CORTISOL AS A BIOLOGICAL MARKER OF DEPRESSION AND MEMORY
DIFFICULTIES IN THE EARLY STAGES OF HUNTINGTON'S DISEASE

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“The disease is distressing to the one suffering from it, or to (their) friends. As the disease progresses the mind becomes more or less impaired, in many amounting to insanity...sometimes that form of insanity which leads to suicide. While in others, the mind and body both gradually fail until death relieves them of their sufferings. I have drawn your attention to this (condition) as a medical curiosity, and as such it may have some interest.”

(George Huntington, M.D.)

Edited excerpt from the original account of what came to be known as Huntington’s disease

"My message for the professionals is keep learning, keep listening to the community, and work longer hours."

(Matt Ellison, HD community member)

“In the confrontation between the stream and the rock, the stream always wins—not through strength but by perseverance.”

(H. Jackson Brown Jr., Author)
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Table of Contents

List of Figures ............................................................................................................................................. V

List of Tables ............................................................................................................................................... VII

Glossary / Definitions / Abbreviations ......................................................................................................... IX

Additional Publications and Conference Proceedings During Candidature ............................................. XII

Abstract ......................................................................................................................................................... XIII

General Declaration ....................................................................................................................................... XV

Acknowledgements ......................................................................................................................................... XVI

Chapter One – Introduction ......................................................................................................................... 1

1.1. Introduction to Huntington’s disease and an overview of the thesis .............................................. 2

1.2. The psychiatric and cognitive profile of HD ..................................................................................... 8

1.2.1. Behavioural changes and depressed mood in HD. ................................................................. 8

1.2.2. Cognitive decline and learning and memory impairments in HD. ............................................ 11

1.2.3. Psychiatric and cognitive changes in HD are independent and the pathological processes underlying these signs are unknown ................................................................. 14

1.3. Using biomarkers to investigate and understand neurodegenerative diseases ....................... 15

1.3.1. Biomarkers: What are they and what makes an ideal biomarker? ........................................... 15

1.3.2. The Hypothalamic-Pituitary-Adrenal Axis (HPA) axis: What is it and how can it be used as a biomarker? ........................................................................................................ 18

1.4. The HPA axis as a biomarker in HD ............................................................................................... 23

1.4.1. HPA axis hormone abnormalities in human HD. ...................................................................... 24

1.4.1.1. HPA axis neuroanatomical changes in human HD ............................................................ 25

1.4.2. HPA axis hormone abnormalities and neuroanatomical change in animal models of HD. ................................................................................................................................. 27

1.4.3. Theories about the mechanisms underlying HPA axis dysfunction in HD. .......................... 29

1.4.4. Evaluating the HPA axis sampling and analysis methodology used in HD research to date ....................................................................................................................................... 32
1.5. Psychiatric and cognitive signs are yet to be properly investigated in the context of HPA axis dysfunction in HD ................................................................. 37
1.6. Phenotypic similarities between HD and non-HD conditions of HPA axis dysfunction ........................................................................................................... 39
  1.6.1. HPA axis dysfunction and mood disorders ................................................................. 39
  1.6.2. HPA axis dysfunction and cognitive decline .............................................................. 42
  1.6.3. HPA axis dysfunction in other chronic and neurodegenerative conditions ............... 44
  1.6.4. The HPA axis in the psychoses .................................................................................. 45
  1.6.5. HPA axis dysfunction and sleep disorders ................................................................. 47
  1.6.6. Summary of the research precedence set by non-HD conditions .............................. 47
1.7. The HPA axis as a therapeutic target for treatment intervention in HD .................... 48
1.8. Summary and aims of the thesis .................................................................................. 50

CHAPTER TWO – METHODS ..................................................................................................... 53

2.1. Participant recruitment ................................................................................................... 54
  2.1.1. Participant consent ..................................................................................................... 55
  2.1.2. Burden of pathology .................................................................................................. 56
  2.1.3. Participant payment ................................................................................................... 56
2.2. Materials ....................................................................................................................... 56
  2.2.1. Motor function rating scale ....................................................................................... 56
  2.2.2. Neuropsychiatric assessment .................................................................................... 57
  2.2.3. Cognitive test battery ............................................................................................... 62
  2.2.4. Administering the psychiatric assessment and cognitive test battery .................... 65
2.3. Cortisol collection and measurement ............................................................................ 67
  2.3.1. A review of different cortisol collection and measurement techniques .................... 67
  2.3.2. The salivary cortisol collection procedure used in this research project .................. 70
  2.3.3. The analysis and assaying of salivary cortisol in this research project ...................... 72
  2.3.4. Blood sampling ........................................................................................................ 73
2.4. Study protocol ............................................................................................................... 73
2.5. Statistical analyses ........................................................................................................ 74
CHAPTER THREE – STUDY 1 ..................................................................................................... 75
Study 1 – Manuscript commentary .................................................................................... 76
Declaration for Thesis Chapter 3 ......................................................................................... 77
Manuscript: "Cortisol and depression in pre-diagnosed and early stage Huntington’s disease" ..................................................................................................................................... 79

CHAPTER FOUR – STUDY 2 ..................................................................................................... 113
Study 2 – Manuscript commentary .................................................................................... 114
Declaration for Thesis Chapter 4 ......................................................................................... 115
Manuscript: "The relationship between cortisol and verbal memory in the early stages of Huntington’s disease" ........................................................................................................... 117

CHAPTER FIVE – GENERAL DISCUSSION ........................................................................... 152
5.1. Summary of Study 1: “Cortisol and depression in pre-diagnosed and early stage Huntington’s disease” ................................................................................................................................... 154
5.2. Summary of Study 2: “The relationship between cortisol and verbal memory in the early stages of Huntington’s disease” ........................................................................................................... 155
5.3. Findings of the thesis in the context of past literature: Addressing the gap in HD HPA axis research ...................................................................................................................................... 157
  5.3.1. Measuring specific clinical signs can help explain the reason behind abnormal cortisol levels in HD and the aetiology contributing to HPA axis dysfunction ........................................... 160
  5.3.2. Different clinical signs of HD may be individually associated with cortisol dysregulation at different times of the day ......................................................................................... 163
  5.3.3. The relationship between cortisol levels and clinical signs of HD changes as people with the HD CAG expansion progress through early stages of the disease .......................................................... 164
5.4. Integrating and interpreting the findings ........................................................................ 166
  5.4.1. A theory about the progressive deterioration of the HPA axis in HD and the cortisol toxicity hypothesis ................................................................................................................................. 167
  5.4.2. Stress and sleep are other factors to consider in the relationship between cortisol concentration and clinical signs in HD ........................................................................................................... 171
5.5. Cortisol is a useful biomarker of HD progression and a pathophysiological correlate of the clinical signs of HD, but more research is required before it can be used in the development of a therapeutic intervention.................................................................173
5.6. Limitations .................................................................................................................176
5.7. Recommendations for future research .................................................................178
5.8. Conclusions .................................................................................................................180

THESIS REFERENCES ...........................................................................................................181
LIST OF FIGURES

Thesis Figures

**Figure 1.1.** An ideal model for therapeutic intervention in HD. 6

**Figure 1.2.** Relationship between estimated years from diagnosis and the Hopkins Verbal Learning Test – Revised *Total learning score* in pre-HD. 14

**Figure 1.3.** The hypothalamic-pituitary-adrenal axis (HPA axis) and negative feedback self-regulation loop. 20

**Figure 1.4.** The normal 24-hour circadian profile. 21

**Figure 1.5.** The volume of brain structures in HD patients as a percent of normal controls. 27

**Figure 2.1.** Distribution of participant IDS–SR *Total* scores. 59

**Figure 5.1.** The rationale for investigating the association between HPA axis dysfunction and psychiatric and cognitive signs in the early stages of HD. 159

**Figure 5.2a.** A model proposing how cortisol levels are related to (a) depression, and **Figure 5.2b.** (b) learning and memory ability as the disease state progresses from pre-HD to early-HD. 175
Manuscript Figures

Chapter 3: Study 1.

Figure 1. Salivary cortisol concentrations for all participants.  

Figure 2a. Salivary cortisol concentrations for (a) IDS–SR Non-depressed participants only, (b) IDS–SR Depressed participants only.  

Figure 3. Association between 8am mean cortisol level and the interaction of diagnostic group and IDS–SR depression status.  

Chapter 4: Study 2

Figure 1. Salivary cortisol concentrations for all participants by diagnostic group.  

Figure 2. Association between CVLT–II Total delayed free recall score and the interaction of diagnostic group and Day 1: 11pm cortisol concentration.  

Figure 3a. Association between (a) CVLT–II Total immediate recall score, and (b) CVLT–II Total delayed free recall score and the interaction of Day 1: 11pm cortisol concentration and TMS.
## List of Tables

### Thesis Tables

**Table 1.1.** Methodology of previous studies investigating HPA axis function in HD  

**Table 2.1.** Levels of depression severity according to the IDS–SR Total score  

**Table 2.2.** Order of test/individual test items to be administered as a part of the wider study protocol  

**Table 2.3.** The availability of cortisol and the collection methodology for cortisol in different biofluids  

**Table 2.4.** Study protocol indicating the order of activities to be undertaken at the study visit
Manuscript Tables

Chapter 3: Study 1.

Table 1. Demographics and clinical information  106

Table 2. Neuropsychiatric assessment scores  108

Chapter 4: Study 2.

Table 1. Demographics and clinical information  147

Table 2. Mean (and Standard deviation) of raw scores for CVLT–II performance by diagnostic group  149

Table 3. Hierarchical standard regression analysis for diagnostic status in comparison to controls and Day 1: 2300hr salivary cortisol concentration correlates of CVLT–II performance  150

Table 4. Hierarchical standard regression analysis for UHDRS TMS and Day 1: 2300hr salivary cortisol concentration correlates of CVLT–II performance  151
GLOSSARY / DEFINITIONS / ABBREVIATIONS

These terms will be italicised upon first use in this thesis:

**Basal/Baseline:** Refers to hormone levels which are sampled in their naturally occurring state, without having been antagonised or exogenously manipulated (i.e., specifically when HPA axis challenge tests have not been applied).

**Biomarker:** ‘A (pathophysiological) characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention’ (Biomarkers Definitions Working Group, 2001, p. 91).

**Clinical marker:** A sign or symptom of how someone functions or feels, which is observed or directly assessed in a clinical setting.

**Cortisol concentration/Cortisol level:** These terms are used interchangeably in this thesis. They both refer to the concentration of circulating cortisol.

**Cortisol Awakening Response (CAR):** A marked spike in cortisol roughly 30 minutes after waking, which reflects the production capacity of the adrenal gland.

**Cortisol toxicity hypothesis:** The ongoing and progressive deterioration of neuroanatomical structures and neurophysiological mechanisms due to toxic cortisol levels, which is self-perpetuated by the inability of these structures and mechanisms to regulate cortisol levels.
**Early Huntington's disease (Early-HD):** Patients who have received a diagnosis of HD within the previous 5 years.

**HD CAG expanded:** An expansion of the polyglutamine CAG repeat sequence in the *HTT* gene, which is long enough to predispose a person to develop Huntington’s disease.

**HD CAG normal (Normal/Control):** A person with fewer than 35 CAG repeats who is therefore unlikely to ever develop HD.

**HPA axis challenge test:** Used to identify the exact point of HPA axis dysfunction by measuring the concentration of CRH, ACTH, or cortisol after administering exogenous substrates that forcibly stress the HPA axis self-regulatory response at each axial feedback point.

**Huntington’s disease (HD):** A clinical diagnosis based on two pieces of evidence, 1) a genetic test result which confirms that a person has the HD CAG expansion; 2) a clinical examination of a collection of motor signs that together are unequivocally indicative of a characteristic HD extrapyramidal movement disorder.

**Glucocorticoid receptors (GR):** Have a low affinity for cortisol, which means that they regulate high cortisol levels (e.g., in the morning or at times of stress) once MR are saturated. GR are distributed ubiquitously throughout the brain. They act through biochemical pathways affecting hypothalamic and pituitary gland hormone release.
Mineralocorticoid receptors (MR): Have a higher affinity for cortisol than glucocorticoid receptors, which allows MR to regulate low basal circadian cortisol levels. MR are predominantly expressed in the hippocampus alone or with glucocorticoid receptors.

Pre-diagnosed HD (Pre-HD): Those with the HD CAG expansion who either do not yet display overt motor signs, or who show clinical signs but these are not severe enough to warrant a clinical diagnosis.

Sign: An objective measurement or observed clinical evidence that is indicative of a disease/condition.

Symptom: A subjective experience or complaint reported by a patient. A symptom can still be a characteristic of a disease/condition.
ADDITIONAL PUBLICATIONS AND CONFERENCE PROCEEDINGS DURING CANDIDATURE

Additional publications:


Conference proceedings:


ABSTRACT

Huntington’s disease (HD) is a genetic neurodegenerative condition which manifests a motor disorder, psychiatric disturbance, and a decline in cognitive functioning. Cortisol dysregulation in HD has been reported in a limited number of human and mouse model studies, but no published research has focussed on investigating the relationship between cortisol levels and the clinical signs of HD. Cortisol is a stress hormone produced by the hypothalamic-pituitary-adrenal (HPA) axis, and it is involved in a broad range of physical, behavioural, and cognitive functions. The concentration of circulating cortisol is a marker of HPA axis integrity and the ability of the HPA axis negative feedback loop to regulate itself. HPA axis dysfunction is associated with psychiatric and cognitive signs in a number of medical conditions, neuropsychiatric disorders, and neurodegenerative diseases. There is strong evidence of relationships between high cortisol, particularly around the time of morning awakening, and depression as well as between high cortisol and poor memory ability. In HD, however, these relationships have not been studied despite the fact that depression and memory decline are prominent early psychiatric and cognitive signs of this disease. A link between a neurobiological marker of HD progression and the first clinical signs of depression and memory decline may provide an objective indication of early pathophysiological changes in HD. Such a relationship may also make it possible to clinically assess the therapeutic benefit of an early intervention. The aim of the current research project was to extend on previous HD research by examining the relationship between cortisol levels and cognitive and psychiatric signs and symptoms in the early stages of HD. This thesis comprises two studies that have been written up as manuscripts and submitted for publication. Individually, the papers aim to examine the relationship between cortisol levels and signs of depression (Study 1) and memory
performance (Study 2) in pre-diagnosed participants (pre-HD) and patients who had received a diagnosis of HD in the past 5 years (early-HD). Salivary cortisol was collected at four time points over a 24-hour period in 57 participants (17 early-HD, 20 pre-HD, 20 gene-normal healthy controls). To place this research project in the context of previous HPA axis studies of HD, cortisol levels within the subject sample of this project were first analysed at four time points across a 24-hour period, without taking into account clinical signs. The results indicated a trend for higher cortisol levels in pre-HD at all four time points. Study 1 of the thesis reports depression symptoms as measured by the Inventory of Depressive Symptomatology – Self-Report (IDS–SR), and Study 2 reports verbal learning and memory ability as measured using the California Verbal Learning Test – Second Edition (CVLT–II). Results from Study 1 indicated that the morning cortisol concentration in early-HD was dependent on the severity of depression, such that higher levels of depression were associated with higher morning cortisol than in non-depressed early-HD. This pattern was not observed in control and pre-HD. Study 2 reports an association between poorer memory encoding and retrieval, and higher evening cortisol in participants with more severe HD motor signs. This association was also not evident in control and pre-HD. Overall, these results indicate that cortisol dysregulation appears to begin well in advance of an HD diagnosis, perhaps resulting in chronic hypercortisolism through the pre-HD phase. Once HD is diagnosed, cortisol may be differentially influenced depending on the presence or absence of depression. Cortisol also appears to be related to memory decline in HD, but only once motor signs have become pronounced. Neuropathological changes related to HPA axis function, which worsen with disease progression, could account for these findings. Cortisol, and therefore HPA axis function, could thus be considered as a candidate biomarker of HD progression which is meaningfully associated with early neuropsychiatric and cognitive signs of HD.
In accordance with Monash University Doctorate Regulation 17/ Doctor of Philosophy and Master of Philosophy (MPhil) 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 two unpublished publications. The core theme of the thesis is the investigation of the association between cortisol levels and signs of depression (Study 1) and verbal learning and memory ability (Study 2) in the early stages of Huntington’s disease. 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 Psychology and Psychiatry under the supervision of Prof. Julie C. Stout and co-supervision of Dr. Phyllis Chua. 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 Chapter 3 and Chapter 4 my contribution to the work involved the following:

