revision of manuscript	N/A
Andrew Churchyard	Provision of HD individuals, discussion of study design and findings	N/A
Paul Fitzgerald	Conception and design of study, discussion and interpretation of findings, critical revision of manuscript	N/A
Nellie Georgiou-Karistianis	Conception and design of study, discussion and interpretation of findings, critical revision of manuscript	N/A

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work.

Student signature:  **Date:** 12/04/2016

Main Supervisor signature:  **Date:** 12/04/2016

Preamble

The following paper comprises a short communication investigating whether sex differences exert an influence on pathophysiology in HD. There are several lines of evidence that support the existence of sex differences in response to particular TMS measures, from both animal and human studies. However, the general consensus from past research is that sex has a negligible impact on the MEP. In fact, an expert panel recently decided that sex is important to report in TMS studies but does not need to be controlled (Chipchase et al., 2012). For this reason, common TMS measures of cortical excitability, such as the recruitment curve, were not considered in the following paper.

Notably, it is well-known that ovarian hormones may interact with molecules involved in mediating responses to certain TMS protocols, particularly $GABA_A$ receptors. Indeed, research into the effect of sex on less common TMS measures, including SICI, is limited. As such, sex differences in response to SICI require further investigation, especially in the context of investigating pathophysiological deficits in neurological disorders like HD.

There is some evidence that disease progression and severity in HD may also be influenced by sex, with more severe phenotypes in females (Zielonka et al., 2013). However, studies that focused on other neurological disorders, such as Alzheimer's disease, have indicated that ovarian hormones may have neuroprotective effects. Clearly, additional research into potential sex differences in HD participants using TMS is required to dissect some of these inconsistencies in the literature, which would also inform research in other neurological disorders.

Given the various lines of evidence supporting possible sex differences in both TMS and HD studies, the interaction between sex and disease was of particular interest in the following study and constitutes a novel avenue of research. This line of research has potentially important implications for the manner in which TMS measures may be employed as biomarkers in HD, as well as the future use of TMS as a therapeutic technique in this population.

Cortical Inhibitory Deficits in Huntington's Disease are Not Influenced by Gender

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Highlights

- Huntington's disease affects GABAergic function in cortico-subcortical pathways.
- TMS was used to study whether sex modulates pathophysiology in Huntington's disease.
- Cortical inhibition was significantly reduced in symptomatic Huntington's disease.
- Females exhibited significantly reduced cortical inhibition compared with males.
- Sex differences do not interact with inhibitory deficits in Huntington's disease.

Abstract

Huntington's disease (HD) affects GABA-mediated inhibitory circuitry in the cortex. As there is evidence that sex hormones affect GABAergic function, we investigated whether gender modulates GABA-related pathophysiological changes in HD. Fifteen premanifest HD, 11 symptomatic HD and 16 healthy control participants were assessed with paired-pulse transcranial magnetic stimulation applied to the primary motor cortex. Cortical inhibition was significantly reduced in symptomatic HD, compared with premanifest HD and controls. There was reduced inhibition in females overall, but no Group-by-Sex interaction. These findings suggest that sex hormones do not exert a direct influence on the mechanisms underpinning cortical inhibitory deficits in HD.

Keywords

Transcranial magnetic stimulation; GABA; Sex difference.

1. Introduction

Transcranial magnetic stimulation (TMS) is a non-invasive brain stimulation technique useful for investigating cortical excitability. Intracortical inhibition may be indexed via paired-pulse TMS protocols, whereby the effect of a conditioning pulse on the motor-evoked potential (MEP) can be quantified through comparison with the single-pulse MEP amplitude (Kujirai et al., 1993). Short-interval cortical inhibition (SICI) is a well-established protocol that uses 1-5msec inter-stimulus intervals to examine the function of $GABA_A$ receptor-mediated circuitry in primary motor cortices (Di Lazzaro et al., 1998; IliĆ et al., 2002). The consensus from previous research, predominantly using corticospinal excitability measures, is that gender has a 'negligible' effect on MEP characteristics (Cuypers et al., 2014; Groppa et al., 2012; Livingston et al., 2010; Pitcher et al., 2003; Wassermann, 2002). Accordingly, TMS studies have generally not controlled for sex. However, several lines of evidence indicate that fluctuations of ovarian hormones during the menstrual cycle may influence measures of cortical excitability and intracortical inhibition (Inghilleri et al., 2004; Smith et al., 2002; Zoghi et al., 2015). Indeed, the neuroactive steroids progesterone and estrogen, released in high amounts from the ovaries, bind with $GABA_A$ receptors (Murphy et al., 1998; Strous et al., 2006). At the group level, SICI could in fact be reduced in females due to poorly synchronised $GABA_A$ receptor-mediated responses as a result of cyclical hormonal variations. However, it is difficult to isolate the influence of individual hormones, which may have opposing/interacting effects (Smith et al., 1999).

TMS has been used numerous times to study participants with Huntington's disease (HD), an inherited neurodegenerative disorder caused by a triplet (CAG) repeat (Berardelli et al., 2008; Philpott et al., 2013). HD neuropathology affects cerebral cortices and basal ganglia, causing motor, cognitive and psychiatric symptoms that typically manifest in middle adulthood (Vonsattel and DiFiglia, 1998). Inhibitory neurotransmission in these cortico-subcortical circuits, densely populated with GABAergic neurons, is therefore likely to be affected in HD (Twelvetrees et al., 2010). There is evidence that SICI is reduced in premanifest and symptomatic HD (pre-HD and symp-HD, respectively) stages compared with controls (Abbruzzese et al., 1997; Schippling et al., 2009). Despite a wealth of

knowledge about gross neuropathology in HD (Domínguez D et al., 2013; Tabrizi et al., 2013), there still remains little mechanistic understanding regarding pathophysiological changes that occur in the brain circuits underlying the disease (Mayer and Orth, 2014). Moreover, no previous study has specifically addressed whether sex differences play a role in HD pathophysiology. This could be possible given the interaction between ovarian hormones and $GABA_A$ receptors, which may impact synaptic plasticity early in disease progression (Orth et al., 2010), and the evidence to suggest greater HD severity in females (Zielonka et al., 2013). In addition, sexual dimorphisms in the brain, as well as in neurotransmitter systems and age-related atrophy, are well-established (Fanelli et al., 2013; Murphy et al., 1996; Savic, 2014). Thus, neurodegenerative changes in pre-HD and symp-HD might interact with such sex differences, which is why further investigation of gender effects is warranted.

Previous TMS findings in HD are mixed, likely due to methodological differences and potentially inadequate control of confounding variables, including gender. Given the literature suggesting that sex differences might influence GABA-mediated cortical inhibition measures, we investigated whether SICI responses are impacted by gender in HD participants and healthy controls. Firstly, we expected that SICI would be reduced in: i) pre-HD and symp-HD, compared with controls, and ii) females overall, compared with males. Secondly, we predicted a significant Group-by-Sex interaction for SICI.

2. Methods

2.1 Participants

The sample consisted of 42 participants across three groups, comprising 15 pre-HD, 11 symp-HD participants and 16 healthy controls (see Table 1 for demographic and clinical information). There were no significant differences in gender across groups. Controls were age-matched to the pre-HD group, but symp-HD was significantly older (range=43-69 years) than both pre-HD (range=26-54) and control groups (range=26-57). CAG expanded repeat lengths were significantly longer in symp-HD (range=41-47) compared with pre-HD (range=38-44). All HD participants underwent Unified Huntington's Disease Rating Scale (UHDRS) motor assessment and inclusion in the pre-HD group required a UHDRS total motor score <5 (Tabrizi et al., 2009). Symp-HD participants had higher

UHDRS scores (range=5-30) than pre-HD (range=0-2). Participants underwent screening of medical history prior to recruitment (Rossi et al., 2009). Medications included selective serotonin/serotonin-norepinephrine reuptake inhibitors (pre-HD: n=1; symp-HD: n=6), risperidone (symp-HD: n=2) and haloperidol (symp-HD: n=1). Females reported taking oral contraceptives (pre-HD: n=1; control: n=3) and hormone replacement therapies (pre-HD: n=1), but were not questioned about their menstrual status/phase. Participants provided consent in accordance with the Monash University Human Research Ethics Committee.

[Insert Table 1 about here]

2.2 Materials

Biphasic TMS pulses were administered to left primary motor cortex via a hand-held 70mm figure-of-eight coil, using a MagVenture MagPro X100 stimulator (Farum, Denmark). The coil was held tangential to the scalp, with the handle angled backwards and 45° away from the midline. Electromyographic (EMG) activity was recorded from the resting right abductor pollicis brevis muscle, using self-adhesive surface electrodes. EMG was recorded through commercial software (LabChart 7, ADInstruments, Bella Vista, New South Wales), and signals were amplified (x1000), filtered (low-pass=1000Hz, high-pass=10Hz) and digitised (10kHz). Resting motor threshold (RMT) was defined as the minimum intensity required to evoke a peak-to-peak MEP >50μV in at least five of ten consecutive trials (Rossini et al., 1994). The protocol for measuring SICI comprised a subthreshold (80%RMT) conditioning stimulus paired with a suprathreshold (120%RMT) test stimulus, with a 10sec interval. This protocol involved a pseudorandomised train of stimuli, comprising 20 paired-pulse with a 3msec inter-stimulus interval and 20 single-pulse (120%RMT).

2.3 Statistical analyses

The primary outcome measure was SICI; RMT was established to determine appropriate stimulation intensities. EMG trials were excluded if baseline muscle activity was >40μV. The degree of inhibition was calculated using a formula that compared average MEP amplitudes following paired-pulse stimuli to single-pulse MEPs. Two-tailed analyses were performed in SPSS Statistics 22. SICI was log-

transformed to satisfy normality assumptions. Group, Sex and interaction effects for SICI were investigated with 3x2 ANCOVA, covarying for age. Fisher's LSD was applied in post-hoc tests. The threshold for significance was $p<.05$.

3. Results

Estimated marginal means and standard errors for the RMT and SICI across the three groups, and for males and females separately, are presented in Table 1. Levene's test indicated homogeneous variances across groups for the RMT and SICI. The RMT did not differ by Group ($F_{2,38}=1.19$, $p>.05$) or Sex ($F_{1,39}=0.98$, $p>.05$). The two-way ANCOVA for SICI revealed a significant main effect of Group ($F_{2,35}=7.08$, $p=.003$, $\eta^2=.29$), with reduced SICI in symp-HD, compared with pre-HD and controls (both $p=.005$; see Table 1). Additionally, there was a significant main effect of Sex ($F_{1,35}=9.63$, $p=.004$, $\eta^2=.22$), with less SICI in females than males overall (see Table 1). The two-way ANCOVA did not show a significant Group-by-Sex interaction for SICI ($F_{2,35}=0.98$, $p>.05$). Removal of participants taking oral contraceptives/hormone replacement therapies did not change the pattern of significant results.