<table>
<thead>
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<th>Thesis chapter</th>
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<th>Nature and extent of candidate’s contribution</th>
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<td>3</td>
<td>Cortisol and depression in pre-diagnosed and early stage Huntington’s disease.</td>
<td>Submitted</td>
<td>Design and conduct study, manage participant recruitment, data analysis, write manuscript for publication. Approx. 80% of total contribution.</td>
</tr>
<tr>
<td>4</td>
<td>The relationship between cortisol and verbal learning and memory in the early stages of Huntington’s disease.</td>
<td>Submitted</td>
<td>Design and conduct study, manage participant recruitment, data analysis, write manuscript for publication. Approx. 80% of total contribution.</td>
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I have not renumbered sections of submitted manuscripts in order to generate a consistent presentation within the thesis.

Signed: ..............................................................................................................

Date: ..............................................
It is done. Over four years of work has been put into this thesis and I am very proud of what has been accomplished. It is a piece of work which would not have been possible to achieve without the input and support of many people, and I would like to acknowledge these people.

The first and foremost are the research participants. Thank you for the generosity of your time and cooperation. In particular to the HD patients and their families who participated; it is not a cliché when I say that it was a privilege to meet you and work with you. The resilience you demonstrate amongst the hardships you endure is a testament to your character. More broadly, I acknowledge members of the international Huntington’s disease community. From patients and families, to the clinicians and researchers, as well as other staff and volunteers involved (a special mention goes to the girls at Huntington’s Victoria), I have been fortunate to be part of an internationally community that is aligned and driven to improve the quality of life for patients and their families. When attending international HD events I have been amazed at both the calibre of minds working together, as well as the openness towards new members. I have always felt welcomed and valued in this international community. It is a good mantra to foster and I have no doubts that this collegiate attitude will only bring prosperity.

To my research supervisors, Prof. Julie C Stout and Dr. Phyllis Chua, my involvement in this area of research would not have been possible in the first place without the opportunity you provided to me. This opportunity extended beyond working on a fascinating project, and included travel and exposure to research and clinical practice on a world scale. At times our professional relationship was tested, but hopefully this is just a natural bi-product of determined and passionate people working together. Julie, the quality of the research and the
thesis could not have been achieved without your guidance, wisdom, experience, and attention to detail. I can now appreciate how you wanted me to develop as a student, researcher, professional, and a thinker; I know that I will be all the better for it. Phyllis, I am very appreciative of the support you provided me throughout. You were always willing to put aside time to assist, you were extremely accommodating in your flexibility with participant testing, and I felt like there was always someone who I could call for “on the ground” advice; this was very reassuring and you helped make the project enjoyable. I also acknowledge those who were instrumental in conceptualising the original foundations of the project and provided advice throughout: Prof. Edmond Chiu, Dr. Andrew Churchyard, Dr. Anthony Hannan, and Dr. Terence Pang.

To those who assisted in the conduct of the research project: Dr Andrew Churchyard, thank you for assistance with participant recruitment and allowing me to attend your clinics. Observing a clinician of your quality was a fascinating and enjoyable experience. Ms. Angela Komiti, you helped so much in so many ways throughout the project and I am extremely grateful. As you predicted, the “thesis war chest” got filled! Ms. Joy Preston and Ms. Jenny McCutchen, thank you for your administrative help at your respective clinics. To those who assisted in the preparation of the manuscripts and the thesis: Prof. Grahame Coleman, thank you for your statistical advice and your patience. Dr Georgia Lowndes, thank you for reading my work and for helping me through the drafting process (as well we the words of wisdom). To Frank Gullone, who made Figure 1.3. look amazing, you did a brilliant job and I am so happy to have a mate’s work in my thesis. Last but not least, thank you to Kate O’Donnell for proof reading my thesis, your eagle eyes were priceless.
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I would like to conclude with a statement of thoughts. It is true that our fullest potential is only restricted by our ability to conquer the obstacles we face and the desire to challenge our own limits. Through my doctoral experience, I found the nature of obstacles that arise fall into three categories: some are valid, some are unavoidable, but some are unproductive and ironically unconducive to the practices we should preach. In terms of the former two, these are necessary and welcomed because they test our ability to manage situations in a productive manner. In relation to the latter, there are many valuable lessons to be learned. I would hope that these learnings are used to advance our educational and work modus operandi across research, clinical practice, and professional management more widely. I welcome the chance to partake in these developments and to embark on the next challenge ... whatever it may be.

A special mention goes to Prof. Jennie Ponsford, and the school’s research degree staff, who do a brilliant job of running the Doctorate of Clinical Neuropsychology course. My candidature was supported by an Australian Postgraduate Award scholarship. The research project was funded by a Pfizer Neuroscience Research Grant and the School of Psychology and Psychiatry, Monash University.
Chapter 1 – Introduction

1.1. Introduction to Huntington’s disease and an overview of the thesis

Huntington’s disease (HD) is an autosomal dominant neurodegenerative condition. Latest figures from the World Health Organisation (2011) estimate that 5–7 people per 100,000 in Western countries suffer from HD, however these estimates are thought to under-represent the true worldwide prevalence (Fisher & Semaka, 2011). HD is caused by an expansion of the polyglutamine CAG repeat sequence in the \textit{HTT} gene locus IT15 (4p16.3) on chromosome 4 (The Huntington's Disease Collaborative Research Group, 1993). The unstable DNA segment produces abnormalities in the ubiquitous protein \textit{huntingtin}. The disease can begin at any age, with onset typically in the early-to-mid 40s. Death generally ensues 10–20 years from the time of diagnosis. A genetic test of the 4p16.3 gene locus can identify those who are \textit{HD CAG expanded} and therefore are predisposed to develop the disease. There is a negative correlation between the age of onset and CAG repeat size, which means that a longer polyglutamine sequence is usually associated with earlier disease onset and a more rapid disease progression (Brandt et al., 1996). In excess of 40 CAG repeats is associated with a high probability that HD will manifest in a person’s lifetime, as estimated by predictive models that indicate an expansion of 41 CAG repeats carries a 90% chance of HD onset by 70 years of age (Langbehn et al., 2004). For a CAG length of 36–40 repeats, there is uncertainty about whether a person will develop HD. Those with fewer than 35 CAG repeats are unlikely to ever develop HD and as a result are considered to be \textit{HD CAG normal}. The accuracy with which age of HD onset can be predicted from the HD CAG repeat length appears to depend on the length of the CAG
repeat sequence itself. To be specific, a long CAG replication (i.e., over 44 repeats) is thought to be more reliable in predicting age of onset than a short CAG repeat sequence (Langbehn et al., 2004). If the expansion has fewer than 44 repeats, the ability for polyglutamine length to predict the age of HD onset is reported to be only 50–70% accurate (Tabrizi et al., 2009). Therefore the prognostic robustness of CAG repeat length has been questioned (Andrew et al., 1993; Duyao et al., 1993; J. L. Li et al., 2003), because there may be other environmental and genetic factors that contribute to the disease start point. These other factors are starting to become understood through HD research.

HD is characterised by a motor disorder, psychiatric disturbance, and a decline in cognitive functioning. Motor abnormalities comprise idiosyncratic hyperkinetic and dystonic movements in combination with a hypokinetic bradykinesia that together can involve almost the whole voluntary musculo-skeletal system (Ruiz et al., 2000; P. D. Thompson et al., 1988; Wild & Tabrizi, 2007). Psychiatric and cognitive signs and symptoms were only briefly discussed in George Huntington’s initial description of the disease: ‘the mind becomes more or less impaired, in many amounting to insanity…sometimes that form of insanity which leads to suicide’ (Huntington, 1872). Neuropsychiatric signs are however reported to occur in 98% of HD patients (Paulsen, Ready, Hamilton, Mega, & Cummings, 2001). This makes them more common in HD compared to most other dementias and subcortical brain disorders, and more detrimental for daily functioning than the HD motor signs for many patients and families. Psychiatric signs and symptoms include severe low mood, dysphoria, agitation, irritability, apathy, obsessive-compulsiveness, and anxiety; hallucinations and delusions are rare (Craufurd, Thompson, & Snowden, 2001; Duff et al., 2007; Paulsen, Ready, et al., 2001;
van Duijn et al., 2008). Cognitive signs and symptoms affect a range of abilities including processing speed, attention and concentration, working memory, executive functioning, and memory (Ho et al., 2003; Lemiere, Decruyenaere, Evers-Kiebooms, Vandenbussche, & Dom, 2004; Snowden, Craufurd, Griffiths, Thompson, & Neary, 2001; Stout et al., 2007). The neuropsychological profile of HD patients eventually progresses to a dementia. To add to the complexity of HD diagnosis and ongoing disease management, the order and severity in which clinical signs and symptoms manifest is not uniform across individuals.

Current clinical protocol for a diagnosis of HD in people with the HD CAG expansion requires a very high degree of clinical confidence that the presenting motor signs are indicative of HD. For this thesis, a patient clinically diagnosed with HD will be referred to as HD. Early-HD will be used to denote HD patients within 5 years post-diagnosis. Those with the HD CAG expansion who do not yet display motor signs sufficient to warrant a clinical diagnosis are referred to as pre-diagnosed HD (herein pre-HD). Contrary to diagnostic standards, which are based entirely on the presentation of HD motor signs, it is now becoming accepted that psychiatric and cognitive signs of HD may start to emerge, on average, 10–20 years prior to motor diagnosis in HD CAG expanded individuals (Duff et al., 2007; Paulsen et al., 2007; Paulsen et al., 2005; Stout et al., 2011). Psychiatric and cognitive signs of HD that manifest early in the disease timeline, even in pre-HD, are now receiving increasing attention in the literature as useful clinical markers of the development and progression of HD (Weir, Sturrock, & Leavitt, 2011). A clinical marker is a sign or symptom of how someone functions or feels which can be observed or directly assessed in a clinical setting. Coincidentally, the development of pre-HD clinical signs mirrors the suggested progression of neuroanatomical
degeneration in HD (Aylward et al., 2004). If structural brain changes are occurring a long time before HD motor diagnosis, it is likely that there are also other markers of biological change that can be measured. These biological markers are referred to as biomarkers. Biomarkers are an objective pathophysiological indicator of a biological or pathological process, and are distinct from clinical markers. The link between biomarkers and clinical markers can provide an understanding of the pathophysiology underlying the clinical manifestation of a disease or condition. Together, biomarkers and clinical markers provide a quantifiable measure of disease progression, ideally beginning early in the disease process. Ultimately, this link allows the efficacy of any therapeutic intervention to be measured and monitored. The biomarkers and their associated physiological systems may themselves even present a promising target for therapeutic intervention. At the moment there is no effective intervention for HD. Current treatments are symptomatic and palliative, in that they only attempt to manage clinical signs once they have already developed into a problem for patients. It is hoped that one day treatment will be able to delay or ultimately prevent disease onset before a pathological and phenotypic decline ensues (see Figure 1.1., page 6). Treatments that slow disease progression or reverse disease effects are also needed for those already diagnosed with HD.
Figure 1.1. An ideal model for therapeutic prevention in HD. The use of an early prophylactic treatment (downward arrow) before neurodegenerative and functional decline begins would slow the course of disease progression. However, currently, clinical diagnosis is only made once motor signs are advanced (upward arrow), by which time the disease process has already began to affect function (Paulsen et al., 2006).

The functional integrity of the hypothalamic-pituitary-adrenal (HPA) axis has received only a small amount of attention as a biomarker in HD, but the findings thus far are encouraging. The HPA axis is described in detail in Section 1.3.2., but briefly, it is a neuroendocrine system which produces cortisol and regulates several functions in the body – including mood and emotions, cognition, energy metabolism, aspects of the immune system, and the response to stress. HPA axis research in HD has been conducted in both rodents and humans. The handful of human studies have primarily investigated HPA axis regulation at the different stages of the disease according to diagnostic status (i.e., HD CAG normal control, pre-HD, diagnosed HD), without specifically examining how it relates to HD signs or symptoms. Where HPA axis function has been linked to HD signs or symptoms, only physical characteristics have received consideration (e.g., weight and body mass). A number of authors have hypothesised that
neuropathological changes to the HPA axis in HD may underlie myriad neurophysiological, neuropathological, and neurocognitive disturbances (Aziz, Swaab, Pijl, & Roos, 2007; Björkqvist et al., 2006; Petersén & Björkqvist, 2006). However, the association between HPA axis abnormalities and the early psychiatric and cognitive signs of HD has not been empirically examined. The best theoretical support for this relationship comes from inferences based on other non-HD neuropsychiatric conditions (e.g., depression, schizophrenia) and neurodegenerative diseases (e.g., Alzheimer’s disease), which display some similar clinical characteristics to HD and also experience HPA axis dysfunction. The relevance of the HPA axis as a biomarker in HD hinges on its relationship with early psychiatric and cognitive markers of disease progression.

The aim of the research project reported by this thesis was to investigate the association between HPA axis function, as reflected by cortisol concentrations, and psychiatric and cognitive signs in the pre-HD and early post-motor diagnosed staged of HD with a specific focus on depression, and verbal learning and memory ability respectively. The first chapter of the thesis presents and reviews the existing literature. It will begin by summarising the characteristics of the early neuropsychiatric and neurocognitive signs in HD. The next section will explain what biomarkers are and define the criteria for an ideal biomarker. The HPA axis will then be introduced and its utility as a biomarker will be assessed. An appraisal will be made about the research into HPA axis function in HD to date, and comparisons will be drawn between the phenotypic similarities of HD and those that are seen more widely in other conditions of HPA axis dysfunction. In regards to whether the HPA axis itself is a potential target for therapeutic intervention to alter disease progression; the purpose of this research

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1 For the purpose of this thesis, the terms (hormone) concentration and (hormone) level are used interchangeably.
Chapter 1 – Introduction

project was not to evaluate disease-modifying therapy, however a brief comment will be made about the potential for the HPA axis to be considered as a therapeutic target for treatment intervention in HD. To establish the rationale for this research project, the literature review will identify the current gap in HD HPA axis research and then outline the steps this project will take to extend our understanding of the relationship between the HPA axis and the early psychiatric and cognitive clinical signs of HD.

1.2. The psychiatric and cognitive profile of HD

1.2.1. Behavioural changes and depressed mood in HD.

HD mutant gene carriers, both pre-HD and diagnosed patients, display a higher prevalence of psychiatric disorders than the general population (van Duijn et al., 2008). Based on factor analysis undertaken by Craufurd et al. (2001), most behavioural features of HD can be ascribed to three clusters: depression, irritability, and apathy. Depressive disorder in particular is very common in HD (van Duijn et al., 2008). Estimates suggest that at least 50% of diagnosed HD patients seek treatment for depression (Paulsen et al., 2005). In fact, depression is considered one of the earliest signs of HD onset (Kingma, van Duijn, Timman, van der Mast, & Roos, 2008), and has been detected over 10 years prior to motor onset in HD gene carriers (Duff et al., 2007). One study found that more than 30% of gene carriers initially present for medical investigation with signs and symptoms of depression in the absence of any diagnostic neurological motor signs (Shiwach, 1994). The severity of depression appears to follow a temporal gradient, with a significant increase in depression severity through the pre-HD phase as the point of motor-based diagnosis draws closer (Julien et al., 2007). Depressive
features of low mood, reduced self-esteem, and heightened anxiety are initially present early in the post-diagnosed stage of HD, but then appear to show a general reduction in severity through the middle and later stages of the disease (Paulsen et al., 2005). It is possible that the apparent diminution of depression severity, which is largely gauged by patient self-report, may be related to a lack of patient self-awareness because of declining cognitive functioning associated with the dementing process. The importance of understanding psychiatric disturbance in HD is made all the more critical by the fact that a depressed mood is highly correlated with suicidality in HD as noted by Hubers et al. (2011), and by George Huntington himself (Huntington, 1872). Suicide is a major problem in HD and it has been detected in up to 20% of people with the HD CAG expansion (Hubers et al., 2011). Furthermore, over 10% of patients report at least one suicide attempt (Paulsen et al., 2005). Suicide has been reported as the third leading cause of known death amongst HD patients. It has been attributed to 4% of deaths, which is significantly higher than the 1% seen in the population without the HD CAG expansion (Schoenfeld et al., 1984).

Clinicians and researchers are beginning to regard “HD depression” as unique, based on the collection of behavioural signs and symptoms which differ, at least partially, from major depressive disorder in the general population (Craufurd et al., 2001). The criteria classically used to define “garden variety” depression – such as a modified appetite, weight loss, reduced thinking ability, and psychomotor agitation or retardation – are clinical features of HD that usually manifest irrespective of whether a mood disturbance is present or not. Using these signs to identify HD depression would be inaccurate, which is why diagnostic tools such as the Diagnostic and Statistical Manual of Mental Disorders – Fourth Edition – Text Revision
Chapter 1 – Introduction

(DSM-IV-TR) are accompanied by a caveat that when diagnosing depression, the clinical signs cannot be accounted for by a medical condition (American Psychiatric Association, 2000).

There is uncertainty about whether the depression in HD is reactive to situational psychosocial stressors, or is related to an endogenous aetiology (Shiwach, 1994). Whilst there is no doubt that stressors associated with being a member of a HD family are burdensome (Wexler, 1979), it is likely that organic factors contribute towards a large portion of the distinctive phenotype of HD depression, because evidence attributing depression to potential situational factors in HD is inconsistent. Van Duijn et al. (2008) found that the impact of the family setting on mood changes is relatively minimal since there is no difference in the psychiatric state between the general population and first-degree HD CAG normal (i.e., those who have a “normal” HD CAG expansion but still shared the environmental experience of growing up in a family of people with the HD CAG expansion). The high rates of affective disorder at close proximity to motor onset is thought to be unrelated to the distress surrounding the manifestation of motor signs because patients are usually subjectively unaware of their emerging soft-neurological abnormalities such as chorea, dystonia, dysarthria, and oculomotor difficulties (Julien et al., 2007). Another obvious psychosocial trigger to consider is the assumption that receiving a positive gene test precipitates the onset of depression (Codori, Slavney, Rosenblatt, & Brandt, 2004). A 5-year follow-up study of those who underwent predictive genetic testing found that depression levels do appear to rise over time in those who receive a positive test result (Almqvist, Brinkman, Wiggins, & Hayden, 2003). However, the findings of this study should be interpreted with caution since the impact of other situational and pathophysiological factors were not discussed, and the number of participants with
clinically diagnosable levels of depression did not actually differ between recipients of a positive or negative genetic test result at any point. In addition, Julien et al. (2007) identified a greater prevalence of depression in HD CAG expanded individuals compared to those who were HD CAG normal, even when participants were unaware of their gene status and were therefore not burdened by knowing that they were likely to develop HD. Therefore, whilst HD psychopathology might be related in part to situational factors, past research findings indicate that depression in HD is not purely reactive. Instead, the literature argues that a predominantly organic aetiology of depression in HD is highly probable (Paulsen, Ready, et al., 2001; van Duijn et al., 2008). The high prevalence of depression in HD, and its early onset, makes depression a potentially good clinical correlate for biomarker change. A further discussion about depressive signs and symptoms in HD is provided in Chapter 3 (Study 1).

1.2.2. Cognitive decline and learning and memory impairments in HD.

Cognitive signs have been found up to 15 years before clinical diagnosis of HD. Prior to this point, pre-HD patients perform relatively similarly to HD CAG normal controls on neuropsychological testing (Robins Wahlin, Lundin, & Dear, 2007; Stout et al., 2011; Stout et al., 2007). In the 10–15 year period before estimated motor-based diagnosis, there is evidence of some mild cognitive weakness (Stout et al., 2011). Timed cognitive tasks, such as the Symbol Digit Modalities Test, are highly sensitive to early cognitive changes, which suggests that psychomotor and information processing speed might be amongst the earliest features of HD and weaknesses in these areas could contribute to deficits in other cognitive domains (Lemiere et al., 2004; Tabrizi et al., 2011). Once pre-HD are within 9 years of the estimated
diagnosis a rapid cognitive decline ensues, so that pre-HD patients perform worse than healthy gene negative controls on many neurocognitive measures (Paulsen et al., 2007). Difficulties are particularly apparent in the areas of memory, and executive-level attentional control and mental flexibility (Stout et al., 2007). The cognitive decline becomes more pronounced within 2 years before estimated diagnosis (Paulsen, Zhao, et al., 2001), and there can be further deficits in verbal word recognition and visual perception as the disease progresses (Lemiere et al., 2004). Over a 3–6 year period post-motor diagnosis, both basic and high level executive skills deteriorate further, particularly skills of attentional control and planning ability (Ho et al., 2003). At this stage verbal fluency and speed of cognitive processing skills continue to suffer, but to a lesser degree than in pre-HD – most likely because they are already at an impaired level. The presence and severity of HD motor signs do not seem to account for the changes in cognitive ability; that is, cognitive signs and motor signs occur independently of each other (Stout et al., 2011).

Cognition is a broad area, so this thesis will focus on the cognitive domain of verbal learning and memory. Verbal learning and memory is a complex system, beginning with the accurate registration and efficient encoding of incoming verbal information. Memory capacity and storage capabilities dictate how much information can be consolidated and then accurately retrieved after a delay. The ability to recognise the original information reflects the integrity of the initial learning process, as well as a susceptibility to interference and distraction (Lezak, Howieson, & Loring, 2004; Squire, 1992). Cues, prompts, repetition, and semantic clustering can assist at each of these memory stages. Repeated word list tasks are often used to assess the integrity of the declarative or episodic memory system in memory studies (Lezak et al., 2004;
Learning and memory dysfunction is considered to be amongst the earliest cognitive signs in HD. As with the other cognitive domains in HD, once learning and memory ability begin to deteriorate, the decline continues to progress at the same time as motor signs become more pronounced (Paulsen et al., 2007; Robins Wahlin et al., 2007; Solomon et al., 2007; see Figure 1.2., page 14). Of the broader verbal learning and memory domain, the initial learning and encoding of verbal information, and then the ability to retrieve this information from memory storage, are particular areas of weakness in pre-HD and early-HD (Lemiere et al., 2004). Assessments using verbal list learning tasks suggest a reduction in memory span, inconsistent recall, an inability to employ strategies of semantic clustering, and high rates of semantic intrusions. However, the rate of forgetting what was learnt is less affected, at least initially in the disease, and the use of externally provided retrieval aids (e.g., semantic prompts) is highly beneficial for information recall (Massman, Delis, Butters, Levin, & Salmon, 1990). Recognition memory, whilst reduced compared to healthy controls (Berrios et al., 2002), is not as impaired as in some other neurodegenerative conditions. Though it too usually declines 2–5 years after a diagnosis is made. Eventually forced-choice recognition performance is comparable to that of Alzheimer’s disease patients (Brandt, Corwin, & Krafft, 1992), which is a neurodegenerative condition that uses marked deficits in recognition memory as a diagnostic clinical marker. The lack of rapid forgetting and relative preservation of recognition ability early in HD, in conjunction with the psychiatric and behavioural features, contributes to a neuropsychological profile of HD that is consistent with a subcortical dementia (Delis, Kramer, Kaplan, & Ober, 2000; Massman et al., 1990; McHugh & Folstein, 1975). Further discussion about learning and memory in HD is provided in Chapter 4 (Study 2).
1.2.3. Psychiatric and cognitive changes in HD are independent and the pathological processes underlying these signs are unknown.

The onset of psychiatric and cognitive signs in HD appears to occur at a similar time point in pre-HD, even though the rate of change might differ. Whilst there is some evidence to suggest that depression in HD is related to poor cognitive performance (Nehl, Ready, Hamilton, & Paulsen, 2001), it is in fact apathy, and not depression, that is more closely aligned with a decline across a range of cognitive domains, including verbal memory performance (Baudic et al., 2006). The reason for the difference between depression and apathy in relation to cognition becomes intuitive when the features of the two constructs are understood. Apathy comprises features of reduced activity and initiative, a lack of perseverance, poor work

Figure 1.2. Relationship between estimated years from diagnosis and the Hopkins Verbal Learning Test – Revised Total learning score in pre-HD (Paulsen et al., 2007). The solid line reflects the predicted memory score over time, broken lines are 95% confidence intervals for the estimated mean response.
quality, impaired judgement, and impulsivity. These are features which are directly related to cognitive performance. Depression, on the other hand, comprises features of low mood and self-esteem, anxiety, and suicidal ideation (J. C. Thompson, Snowden, Craufurd, & Neary, 2002), which are themselves not problems of cognitive ability. The features of depression in HD have not been found to be related to cognitive ability in HD (Baudic et al., 2006).

Because there is independent variability in the effects of depressed mood and memory decline in HD, it is unlikely that there is a single pathological region that can fully account for the HD phenotype. There are suggestions that basal ganglia damage and sub-cortical degeneration affecting fronto-thalamic subcortical circuitry might contribute to signs of neuropsychiatric and cognitive change in HD (Massman, Delis, Butters, Dupont, & Gillin, 1992; Paulsen et al., 2005; Paulsen, Ready, et al., 2001). However, in comparison to other subcortical dementias such as Parkinson’s disease in which pathology is also centred around the basal ganglia, HD patients present a collection of symptoms that are relatively more severe (Massman et al., 1990). Other pathology and processes may be involved in HD, especially in the early stages of the disease. Biomarkers of the HPA axis could help elucidate what these underlying precipitants may be.

1.3. Using biomarkers to investigate and understand neurodegenerative diseases

1.3.1. Biomarkers: What are they and what makes an ideal biomarker?

A biological marker or biomarker is defined as ‘a (pathophysiological) characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic
processes, or pharmacologic responses to a therapeutic intervention’ (Biomarkers Definitions Working Group, 2001, p. 91). These are different from clinical markers, which reflect signs and symptoms of how someone functions or feels. Biomarkers offer an insight into the functionality or dysfunctionality of the body’s physiological systems, and hence provide a valuable understanding of the mechanism involved in the presentation of clinical signs and symptoms. They are an important key in the drug-development process and clinical trials pathway because they indicate relationships between pathophysiological actions of a drug, and the therapeutic outcome. As such, a good biomarker can reduce the cost of drug development whilst improving drug safety and efficacy (Frank & Hargreaves, 2003; Katsuno et al., 2008).

Ideally, biomarkers correlate with known indices of clinical markers longitudinally across the course of the disease (Frank & Hargreaves, 2003). Biomarkers therefore deliver the ability to diagnose diseases at an early stage, monitor progression, estimate disease prognosis, and gauge the most efficacious point to begin therapeutic intervention. As a result of their properties, biomarkers are now recognised as a valuable method of investigating chronic neurodegenerative diseases (Henley, Bates, & Tabrizi, 2005). Henley et al. (2005) defined an ideal biomarker for neurodegenerative diseases according to the following criteria:

1. Easy to quantify in accessible tissue or biofluid.
2. Not subject to wide variation in the general population if used as a diagnostic biomarker.
3. Unaffected by unrelated conditions and comorbid factors.
4. Measurement is reliable and quick.
5. Measurements are reproducible at a different time in a different location.
Chapter 1 – Introduction

6. The biomarker changes linearly (either negatively or positively) with disease progression.

7. The biomarker changes in response to a disease-modifying therapeutic intervention that closely correlates with established clinico-pathological parameters of the disease (Henley et al., 2005, p. 703).

Aside from finding a cure for HD, the ultimate goal in HD research is arguably the need to identify the best possible biomarkers, especially in pre-HD individuals who have not yet received a clinical diagnosis (Weir et al., 2011). Whilst genetic testing can indicate if a person has an expanded number of HD CAG repeats, which in turn can be used to estimate the likely age of onset, it is not indicative of the clinical state of the disease. Current “gold-standard” clinical measures, such as the Unified Huntington’s Disease Rating Scale (UHDRS) Motor Scale (Huntington Study Group, 1999), can detect subtle motor abnormalities many years before more severe diagnostic choreiform movements manifest (Paulsen et al., 2006), however they are insensitive to non-motor signs and changes over short amount of time. Scales such as these are also susceptible to subjectivity by the rater (Borovecki et al., 2005; Henley et al., 2005; Runne et al., 2007).