4. Discussion

This study investigated whether gender differentially influences cortical inhibition in HD participants and healthy controls. In line with the primary hypotheses and prior research (Abbruzzese et al., 1997; Berardelli et al., 2008; Schippling et al., 2009), our findings demonstrated reduced SICI in symp-HD participants, compared with pre-HD and controls. Furthermore, females showed less inhibition overall than males. However, contrary to our secondary hypothesis, there was no significant Group-by-Sex interaction for SICI. Based on these findings, sex hormones may affect the neural mechanisms underpinning cortical inhibition generally, but independent of the pathophysiological processes in HD.

Our finding of reduced overall SICI in females is novel, but supported by previous literature. For example, differences in SICI according to menstrual cycle phase have been reported, indicating disrupted GABAergic transmission (Smith et al., 2002; Smith et al., 1999). However, conflicting results have led most researchers to conclude that it is not necessary to control for gender in TMS

studies (Chipchase et al., 2012). Although the mechanisms driving our finding of reduced SICI in females remain unclear, it nonetheless would be consistent with a progesterone-mediated disturbance of $GABA_A$ receptor function (Strous et al., 2006). Given that there was no Group-by-Sex interaction, we suggest that progesterone-mediated effects and the progressive pathological HD effects might impact on separate functions (or sites) of the $GABA_A$ receptor. Indeed, our finding, of reduced SICI in symp-HD, suggests that $GABA_A$-mediated inhibition remains relatively unaffected by the early progressive pathology during pre-HD stages (Abbruzzese et al., 1997). The impact of other sex hormones on SICI responses (e.g., oestrogen-mediated effects on glutamate transmission), as well as non-hormonal factors, must also be considered. However, the former is less likely to have contributed to our findings, given that the RMT did not show sex differences (Inghilleri et al., 2004).

To our knowledge there are no previous studies investigating whether gender influences TMS responses in HD participants. However, several lines of evidence suggest that this question should be further explored, despite our null findings. For example, sexual dimorphisms in the basal ganglia and cortex are well-established (e.g., Savic, 2014). Furthermore, sex differences in HD severity have been reported, albeit infrequently, in the literature. Previous reports include poorer UHDRS scores and faster progression in females (Zielonka et al., 2013). In contrast, numerous studies indicate that oestrogen may exert neuroprotective effects in females (e.g., Smith and Dahodwala, 2014). We contend that further research in this area may provide important insights into underlying pathophysiological mechanisms, synaptic plasticity and phenotypic heterogeneity in HD. Indeed, reduced SICI could be intrinsically linked to metaplastic changes with progression of HD via ‘gating’ of motor cortex excitability (Lorenzano et al., 2006; Ziemann and Siebner, 2008).

With regards to study limitations, our sample was relatively small. Moreover, while the symptom groups were matched for gender ratio, overall there were more females than males. We did not inquire about the menstrual status/phase of female participants, a limitation as it precluded analyses of cyclical effects. In addition, differing levels of cortical atrophy may have affected the stimulation strength, particularly in symp-HD participants (Berardelli et al., 2008). Furthermore, it is possible that our finding of reduced net inhibition in symp-HD reflects a state of perpetual movement preparation

(Reynolds and Ashby, 1999), or contamination by short-interval intracortical facilitation (Abbruzzese et al., 2000; Peurala et al., 2008). Lastly, medication types are also known to affect responses to TMS, a factor we did not control for.

To conclude, the present study indicates that sex differences in cortical inhibition are important to consider in future research involving both healthy and clinical populations. Our results may partially explain the heterogeneity of findings from past TMS research, which rarely controls for gender. However, we cannot draw conclusions about the influence of specific ovarian hormones on neurophysiology. We suggest that future studies account for sex differences when investigating SICI through statistically covarying for gender, testing female participants in the same menstrual cycle phase and/or recruiting samples of only male and/or post-menopausal women.

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Contributors

April Philpott – Research project: conception, organization, execution; Statistical analysis: design, execution; Manuscript preparation: writing of the first draft, review & critique.

Tarrant Cummins - Research project: conception, organization; Statistical analysis: design, review & critique; Manuscript preparation: review & critique.

Neil Bailey - Research project: organization, execution; Statistical analysis: design, execution, review & critique; Manuscript preparation: review & critique.

Andrew Churchyard - Research project: conception, organization, execution; Manuscript preparation: review & critique.

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Nellie Georgiou-Karistianis - Research project: conception, organization; Statistical analysis: design, review & critique; Manuscript preparation: review & critique.

Conflict of Interest

This project was funded by the School of Psychological Sciences, Monash University. Professor Fitzgerald is supported by a NHMRC Practitioner Fellowship, has received equipment for research from MagVenture, Brainsway, Cervel Neurotech and Neuronetics, and funding for research from Cervel Neurotech. The funding sources had no role in the design of the study, preparation of the manuscript or the decision about submitting the article for publication. The authors have no further conflicts of interest to declare.

Table 1. Demographic, clinical and neurophysiological data for each group and for males and females overall

Measure	Control	Pre-HD	Symp-HD	p_1	Male	Female	p_2
n	16	15	11	-	17	25	-
Gender (M/F)	5/11	5/10	7/4	>.05	-	-	-
Age (M ± SD)	41.92 ± 11.74	41.28 ± 7.88	54.44 ± 8.69	<.01[a]	49.12 ± 11.05	42.15 ± 10.38	.04[c]
CAG (M ± SD)	-	41.67 ± 2.13	43.27 ± 1.68	.05[b]	-	-	-
UHDRS (M ± SD)	-	0.20 ± 0.56	17.36 ± 8.05	<.01[b]	-	-	-
RMT (EMM ± SE)	53.41 ± 2.41	49.14 ± 2.50	54.86 ± 3.20	>.05	50.41 ± 2.37	53.52 ± 1.94	>.05
SICI (EMM ± SE)	-0.91 ± 0.17	-0.91 ± 0.17	0.06 ± 0.22	<.01[a]	-0.92 ± 0.16	-0.26 ± 0.14	<.01[d]

Pre-HD = premanifest Huntington's disease; Symp-HD = symptomatic Huntington's disease; M ± SD = mean plus or minus standard deviation; EMM ± SE = estimated marginal mean, after controlling for age, plus or minus standard error; CAG = repeat length in expanded allele; UHDRS = Unified Huntington's Disease Rating Scale total motor score; RMT = resting motor threshold (percent of stimulator output); SICI = degree of short-interval cortical inhibition. For the SICI measure, negative values signify that inhibition occurred and positive values signify facilitation. AN(C)OVA/χ^2/*t*-test results from significance testing of group differences: p_1 = significance level of difference between pre-HD, symp-HD and control groups; [a]Symp-HD > Pre-HD, Control; [b]Symp-HD > Pre-HD; p_2 = significance level of difference between males and females; [c]Males > Females; [d]Males < Females.

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Chapter five

Monash University

Declaration for thesis chapter five

Declaration by candidate

In the case of chapter five, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
The candidate planned the study and reviewed relevant literature, attained ethics approval and met ongoing reporting requirements, recruited participants, collected, analysed and interpreted data, and planned and wrote the manuscript, with guidance and feedback from co-authors	70

The following co-authors contributed to the work. If co-authors are students at Monash University, the extent of their contribution in percentage terms must be stated:

Name	Nature of contribution	Extent of contribution (%) for student co-authors only
Paul Fitzgerald	Conception and design of study, discussion and interpretation of findings, critical revision of manuscript	N/A
Neil Bailey	Collection and analysis of data, discussion and interpretation of findings, critical revision of manuscript	N/A
Andrew Churchyard	Provision of HD individuals, discussion of study design and findings	N/A
Nellie Georgiou-Karistianis	Conception and design of study, discussion and interpretation of findings, critical revision of manuscript	N/A
Tarrant Cummins	Conception and design of study, analysis of data, discussion and interpretation of findings, critical revision of manuscript	N/A

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work.

Student signature:  **Date:** 12/04/2016

Main Supervisor signature:  **Date:** 12/04/2016

Preamble

The following paper builds upon the findings of pathophysiological changes in HD presented in chapter three by considering genetic modifiers of HD, both in terms of underlying pathophysiology and phenotypic manifestation. This paper investigates genetic associations between the different TMS measures and variation within particular receptor genes in pre-HD and symp-HD participants for the first time. Associations between gene variants and age at onset in symp-HD were also investigated, in order to provide additional insights into phenotypic heterogeneity. Candidate genes were selected based on evidence that they are involved in the key cortico-subcortical pathways activated by specific TMS protocols, and are also known to be affected in HD. Given that CAG repeat length is the major driving force on pathogenic processes and clinical phenotype in HD, and that the healthy controls in our sample were not tested for the CAG expansion, the following paper presents data from pre-HD and symp-HD participants only. Furthermore, the AMT and one of the CSP measures were not included due to strong correlations with other TMS measures, leaving six TMS measures as the focus of this paper.

This chapter addresses an important part of the puzzle in understanding the complex pathway from genotype to phenotype in HD. At this stage, robust evidence for genetic modifiers is lacking and little is known about the functional impact of potential modifiers on pathogenic processes at the core of HD (Arning & Epplen, 2012). Identifying genetic modifiers of HD, whether directly related to pathogenesis or clinical signs, is important for improving diagnostic and prognostic sensitivity, and discovering novel targets for therapeutic interventions.

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Research paper

A *GABBR2* gene variant modifies pathophysiology in Huntington's disease

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HIGHLIGHTS

- Genetic modifiers of pathophysiology in HD have not been previously investigated.
- SNP mapping of GABA and dopamine receptor genes was performed in 29HD participants.
- A putatively functional *GABBR2* variant was associated with cortical excitability.
- *GABBR2*, *GABRA2* and DRD2 SNPs were associated with pathophysiology and age at onset.
- Uncovering genetic modifiers helps to identify novel targets for clinical trials.