A difficulty for biomarker researchers and drug trial studies in HD is that the disease has a slow progression (Snowden et al., 2001) and the nature of the HD phenotype is highly variable between patients. It is unlikely that a single measure can fulfil all the criteria of a biomarker for a neurodegenerative disease such as HD (Henley et al., 2005). Therefore, multiple biomarkers may be needed to serve as early diagnostic indicators and measures of disease progression (Henley et al., 2005). In HD, a number of such markers have already been
investigated through neuroimaging, clinical examinations and tests, as well as biochemical and molecular analyses in blood, urine, cerebrospinal fluid (CSF), and saliva (Katsuno et al., 2008; Weir et al., 2011). Whilst it would be ideal to identify sensitive biomarkers in pre-HD, it appears that the present range of biomarkers are instead most sensitive in early-HD (Tabrizi et al., 2011). HD research is now shifting focus to try and understand biomarkers in early-HD, with the hope that a better understanding of pre-HD will follow.

1.3.2. The Hypothalamic-Pituitary-Adrenal Axis (HPA) axis: What is it and how can it be used as a biomarker?

The HPA axis is a neuroendocrine system responsible for regulating the body’s response to stress and arousal. It has been implicated in the sleep-wake cycle, mood and emotions, cognition, energy usage and storage, digestion, sexuality, and the immune system. As such, the HPA axis is involved in a number of biochemical processes, ultimately through the effects of cortisol. This thesis will focus on the HPA axis pathway regulating cortisol levels. Cortisol control begins with the nervous system release of the peptide Corticotropin-Releasing Hormone (CRH) along with Argine Vasopressin (AVP, also known as Vasopressin or Anti-Diuretic Hormone, ADH [AVP is not directly related to this thesis so will not be discussed further]) from the Paraventricular nucleus (PVN) of the hypothalamus, through the hypophyseal portal system, to act on the anterior pituitary gland (also known as the adenohypophysis). Adrenocorticotropic hormone peptide (ACTH, also known as corticotropin) is released from the anterior pituitary gland into the peripheral blood circulation, stimulating cortisol release from the adrenal gland. Cortisol, called corticosterone in rodents, is a glucocorticoid steroid stress hormone produced by the zona fasciculata of the adrenal
gland cortex. It circulates throughout the body to induce a physiological response on the target organ. Negative feedback by cortisol on the pituitary gland, hypothalamus, as well as the hippocampus, then acts to suppress CRH and ACTH release and thereby controls circulating cortisol levels (see Figure 1.3., page 20). There are two receptor sub-types that mediate the negative feedback system: Type I *Mineralocorticoid Receptors* (MR), and Type II *Glucocorticoid Receptors* (GR). It is believed that MR has a comparatively higher affinity for cortisol, which allows for the regulation of low circadian glucocorticoid levels. MR are predominantly expressed in the hippocampus alone or with GR. GR regulate inflated circadian or stress-induced glucocorticoid levels once MR are saturated. GR are ubiquitously distributed throughout the brain, with a high density in the paraventricular nucleus of the hypothalamus and in the dentate gyrus of the hippocampus. (Reul & de Kloet, 1985; Spencer, Kim, Kalman, & Cole, 1998). Circulating levels of cortisol follow a cyclical circadian pattern with the highest levels of ACTH and cortisol in the morning and the lowest levels at midnight (see Figure 1.4., page 21; Weitzman et al., 1971). The naturally occurring cortisol awakening response (CAR) produces a marked spike in cortisol 30 minutes after waking and reflects the production capacity of the adrenal gland (Pruessner et al., 1997); as such the CAR is also indicative of the overall HPA axis production capacity. A number of factors can affect HPA axis functioning, including emotional state, sleep activity, physical activity, medications, and pathophysiological conditions. In turn, a dysfunctional HPA axis can have wide-reaching effects on the body’s physical, cognitive, and mood state.
Figure 1.3. The hypothalamic-pituitary-adrenal axis (HPA axis) and negative feedback self-regulation loop. ACTH, Adrenocorticotropic hormone; CRH, Corticotropin-Releasing Hormone; GR, Glucocorticoid Receptors; MR, Mineralocorticoid Receptors.
Figure 1.4. The normal 24-hour circadian profile averaged across a sample of healthy people. The illustration accounts for the light zeitgeber, and is broken down into one hour intervals showing (a) the mean and standard deviation plasma cortisol concentration, (b) mean hourly cortisol output, (c) and the mean amount of secretory minutes in each hour (Weitzman et al., 1971, p. 17).

Constituents of the HPA axis have the potential to act as very precise biomarkers. HPA axis neuropeptides and glucocorticoids, as well as their metabolites, are present in cerebrospinal fluid (CSF), urine, blood, and saliva. Samples of urine, blood, and saliva in particular are available via rapid, accessible, and non-invasive collection techniques (see Chapter 2 – Methods for a review of cortisol collection and measurement). The relative ease of collecting
samples of these biofluids increases the possibility of developing measures of HPA axis biomarkers (Katsuno et al., 2008; Raff, 2009). The initial step in understanding the integrity of the HPA axis is baseline or basal measurement of HPA axis neuroendocrines, in which hormone levels are sampled in their naturally occurring state, without having been antagonised or exogenously manipulated. This “first step” effectively indicates whether there are any abnormalities in the HPA axis that require further investigation. To then enhance the specificity of urinary, blood, and salivary sampling methods, the administration of exogenous substrates are employed to identify the exact point of HPA axis dysfunction. HPA axis challenge tests, as they are referred to in the endocrinological literature, take advantage of the negative feedback loop by forcibly stressing the integrity of the self-regulatory response at each feedback point, whilst measuring the levels of different glucocorticoids. The process involves administering dexamethasone (DEX, a synthetic glucocorticoid that mimics the actions of cortisol to inhibit ACTH secretion as a part of the dexamethasone suppression test, DST), animal-derived ovine CRH (oCRH, used to inhibit CRH release), or a combination of both. These techniques provide a more accurate understanding of HPA axis dysfunction, and as a result the differential diagnoses of the associated condition can be determined (McLean & Smith, 1995; Newell-Price, Trainer, Besser, & Grossman, 1998). However, undertaking HPA axis challenge tests are very demanding for researchers and participants. They require an intensive study design and should only be performed once there is evidence to suggest that there is a problem in HPA axis functioning. Cortisol is the end product of the HPA axis and as such is an ideal biomarker for investigating the integrity of the HPA axis in HD. For exploratory studies examining HPA axis functioning, such as those undertaken in this thesis, an analysis of baseline cortisol levels at multiple time points is a sufficient biological measure
to use (Carroll, Raff, & Findling, 2008; Viardot et al., 2005; H. Raff, personal communication, 05 September, 2011).

A demonstration of the utility of the HPA axis as a biomarker comes from the extensive amount of research in Cushing’s syndrome, which is an extreme and relatively common manifestation of HPA axis malfunction. Cushing’s syndrome is a clinical state resulting from a prolonged and abnormal exposure to heightened cortisol as a result of a dysfunctional hormone feedback loop and irregularities in circadian cortisol secretion. It is the classic paradigm of HPA axis dysfunction. Abnormally high levels of neuroendocrine biomarkers at every level of the HPA axis form the primary hallmark diagnostic criteria of Cushing’s syndrome (Newell-Price et al., 1998; Raff, 2009). Changes in the concentration of these neuroendocrine biomarkers are associated with the clinical signs of Cushing’s syndrome, which include physical changes and neuropsychiatric abnormalities of depression, anxiety, and a cognitive decline particularly in the domain of memory performance (Kelly, Kelly, & Faragher, 1996; León-Carrión et al., 2009; Newell-Price et al., 1998; Sonino & Fava, 2001; Starkman, Giordani, Berent, Schork, & Schteingart, 2001; Starkman, Schteingart, & Schork, 1981).

1.4. The HPA axis as a biomarker in HD

Due to its accessibility and proven utility, the HPA axis has been endorsed as a potentially very suitable biomarker system in HD (Petersén & Björkqvist, 2006). However, with only a handful of reports on this topic, there are significant gaps in what is known about the HPA axis in HD. The limited volume of research to date, which will be reviewed and interpreted
below, has investigated the patho-physiological processes in HPA axis dysfunction by measuring baseline hormone levels in both human HD and HD rodent models, using challenge tests to stress the system through the administration of exogenous hormones, as well as structural and functional analysis of the hypothalamus, pituitary gland, adrenal gland, and hippocampus.

1.4.1. HPA axis hormone abnormalities in human HD.

A number of biofluids have been sampled for measurement of HPA axis function in HD and these include CSF, urine, blood, and saliva. The following is a review of findings from studies that have collected each of these respective biofluids to analyse HPA axis function in HD.

CSF has only been used in one study, which showed that mid-afternoon CRH concentration was significantly higher in early-HD patients compared to healthy participants (Kurlan et al., 1988). Total urinary cortisol concentration (i.e., urine sampling undertaken over an extended duration of time) was also found to be elevated in early-HD compared to controls over a 24-hour period (Aziz et al., 2009), and significantly higher over a 3-hour mid-afternoon time period (14:00p.m.–17:00p.m.) in the moderate–advanced stage of HD (Björkqvist et al., 2006). Blood serum measurement has been the most widely used sampling medium for ACTH and cortisol, as it allows hormonal fluctuations to be measured at different times of the circadian cycle, compared to urine sampling, which only gives an indicator of total production over a period of time. Heuser, Chase, and Maral Mouradian (1991) found basal blood serum ACTH was significantly higher in HD patients, compared to healthy controls. Saleh et al.
(2009) also reported elevated serum ACTH in HD patients but this was not statistically significantly different from controls. Baseline serum cortisol on the other hand, whether measured at one time point or over multiple time points, is consistently reported as being significantly higher in HD patients compared to healthy controls (Heuser et al., 1991; Leblhuber et al., 1995; Saleh et al., 2009). Aziz et al. (2009) found total 24-hour cortisol secretion rates were significantly higher in early-stage HD compared to controls, and the oscillation, or variability, of cortisol levels around the mean was also significantly greater in HD participants. Whilst both healthy controls and HD patients demonstrated the expected maximum cortisol concentration around 08:30a.m., mean plasma cortisol concentration was significantly higher for the 08:30a.m.–16:30p.m. time period in HD. During this time period, HD pulsatile and total secretion rates were almost two times greater than in the control group (Aziz et al., 2009). Finally, in terms of salivary cortisol measurement, morning and evening salivary cortisol analysis revealed the CAR in pre-HD was higher than diagnosed HD and HD CAG normal controls (van Duijn et al., 2010). Evening cortisol levels and morning cortisol, after low dose DEX administration the night before did not differ between HD, pre-HD, or control groups. In summary, there is generally consistent evidence of heightened and dysregulated HPA axis activity in HD CAG expanded pre-HD and HD patients, particularly in the morning and afternoon, in all biofluid samples.

1.4.1.1. HPA axis neuroanatomical changes in human HD.

Aside from striatal degeneration, which has been well-characterised in HD and may precede motor sign onset by 10 years in pre-HD (Aylward et al., 2004), there is evidence of
hypothalamic and hippocampal change which suggests that HPA axis pathology may also be a key neuroanatomical feature of HD. Magnetic resonance imaging (MRI) has shown a significant reduction in hypothalamic grey matter volume of HD patients (Kassubek et al., 2004), and pre-HD beginning around 10 years before clinical diagnosis (Soneson et al., 2010). Studies report neuronal atrophy of up to 90% in the lateral hypothalamus in post-mortem HD brains, as well as an increase in neuroglial cells compared to controls (Kremer et al., 1991; Kremer, Roos, Dingjan, Mariani, & Bots, 1990; Petersén et al., 2005). Positron emission tomography (PET) has also been used in HD and has identified dopamine receptor dysfunction and increased microglial activity in the hypothalamus of both diagnosed HD and pre-HD (Politis et al., 2008). In this study there was no significant relationship between hypothalamic dopaminergic and microglial change and striatal degeneration, thereby suggesting hypothalamic dysfunction might occur independently of striatal abnormalities. Despite the evidence available it is still not clear at what point of the disease hypothalamic changes begin.

There does not appear to be any literature commenting on the anatomy of the pituitary gland or adrenal gland in human HD. However, there is evidence of hippocampal neuronal loss and volume reduction in HD compared to control brains (see Figure 1.5., page 27; Rosas et al., 2003; Spargo, Everall, & Lantos, 1993). The hippocampus is an important neuroanatomical site to consider in HD and in the current research project. Functionally, it plays a role in the negative feedback of the HPA axis and damage to the hippocampus could disturb HPA axis regulation. It is also an integral structure involved in cognitive ability – specifically learning and memory ability – and thereby, changes to the hippocampus are a potential cause of cognitive impairment in HD (Spargo et al., 1993).
Figure 1.5. The volume of brain structures in HD patients as a percent of normal controls. Disease severity for HD patients was not specified. A 9% reduction in the volume of the hippocampus was found to be a significant difference (Rosas et al., 2003).

1.4.2. HPA axis hormone abnormalities and neuroanatomical change in animal models of HD.

As with to human studies, rodent HD models also reveal HPA axis dysregulation and pathological changes. Using the R6/2 mouse model, which develops a severe and advanced HD phenotype, Bjorkqvist et al. (2006) reported significantly higher serum ACTH, and significantly higher serum and urine corticosterone compared to wild-type mice. It is, however, worth noting that the time and duration of blood and urine sampling was not...
specified by Björkqvist et al. In this same study, R6/2 tissue showed abnormal morphology and pathophysiology in three major anatomical sites across the HPA axis. Histological analysis indicated a 62% reduction in hypothalamic CRH content. In the pituitary gland, there was a 42% increase in the cross-sectional area of intermediate lobe, as well as an increase in ACTH-immunoreactive cells and a 50% decrease in the number of D2 receptors. A loss of D2 receptors is a key finding, since they are thought to repress ACTH expression in the intermediate lobe. Finally, adrenal hypertrophy was accompanied by fusion of adrenal cortex zona (Björkqvist et al., 2006). These changes were all considered to be signs of HPA axis hyperactivity.

Other neuroanatomical changes reported in R6/2 mice include a loss of neurons in the lateral hypothalamus (Petersén et al., 2005), which is similar to the pathology reported in human post-mortem brains and suggests that hypothalamic neuronal degeneration is a consistent feature of HD in both humans and animals. Finally, the argument for hippocampal damage in HD is strengthened by evidence from a range of HD murine transgenic models, including R6/1, R6/2, YAC128, which each express different HD phenotypes but all consistently display reduced neurogenesis and cell proliferation in the hippocampus (Fedele, Roybon, Nordström, Li, & Brundin, 2011; Gil et al., 2005; Grote et al., 2005; Lazic et al., 2004; Simpson et al., 2011). Collectively, the evidence of HPA axis dysfunction and pathological change provided by a number of HD models attests that it is highly likely that HPA axis integrity is affected by the HD CAG expansion.
1.4.3. Theories about the mechanisms underlying HPA axis dysfunction in HD.

The theories that attempt to explain HPA axis change in HD are varied, which is possibly because the point of axial dysfunction is not conclusively known, and the evidence of HPA axis dysregulation in HD is somewhat conflicting. Each neuroanatomical structure along the HPA axis has the potential to be dysfunctional, according to the HD literature, and these structures will be discussed in turn.

In support of the hypothalamus as the site of HPA axis malfunction, Heuser et al. (1991) reported that in HD patients with high levels of basal cortisol, the addition of exogenous ovine CRH (oCRH) reduced secretion of ACTH in comparison to controls. This ACTH blunting response suggests that in the context of persisting basal hypercortisolism (high baseline cortisol levels), the HPA axis is hypersensitive to elevated CRH as it attempts to counteract the excessive push for increased ACTH secretion, and therefore increased cortisol levels, by the hypothalamus on the pituitary gland. In other words, the pituitary-adrenal negative feedback loop is working effectively but the problem lies further back in the system with a CRH overdrive, possibly due to a loss of inhibitory GABAergic neurons (Heuser et al., 1991). Excessive CRH production as a first-point of dysfunction in HD is consistent with reports of elevated CRH in the CSF of HD patients (Kurlan et al., 1988). It could explain why CRH levels in the post-mortem HD brain are relatively high compared to other neurodegenerative disorders (De Souza, Whitehouse, Folstein, Price, & Vale, 1987). Uncontrolled CRH levels point to hypothalamic abnormalities as a potential causal factor for HPA axis hyperactivity. This strengthens the suggestion by Aziz et al. (2009) that dysregulation of the HPA axis in HD is attributable to impaired glucocorticoid receptor (GR) function in the paraventricular nucleus.
(PVN) of the hypothalamus. As a reminder, glucocorticoid receptors are the low-affinity receptors which only become active after high-affinity mineralocorticoid receptors (MRs) are saturated. The theory of hypothalamic dysfunction also helps explain Aziz et al.’s finding of excessive cortisol in peak periods, such as the morning, when HPA axis activity is naturally high and glucocorticoid receptors should be controlling inflated cortisol secretion. However it does not explain the reasons underlying hypercortisolism in the early-mid afternoon period when cortisol levels are normally low and mineralocorticoid receptors should dictate cortisol regulation (Spencer et al., 1998).

There is evidence of a pituitary-related HPA axis disturbance in HD. This evidence comes from the R6/2 mouse, which shows excessive and unopposed serum ACTH levels that induce adrenal hypertrophy and morphological abnormalities, which in turn promote corticosterone overproduction (Björkqvist et al., 2006). These murine models displayed a significant reduction in hypothalamic CRH content, which is possibly an attempt to counteract the hyperactivity downstream of the hypothalamus. Compensatory mechanisms that are supposed to address increased levels of HPA axis hormones at the level of the adrenal gland may also be failing in HD, with a significant reduction in the level of the anti-glucocorticoid dehydroepiandrosterone sulfate (DHEAS) in HD (Leblhuber et al., 1995). DHEAS is an enzymatic convert of dehydroepiandrosterone (DHEA). DHEA is an anti-glucocorticoid produced by the zona reticularis of the adrenal gland cortex and is thought to have an antagonistic influence on the metabolic effects of glucocorticoids. Bruyn, de Yong, and van der Molen (1972) speculate that low DHEAS levels are due to a malfunction in the link between the adrenal gland–sympathetic nervous system and striatal dopaminergic
catecholamine system. Finally, theories about the role of the hippocampus in HPA axis functioning in HD have not been broached.

A non-specific global breakdown in the HPA axis system could also be occurring in HD, as opposed to localised hypothalamic, pituitary, adrenal, or hippocampal dysfunction. In the study by Hueser et al. (1991), despite a hypersensitive feedback reaction to excessive CRH, which caused a reduction in ACTH levels, circulating cortisol concentration remained elevated. Therefore, whilst ACTH was regulated, cortisol was not. In addition, after administering DEX as a part of the dexamethasone suppression test, cortisol levels in HD patients remained higher than controls whilst DEX levels were comparatively lower in the patient group. Effectively this means that even though excessive glucocorticoid levels were being corrected through metabolic clearance mechanisms in HD, they were not being as efficiently regulated from the secretion standpoint because cortisol was still being released as DEX levels were falling. According to Hueser et al., this also occurred in some HD CAG normal depressed patients. The results of the study by Hueser et al. are therefore somewhat conflicting. The conflict occurs because some findings point to a localised point of HPA axis dysfunction in HD, whereas other findings challenge a site-specific disturbance to the HPA axis in HD and instead suggest that the HPA axis as a whole could be affected.

There are a number of other potential factors that could increase cortisol levels in HD patients. These include stress – either emotional or physical, due to the continuous hyperkinesias (Leblhuber et al., 1995; Saleh et al., 2009; van Duijn et al., 2010), neuroleptic treatments
(Saleh et al., 2009), or age which can impact the metabolic rate of glucocorticoids (Heuser et al., 1991).

1.4.4. Evaluating the HPA axis sampling and analysis methodology used in HD research to date.

Table 1.1. (see page 34) presents a summary of the protocols used in the eight published studies which have investigated the HPA axis in HD. These all demonstrated abnormal HPA axis activity in HD. However, there are inconsistencies in the methodology used across these studies, which might contribute to the conflicting theories about HPA axis change in HD to date. Despite the HPA axis being a fluctuating physiological system that changes over the circadian cycle, only Aziz et al. (2009) measured cortisol at multiple time-points over a 24-hour period. The other studies only reported total mean cortisol levels averaged across a two or three hour period of time, as in urine collection studies; or they measured levels of cortisol and other HPA axis hormones by taking CSF, blood, and saliva samples at a time during the day that was not consistent with collection times of other HD HPA axis studies. These sampling methods do not capture the cyclical nature of the HPA axis at multiple points throughout the day. In addition, the measurement epochs are inconsistent across studies and vary between morning, afternoon, and evening. Another limitation in these studies is small sample sizes, which are an inherent difficulty in all HD research, partly because of the low population prevalence of HD but also because the debilitating effects of HD for both the patient and carers restricts participation in research. It also appears that tracking HPA axis

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2 Neuroendocrinological studies in HD that did not directly test for cortisol or HPA axis hormones involved in cortisol secretion were not included in Table 1.1.
Chapter 1 – Introduction

integrity across disease progression – that is, from pre-HD many years before diagnosis to late stage diagnosed HD – has not been a research focus. As a result, it is hard to know the critical points of HPA axis change across the disease. There were studies that accurately identified pre-HD (van Duijn et al., 2010) and early-HD as important stages at which to characterise HPA axis abnormalities (Aziz et al., 2009; Kurlan et al., 1988). However, only two studies measured cortisol levels across both pre-HD and diagnosed disease states in comparison to healthy controls (van Duijn et al., 2010), with Björkvist et al. (2006) even differentiating between early-HD and late-stage HD. The other five of the eight studies did not specify the disease stage of the HD participants (Heuser et al., 1991; Leblhuber et al., 1995; Markianos, Panas, Kalfakis, & Vassilopoulos, 2007; Saleh et al., 2009; van Duijn et al., 2010). Therefore, due to methodological limitations or inconsistencies, the utility of HPA axis hormones as a biomarker in HD is yet to be explored to its fullest potential, both in pre-HD and early-HD stages.
### Table 1.1.

*Methodology of previous studies investigating HPA axis function in HD*

<table>
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## Chapter 1 – Introduction

### Protocol

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<td>Blood</td>
<td>Saliva</td>
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| Stress test undertaken | oCRH | DST | - | - | - | - | - | - |

| Collection period | 15:00 – 17:00 | 18:30 – 21:00 | 19:00 – 21:00 | 16:00 | 8:00 | 14:00 – 17:00 | 8:00 – 10:00 | 24hr | 24hr | Morning | Waking; DST | Waking | 22:00 – 23:00 |

### Variables measured

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### Measures used

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<tr>
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<tr>
<td>Other</td>
<td>Diagnostic interview; LIFE - - - - - - -</td>
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<table>
<thead>
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<th>Assessment of cognitive symptoms</th>
</tr>
</thead>
<tbody>
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<td>Y/N</td>
<td>N  N  N&lt;sup&gt;b&lt;/sup&gt;  N  Y  N  N  Y</td>
</tr>
<tr>
<td>MMSE</td>
<td>-  -  -  -  √  -  -  √</td>
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</tbody>
</table>

**Note.** BDI, Beck’s Depression Inventory; BMI, Body Mass Index; CSF, Cerebrospinal Fluid; CIDI; Comprehensive International Diagnostic Interview v.2.1; DSM, Diagnostic and Statistic Manual of Mental Disorders; DST, Dexamethasone Suppression Test; LIFE, Longitudinal Interval Follow-up Evaluation psychiatric status scale; MMSE, Mini Mental State Examination; oCRH, ovine Corticotropin-Releasing Hormone; TFC, Total Functional Capacity; UHDRS, Unified Huntington’s Disease Rating Scale. Cells with a dash (-) indicate that data was not obtained or was not reported by the study.

<sup>a</sup>“Functional incapacity” was used by Hueser, et al. (1991) to categorise the stage of disease severity according to Shoulson and Fahn (1979).

<sup>b</sup>Patients with dementia or depression were excluded by Leblhuber, et al. (1995).

<sup>c</sup>Mood was not assessed in participants who produced a MMSE score <18 points.

Studies investigating HPA axis physiology that did not directly test for cortisol levels or HPA axis hormones involved in cortisol secretion were not included in this table. These studies are Bruyn, de Yong, & van der Molen, 1972; Goodman et al., 2008; and Wood et al., 2008.
1.5. Psychiatric and cognitive signs are yet to be properly investigated in the context of HPA axis dysfunction in HD

Investigations into the association between HPA axis neuroendocrine dysfunction and the HD phenotype have thus far focused on the physical effects of the disease, specifically weight loss, metabolism, and sleep disturbance (Aziz et al., 2009; Aziz et al., 2007; Petersén & Björkqvist, 2006). Bjorkqvist et al. (2006) likened the physical presentation of the R6/2 mouse to a severe HPA axis disturbance, such as a Cushing’s-like phenotype of muscle atrophy, reduced body mass, reduced bone density, insulin resistance, and an increase in intra-abdominal fat deposits. In human studies, serum cortisol concentration and a number of cortisol functional parameters have been found to be related to CAG size, UHDRS behavioural and motor scores, UHDRS Total Functioning Capacity score, and Body Mass Index (Aziz et al., 2009; Saleh et al., 2009).

A relationship between HPA axis dysfunction and psychiatric and cognitive abnormalities in HD has been alluded to in the literature (Aziz et al., 2007; Björkqvist et al., 2006), though little research has actually investigated this link. When mood and cognitive functioning have been measured in the context of HPA axis function this has not been a primary research focus, and the tests used have largely been limited to screening tools (see Table 1.1.). In terms of mood, Hueser et al. (1991) found that a past diagnosis of major depression was not associated with basal cortisol levels or hormone response to oCRH. Van Duijn et al. (2010) did not find a difference in the presence of a depressive disorder between controls, pre-HD, and HD groups when using the Comprehensive International Diagnostic Interview (CIDI), which is a screening tool that can diagnose the presence of mental disorders but not the severity of the condition. In the study by van Duijn et al., the number of diagnosed depressed participants was
low across all groups and this may be why the authors did not consider depression as a variable in the analysis of HPA axis function. Only two studies have examined the relationship between ratings of current mood severity and corticotropic hormone levels in HD (Aziz et al., 2009; Kurlan et al., 1988). Aziz et al. (2009) did not identify any association between mood and cortisol levels. Kurlan et al. used the DSM-III, a diagnostic measure of mood disturbance, and found that CSF CRH levels were not significantly different between depressed and non-depressed HD patients. There was, however, a significant positive correlation between scores on the Longitudinal Interval Follow-up Evaluation (LIFE) psychiatric status scale\(^3\) and CRH levels in early stage HD patients who met the diagnostic criteria for major depression (Kurlan et al., 1988). The findings of Kurlan et al. have not been replicated.

The association between the HPA axis and cognition in HD has received even less attention than the HD psychiatric disturbances. In fact, this has only been analysed in one study where the Mini-Mental State Examination (MMSE) – which is a brief screening measure that produces a single score to indicate the state of global cognitive functioning – score was not found to be associated with DHEAS\(^4\) levels or cortisol concentration in female HD patients (Markianos et al., 2007). Therefore, whilst a picture of the role of the HPA axis in the physical phenotype of HD is beginning to form, there is almost nothing known about the relationship between the integrity of the HPA axis, specifically cortisol levels, and psychiatric and cognitive functioning in HD.

\(^3\) The LIFE is a semi-structured interview which measures the severity of affective disorders.

\(^4\) As a reminder, DHEAS is an enzymatic convert of the anti-glucocorticoid DHEA.
1.6. Phenotypic similarities between HD and non-HD conditions of HPA axis dysfunction

The findings from research that has investigated HPA axis dysfunction in non-HD medical and neuropsychiatric conditions is also relevant for informing research on HPA axis function in HD. Inferences from disorders which display a similar clinical phenotype to HD set a precedent that supports the rationale for investigating the relationship between the HPA axis and cognitive and psychiatric signs in HD. In order to evaluate this link, the literature review will now briefly diverge from HD to explore relevant non-HD conditions. Although Cushing’s syndrome provides a strong premise for understanding the HPA axis, it will not be directly compared to HD. Cushing’s syndrome is almost purely a disorder of the HPA axis, whereas in HD there are a complex array of processes that are likely to be occurring; many of these are unrelated to HPA axis abnormalities, thus for drawing parallels between HD and disorders that display HPA axis dysfunction, more comparable conditions will be assessed.

1.6.1. HPA axis dysfunction and mood disorders.

Depressive mood disorders are very good models of HPA axis dysfunction to compare HD against, because of the phenotypic similarity between the two conditions. Plasma sampling by Sachar et al. (1973) indicated that patients with a depressive illness secreted almost twice as much cortisol per day as controls and they were significantly elevated on most secretory characteristics, including: significantly higher maximum and minimum cortisol concentrations at almost all phases of the day, significantly more time spent secreting cortisol, significantly more cortisol production per minute, more major cortisol secretory episodes, and significantly more cortisol production in these secretory episodes. The authors concluded that cortisol
Chapter 1 – Introduction

hypersecretion occurred through the nocturnal and daytime waking hours in depressed patients. Abnormal cortisol levels in saliva have also been reported at a number of time points in patients with depression (Alhaj, Massey, & McAllister-Williams, 2007; Hinkelmann et al., 2009). Den Hartog et al. (2003) found that salivary cortisol was elevated in the evening in depressed patients and, in comparison to non-depressed healthy controls, this actually resulted in an overall flattening of the cortisol curve. This finding is concerning for the patient because if cortisol concentration is elevated at a time point where it traditionally should be very low, there is no reprieve from hypercortisolism at any time during the day. It is, however, the cortisol awakening response that can best highlight the relationship between HPA axis dysfunction and depressed mood. Because the cortisol awakening response reflects the production capacity of the HPA axis (Pruessner et al., 1997), the spike in cortisol is a consistent indicator of the body’s maximum stress response. Abnormal zenith cortisol levels shortly after waking in depressed patients are therefore a useful biomarker of depression (Bhagwagar, Hafizi, & Cowen, 2005; Hinkelmann et al., 2009; Sachar et al., 1973).