GRAPHICAL ABSTRACT

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ABSTRACT

Striatal degeneration in Huntington's disease (HD) causes changes in cortico-subcortical pathways. Transcranial magnetic stimulation (TMS) is a valuable tool for assessing pathophysiology within these pathways, yet has had limited application in HD. As cortico-subcortical pathways are largely mediated by GABA and dopamine receptor genes, understanding how these genes modulate neurophysiology in HD may provide new insights into how underlying pathology maps onto clinical phenotype. Twenty-nine participants with HD underwent motor cortex stimulation, while corticospinal excitability, cortical inhibition and intracortical facilitation were indexed via peripheral electromyography. Single-nucleotide polymorphism mapping was performed across six genes that are known to modulate cortico-subcortical pathways (*GABRA2*, *GABRB1*, *GABBR2*, *DRD1*, *DRD2*, *DRD4*). Genetic associations with six TMS measures and age at onset were investigated. Our hierarchical multiple regression analysis, controlling for CAG and age, revealed that a *GABBR2* variant, predicted to be disease-causative, was significantly associated with corticospinal excitability at corrected levels. A subsequent uncorrected exploratory analysis revealed associations between *GABBR2*, *GABRA2* and DRD2 variants with TMS measures of corticospinal excitability and cortical inhibition in HD, as well as with age at onset. Our

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findings support the notion that uncovering genetic associations with pathophysiological measures and age at onset is an important way forward in terms of generating meaningful biomarkers with diagnostic and prognostic sensitivity, and identifying novel human-validated targets for future clinical trials.

1. Introduction

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by a CAG repeat expansion in the huntingtin (HTT) gene. HD pathogenesis involves cortical and subcortical brain regions, with marked loss of dopamine-innervated GABAergic striatal cells [43]. Striatal degeneration disrupts activity in cortico-subcortical pathways and causes a constellation of motor, cognitive and psychiatric symptoms [8]. Individuals with HD show heterogeneity in phenotypic manifestation, thought to be modified by environmental and genetic factors [45]. Numerous genetic factors are known to influence cellular function and plasticity, and are likely to drive inter-individual differences in response to pathogenic changes in HD [42]. Accordingly, single-nucleotide polymorphisms (SNPs) associated with age at onset (AO) have been reported in the HD literature. However, none have been adequately replicated or understood in terms of their functional significance. A better understanding of how genetic variation modulates neuronal function may help tease apart the complex biobehavioral relationships in HD. Further, identification of additional genetic modifiers of the HD phenotype will enhance prediction of disease onset and may provide new targets for therapeutic interventions based on genetic substrates for underlying pathology [17].

Given the body of evidence detailing neurophysiological decline in HD [25,28], the investigation of genetic modifiers using transcranial magnetic stimulation (TMS) may help bridge the gap between genotype and phenotype. While neuronal function can be assessed non-invasively and with good spatio-temporal resolution using TMS, it has had limited application in HD to date [33]. Following TMS to motor cortex, amplitudes of motor-evoked potentials (MEPs) can be recorded through electromyography (EMG) in a peripheral muscle, and manipulated via changes in stimulus intensity and other parameters. Primary measures of corticospinal excitability following TMS include the resting motor threshold (RMT) and the slope of the recruitment curve (SRC), which shows the relationship between stimulus intensity and MEP amplitude [11]. Both measures reflect net excitability and depend on numerous factors, including neurotransmission across synapses mediated by various receptor types [14]. The duration of post-excitatory inhibition, which is produced by corticospinal and intracortical inhibitory mechanisms and primarily mediated by $GABA_B$ receptors, can be indexed by the cortical silent period (CSP; [19]). Several additional measures can be generated by paired-pulse TMS techniques, which activate distinct neurotransmitter systems [46]. For example, short-interval cortical inhibition (SICI) is mediated by $GABA_A$ receptors, while long-interval cortical inhibition (LICI) depends on $GABA_B$ neurotransmission [16]. Importantly, there is substantial interaction between neurotransmitter and neuromodulator systems. Indeed, paired-pulse measures may be disturbed in participants with disorders associated with dopaminergic dysfunction, including Parkinson's disease [6]. As such, TMS is a valuable tool for probing the function of neurons within targeted cortico-subcortical pathways to understand the pathophysiology of neurological disorders, including HD [21].

Furthermore, genetic variants of some neurotransmitters (e.g., GABA) and neuromodulators (e.g., dopamine), and their receptors (e.g., $GABA_B$ and D_1), may affect corticospinal plasticity [9]. In addition, variation of dopamine D_2 receptors may affect their capacity to modulate intracellular calcium levels in striatal cells [5]. Thus, genetic variation may influence neurophysiological responses, as indexed by TMS, and the pathogenesis of neurological conditions. In line with this proposal, a study that administered transcranial direct current stimulation to the prefrontal cortex of healthy participants, during performance of an executive task, demonstrated differential responses according to variants of the COMT gene [which codes for an enzyme involved in dopamine regulation; [35]]. As such, the investigation of genetic variation can help elucidate the complex relationships between brain function, stimulation effects and behavior, and offer important new insights in our understanding of inter-individual differences in HD.

In this study, we build upon strong evidence for the modulation of corticospinal excitability and neuroplasticity by GABAergic and dopaminergic receptors to predict, a priori, targets for genetic association. We investigated genetic associations with responses to different TMS protocols in premanifest (pre-HD) and symptomatic HD (symp-HD), and their associations with AO in symp-HD. Six TMS measures were investigated, specifically: RMT, SRC, CSP, SICI, LICI and intracortical facilitation. SNP mapping was performed across six candidate genes, namely: *GABRA2*, *GABBR1*, *GABBR2*, *DRD1*, *DRD2* and *DRD4*. The influence of genotypic variation at each SNP on TMS measures and AO was assessed with dominant regression models. A primary analysis investigating only the putatively functional variants likely to be disease-causative was followed up with an exploratory analysis including all SNPs.

2. Materials and methods

2.1. Participants

The total sample comprised 16 pre-HD and 13 symp-HD individuals. An independent-measures *t*-test revealed that the symp-HD group was significantly older than pre-HD. There were no group differences in gender or caffeine consumption. The symp-HD participants, however, had significantly fewer years of formal education. Participants underwent HTT gene testing and CAG repeat lengths in the expanded alleles ranged from 38 to 47. Participants were also clinically assessed by a neurologist (A.C.) and underwent a Unified Huntington's Disease Rating Scale (UHDRS) motor assessment. Inclusion in the pre-HD group [40] required a UHDRS total motor score <5 (range= 0–2). Estimated years to clinical onset for pre-HD participants ranged from 0 to 44, according to a widely-used formula [22]. Symp-HD participants had UHDRS motor scores ranging from 5 to 30 and duration of diagnosed disease between 1 and 12 years; AO ranged between 40 and 63 years. Disease burden scores were also calculated [30] and were significantly higher for symp-HD.

Participants underwent rigorous screening prior to recruitment. All participants were right-handed, of Caucasian background and free from brain injury, neurological and/or severe diagnosed psychiatric conditions other than HD. Participants remained on their normal medication regimen, which included reuptake inhibitor antidepressants (n = 7), anxiety/mood stabilizers (n = 2) and neuroleptics (n = 2). The study was approved by the Monash University Human Research Ethics Committee and informed consent was

Table 1
Demographic and clinical data for each group in the final sample.

Measure	Pre-HD (n = 15)	Symp-HD (n = 12)	p
	Mean ± standard deviation		
Age	41 ± 8	50 ± 9	<0.01
Gender (M/F)	6/9	7/5	>0.05
Education	15 ± 4	12 ± 3	0.03
Caffeine	2 ± 2	2 ± 2	>0.05
CAG	41 ± 2	43 ± 2	>0.05
Disease burden	230 ± 82	408 ± 65	<0.01
UHDRS motor	0 ± 1	16 ± 7	<0.01
Years to onset	20 ± 11	–	–
Duration	–	4 ± 3	–
Age at onset	–	52 ± 8	–

p = *t*-test/χ^2 results from significance testing of group differences; pre-HD = premanifest Huntington's disease participants; symp-HD = symptomatic HD participants; education = years of formal education; caffeine = typical daily consumption of caffeinated drinks; CAG = number of repeats on the expanded allele; disease burden = score calculated using the Penney et al. [30] formula; UHDRS motor = unified Huntington's disease rating scale (total motor score); years to onset = score calculated using the Langbehn et al. [22] formula; duration = years since diagnosis of symp-HD.

obtained in accord with the Helsinki Declaration. Demographic and clinical details for the final sample are presented in Table 1.

2.2. Genotyping procedure

Saliva samples were obtained using the Oragene-DNA self-collection kit (DNAgenotek, Ontario, Canada). Genomic DNA extraction from saliva was successful for all samples. SNPs were selected from GABA and dopamine receptor genes (*GABRA2*, *GABBR1*, *GABBR2*, *DRD1*, *DRD2*, *DRD4*). Haplotype-tagging SNPs were chosen to provide >80% coverage of each gene, using the HapMap project database of European Caucasians. Selection criteria for haplotype-tagging SNPs were a minor allele frequency of >0.1 and a correlation to surrounding known variants of r = 0.9. The criteria resulted in the selection of 161 SNPs. Genotyping was performed by the Australian Genome Research Facility (AGRF: St Lucia, Queensland). Eighteen SNPs failed the AGRF's quality control protocol. Further quality control procedures led to the removal of two participants with successful genotyping rates <80% (all other participants: >98%). Exclusion criteria for the removal of SNPs included SNP call rates (across samples) <90% and deviation from Hardy–Weinberg equilibrium. Only one variant was removed under these criteria. Thus, 142 SNPs from six genes remained, with a final sample of 27 participants (see Table 1). The Combined Annotation Dependent Depletion (CADD, v1.2; 20) tool was used to quantitatively prioritize variants highly likely to be functional and disease-causative (CADD score > 10, top 10% most deleterious variants genome-wide). Based on these results, our primary analysis, which corrected for multiple comparisons, investigated genetic associations between 16 putatively functional variants and both TMS measures of pathophysiology and AO. This analysis was followed up with an uncorrected exploratory analysis that included all 142 SNPs.

2.3. TMS administration and measures

Biphasic TMS pulses were administered to left primary motor cortex via a hand-held, 70 mm figure-of-eight coil, using a MagVenture MagPro X100 stimulator (Farum, Denmark). The coil was held tangential to the scalp, with the handle angled backwards and 45° away from the midline. EMG activity was recorded from the right abductor pollicis brevis muscle, using self-adhesive electrodes in a tendon-belly montage. The site of optimal motor response was located that produced the largest MEP at a modestly suprathreshold intensity. This position was marked on the scalp and used throughout the TMS procedures. Participants were seated in a comfortable armchair with a headrest, with their eyes open and hand resting on a pillow. Participants were asked to refrain from consuming caffeine. No adverse events were reported following TMS. EMG was recorded through commercially-available software (LabChart 7, ADInstruments, Bella Vista, New South Wales), and signals were amplified (×1000), band-pass filtered (low-pass = 1000 Hz, high-pass = 10 Hz) and digitized (10 kHz).

Six TMS measures were investigated, specifically: RMT, SRC, CSP, SICI, LICI and intracortical facilitation. The RMT was defined as the minimum stimulation intensity required to evoke a peak-to-peak MEP > 50 μV in at least five of ten consecutive trials [38]. To calculate the SRC, corticospinal excitability at rest was determined through 12 pulses administered at four suprathreshold intensities (110, 120, 130 and 140%RMT) in a pseudorandomized manner with a 10 s inter-pulse interval. Active motor threshold (AMT) was defined as the minimum stimulation intensity required to produce an MEP of approximately 200 μV in at least five of ten trials during voluntary muscle contraction. The CSP was determined in the active muscle through 12 suprathreshold stimuli (140%AMT) administered in a single train with a 10 s inter-pulse interval.

A paired-pulse TMS paradigm investigating SICI and intracortical facilitation consisted of a subthreshold (80%RMT) conditioning stimulus followed by a suprathreshold (120%RMT) test stimulus in the resting muscle. This paradigm involved a pseudorandomized train of 60 trials with a 10 s inter-pulse interval, comprising 20 paired-pulse with a 3 ms inter-stimulus interval, 20 paired-pulse with a 10 ms inter-stimulus interval and 20 single-pulse TMS at 120%RMT. A paired-pulse TMS paradigm investigating LICI was administered separately and involved a pseudorandomized train of 150 trials with a 5 s inter-pulse interval. This paradigm comprised 75 single pulses at 120%RMT and 75 paired-pulses with both conditioning and test stimuli at 120%RMT and a 100 ms inter-stimulus interval.

2.4. Statistical analysis

A non-randomized cross-sectional design was employed. Relatively small samples in previous case/control TMS studies of HD [e.g., n = 11; [1]] have revealed medium-to-large effect sizes [see also Refs.: [26,27,36,37,39,41]]. Moreover, genetic modifiers of AO from previous studies have also shown medium-to-large effect sizes [2,4]. Thus, our sample size of 29 participants should be ample to reveal the effect sizes typically observed in these cohorts. Notably, this sample size represents the largest sample of HD participants studied with TMS to date and is the first to consider genetic associations with pathophysiological measures.

EMG data was examined offline using LabChart 7. Trials were excluded if baseline muscle activity was >40 μV prior to TMS at rest. The AMT was measured to determine the stimulus intensity for the CSP protocol and was not included as a separate variable because it is strongly related to RMT [44]. CSP duration was measured as the time between MEP onset and resumption of voluntary activity, and average peak-to-peak amplitudes of MEPs were determined for the other measures. Corticospinal excitability was expressed as a function of the difference between average MEP amplitudes at 110 and 140%RMT. Degree of inhibition and facilitation was expressed as a function of the average MEP amplitude generated by paired-pulse TMS, compared to that generated by the test stimuli alone (whereby negative values signify inhibition and positive signify facilitation). Distributions of TMS measures were assessed for normality and log-transformed where appropriate.

The primary analysis involved the investigation of genetic associations between each putatively functional variant with TMS

measures and AO (corrected for multiple comparisons). This was followed by an uncorrected exploratory analysis that involved all SNPs. Numerous variants showed low minor allele homozygote counts, thus dominant regression models were employed. Participants were classified as possessing either 0|1 or 2 copies of the common allele for each SNP. Separate two-step hierarchical multiple regression analyses were conducted to investigate the associations between each variant and TMS measures. For each TMS measure, CAG and age were entered at step 1 and the SNP was entered at step 2. Additionally, separate two-step hierarchical multiple regression analyses were conducted to investigate the associations between each variant and AO in symp-HD participants. For these models, CAG was entered at step 1 and the SNP was entered at step 2.

For the primary analysis, the threshold for statistical significance was determined through SNP spectral decomposition and matrix spectral decomposition [12,24,29]. Briefly, the effective number of independent functional loci was determined across all genes (Meff[]/Meff[]= 15) and the effective number of independent variables was determined for TMS measures (Veff[]/Veff[]= 5). For the regression analyses involving TMS measures, the critical significance threshold was calculated as 0.05/(Meff[]/Meff[] × Veff[]/Veff[]: 75), resulting in an adjusted $\alpha = 0.0007$. For the AO analyses, the critical significance threshold was calculated as 0.05/Meff[]/Meff[], resulting in an adjusted $\alpha = 0.003$. Lastly, the threshold for statistical significance in the exploratory analysis including all SNPs was corrected only for the number of effectively independent variables (but not for the genetic markers). For the TMS measure analyses, this resulted in an adjusted $\alpha = 0.01$, and for the AO analyses an $\alpha = 0.05$.

3. Results

3.1. Genetic associations with TMS measures

Results of the primary analysis involving functional variants and TMS measures revealed that one SNP, rs117809969 from *GABBR2*, was significantly associated with the SRC (log transformed) at corrected significance levels, after accounting for the influence of CAG and age ($\Delta R^2 = 0.31$, $p = 0.0002$, standardized $\beta = 0.59$). Carriers of the uncommon C allele for this variant (genotype frequency = 0.21: all were TC heterozygotes) exhibited reduced corticospinal excitability (estimated marginal mean ± standard error: 0.06 ± 0.35, $\eta^2 = 0.44$) compared with common TT homozygotes (1.67 ± 0.18). The CADD tool revealed a scaled C-score of 10.43 for this SNP, thus it is highly likely to have a functional impact on either the protein product of the gene or its regulation [20].

In the exploratory analysis, several other SNPs from *GABBR2*, as well as *GABRA2* and *DRD2* variants, showed significant associations with at least one of the TMS measures (the SRC, SICI and LICI) at $\alpha = 0.01$ (see Table 2). CADD scores indicated that three of these variants, all from *GABBR2*, were predicted to have a functional impact (rs10121587: scaled C-score = 14.22; rs2900512: 17.91; rs7847809: 19.86).

3.2. Genetic associations with age of onset

AO was log-transformed due to its known curvilinear relationship with CAG [45]. Consistent with previous studies including a similar range of repeats, CAG explained 42% of the variance in AO at step 1. No significant associations between putatively functional SNPs and AO were observed in the two-step hierarchical model at corrected levels (adjusted $\alpha = 0.003$).

In contrast, a number of SNPs from *GABBR2*, *GABRA2* and *DRD2* showed significant associations with AO at $\alpha = 0.05$ in the exploratory analysis (see Table 2). One of these variants, rs10986125 from *GABBR2*, was predicted to have a functional impact (scaled C-score = 17.47).

4. Discussion

For the first time, the present study sought to investigate genetic associations between variation in GABA and dopamine receptor genes with TMS measures and AO in HD. Our results showed that the *GABBR2* gene variant rs117809969, predicted to be disease-causative, significantly influenced corticospinal excitability (i.e., the SRC measure). No other significant associations were observed amongst the putatively functional SNPs. However, the subsequent uncorrected exploratory analysis revealed that a number of *GABBR2*, *GABRA2* and *DRD2* gene variants were associated with at least one of the TMS measures (the SRC, SICI and LICI), and with AO. The implications of these novel findings are three-fold. Firstly, neurotransmission via $GABA_B$ receptors may be crucial for the regulation of corticospinal excitability, and could represent a therapeutic target for new interventions in HD. Secondly, genotypic variation in GABA and dopamine receptor genes may affect the progression of HD and development of symptoms, indicating that neurotransmission in such circuits might be central to HD pathogenesis. Thirdly, genetic variation may explain some of the inter-individual heterogeneity in TMS responses and phenotypic manifestation.

The association between a $GABA_B$ receptor gene variant and corticospinal excitability is unexpected given the lack of robust associations between GABA receptor variants and measures of cortical inhibition. However, altered function within GABAergic pathways might influence measures of net excitability by disturbing the balance or synchronization of facilitatory and inhibitory processes [10]. Furthermore, striatal neurons express both GABA and dopamine receptors, and neurotransmission of GABA may be modulated by dopamine receptor activity [31], suggesting that these systems are likely to be related in HD: indeed, this notion was supported by our exploratory analysis. The SRC is able to capture the distribution of excitability within the corticospinal motor system, reflecting the discharge probability of individual neurons, recruitment of neurons with larger motor unit potentials and increasing synchronization of discharges [14]. In line with this, $GABA_B$ receptors are located pre- and post-synaptically and may modulate glutamatergic inputs [13]. It is possible that the lack of strong associations between *GABBR2* and other TMS measures is due to the inability of these measures to capture the complex modulatory role of $GABA_B$ receptors. There is some evidence that the RMT is increased and the SRC, SICI and LICI are decreased in pre-HD and symp-HD, suggesting that overall corticospinal excitability might be decreased [32,33]. Lower SRC in carriers of the uncommon C allele of rs117809969 could be caused by numerous factors, including fewer excitable cells, axonal changes or altered synaptic neurotransmission [18,23]. However, the fact that this SNP is likely to have a disease-causative impact suggests that altered $GABA_B$ receptor function might be driving the association. Signal transduction by $GABA_B$ receptors requires the heteromeric assembly of two subunits [7], thus genetic variation in *GABBR2* could impact normal transmission and thereby influence TMS measures. Indeed, previous research has shown that point mutations in the $GABA_B$ receptor 2 subunit can affect G-protein activation [34]. Despite this, it remains unclear from the present results which pathogenic processes are responsible for the SRC differences. Future TMS studies with concurrent neuroimaging techniques, such as TMS-EEG, would be beneficial for measuring the function of targeted neural circuits with greater spatio-temporal resolution. Moreover, longitudinal studies will be necessary to

Table 2
Results from the exploratory analysis showing significant associations[a] of gene variants with TMS measures and age at onset in HD participants.

Gene	C-score[b]	SRC	SICI	LICI	AO
GABRA2	>10	–	–	–	–
	<10	rs1442060	–	–	rs13152740 rs17537141 rs3822051
GABBR2	>10	rs10121587 rs11789969 rs2900512 rs7847809	–	–	rs10986125
	<10	rs10760389 rs1182885 rs12236069 rs16917019 rs2095121 rs2418085 rs2808534 rs2808539 rs518655 rs585325 rs648085 rs884886	rs10760389 rs117893322 rs2095121	rs774342	rs10125760 rs1930135 rs2779543 rs513763 rs642771 rs7020190 rs7037275 rs812855
DRD2	>10	–	–	–	–
	<10	rs7131056	–	–	rs4936270

GABRA2 – $GABA_A$ receptor alpha 2; *GABBR2* – $GABA_B$ receptor 2; *DRD2* – dopamine receptor D_2; SRC – slope of the recruitment curve; SICI – short-interval cortical inhibition; LICI – long-interval cortical inhibition; AO – age at onset.