The course and cause of abnormal HPA axis functioning in depression, in particular the cortisol awakening response, is unknown, which makes the task of understanding the relationship between the HPA axis and mood difficult. It is likely that HPA axis disturbances are a long-term dysfunction. A higher cortisol awakening response in both current and remitted major depressive disorder participants suggests HPA axis abnormalities are a chronic trait marker, and not a transient state marker, of depression because elevated morning cortisol levels can continue even after clinical recovery (Bhagwagar, Hafizi, & Cowen, 2003; Vreeburg et al., 2009). An alternate argument is that altered cortisol levels may only be an acute reaction to depression. Watson et al. (2002) found that chronically depressed patients do
not show significant abnormalities in cortisol secretion or the HPA axis suppression response to challenge tests. This could reflect the normalisation of the HPA axis over time or the adjustment to long-term treatment. However, this study focused on afternoon cortisol levels only – which by nature are inherently low – and it did not investigate cortisol concentration at time points that are most sensitive to HPA axis abnormalities (i.e., in the morning).

As in the HD literature, the neuropathology of the HPA axis has not been thoroughly investigated in depression research (Sheline, Mittler, & Muntin, 2002). In saying that, an organic basis to abnormally elevated cortisol levels in depression is likely. Melancholic depression, which is considered to be an idiopathic organic form of depressed mood is, in part, characterised by high morning cortisol (Gold & Chrousos, 2002). This suggests that there is a biological underpinning to HPA axis dysfunction in depressive mood disorders, which is not purely a reaction to psychosocial events. Studies have shown that glucocorticoid receptors are reduced in depression and can be increased with anti-depressant medication (Barden, 1996; Vreeburg et al., 2009). Therefore, the hypothalamus is a potential aetiological site for HPA axis disturbance in depression. The hippocampus may also be involved, with evidence of hippocampal atrophy in depressed patients (Colla et al., 2006; Sheline et al., 2002; Sheline, Wang, Gado, Csernansky, & Vannier, 1996). On the other hand, irregularities within the HPA axis per se might not be directly involved. DHEA, the glucocorticoid antagonist, has been shown to be unbalanced against cortisol in patients with major depression causing a high cortisol-DHEA ratio\(^5\) to exist (A. H. Young, Gallagher, & Porter, 2002).

\(^5\) A high cortisol-DHEA reflects high cortisol in relation to low levels of the anti-glucocorticoid DHEA.
In summary, one of the principal complexities in understanding the relationship between high cortisol levels and depression, which also makes it hard to apply this research to HD, is encapsulated by the conundrum; “What came first ... the chicken or the egg?” It is not clear whether hypercortisolism is indicative of a biological predisposition to depressed mood, or whether hypercortisolism is a biological vulnerability that persists following the onset of a mood disturbance (Bhagwagar et al., 2005). Further discussion about the link between HPA axis dysfunction and depression is provided in Chapter 3 (Study 1).

1.6.2. HPA axis dysfunction and cognitive decline.

Depression is also relevant when trying to understand the consequence of abnormal HPA axis function on cognition because of the association between heightened cortisol in depressive disorder and reduced cognitive ability in the domains of verbal memory, visuospatial memory, attentional control, and speed of cognition (Alhaj et al., 2007; den Hartog et al., 2003; Gomez et al., 2009; Hinkelmann et al., 2009). However, it is unclear as to whether the mood and cognitive changes in depression occur via similar neuroendocrinological mechanisms (den Hartog et al., 2003; A. H. Young, Gallagher, & Porter, 2000). In mild cognitive impairment (MCI), which is a clinical state of impaired memory functioning, considered in many cases to be a prodromal dementia phase, higher waking salivary cortisol levels have been reported in patients that have a lower score global cognitive functioning (MMSE) score than healthy controls (Lind, Edman, Nordlund, Olsson, & Wallin, 2007). In normal aging, hypercortisolism is also related to reduced performance on tasks of language, processing speed, eye-hand coordination, executive functioning, verbal memory and learning, and visual memory (Comijs et al., 2010; Lee et al., 2007; G. Li et al., 2006). Elderly people whose cortisol levels have
increased over time and then rise even further show significant cognitive difficulties. In contrast, elderly people whose cortisol levels decline from an elevated level over time perform similarly to healthy younger people (Lupien et al., 1994).

The effect of HPA axis hyperactivity on the cognitive domain of verbal learning and memory performance is of particular interest to this thesis. The profound impact on memory function in people with long-term elevated and rising cortisol has been likened to amnestic patients with hippocampal damage or temporal lobe removal (Lupien et al., 1994). The hippocampus, a structure widely regarded as playing a fundamental role in learning and memory, is susceptible to neuroendocrine toxicity. As mentioned previously, not only is this important for the cognitive state of the patient but also for the role that the hippocampus plays in regulating the HPA axis. Hippocampal pathology is thought to occur in a number of conditions and states that display HPA axis disturbance (Bremner et al., 1995; Giubilei et al., 2001; Lupien et al., 1998; Sapolsky, 2000; Sheline et al., 1996; Starkman, Gebarski, Berent, & Schteingart, 1992). These conditions and states are discussed throughout Section 1.6 of the Introduction chapter of this thesis. HD could also be added to this list. There is evidence that the volume and function of the hippocampus changes in HD, and these pathophysiological changes could contribute to the clinical profile of neuropsychological deficits as discussed previously, in Section 1.2.2. of the Introduction chapter of this thesis. Further discussion about the link between HPA axis dysfunction and learning and memory change is provided in Chapter 4 (Study 2).
1.6.3. HPA axis dysfunction in other chronic and neurodegenerative conditions.

Abnormal and inconsistent patterns of the HPA axis have been described in people infected with the human immunodeficiency virus (HIV). Patients that display an upward trend in salivary cortisol throughout the day record the highest depression, anxiety, fatigue, and HIV viral load (Barroso, Burrage, Carlson, & Carlson, 2006). Post-traumatic stress disorder (PTSD) is a condition of prolonged exposure to physical and/or emotional stress in which the HPA axis can eventually become dysfunctional and poorly regulated. After experiencing chronic hypercortisolism, because of high stress, PTSD patients commonly display hypocortisolism (low baseline cortisol levels) throughout the circadian cycle (Raison & Miller, 2003; Yehuda, Teicher, Levengood, Trestman, & Siever, 1994). Repeated episodes of acute stress can, however, still induce an exaggerated response of hypercortisolism in PTSD patients with hypocortisolism (Bremner et al., 2003; Heim et al., 2000). The pathological and functional changes that occur to the HPA axis in PTSD are unclear. There are suggestions it could be a consequence of HPA axis burnout, disrupted cortisol receptor signalling pathways, or receptor hypersensitivity and negative feedback over-regulation. Clinically, mood complications are intertwined with PTSD, and there is a relationship between dysregulation of high cortisol concentration and verbal declarative memory deficits (Grossman et al., 2006). Finally for this section, chronic heightened glucocorticoid levels are also associated with neurodegenerative diseases and severe forms of dementia. Elevated cortisol has been detected in the blood, saliva, and CSF of Alzheimer’s disease patients both alive and deceased, and both pre-senile and senile (Davis et al., 1986; Hatzinger et al., 1995; Peskind, Wilkinson, Petrie, Schellenberg, & Raskind, 2001; Wahbeh, Kishiyama, Zajdel, & Oken, 2008). What is
particularly interesting is that a number of these studies found cortisol levels to be abnormally high in comparison to controls in the morning hours.

1.6.4. The HPA axis in the psychoses.

Considerable inroads have been made into the understanding of the HPA axis through research involving disorders of extreme psychosis. These findings are very relevant to HD because pre-HD individuals and HD patients can display similar neuropsychiatric features to psychotic depression and schizophrenia. As an example, these features can include signs of hallucinations, delusions, mania, insomnia, blunted affect, and suicidal ideation (Craufurd et al., 2001; Paulsen, Ready, et al., 2001; van Duijn et al., 2008). Psychotic mood disturbance, including severe affective psychosis and suicidality, has been linked to a marked hyperactivation of the HPA axis (Bunney & Fawcett, 1965; Sachar, Kanter, Buie, Engle, & Mehlman, 1970) in what Sachar, et al. (1970) described as a “neuroendocrine storm” (p. 1075). Onset of first episode schizophrenic psychoses provides a transitional state to study changes in the stress hormone system, and the findings strengthen the likelihood of a link between psychiatric disturbances and HPA axis dysfunction. Higher levels of neuropsychiatric signs and worse cognitive function are associated with abnormal cortisol regulation in schizophrenia (Newcomer, Faustman, Whiteford, Moses, & Csernansky, 1991). Corticosteroid levels in acute schizophrenia have been measured at two-and-a-half times above baseline levels. These increases are above the normal fluctuating stress levels of 25%–30% seen in healthy humans (Sachar et al., 1970).
Anatomical imaging suggests that pituitary hyperplasia is a key feature of first episode psychosis patients. Volumetric analysis can differentiate between those who will and will not develop a psychosis in the high-risk patient population with a reasonable degree of accuracy. Pituitary size increases as onset becomes more proximal, and patients transition to their first psychotic episode (Garner et al., 2005; Pariante et al., 2005; Pariante et al., 2004). The type of psychosis that is experienced has an effect on pituitary volume, with a significant increase in pituitary volume of 24% in schizophrenia or schizophreniform psychosis and a 17% increase in affective psychosis compared to healthy controls (Pariante, et al., 2005). Enlargement most likely occurs in the anterior pituitary, as there are no known conditions of posterior pituitary enlargement, except tumours (Garner et al., 2005; Pariante et al., 2005). Furthermore, it is independent of neuroleptic medications, apart from typical antipsychotic treatment, which may possibly contribute to increasing pituitary size (Pariante et al., 2005).

The theories about the mechanisms underlying HPA axis activation in psychosis are speculative. A hypothesis is that a stressful state of psychiatric disorganisation in the prodromal phase induces HPA stress response activation and, in particular, pituitary ACTH production (Garner et al., 2005). Elevated corticosteroid levels then only decline upon recovery, or during periods of psychiatric stability (Sachar et al., 1970). An anomaly is that non-converting patients at a high risk of psychosis have been found to have smaller pituitary volumes than controls and, in addition, the pituitary volumes of patients with chronic diagnosed schizophrenia of at least 5 years are significantly smaller than controls (Garner et al., 2005; Pariante et al., 2004). The proposed explanation for this is that, in general, those who are at risk of psychiatric illness have an abnormally small pituitary gland, possibly because of a neurodevelopmental irregularity. In those who go on to develop a psychosis, their
pituitary grows larger as they progress to the point of transition, thereby surpassing the “normal” volume until it becomes significantly larger around the time of onset. Reduced stress response activity can protect those who are at risk of psychosis against converting to their first psychotic episode (Garner et al., 2005). For patients who do convert to a psychosis, the chronicity of mental illness may induce pathophysiological mechanisms, which eventually leads to pituitary hypoplasia (Pariante et al., 2004).

1.6.5. HPA axis dysfunction and sleep disorders.

Sleep disorders are also relevant to this thesis. Hyperactivation of the HPA axis and heightened glucocorticoid levels are associated with arousal and sleeplessness (Vgontzas & Chrousos, 2002). Buckley and Schatzberg (2005) suggest that poor sleep and HPA axis dysfunction are related in an inextricable cycle so that excess corticosteroid hormones reduce slow wave sleep and shortens sleep duration via glucocorticoid receptor stimulation. In turn, extreme conditions of poor sleep quality can provoke HPA axis dysfunction, and therefore propagate a detrimental cycle. Sleep quality has received minimal research attention in HD, despite clinical anecdotes and a small volume of research indicating that sleep disturbance is a problem in HD and could begin early in the disease process (Shannon & Moore, 2001).

1.6.6. Summary of the research precedence set by non-HD conditions.

There are a range of mood disorders, neurodegenerative dementias, neuropsychiatric conditions, medical diseases, and disrupted homeostatic states that manifest signs of a depressed mood and memory decline which are similar to HD, in the context of HPA axis
dysfunction. The collective finding from the non-HD literature is that HPA axis biomarkers and phenotypic clinical markers are related. Given that there is no known association between these signs and HPA axis changes in HD, the non-HD literature is useful for considering this link. There is also a better understanding of the pathophysiology underlying the relationship between HPA axis dysfunction and the phenotype in these disorders. Insight into the cause of biomarker change and clinical signs translates to a better knowledge for developing treatment interventions. If this understanding could be applied to HD, it is possible that the treatment development pathway could benefit.

1.7. The HPA axis as a therapeutic target for treatment intervention in HD

If HPA axis dysfunction is found to be a key physiological feature of HD and it is shown to be associated with HD clinical signs, there is no reason to suggest why the HPA axis should not be considered as a target for therapeutic intervention. As yet this has not been investigated in HD. There are a range of options for the treatment of HPA axis dysfunction, and for addressing depression and memory signs. The treatment of extreme HPA axis dysfunction in Cushing’s disease often requires dramatic surgical intervention. For management of a relatively mild HPA axis disturbance, studies investigating antidepressant medications show that mood medications may ameliorate excessive and unregulated CRH release. Eventually this is associated with correction of cortisol levels and the remission of depression (Holsboer, 2001; Holsboer & Barden, 1996). It has been suggested that antidepressant selective serotonin reuptake inhibitors (SSRIs) may not reduce cortisol levels through a direct effect on the HPA axis system, but instead because of adaptive changes in serotonergic (5-HT) receptors after chronic administration (Raap & Van de Kar, 1999). A combination of three pieces of evidence
from HD and non-HD literature focus on the hippocampus to explain how this phenomenon could apply to HD. Firstly, Pang, Du, Zajac, Howard, and Hannan (2009) found a reduction in 5-HT receptor gene expression was associated with depression-related behaviour in a transgenic HD mouse model. Secondly, 5-HT receptor loss has been identified in the hippocampus of depressed patients (Sheline et al., 2002) and HD mice (Pang et al., 2009). Third, hippocampal neurogenesis can be rescued and behavioural and cognitive signs improve in HD mice following treatment with SSRIs (Grote et al., 2005). A consideration across all of these findings is that the effects reported may be gender-specific (Pang et al., 2009). One missing link amongst this collection of evidence which remains to be examined is whether SSRIs affect hippocampal 5-HT receptors in HD and if, in turn, this alters cortisol levels.