[a] For the exploratory genetic associations with transcranial magnetic stimulation (TMS) measures an $\alpha = 0.01$ (0.05/VeffVeffLi) was used. For the exploratory genetic associations with age at onset an $\alpha = 0.05$ was used.

[b] Variant highly likely to have a functional disease-causative impact if scaled C-score > 10 [20].

investigate how pathophysiology changes with each genotype and as the disease progresses.

To further examine phenotypic heterogeneity in HD, we investigated whether genetic variation of receptors involved in TMS-activated cortico-subcortical pathways modified AO. Previous research has reported genetic modifiers of AO within dozens of genes [3]. Our finding regarding the potential modulation of AO by *GABBR2*, *GABRA2* and *DRD2* SNPs is novel. Given the central involvement of dopamine-innervated GABAergic cells in corticostriatal function, it is not surprising that variation of these genes is implicated in modifying disease progression in HD. Indeed, alterations in $GABA_B$ receptor function have been associated with various neuropsychiatric disorders, including epilepsy and schizophrenia [15]. Although functional analysis suggested that only one of these variants (rs10986125 from *GABBR2*) is likely to be functional, the underlying mechanisms driving the additional associations remain unknown. Regardless, genotypic stratification of participants in future studies may be advantageous to further investigate phenotypic heterogeneity. These findings highlight the potential role of intracortical pathways in contributing to symptomology, and underline the importance of exploring genetic associations to understand pathophysiological mechanisms in HD.

There are several limitations that should be considered. For example, many of the expected associations did not show significance. There is strong evidence from prior research that SICI and LICI are mediated by $GABA_A$ and $GABA_B$ receptors, respectively [16]. The lack of significant results could be reflective of inadequate power. Furthermore, while our analyses with the putatively functional variants are corrected for multiple comparisons, our exploratory analyses across all SNPs are not and must be interpreted with caution. Future studies should employ larger sample sizes as this may allow for well powered additive analyses. They may also benefit by stratifying the sample by gender/ethnic background. Notably, the present sample size is greater than previous studies using TMS in HD and our results suggest important new directions for further research. The range of responses to TMS may have been limited due to the lack of a control group. Furthermore, findings may be more robust in future studies if the full range in CAG repeat length is captured. Lastly, it is possible that medications affected responses to TMS, a factor we did not control for.

In sum, we have demonstrated that investigating genetic variation in genes other than *HTT* has potential utility for understanding the pathogenic mechanisms underpinning neurophysiological differences and variation in AO in HD. In particular, the association between a *GABBR2* gene variant and corticospinal excitability is significant and novel. Importantly, these findings offer new research avenues that might help to bridge the gap between genotype and phenotype. However, the exact mechanisms that operate to alter neurophysiology, and potentially trajectories of disease progression, remain unclear. Identifying gene variants that might modulate relationships between pathogenic mechanisms, neurophysiological responses and clinical severity is potentially beneficial in terms of establishing new targets for pharmaceutical interventions, identifying TMS measures sensitive for use as endophenotypic biomarkers, selecting participants for inclusion in clinical trials and developing individualized treatments.

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Conflict of interest

Prof Fitzgerald is supported by a NHMRC Practitioner Fellowship, and has received equipment for research from MagVenture, Brainsway and Neuronetics. The authors have no further conflicts of interest to declare.

Author contributions

April Philpott—research project: conception, organization, execution; Statistical analysis: design, execution; Manuscript

preparation: writing of the first draft, review & critique. *Paul Fitzgerald*—research project: conception, organization: Statistical analysis: design, review & critique: Manuscript preparation: review & critique. *Neil Bailey*—research project: organization, execution: Statistical analysis: design, execution, review & critique: Manuscript preparation: review & critique. *Andrew Churchyard*—research project: conception, organization, execution: Manuscript preparation: review & critique. *Nellie Georgiou-Karistianis*—research project: conception, organization: Statistical analysis: design, review & critique: Manuscript preparation: review & critique. *Tarrant Cummins*—research project: conception, organization: Statistical analysis: design, execution, review & critique: Manuscript preparation: review & critique. All authors have approved the final article.

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Chapter six

Preamble

While the previous three chapters focused on TMS administered to the motor cortex, with responses measured using EMG, the following discussion broadens the scope of research through its consideration of the TMS-EEG technique in HD. For the first time, we attempted to investigate cortical inhibitory function in HD participants following prefrontal stimulation, and compare this to cortical inhibition within the motor cortex. There are a myriad of analyses that could be conducted with this novel and rich TMS-EEG data. However, the overarching aim was to determine whether TMS-EEG was a sensitive tool for investigating pathophysiology in HD, in motor and non-motor cortices, and whether such measures were associated with the development of symptomology. Thus, the investigation was originally focused on TEPs measured from the motor cortex and DLPFC, and also their clinical, neurocognitive and psychiatric correlates.

Unfortunately, there were significant issues with the quality of the EEG data that was collected. This was caused by an unforeseen technical problem despite initial checking of data quality. This meant that the data could not be analysed for this thesis. Various methods were attempted to recover the data, which will be described below, but these were not sufficiently reliable or valid. As such, the following chapter provides a brief literature review and rationale for the intended study. It describes the data analysis techniques that were attempted and considers future directions for studies of this nature.

Literature review

LICI is a paired-pulse TMS technique in which the application of a conditioning stimulus 100 msec prior to a test stimulus leads to suppression of the MEP (Valls-Solé et al., 1992). It is well-established that LICI is mediated by $GABA_B$ receptors and may be affected in several neurological disorders, including Parkinson's disease and dystonia (Berardelli et al., 2008). $GABA_B$ receptors require the heteromeric assembly of two subunits in order to

activate second messenger systems, producing a wide-ranging regulatory influence (Kaupmann et al., 1998). Following post-mortem investigations of brain protein levels, $GABA_B$ receptor function has been found to be disturbed in a number of neuropsychiatric disorders, including bipolar disorder, major depression and schizophrenia (Fatemi, Folsom, & Thuras, 2011). Taken together, these findings suggest that the activity of $GABA_B$ receptors within neural networks is important for normal brain function.

While TMS-EMG can provide insight into pathophysiology in the motor system, an increasing focus for research has been on neurophysiology in non-motor regions. Accordingly, TMS-EEG is a novel technique capable of investigating the function of neurons in diverse brain regions with increased spatio-temporal sensitivity (Premoli et al., 2014). Recent technological advances have largely overcome the complications that prevented earlier use of the technique (Rogasch, Thomson, et al., 2014). For example, traditional EEG amplifiers were saturated by the TMS pulse, which meant that neural signals could not be recovered for several hundred milliseconds after the pulse. This problem can be addressed with a number of hardware solutions, including “sample-and-hold” circuits, which block the large voltage peaks and prohibit any residual voltage from being collected (Virtanen, Ruohonen, Näätänen, & Ilmoniemi, 1999). TMS-EEG is able to assess time-varying TMS-evoked activations at particular oscillatory frequencies, allowing for the causal investigation of functionally interconnected networks (Miniussi & Thut, 2010). Importantly, TMS protocols established for motor cortex assessments have been validated for use in non-motor regions (Fitzgerald et al., 2008). For example, LICI may be generated following stimulation of the DLPFC and such measurements correlate with motor cortex LICI (Farzan et al., 2010b). A number of methods have been suggested to localise the DLPFC for stimulation but the most practical and reliable method (in the absence of MRI coregistration) involves stimulation at specific electrodes of the EEG cap (Fitzgerald et al., 2009).

The DLPFC has been demonstrated to show structural and functional changes early in HD progression (Georgiou-Karistianis, Poudel, et al., 2013; Wolf et al., 2007). While some

changes decline longitudinally and are associated with the development of symptomology, others appear to reflect compensatory mechanisms in response to HD pathogenesis (Poudel et al., 2013; Rosas et al., 2005). Similarly, LICI is affected early in HD progression; it seems to be the only TMS measure affected in pre-HD participants and is associated with the length of the CAG repeat as well as psychiatric and behavioural symptoms (refer to chapter three; Philpott, Cummins, et al., 2016). Moreover, there is further evidence of HD-related EEG deficits, using various approaches, including reduced alpha band power during memory activation in pre-HD (Van Der Hiele et al., 2007). Painold et al. (2011) also reported increased prefrontal delta power in symp-HD, together with a global decrease of alpha and theta power, which was correlated with increasing motor and cognitive decline. Such power changes are usually interpreted to reflect an overall slowing of oscillatory activity due to the disruption of cortico-subcortical circuitry, and may therefore have utility as sensitive biomarkers in HD (Beste et al., 2007; Nguyen et al., 2010; Painold et al., 2010; Van Der Werf, Sadikot, Strafella, & Paus, 2006). Indeed, oscillatory activity within the gamma band is thought to be a consequence of GABA-mediated interneuronal function (Whittington, Traub, Kopell, Ermentrout, & Buhl, 2000). Further, associations between gamma activity and DLPFC-mediated cognitive abilities are well-established (Cummins, Broughton, & Finnigan, 2008). Finally, investigation of genotypic variation within *GABBR2* (the gene coding for the $GABA_B$ receptor subunit 2) revealed that HD individuals carrying rare alleles for particular SNPs exhibited reduced corticospinal excitability and earlier age at onset (refer to chapter five). In sum, these findings indicate that altered function within $GABA_B$-mediated frontostriatal networks may be associated with the pathogenesis, pathophysiology and phenotypic manifestation of HD, which might reflect an underlying mechanism driving the genotype-phenotype relationship.

On balance, these lines of evidence suggest that $GABA_B$-mediated cortical inhibition in the DLPFC may be impaired early in HD, yet to our knowledge, it has not been investigated to date. The present study was broadly modelled on previous TMS-EEG

research conducted by members of our group (Daskalakis, Farzan, Barr, Rusjan, et al., 2008; Farzan et al., 2010a). We sought to determine whether LICI generated from the DLPFC is affected in pre-HD and symp-HD participants, and whether prefrontal pathophysiology is related to the development of symptomology.

Data analysis and technical difficulties

The sample for this aspect of the study comprised 41 participants, consisting of 14 pre-HD (age range=26-54 years, 5 males), 12 symp-HD (age=43-69, 7 males) and 15 healthy controls (age=26-57, 6 males). The reduced sample size was a result of a high RMT (n=3) and technical issues (n=2). The motor cortex LICI protocol was administered before the DLPFC LICI. Participants were given a trial of prefrontal stimulation before the DLPFC LICI protocol and five further participants (pre-HD: n=1; control: n=4) withdrew at this stage due to discomfort (e.g., excessive facial muscle activation).

Initially, when setting up the TMS-EEG protocol and collecting pilot data, there were some concerns about the artefact that was being generated. This artefact was time-locked to the TMS pulse, but was far larger than the typical artefact generated by TMS-EEG (Veniero, Bortoletto, & Miniussi, 2009). A number of approaches were attempted to remove or reduce the artefact in the data before commencing data collection. Such approaches included altering the recharge delay of the TMS machine, and holding a piece of foam between the TMS coil and the EEG cap in order to buffer the electrodes from direct contact with the electromagnetic activity generated by the coil (Ilmoniemi & Kičić, 2010; Rogasch, Thomson, et al., 2013). These methods were not found to be effective.

However, when analysing the files from the first five participants that were tested, it was found that the usual methods of independent component analysis correction were able to remove the artefact and leave analysable data (Hernandez-Pavon et al., 2012; Rogasch, Thomson, et al., 2014). Typically, two runs of an independent component analysis are performed to remove the artefact generated by the TMS pulse from the EEG data, so that the data that remains after this process reflects only brain activity. The first run removes the

large muscle artefact related to the TMS pulse, while the second run removes other typical EEG artefacts (e.g., eye blinks) and decay artefacts from the TMS pulse. Therefore, data collection was continued until the desired sample size was reached.

Unfortunately, subsequent to ascertaining the quality of the data, another more severe artefact began to be generated that was not detected online as being a different form of artefact. This square, step-like artefact was present through the majority of the files collected thereafter, and was of a semi-consistent shape but with inconsistent timing and size. Similar 'step-wise' EEG activity is observed when the reference or ground electrodes are not plugged in or are faulty. Without an adequate reference or ground electrode, EEG data shows significant drifts and step-like jumps in voltage because electrical voltages are measured as the difference between two points (Light et al., 2010). In addition to this issue, most of the electrodes were significantly shifted from the zero point, making the data more difficult to analyse as the usual analysis method does not typically cater for significant deviations from a baseline of zero (Rogasch, Thomson, et al., 2013). As such, the usual methods of artefact rejection for TMS-EEG data did not work.

A series of alternative methods were then attempted to recover the data to an analysable condition. These custom methods focused on the continuous data from each component generated, as opposed to the topography, frequency spectrum and averaged epoch, which more easily allowed for the determination of which components were reflective of artefact. However, even after removing all the components that seemed to be affected by the artefact (frequently more than half of all components), the artefact still remained in the next run of analysis. This seemed to be due to the scale of the artefact; it was so large that although some components were apparently unaffected upon first inspection, they were affected after removing the largest artefacts. Indeed, the artefact was still present after a third and fourth run of independent component analysis were performed. Average re-referencing the data, extracting different epochs and baseline correcting the data in alternate ways also did not resolve the artefact (Litvak et al., 2007).

Lastly, a novel method was attempted, which involved cutting out the data contained in the ‘step’ of the artefact (the section where the data showed a large jump in voltage), baseline correcting the non-shift data and interpolating the two remaining sections together (Virtanen et al., 1999). However, the artefact was too complex and variable (across electrodes, epochs and participants) to be able to apply any consistent procedures. Furthermore, data analysed using this method may not have been valid, as the method considerably manipulates the data and has never been used before.

Therefore, it was decided that the data collected for this study could not be analysed in a sufficiently reliable and valid manner. Each of the methods tested took a considerable amount of time, as a new analysis script had to be written each time, then the manual parts had to be processed and the results of the analyses examined across a number of participants. As such, it took several months of work before we could confidently ascertain that the data could not be retrieved.

Concluding statements

In general, it is impossible to be certain about the origin of the artefacts in the data after the data has been collected. However, if one of the ground or reference electrodes is not plugged in, the EEG activity can jump and vary considerably because it has no reference point as a baseline. Given that the electrical impedances were monitored using Curry 7 before and during data collection, we can conclude that the reference and ground electrodes were plugged in.

Therefore, a workable conclusion to explain the extreme artefact in this data seems to be that the jumper cable was faulty. This cable is responsible for transmitting the signal from the reference electrode plug to the reference electrode socket in the EEG amplifier and is essential to obtain high quality and analysable data. Furthermore, if this cable had an intermittent fault, the artefact that was recorded in this study might have resulted. Regular testing of TMS-EEG equipment is not habitually carried out as this would be too time-consuming because there are so many variables that could show faults. For instance, each

individual electrode, lead and plug would need to be tested to ensure they are each functioning appropriately. Moreover, poorly functioning components and data quality issues are typically identified and reported by individual researchers with significant experience collecting data of this nature. This did not occur, however, because the present study was the only one being carried out at the facility using the apparently faulty equipment, and there was thus no prior knowledge of this fault.

The technical difficulties we experienced serve to highlight the complexities of this type of research but should not discourage studies from using the TMS-EEG technique in future. With stringent checks in place, including regular quality control of data throughout the collection phase, problems such as these may be avoided. Of benefit for future studies, we found that prefrontal TMS-EEG was generally well-tolerated by HD participants and did not cause any discomfort beyond that reported by healthy control participants. TMS-EEG is a valuable methodology for investigating pathophysiology in non-motor brain circuits in HD, and other neurological disorders, given the direct, objective and spatio-temporally sensitive nature of the data. It remains to be seen whether the cortical inhibitory deficits measured from the primary motor cortex in pre-HD and symp-HD (refer to chapter three; Philpott, Cummins, et al., 2016) also exist in the prefrontal corticostriatal circuits. This is of particular interest in the context of frequent DLPFC-mediated cognitive and behavioural disturbances in HD individuals and the limited insight at present into pathophysiological mechanisms in this regard.

Chapter seven

Summary and interpretations

Overview of findings

In HD research, TMS represents a novel, data-rich and valuable, yet vastly under-utilised, tool. This thesis presents new and important findings that will inform and facilitate future research in this field. We sought to investigate the pathophysiological deficits in pre-HD and symp-HD participants, across a number of TMS measures of corticospinal excitability, cortical inhibition and intracortical facilitation, and their associations with clinical severity, neurocognitive performance, psychiatric symptoms, sex differences and genetic variants. TMS was applied to the primary motor cortex and DLPFC, and outcomes measured via peripheral EMG and EEG, in order to investigate neurophysiological changes in two different corticostriatal circuits relevant to HD pathology.

Our findings in chapters three through five predominantly describe possible disturbances associated with GABAergic neurotransmission in cortico-subcortical pathways. Across the three experimental papers, this thesis has shown that GABAergic inhibitory function may be disturbed in pre-HD and symp-HD participants, and this is associated with both the underlying pathological burden and the development of symptomology, but is not modulated by sex differences. Furthermore, we have shown that $GABA_B$-mediated inhibitory function may be the earliest pathophysiological deficit to emerge in HD, and that polymorphic variation in the $GABA_B$ receptor might modulate cortical excitability and the age at onset of HD. Taken together, these findings are highly significant and novel.

Findings from specific TMS protocols

Our findings of pathophysiology in HD indicated that LICI, investigated in the resting muscle, was reduced in both pre-HD and symp-HD, compared with controls. Li and Chen (2015) recently studied motor cortex excitability in participants with hyperglycemic chorea, administering various TMS protocols during states of muscle contraction and at rest. The

authors suggested that LICI, the only TMS measure that was affected in these participants, results from interactions between cortical inhibitory processes and voluntary movement, and might represent a compensatory response to reduce symptoms (Jie-Yuan Li & Chen, 2015). However, our results for premanifest non-choreic individuals, as discussed in chapter three, seem to contradict those of the aforementioned study. Our findings, in line with those of Gu et al. (2005), may imply that cortical inhibitory deficits are a more primary pathogenic mechanism in HD, and that their pathophysiology might differ from other movement disorders. Indeed, both LICI and SICI were correlated with the CAG repeat length, and SICI was not modulated by gender, thus supporting the notion they may represent primary pathogenic features of HD. The sensitivity of these two measures to group differences in this sample might also reflect their capacity to isolate a particular aspect of neuronal function. In contrast, the other TMS measures that were investigated reflect more 'global', or generalised, responses (Rossini & Rossi, 2007). As such, they may be less likely to exhibit group differences, particularly in a sample with considerable heterogeneity, as they are dependent on various types of cells and synapses (Curra et al., 2002).

With regards to underlying compensatory processes that might influence TMS responses, the findings in chapter three showed a non-significant trend for the CSP to be prolonged in pre-HD, compared with symp-HD and controls, particularly at the higher stimulation intensity. The RMT also showed a non-significant trend, with slightly lower thresholds in pre-HD, compared with symp-HD and controls. Indeed, our results from a pilot sample of 8 symp-HD, 12 pre-HD participants and 8 controls presented as a poster (see: Philpott, Fitzgerald, Cummins, Churchyard, & Georgiou-Karistianis, 2014) indicated a significantly lower RMT in pre-HD, compared with the other two groups. Whilst tentative trends in the data such as these cannot be interpreted with any level of certainty, they highlight areas for future TMS research. If replicated in larger samples, they could be indicative of compensatory processes in response to early neurodegeneration in pre-HD stages, which might mask symptoms for some time but decline as individuals begin to exhibit

overt symptomology (Klöppel et al., 2009; Papoutsi, Labuschagne, Tabrizi, & Stout, 2014). Another hypothesis is that an excitatory phase may precede neurodegenerative processes in pre-HD (Ljubisavljevic et al., 2013); a suggestion that is not supported by the present findings. Indeed, the gross functional changes observed using MRI and PET techniques in pre-HD participants have been interpreted in terms of compensatory effects and would explain the phenotypic heterogeneity due to inter-individual differences in cognitive reserve (Borroni et al., 2012; Georgiou-Karistianis, 2009; Poudel, Egan, et al., 2014). However, such compensatory effects are observed in many regions outside the primary motor cortex, which suggests they may be better investigated with multimodal techniques, such as TMS-EEG.

Clinical correlates of pathophysiology

Taken together, the findings of this thesis suggest that cortical inhibitory deficits might contribute to the development of HD symptomology, which is particularly novel. Given the correlations between cortical inhibitory deficits and a range of clinical signs described in chapter three, it is likely that similar underlying mechanisms drive both SICI or LICI and the HD phenotype. Indeed, this would be consistent with reports from Simonetta-Moreau et al. (2006) of impaired SICI in participants with focal dystonia. On the other hand, this is in contrast to the idea proposed by Cantello (2002), that TMS responses may vary according to specific symptomology in some neurological conditions. However, we cannot rule out that this suggestion could be the case for the other TMS measures that did not show overall group differences in our sample. We decided to only investigate the clinical correlates of SICI and LICI due to concerns about multiple comparison issues.