Aside from SSRIs, cortisol synthesis inhibitors can be useful for treating hypercortisolism, but they are accompanied by a range of side-effects. The antagonistic effects of synthetic glucocorticoids, such as mifepristone, are another option to treat HPA axis dysfunction, though these have not been extensively studied (Rothschild, 2003). Therapeutic treatment of HD is a clinical priority. Understanding HPA axis dysfunction does offer some options for how to proceed – by either targeting the HPA axis directly, or monitoring HPA axis biomarker levels and the associated change in clinical markers after administering an intervention. Whilst there is the potential that the HPA axis may be involved in the treatment of HD in some way, HD therapy is not a major focus of this thesis and the clinical relevance of the involvement of the HPA axis in HD, and any implications for treatment, will be only briefly addressed in Chapter 5 (General discussion).


1.8. Summary and aims of the thesis

There is a strong body of evidence indicating that the HPA axis is abnormal in HD. Animal studies have indicated hypercortisolism in HD mouse models; human studies have also found significantly heightened cortisol in pre-HD and HD and evidence of an impaired HPA-axis negative feedback loop. Furthermore, HD neuropathological studies have identified abnormalities in the hypothalamus, pituitary gland, adrenal gland, and the hippocampus. However, the understanding of HPA axis function in HD is primarily limited to a descriptive analysis of the hormone profile and how it might differ between pre-HD and HD in comparison to normal controls. The relationship between HPA axis function and the early signs of the HD clinical phenotype has not been thoroughly tested. Where psychiatric or cognitive measures have been included in HD studies they have not been a primary research focus, and therefore the analysis of these clinical domains has been non-specific and suggestive at a high level only. Much of the rationale for the pursuit to undertake the research in this thesis comes from non-HD conditions because they show a similar psychiatric and cognitive profile to HD, and in these conditions, the clinical signs are associated with HPA axis dysfunction. There is an opportunity to gain a better understanding of the integrity of the HPA axis in HD, and the relationship that abnormal corticotropic hormone levels have to the psychiatric and cognitive signs seen in pre-HD and early-HD. Identifying a link here would lend to support the possibility of using the HPA axis as a biomarker of HD onset and disease progression, and even open the pathway for the development of treatment in the future.

The aim of this research project was to extend the current understanding of how the integrity of the HPA axis changes in HD, by investigating whether there is a relationship between HPA
axis function and clinical signs across the early stages of HD. Specifically, the focus was on
the association between cortisol concentration, as a marker of HPA axis function, and the
psychiatric syndrome of depression, as well as the cognitive ability of learning and memory.
These two domains were selected based on the literature indicating that they are affected early
in HD and they are implicated in non-HD conditions that also develop HPA axis
abnormalities. The association between cortisol levels and the severity of signs in each of
these clinical domains will be investigated separately, and the findings are reported in two
separate manuscripts that have been submitted for publication. Chapter 3 (Study 1) reports an
examination of the relationship between HPA axis function, in particular morning cortisol
levels, and depression in the pre-HD and early post-diagnosed stages of HD. Chapter 4 (Study
2) reports an examination of whether there is a relationship between HPA axis function,
foecussing on evening cortisol levels, and the verbal learning and memory profile in the pre-
HD and early stages of diagnosed HD. Stress and sleep quality are also measured and analysed
as a part of this research project because these are factors known to be associated with cortisol
levels, as well as with mood and neuropsychological performance.

Chapter 2 (Methods) describes the experimental methodology used in the research project
which is either not presented in detail in the manuscripts submitted for publication, or which
requires further elaboration. The research protocol undertaken was designed to determine
relationships between cortisol concentrations and the psychiatric and cognitive signs, and the
point in the 24-hour cortisol cycle at which these associations are the strongest. Selected
psychiatric and neuropsychological measures will be employed to specifically assess how
depression, and learning and memory are associated with HPA dysregulation. Cortisol
sampling at multiple time points over a 24-hour period used in this project will consider the
fluctuating cortisol profile over the circadian cycle, and this is a methodological advancement compared to previous HD research. A participant cohort that spans the pre-HD to early-HD population will allow a comparison of HPA axis activity across different stages in disease progression. In addition, Chapter 2 (Methods) also contains a brief description of measures that formed part of the wider research protocol, but which are not presented in either manuscript. These tests were designed to assess processing speed, visual scanning, as well as selective, sustained, and divided attention. Results of these measures were not analysed or reported because they detract focus from the primary objectives of the thesis. The thesis concludes by discussing the link between the findings of Study 1 and Study 2 in Chapter 5 (General discussion). The General discussion will evaluate what the findings mean for the understanding of HD and the early disease process, how the findings extend on the literature, the utility of the HPA axis as a biomarker in HD, and the clinical relevance and functional application of the results. In the final parts of the General discussion chapter, consideration for the limitations of the study and future directions for this field of research in HD are then discussed.
CHAPTER TWO

- METHODS -
Chapter 2 – Methods

Methodology for Study 1 and Study 2 is presented in Chapter 3 and Chapter 4 of this thesis, respectively. This chapter provides additional methodological details not included in Chapter 3 and Chapter 4. The research project was approved by the St. Vincent’s Hospital Human Research and Ethics Committee, and Monash University Human Research Ethics Committee.

2.1. Participant recruitment

Over a period of 17 months (September 2009–January 2011) a total of 134 people (37 early-HD, 41 pre-HD, and 56 HD CAG normal [controls]) were invited to participate. Potential participants were provided with a copy of the Participant Information and Consent Forms (PICF) pack (see Appendix 1), which contained an explanatory statement of the study, a Participant Response Form, and two consent forms to be signed at the study visit. Those interested in participating were asked to return the Participant Response Form in the reply-paid envelope provided. Potential participants who did not reply were followed-up by phone to ensure they received the invitation. Sixty-six people (21 early-HD, 21 pre-HD, and 24 controls) expressed an interest in participating, and their eligibility was assessed through a phone-screen (see Appendix 2). Fifty-nine participants (19 early-HD, 20 pre-HD, 20 controls) met the eligibility criteria and were enrolled in the study. Attempts were made to recruit a similar proportions of male and female participants in each group in order to account for any gender-related dimorphism in HPA axis functioning.
Inclusion and exclusion criteria are detailed in Chapter 3 and Chapter 4. A pathologist from Healthscope Pathology Ltd. (Melbourne, Australia) was consulted if there was a query about the eligibility of a potential participant to participate (usually this was due to their medication regimen or physical state), and whether this would affect their cortisol levels.

2.1.1. Participant consent.

Participants were asked to provide written consent to participate by signing two forms at the time of the study visit. These were included in the original PICF pack (see Appendix 1), but needed to be signed in the presence of the researcher and a witness. These forms related to:

- Participation in the study; and
- Contacting the participant’s neurologist/psychiatrist or testing laboratory in order to obtain their CAG repeat numbers.

St. Vincent’s Hospital Human Research and Ethics Committee project approval stipulated that competence to consent must be determined by one of the senior investigators: Prof. Ed Chiu, Psychiatrist; Dr Phyllis Chua, Psychiatrist; Dr Andrew Churchyard, Neurologist. A person was deemed competent to give consent if they could give consent freely, and could demonstrate that they comprehended the study risks and benefits by reading the PICF and explain to the researcher in their own words what they understood the study to be about.
2.1.2. Burden of pathology.

CAG repeat lengths were used to compute the burden of pathology score for demographic profiling of the sample. The burden of pathology score is a continuous variable used to provide an estimate of disease burden across both pre-HD and early-HD. It is only calculated for participants who have undergone DNA testing and received confirmation that they have the HD CAG expansion. The formula used to calculate the burden of pathology score is: age \( \times \frac{\text{CAG}}{35.5} \) (Penney, Vonsattel, MacDonald, Gusella, & Myers, 1997).

2.1.3. Participant payment.

Participant travel expenses were reimbursed. In addition, participants and carers were offered a meal or snack allowance if they had travelled a substantial distance to attend testing, or if testing took longer than expected.

2.2. Materials

2.2.1. Motor function rating scale.

All participants were administered the Unified Huntington’s Disease Rating Scale (UHDRS). The UHDRS is a standardised clinical rating scale that assesses four components of HD: motor function, cognition, behaviour, and functional abilities (Huntington Study Group, 1996). Only the motor component of the UHDRS was performed in this research project (see Appendix 3) because cognition, mood, and behaviour were investigated through
Chapter 2 – Methods

alternate measures. The motor scale comprises 31 items used to assess 15 extrapyramidal motor signs that can manifest throughout the body. Each item is scored from 0 (normal) to 4 (terminology varies for each item, but it denotes a severe irregularity of the respective motor ability). In addition, there is a diagnostic confidence level item for which the rater assigns a rating of 0–4 representing the likelihood that the observed motor presentation is indicative of HD. Only the summed total from the 15 motor items (Total Motor Score [TMS]) was used in Chapter 3 and Chapter 4.

The authors of the UHDRS report internal consistency of the UHDRS – Motor scale as Cronbach’s $\alpha = 0.95$, and an inter-rater reliability correlation coefficient of 0.94. For this study, all UHDRS – Motor scale assessments were performed by clinicians formally trained in using the UHDRS – Motor scale.

### 2.2.2. Neuropsychiatric assessment.

The neuropsychiatric assessment battery was used to measure depression, sleep quality, and stress, which are clinical signs that can manifest early in the HD process and are also associated with HPA axis functioning. The Inventory of Depressive Symptomatology – Self-report, Primary Care Evaluation of Mental Disorders, Pittsburgh Sleep Quality Index, and Perceived Stress Scale were administered and analysed as a part of Study 1, and all except the Primary Care Evaluation of Mental Disorders were administered and analysed as a part of Study 2. A description of these measures is provided in Chapter 3 and Chapter 4. Additional
scoring procedures and psychometric properties not detailed in the manuscripts are provided below.

The Inventory of Depressive Symptomatology – Self-report was selected as the primary measure of depression in this research project, instead of the Primary Care Evaluation of Mental Disorders. Whilst the sample size was too small for either depression assessment tool to identify a significant difference in the number of depressed participants between diagnostic groups, the Inventory of Depressive Symptomatology – Self-report was chosen because it considered depression severity when differentiating the proportion of depressed participants from non-depressed participants.

**Inventory of Depressive Symptomatology – Self-report (IDS–SR)** (see Appendix 4): The IDS–SR items assess all symptom domains for major depressive disorder according to DSM-IV criteria, and thus can be used to screen for depression (Rush, Gullion, Basco, Jarrett, & Trivedi, 1996). The IDS–SR was not developed as a HD-specific measure of depression, but it was selected for this project because it assesses depressive features of irritability, anxiety, somatic symptoms, sleep disturbance, and cognitive difficulties, which are common in HD. In addition, the IDS–SR de-emphasises psychomotor features of depression, which participants could misinterpret as referring to their HD motor signs.

Table 2.1. (see page 59) shows the cut off score and descriptions for levels of depression symptom severity. As explained in Chapter 3, for the purpose of this study, participants with an IDS–SR total score greater than 14 were considered to be depressed. Figure 2.1. (see page 59) displays the distribution of IDS–SR Total scores for participants in this research project.
IDS–SR authors report the internal consistency of the IDS–SR for their entire cohort as

\[ \text{Cronbach’s } \alpha = 0.94, \] and for diagnosed major depressive disorder patients as \( \text{Cronbach’s } \alpha = 0.77. \) The inter-rater reliability is not provided since this is a self-report questionnaire.

Table 2.1.

*Levels of depression severity according to the IDS–SR Total score*

<table>
<thead>
<tr>
<th>IDS–SR total score</th>
<th>Severity rating</th>
<th>Depression severity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–13</td>
<td>0</td>
<td>None</td>
</tr>
<tr>
<td>14–25</td>
<td>1</td>
<td>Mild</td>
</tr>
<tr>
<td>26–38</td>
<td>2</td>
<td>Moderate</td>
</tr>
<tr>
<td>39–48</td>
<td>3</td>
<td>Severe</td>
</tr>
<tr>
<td>49–84</td>
<td>4</td>
<td>Very severe</td>
</tr>
</tbody>
</table>

*Figure 2.1. Distribution of participant IDS–SR Total scores. Participant groups are denoted by colour. The cut off score for at least *mild depression* severity is 14.*
Primary Care Evaluation of Mental Disorders (PRIME–MD) (see Appendix 5): The PRIME–MD is a diagnostic measure for the five most common primary mental health disorders (mood, anxiety, somatoform, eating, and alcohol-related; Spitzer et al., 1994). Psychometric properties are reported as indices of the agreement between a gold-standard assessment performed by a mental health professional (MHP), who administered a long diagnostic interview schedule, and the result obtained by a primary care physician (PCP), who used the PRIME–MD. For the current research project, only the mood disorders component of the PRIME–MD was used. In the published normative sample, the indices of agreement for the diagnosis of a mood disorder were: sensitivity (i.e., proportion of cases given a MHP diagnosis and correctly identified by the PCP) = 67%, specificity (i.e., proportion of cases not given a diagnosis by a MPH and also correctly not diagnosed by the PCP, i.e. true negative diagnosis) = 92%, and overall accuracy rate (i.e., proportion of patients that the PCP correctly identified as having or not having the diagnosis) = 84%.

Pittsburgh Sleep Quality Index (PSQI) (see Appendix 6): The PSQI comprises 19 items that ask about seven components of sleep in the past month: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbance, the use of sleep medication, and daytime dysfunction (Buysse, Reynolds 3rd, Monk, Berman, & Kupfer, 1989). The sum of the component scores provides a PSQI Global score ranging from 0–21. A PSQI Global score above 5 is considered indicative of poor sleep quality. PSQI authors report a high internal consistency of Cronbach’s $\alpha = 0.83$ for the individual items and for the seven component scores. Test-retest reliability of PSQI Global scores was reported as $r = 0.85$. Good index validity was determined in a normative sample by high levels of discriminability between
controls and patients with a range of sleep disorders based on the PSQI scoring profile. The
PSQI is a widely used and accepted measure of sleep quality in studies of neurological
conditions, and it has been previously been used in HD research (Aziz, Anguelova, Marinus,
Lammers, & Roos, 2010; Shannon & Moore, 2001; Stephen et al., 2005).

**Perceived Stress Scale (PSS)** (see Appendix 7): The PSS assesses the degree to which
participants appraise their life as being unpredictable, uncontrollable, overloaded, and stressful
during the past month (Cohen, Kamarck, & Mermelstein, 1983). To obtain the PSS Total
score, positive stated questions at items 4, 5, 6, 7, 9, 10, and 13 require reverse scoring. The
**PSS Total score** ranges from 14–70. In terms of psychometric properties, internal reliability
alpha coefficients for samples of freshmen students, psychology students, and participants of a
smoking-cessation program have been reported as $\alpha = 0.84$, $\alpha = 0.85$, and $\alpha = 0.86$
respectively. For the freshman students, the test-retest correlation was $r = 0.85$, whereas it was
weaker for the smoking study subjects, $r = 0.55$ – possibly due to variable effects of the
treatment program they were undertaking. Concurrent validity was established between **PSS**
**Total score** and measures of anxiety, and **PSS Total score** was a good predictor of the outcome
of dealing with stressful life events. The PSS has been used in one HD study to differentiate
the stress levels in pre-HD at different time points with respect to the estimated time to motor
onset (Downing et al., 2011). Outside of HD, the PSS has been used to investigate the
association between stress and changes in cortisol levels (Wahbeh et al., 2008).
2.2.3. Cognitive test battery.