The correlations between LICI and psychiatric measures that were presented in chapter three emerged in the opposite direction to what was hypothesised; a counter-intuitive finding that was not discussed in detail. This finding indicated that HD participants who were responding more in line with the control participants actually showed increased psychiatric symptoms, compared with HD participants with reduced LICI. One perspective that could assist with understanding the negative correlations derives from the concept of

‘positive’ and ‘negative’ psychiatric symptoms. That is, symptoms that represent a lack of a normal trait or behaviour, such as those measured by the Frontal Systems Behaviour Scale (i.e., apathy, disinhibition, executive dysfunction), may be expected to operate differently to those signifying the gain of a psychiatric behaviour. As such, the predominance of psychiatric inventories measuring negative symptoms in this thesis represents a methodological weakness. This issue could be addressed in future research so that more meaningful conclusions can be drawn in this regard. Our previous suggestion that the reliance on self-report measures in participants with cognitive impairment likely contributed to the unexpected findings also warrants further consideration.

Genetic modifiers of HD pathophysiology

Our novel approach in chapter five, considering genetic modifiers of pathophysiology in HD, further strengthened the conclusions drawn from chapter three. The genetic findings serve to underscore the suggestion that $GABA_B$ receptor function is intrinsically linked to disease progression and onset in HD. They also provide additional empirical support for the complex interplay between GABAergic and dopaminergic functional regulation of corticostriatal pathways (André et al., 2010). As such, these findings establish an important connection between synaptic transmission, cortical excitability and symptomology in HD, which emerged after accounting for the large influence of the CAG repeat on such variables. Whilst these genetic findings must be interpreted with utmost caution, due to the sample size and candidate gene approach (compared with seminal papers like the GeM-HD Consortium study; Genetic Modifiers of Huntington's Disease Consortium, 2015), this aspect of our study enabled the identification of additional targets for future research into genetic modifiers of HD.

Underlying disease mechanisms

In terms of the mechanisms underlying the cortical inhibitory deficits in HD participants described in this thesis, one possibility is that the effect is caused by altered synaptic transmission of GABA, driven by reduced inhibitory neurotransmitter release, fewer release

sites or disturbed reuptake (Cepeda et al., 2007; Klapstein et al., 2001; Levine, Cepeda, Hickey, Fleming, & Chesselet, 2004; Miller, Walker, Shah, Barton, & Rebec, 2008; Walker et al., 2008). Hypoactivity in terms of cerebral blood flow in frontal cortical regions involved in the motor corticostriatal circuit, as previously demonstrated using PET, is consistent with this notion (Bartenstein et al., 1997; Weeks et al., 1997). This hypothesis implies that the functional integrity of the cortex is central to HD pathogenesis, in addition to the widespread atrophic changes, reflecting findings from prior research (Gu et al., 2005; Spampanato, Gu, Yang, & Mody, 2008; Strand et al., 2007; Zuccato et al., 2001). Indeed, a recent study provided evidence that the trafficking of GABA receptors to synapses in the cortex is disrupted by mutant huntingtin, which significantly reduces the amplitude of post-synaptic inhibitory potentials (Twelvetrees et al., 2010). The latter finding is in keeping with our findings. Furthermore, the altered synaptic transmission of GABA hypothesis is corroborated by our genetic findings in chapter five, indicating that several *GABBR2* variants modulated SICI and LICI in pre-HD and symp-HD participants and that a putatively functional *GABBR2* variant modulated age at onset. Previous work suggestive of compensatory processes in pre-HD stages, together with cognitive reserve paradigms, would also fit with this hypothesis, given that neuroplasticity is primarily driven by changes at the level of the synapse (Pascual-Leone et al., 1999). Our TMS findings thus support the body of MRI and PET research indicating that inter-individual differences in response to early neurodegenerative changes in GABAergic pathways may explain the heterogeneity across functional domains in HD (e.g., Georgiou-Karistianis, Poudel, et al., 2013).

We therefore propose that specific synaptic changes, due to alterations in the function of $GABA_B$-mediated pathways, could explain the pathophysiological findings in our sample. This notion is in conflict with the findings of Schippling et al. (2009), who investigated a similar range of TMS measures of both excitability and inhibition and proposed that axonal changes were more likely to account for HD pathophysiology. It is difficult to reconcile the differences in findings between the Schippling et al. study and our own due to the significant

methodological differences (see chapter three for a more detailed discussion). However, the findings of this thesis, together with other prior research (e.g., J.-Y. Li et al., 2003), indicate that synaptic changes should not be ruled out as a potential disease mechanism in HD. Indeed, our use of a 3 msec inter-stimulus interval for the SICI protocol would likely align with the second phase of intracortical inhibition (Fisher, Nakamura, Bestmann, Rothwell, & Bostock, 2002; Roshan, Paradiso, & Chen, 2003). It has been established that this second phase is associated with synaptic inhibitory processes, as opposed to excitatory or axonal processes, and may be modulated by pharmacological interventions (Chen et al., 2008). Furthermore, given the time courses of different $GABA_B$ effects, our results following 100 msec LICI more likely reflect disturbances of pre-synaptic receptors in early HD; for example, the altered release of synaptic vesicles (Chu, Gunraj, & Chen, 2008). Such disturbances might also explain the later reduction of SICI in symp-HD stages, because LICI may reduce SICI via presynaptic $GABA_B$ autoreceptors (Sanger et al., 2001). Moving forward, our findings provide a possible link between pathophysiological inhibitory changes and the abnormal neuroplasticity that is now well-established in pre-HD and symp-HD from both animal and human research (Crupi et al., 2008; Cybulska-Klosowicz et al., 2004; Höhn et al., 2011; Lorenzano et al., 2006; Lynch et al., 2007; Orth et al., 2010). As Ziemann and Siebner discuss (2008), homeostatic metaplasticity is likely to be associated with motor learning via changes in cortical excitability, a theory which has a significant bearing on future TMS research in HD.

Deficits in cortical inhibitory function are frequently reported in neurological and psychiatric disorders (Radhu et al., 2013). Better characterisation of inhibitory profiles may shed light on the specific pathophysiological deficits underlying various disorders, whether related to axonal, synaptic or other factors. For instance, the fact that LICI was reduced in pre-HD and symp-HD participants, SICI was reduced in symp-HD only and the CSP was not affected in either group in our sample provides some additional evidence to support the dysfunction of particular GABA receptors at different disease stages. It may be that a

$GABA_B$-related impairment specific to LICI is reflective of underlying pathogenic processes in HD, whereas the emergence of reduced SICI in symp-HD stages is associated with a movement-related inhibitory deficit (Stinear & Byblow, 2003). This notion is substantiated by our finding that SICI was no longer significantly correlated with finger tapping task performance after controlling for the UHDRS motor score in HD participants (secondary analyses in chapter three; Philpott, Cummins, et al., 2016). Moreover, CSP is not purely a measure of intracortical inhibition, as the early component also depends on spinal inhibitory mechanisms (Ziemann, 2004). On the other hand, evidence of an altered CSP in Parkinson's disease suggests that this measure might also be contingent upon dopaminergic function, and its modulation of GABA transmission (Berardelli, Rona, Inghilleri, & Manfredi, 1996). Such evidence could explain why we did not find the CSP to be affected in HD. Our findings highlight the fact that future research should not seek to investigate TMS measures in isolation (i.e., from each other, or from other neuroimaging measures), because inferences about pathophysiology may be limited. In addition, this also emphasises the significance of being able to study non-motor corticostriatal circuits in HD, in order to differentiate between changes in cortical excitability specific to the primary motor cortex and those driven by subcortical structures common to other corticostriatal loops.

Implications and future directions

Diagnostic, prognostic and therapeutic implications

Firstly, the findings of this thesis have important implications for pharmacological treatments of HD. For instance, the results presented in chapters three and five indicate that drugs targeting neurotransmission mediated by $GABA_B$ receptors may be able to provide symptom relief and potentially also slow the onset of HD. The findings of chapters three and four further reinforce the central involvement of GABAergic transmission in HD pathogenesis, suggesting that the function of $GABA_A$ receptors is affected later in the disease during symp-HD stages and is not modulated by sex differences. As such, interventions targeting $GABA_A$ receptors may also be beneficial for HD individuals in terms of symptom relief and could

show different efficacies between males and females. Indeed, many of the $GABA_B$ agonists approved for use in various other neuropsychiatric conditions, including pregabalin and phenibut, are not selective for $GABA_B$ receptors and may also exert an influence on $GABA_A$ receptor activity. Therefore, these drugs deserve additional study in the context of neuroprotective and symptomatic HD treatments, especially given the null findings of trials involving anti-excitotoxic drugs to date (e.g., Landwehrmeyer et al., 2007), with particular consideration of their potential side effects on motor and cognitive function across disease stages (Enna & Bowery, 2004). Furthermore, whilst pharmacological treatments targeting GABAergic function are already used in HD individuals in some cases, including baclofen and clonazepam, a better understanding of their mode of action and optimal timing of treatment in each sex may improve their effectiveness. The treatment of psychiatric and behavioural symptoms in particular, related to $GABA_B$-mediated inhibitory function, might have large, clinically-meaningful effects in terms of improving quality of life for people with HD and their families (Roos, 2010).

Given the associations between TMS measures and the motor, neurocognitive and psychiatric symptoms, there is also potential future utility for rTMS techniques to improve HD symptomology. As discussed in chapter one, rTMS may be able to generate lasting changes in neuronal activity and can be employed to pinpoint specific cortico-subcortical pathways with disturbances to the synchronisation or balance of excitatory and inhibitory function (Fitzgerald, Fountain, & Daskalakis, 2006). In fact, previous work has indicated that subthreshold motor cortex rTMS at low (1 Hz) or high (20 Hz) frequencies may increase cortical inhibition in healthy individuals with lower initial levels of inhibition (Daskalakis et al., 2006). This thesis did not seek to specifically build upon preliminary studies showing symptomatic improvements following treatment with rTMS in symp-HD participants (e.g., Brusa et al., 2005). Nevertheless, our findings are promising for the future use of this technique to modulate brain dysfunction in this population and suggest that symptomatic

improvement may result due to metaplastic changes (Mockett & Hulme, 2008; Ziemann & Siebner, 2008).

The second noteworthy implication from this research is generated by the findings presented in chapter five regarding potential genetic modifiers of HD. Our novel investigation of candidate genes coding for GABA and dopamine receptors suggests that genotyping HD individuals for genes other than the HD mutation might be able to provide increased prognostic sensitivity for gene carriers (Philpott, Fitzgerald, et al., 2016). At present, the best prognostic tools that are available, which are not habitually used in clinical settings, involve simple calculations based on the CAG repeat length of the expanded allele and the individual's current age (Langbehn et al., 2004). If prognostic tools were available that had increased accuracy and were based on a number of pathogenic measures, they may be used clinically to allow HD individuals to make more realistic and timely decisions about their future.

We also suggest that this genotyping approach could be employed to identify individuals with particular genetic risk-factors to undergo neuroprotective or early interventional therapies that could slow the onset of HD and prolong the period of relative health (Hersch & Rosas, 2008). Furthermore, a better understanding of the effects that particular gene variants may have on pathophysiology and symptom onset in HD allows for the implementation of more individualised treatments targeting specific underlying deficits. The putatively functional variant rs11789969 from *GABBR2* is particularly worthy of research attention as a potential moderator of pathophysiology in HD, especially given the $GABA_B$-mediated TMS deficits identified in pre-HD and symp-HD participants (Philpott, Cummins, et al., 2016; Philpott, Fitzgerald, et al., 2016). However based on our findings, we cannot determine whether these genetic modifiers of neurophysiology are specific to HD individuals. Therefore, investigation of these candidate genes coding for GABA and dopamine receptors may provide additional insights into pathophysiology in other neurological and psychiatric disorders as well.

TMS measures as endophenotypic biomarkers

The findings of this thesis reinforce the notion that TMS measures could have potential utility as endophenotypic biomarkers in HD. There are several important and desirable features for any biomarker, which have been reviewed in depth elsewhere (e.g., Weir, Sturrock, & Leavitt, 2011). Of note, a biomarker for tracking disease progression in HD should be a relatively stable and consistent characteristic in the general population (Weir et al., 2011). Aylward (2007) further identifies that biomarkers must be predictably related to clinical and functional signs of the disease, as well as the underlying mechanisms of pathology. In terms of sensitively tracking treatment efficacy in future clinical intervention trials, the biomarker would preferably be non-invasive in order to conduct repeated investigations over time. It would also need to show relatively rapid, linear change to ensure that trials remain manageable in both financial and temporal terms (Aylward, 2007; Henley, Bates, & Tabrizi, 2005).

With these recommendations in mind, it seems clear that the possible candidate biomarkers identified in this thesis, namely SICI and LICI, would satisfy most criteria and warrant further investigation in this population. Notably, SICI and LICI were both significantly correlated with clinical signs (i.e., cognitive and psychiatric symptoms), as well as measures of underlying mechanisms of pathology (i.e., the CAG repeat length and disease burden score). Moreover, Bohanna et al. (2008) noted that a set of biomarkers may be more realistic to capture the short- and long-term neurobiological outcomes of interventional therapies. Indeed, this would be relatively straightforward to accomplish using TMS, given that a number of protocols measuring different aspects of neuronal function can be administered within a short amount of time. A set of TMS biomarkers, or ‘cortical signature’, may be necessary in order to ensure that the pathophysiological measures are highly specific to HD pathogenesis and able to track the clinical and functional outcomes over time (Dickerson et al., 2009; Rizk-Jackson et al., 2011).

TMS has several advantages over other neuroimaging methodologies currently employed as biomarkers in HD (Andre, Scahill, Haider, & Tabrizi, 2014). For instance, it is relatively inexpensive, portable, well-tolerated by participants in general, and has superior combined spatial and temporal sensitivity (Philpott et al., 2013). Although measures such as caudate volume have been identified as sensitive markers of neurodegenerative processes in HD, pharmacological interventions would likely take considerable time to show significant effects on subcortical structural MRI (Aylward, 2007, 2014; Henley et al., 2005). In contrast, it is expected that TMS measures would show positive brain changes much faster, as pathophysiological processes may be reversed more quickly (Beste et al., 2013; Esmaeilzadeh, Ciarmiello, & Squitieri, 2011). Compared with other clinical markers, TMS outcomes are also more proximal to the gene product and may generate larger statistical effect sizes, leading to more cost-effective trials due to smaller sample sizes (Tabrizi et al., 2012). Furthermore, SICI and LICI are promising candidate markers of pathophysiology given that they inherently control for inter-individual differences in corticospinal excitability (Orth, Snijders, & Rothwell, 2003).

However, certain complexities must be considered. TMS is likely to activate a number of pathways beneath the coil with widespread effects across the brain, which are not fully captured by EMG measurements. Moreover, HD is associated with a multitude of pathological and compensatory processes causing hypoactivation in some brain regions, with concomitant hyperactivation in others and functional connectivity changes (Bartenstein et al., 1997; Georgiou-Karistianis, Poudel, et al., 2013; Klöppel et al., 2009; Ljubisavljevic et al., 2013; Quarantelli et al., 2013; Thiruvady et al., 2007; Unschuld et al., 2012; Wolf et al., 2011). These non-linear processes contribute to inter-individual heterogeneity and may prohibit finding group differences overall. Such complexities might also explain why, for example, reduced SICI has also been reported in Parkinson's disease, which has contrary effects to HD on the basal ganglia pathways (Ridding, Rothwell, & Inzelberg, 1995). It may be that cortico-subcortical excitability lies in precise balance, and that basal ganglia

impairments invariably cause dysfunction in intracortical inhibitory pathways (Hanajima, Ugawa, Terao, Ogata, & Kanazawa, 1996). Indeed, the $GABA_A$-mediated interneuronal pathways that produce SICI are credited with controlling the flow of cortical activity in both time and space (Hasenstaub et al., 2005). As such, SICI may be inevitably connected with neurocognitive disturbances in human disorders due to the role of the underlying $GABA_A$ receptors in propagating higher frequency signals in the cortex. Nevertheless, such complexities could be overcome with additional research attention in order to ascertain the optimal parameters for measurement of cortical inhibition in pre-HD and symp-HD participants.

Future research avenues

It is suggested that TMS measures of intracortical inhibition, namely SICI and LICI, be further studied to determine their capacity to serve as sensitive and specific endophenotypic biomarkers in HD. Such research should ideally investigate these measures longitudinally, in a larger sample of pre-HD and symp-HD participants, across a number of different conditioning stimulus intensities, inter-stimulus intervals, target muscles and quantity of trials (Fisher et al., 2002). This approach, in conjunction with ongoing studies investigating the reliability of TMS data, may generate increased specificity for these measures for potential future applications as diagnostic biomarkers (i.e., marking progression from pre-HD to symp-HD stages). Indeed, further study of TMS through large-scale longitudinal studies, similar to TRACK-HD, PREDICT-HD and IMAGE-HD, would be ideal in order to map the trajectories of different TMS paradigms in terms of neuroanatomical, cognitive, psychiatric and functional decline. It will be particularly important to include meaningful clinical measures, such as those assessing activities of daily living, in order to ascertain the range of potential benefits for TMS biomarkers in future clinical trials.

Furthermore, clinical, neurocognitive and psychiatric measures should be carefully selected in order to establish whether SICI and LICI are associated with the development of specific symptomology. The TMS measures that did not show group differences in the

present studies also warrant longitudinal investigation with larger sample sizes, because our results may have been underpowered to detect group differences. Reconsidering measures such as the RMT and CSP in studies with greater statistical power might elicit significant group differences suggestive of additional pathological or compensatory processes. This is an exciting area for future research and would provide important additional insights into pathophysiological changes with disease progression.

Although we conducted secondary analyses excluding HD participants taking psychoactive medication, and found that the results were comparable, we cannot rule out the potential impact of these medications on neuronal function. For example, there is some evidence that treatment with selective serotonin reuptake antidepressants normalises GABAergic deficits by increasing SICI and also reducing ICF (Minelli et al., 2010). This may have affected our ability to detect some groups differences on these measures or influenced the other findings and as such, it could be worth excluding participants affected by certain medication types in future studies.

Despite difficulties with our TMS-EEG data, discussed in chapter six, further investigations into the pathophysiological deficits in non-motor brain regions in HD are essential. More work must be done to characterise the pathophysiological profiles of different corticostriatal circuits in HD participants across disease stages. Validation of the TMS-EEG technique in HD would unlock almost the entire brain for investigation with TMS. Based on the findings we report, we cannot verify whether cortical inhibitory deficits in HD represent a primary disease outcome, possibly caused by the dysfunction of interneurons. Alternatively, the possibly that these deficits occur as a secondary consequence of the striatal degeneration, thus leading to similar deficits between cortical regions involved in corticostriatal pathways, cannot be excluded at present. TMS-EEG research in non-motor circuits in HD would therefore be extremely valuable, particularly regarding the emergence of cognitive and psychiatric symptoms and the mechanisms of neuroplastic changes. Indeed, better clinical characterisation of the disease onset would also be important for sensitive

measurement of progression and identification of biomarkers. This is because some individuals present clinically with significant cognitive or psychiatric changes, consistent with HD-related symptomology, before the onset of any overt motor symptoms (Paulsen & Long, 2014). Monitoring of age at cognitive or psychiatric onset in future cohorts will be important and may provide altered views on which TMS measures could represent candidate biomarkers in HD.

Conclusions

In conclusion, TMS is a valuable tool for investigating pathophysiology in HD. Increasing the understanding of neurophysiology in HD is important for bridging the gap between the direct effects of the HD mutation and the eventual manifestation of heterogeneous symptoms. This thesis has interpreted the TMS findings in light of other cortico-subcortical changes in HD, and provided significant new insights into the complex biobehavioural relationships that modulate the pathway from genotype to phenotype. For the first time, we have provided convincing evidence supporting a central role for GABAergic dysfunction at the level of synaptic transmission in pre-HD and symp-HD individuals. Due to the various relationships between TMS measures, pathological burden and symptomology, this thesis has demonstrated that TMS represents a viable methodology for establishing potential sensitive and specific endophenotypic biomarkers in HD.

This line of research offers several new avenues for future research to better understand the pathogenesis of HD from a neurophysiological point of view, and its associations with metaplastic changes and decline across meaningful functional domains. We suggest that further work particularly focuses on establishing a pathophysiological signature based on multiple/multi-modal TMS measures at different disease stages for optimal specificity. It is anticipated that continued research in this area will culminate in increased prognostic accuracy for clinicians working with pre-HD individuals, as well as novel therapeutic targets for use in future clinical trials of neuroprotective and symptomatic treatments.

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