The cognitive test battery was used to assess a number of cognitive domains that show signs of a decline early in the HD process, but the focus of this thesis was on verbal learning and memory ability. Where possible, measures with a minimal motor demand were selected in the cognitive test battery to reduce the risk of HD motor signs confounding performance. To measure verbal learning and memory ability, which is also associated with HPA axis dysfunction, the Wechsler Test of Adult Reading and the California Verbal Learning Test – Second Edition were administered and analysed as a part of Study 2. A description of these measures is provided in Chapter 4. Additional scoring procedures and psychometric properties not detailed in Chapter 4 are provided below. The Ruff 2 & 7 Selective Attention Test, Benton Visual Form Discrimination Test, and Symbol Digit Modalities Test were also administered as part of a wider project protocol but the results were not included in Chapter 4, or this thesis, because they were designed to assess aspects of cognition that did not directly relate to the aims and hypotheses of this research project. However, because they were a part of the project protocol a brief description of these tests is provided below.

**Wechsler Test of Adult Reading (WTAR)** (see Appendix 8): The WTAR is a verbal-based reading measure that estimates pre-morbid intellectual functioning based on a reading-recognition paradigm that is relatively resistant to cognitive decline associated with normal ageing and brain insult (The Psychological Corporation, 2001). The WTAR can be used to calculate a range of estimated intelligence indices. However, in the current research project the WTAR was used to estimate verbal intelligence (VIQ), given that the WTAR itself is primarily a verbal reading measure. In addition, Chapter 4 of this thesis was focused on verbal
learning and memory ability, and using the WTAR to screen for a verbal intellectual-based deficit helped ensure that there was no adverse pre-morbid impact on CVLT–II performance.

To administer the WTAR, participants were asked to read 50 irregularly spelled words aloud, and the number of correctly pronounced words was summed to obtain a raw score. To calculate an estimated VIQ, U.S. Standardisation Sample and Reference Group standard score conversion was used, because this has a broader stratification of age ranges than the U.K. standard score conversion tables. The authors report an internal consistency coefficient of $\alpha = 0.93$. The WTAR correlates highly, $r = 0.78$, with the National Adult Reading Test, which is another measure of reading recognition, thereby indicating good convergent validity. The WTAR VIQ estimate also has a high positive correlation with VIQ as measured by the Wechsler Adult Intelligence Scale – III (which is the gold-standard measure of VIQ) across age groups, $r = 0.66$ to $r = 0.80$.

**California Verbal Learning Test – Second Edition (CVLT–II)** (see Appendix 9):

Psychometric properties of the CVLT–II are based on the *Total immediate recall* score, which has an internal consistency reliability of $\alpha = 0.94$ (Delis et al., 2000). An estimate of test validity was evaluated in relation to the first edition of the CVLT. CVLT–II authors report high positive correlations between both CVLT versions, and no statistically significant differences between the two. CVLT measures have been used in numerous published HD studies (Hamilton, Murphy, & Paulsen, 1999; Lundervold, Reinvang, & Lundervold, 1994; Massman et al., 1990; Rosenberg, Sørensen, & Christensen, 1995; Stout et al., 2007).
Ruff 2 & 7 Selective Attention Test (2 & 7 Test): The 2 & 7 Test is a non-verbal test which measures the constructs of processing speed, sustained attention, selective attention, and the discrepancy between these (Ruff & Allen, 1996). It is a pencil-and-paper cancellation task that consists of a set of 20 trials administered consecutively in 15-second intervals. For each trial the participant is required to mark out 2 and 7 targets whilst ignoring other letters (Automatic detection) or numbers (Controlled search). The total administration time is 5 minutes. This test yields score of speed and accuracy, after considering the number of errors. This measure has not yet been reported on in HD research.

Benton Visual Form Discrimination Test (VFDT): The VFDT is a multiple-choice test of visual recognition without the confound of heavy motor requirements (Benton, Sivan, Hamsher, Varney, & Spreen, 1994). Participants are required to choose which of four stimuli correctly matches a target diagram. The other three options contain small variations to the image, such as a graphical displacement, rotation, or distortion. A correct response (i.e., exact match) is awarded two points, a peripheral error (i.e., displacement or rotation of the peripheral aspect of the diagram) is awarded one point, and a rotation or distortion of a major component of the diagram, along with an absent response, is awarded no points. There are 16 trials with a maximum possible score of 32. Benton et al. set a cut-off score below 23 for severely impaired performance.

Symbol Digit Modalities Test (SDMT) – Oral version: The SDMT is used to assess divided attention, visual scanning, tracking, and processing speed (Smith, 1973). The test stimulus contains a series of abstract symbols, each paired with a vacant box below. A coding key is also presented, which consists of the nine symbols used throughout the test stimulus, each
paired with a number in a box below. The participant is instructed to say the number corresponding to each symbol in the test stimulus, as indicated in the coding key, as rapidly as possible until they are instructed to stop. Participants are given a 90-second time limit in which to undertake the task, but they are not pre-informed of this duration. Scoring is based on the number of correct responses. The oral version of the SDMT was selected, instead of the conventional written version, to limit the motor demands on participants. The SDMT is reported to be a valid and sensitive measure in pre-HD and in the early stages of diagnosed HD (Paulsen, Zhao, et al., 2001). Participants can show a strong practice effect in the written version of the SDMT. The written version has been used in a number of HD studies, of which some of the participants of this research project had previously partaken.

2.2.4. Administering the psychiatric assessment and cognitive test battery.

The psychiatric and cognitive component of the test battery was estimated to take approximately 2 hours. To encourage optimal cognitive performance, the cognitive test battery was administered before the psychiatric assessment. This also precluded any psychosocial stressors, which might have been generated by the psychiatric assessment, from influencing cognitive performance. The order of test administration and the delays which were built into this battery, as required by the CVLT–II, are listed in Table 2.2. (see page 66). To ensure the test battery was administered in the correct order and the appropriate delay-times were adhered to, the researcher used a check-list called the Cognitive & Mood & Sleep Assessment Summary for each participant (see Appendix 10).
Table 2.2.

*Order of test/individual test items to be administered as a part of the wider study protocol (see Table 2.4. for study protocol)*

<table>
<thead>
<tr>
<th>Order</th>
<th>Test or test item</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CVLT–II – Immediate free recall x 5</td>
</tr>
<tr>
<td>2</td>
<td>CVLT–II – Interference trial – Immediate free recall</td>
</tr>
<tr>
<td>3</td>
<td>CVLT–II – Short delay free recall</td>
</tr>
<tr>
<td>4</td>
<td>CVLT–II – Short delay cued recall</td>
</tr>
<tr>
<td>5</td>
<td>Ruff 2 &amp; 7 Test</td>
</tr>
<tr>
<td>6</td>
<td>VFDT</td>
</tr>
<tr>
<td>7</td>
<td>CVLT–II – Long delay free recall</td>
</tr>
<tr>
<td>8</td>
<td>CVLT–II – Long delay cued recall</td>
</tr>
<tr>
<td>9</td>
<td>CVLT–II – Long delay Yes/No recognition</td>
</tr>
<tr>
<td>10</td>
<td>SDMT – Oral</td>
</tr>
<tr>
<td>11</td>
<td>CVLT–II – Long delay forced-choice recognition</td>
</tr>
<tr>
<td>12</td>
<td>WTAR</td>
</tr>
<tr>
<td>13</td>
<td>PRIME–MD</td>
</tr>
<tr>
<td>14</td>
<td>IDS–SR</td>
</tr>
<tr>
<td>15</td>
<td>PSQI</td>
</tr>
<tr>
<td>16</td>
<td>PSS</td>
</tr>
</tbody>
</table>

20 minute delay

10 minute delay
2.3. Cortisol collection and measurement

2.3.1. A review of different cortisol collection and measurement techniques.

As described in Chapter 1, there are four biofluids which can be collected for cortisol analysis: cerebrospinal fluid (CSF), urine, blood, and saliva. The majority of the studies into HPA axis function in HD have used blood as the medium for HPA axis hormone measurement (Aziz et al., 2009; Heuser et al., 1991; Leblhuber et al., 1995; Markianos et al., 2007; Saleh et al., 2009). Two studies have used urine samples (Aziz et al., 2009; Björkqvist et al., 2006). CSF (Kurlan et al., 1988) and saliva (van Duijn et al., 2010) have only been investigated by one study each.

The biofluid that is collected, and the collection technique that is used, can dictate the accessibility of the sample, the convenience for the participant, accuracy of the results, organisation and efficiency of the project protocol, and the financial cost to the project. A number of factors were considered in designing the cortisol collection protocol for this study, including the invasiveness required to obtain the sample, the ability to measure fluctuations in cortisol over the circadian cycle, and the biochemical state of cortisol in the biofluid (see Table 2.3., page 68). In regard to the latter point, cortisol exists in a bound and unbound state in the circulation. Cortisol primarily binds to cortisol binding protein (CBP) in the CSF and blood (Dunn, Nisula, & Rodbard, 1981; Predine, Brailly, Delaporte, & Milgrom, 1984). Once the available CBP becomes saturated, cortisol then largely circulates in an unbound or free state. Only this free cortisol is considered active and able to induce the respective physiological effects. The ratio of bound cortisol to free cortisol can fluctuate. It is estimated
that 90–97% of circulating cortisol is bound, and 3–10% is free (Arafah, Nishiyama, Tlaygeh, & Hejal, 2007; Dunn et al., 1981; Levine, Zagoory-Sharon, Feldman, Lewis, & Weller, 2007; Predine et al., 1984).

Table 2.3.

*The availability of cortisol and the collection methodology in different biofluids* (Hellhammer, Wüst, & Kudielka, 2009; Predine et al., 1984)

<table>
<thead>
<tr>
<th>Biofluid</th>
<th>CSF</th>
<th>Urine</th>
<th>Blood (serum/plasma)</th>
<th>Saliva</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sampling factors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Accessibility</td>
<td>Invasive</td>
<td>Non-invasive</td>
<td>Invasive</td>
<td>Non-invasive</td>
</tr>
<tr>
<td>Biochemical state of cortisol</td>
<td>• Bound</td>
<td>Free</td>
<td>• Bound</td>
<td>Free</td>
</tr>
<tr>
<td></td>
<td>• Free</td>
<td></td>
<td>• Free</td>
<td></td>
</tr>
<tr>
<td>Collection points</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single sample</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Pooled duration</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Multi-time point</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Salivary cortisol collection and analysis was selected as the preferred technique in which to sample and measure cortisol in this project for reasons of pragmatics, the ability to measure free active cortisol, and pricing. This decision is supported by literature, which indicates that salivary cortisol analysis is preferable to blood sampling for measuring the functional integrity of the HPA axis (Vining, McGinley, Maksvytis, & Ho, 1983). Salivary cortisol collection and
analysis is considered to be a useful, reliable, and accurate measure of HPA axis function in a number of illnesses and disease states (Arafah et al., 2007; Castro et al., 2000; Raff, 2009; Raff, Raff, & Findling, 1998) as well as in neuropsychiatric conditions (Kirschbaum & Hellhammer, 1989). Collection of saliva is non-invasive, thus allowing for cortisol to be sampled at numerous time points over a 24-hour period, and importantly it is convenient for participants because they can collect their own saliva away from the research setting. Blood sampling also affords the option of sample collection at range of time points; however, it is less convenient because it requires participants to be present at the research site for the full sampling period. An important factor in deciding between blood and saliva collection, aside from participant convenience, was the ability to measure the fraction of biologically active cortisol. Analysis of blood indicates the total amount of circulating cortisol (bound + unbound), whereas saliva only reflects the proportion of free circulating cortisol because conjugated cortisol is unable to pass freely from the blood through salivary gland acinar cells (Kahn, Rubinow, Davis, Kling, & Post, 1988; Laudat et al., 1988). It is widely reported in the literature that salivary cortisol concentration is a very good indicator of blood cortisol concentration, both in the serum and plasma. Cortisol concentration levels in the blood and saliva correlate strongly (Bolufer, Gandia, Rodriguez, & Antonio, 1989; Kahn et al., 1988; Laudat et al., 1988; Lo, Ng, Azmy, & Khalid, 1992), and changes in blood cortisol concentration usually equilibrates with saliva within 5 minutes (Vining et al., 1983). Finally, blood collection and analysis is comparatively more expensive than cortisol collection and analysis. One consideration for salivary cortisol concentration is that in the saliva, cortisol is subject to a small amount of enzymatic breakdown by 11β-hydroxysteroid dehydrogenase type 2 (Levine et al., 2007).
In relation to the other two alternative biofluids, drawing CSF from spinal tap is invasive, laborious, carries risks, and therefore reduces the number of samples which can be obtained. Urine analysis is non-invasive, and cortisol metabolites in urine do reflect free cortisol levels. However, urine collection can cause the participant undue discomfort and many people cannot produce urine “on demand”, which means the ability to collect multiple urine samples at desired time points is limited; hence the reason why majority of urine sampling studies report urinary free cortisol (UFC) levels as an average across a long period of time rather than using multiple single samples.

It was decided that HPA axis challenge tests, which were briefly reviewed in Chapter 1 (Introduction) of this thesis, would not be used to investigate HPA axis integrity in this research project. Challenge tests are usually employed to determine the specific point of axial dysfunction only after substantial evidence has indicated that there is a problem with the functional integrity of the HPA axis. Because this research project was a first-step investigation into the relationship between psychiatric and cognitive symptomatology and HPA axis function in pre-HD and early-stage HD, it was decided that the measurement of baseline cortisol concentration would be performed. Taking baseline cortisol measurements is a recommended procedure for diagnostic studies such as the current research project (Carroll et al., 2008; Viardot et al., 2005; H. Raff, personal communication, 05 September, 2011).

### 2.3.2. The salivary cortisol collection procedure used in this research project.

Saliva samples are stable and easy to collect (Clements & Parker, 1998). Though, as with all biofluid collection techniques, there were a number of methodological risks that had the
potential to affect cortisol concentrations and therefore the reliability of the results (Adam & Kumari, 2009). To minimise these risks, participants were instructed on a standardised collection protocol, as detailed in Chapter 3 and Chapter 4. Whilst morning cortisol concentration is a fundamental indicator of HPA axis integrity, sampling morning cortisol levels are alone is not sufficient to understand HPA axis functioning (Raff, 2009) because they need to be considered within the context of the circadian cycle. Therefore, in this study, salivary collection at four time points over a 24-hour period was considered sufficient to establish an overview of circadian cortisol fluctuations without unduly burdening participants.

The specified collection times were:

- Day 1: 23:00p.m.,
- Day 2: 08:00a.m. (about 30 minutes after waking), 16:00p.m., 23:00p.m.

Participants were asked to collect samples as close as possible to the specified times. The exact date and time of collection was to be recorded on completed collection tubes.

A number of different commercial saliva collection kits are available. This study employed two: a Passive Dribble Kit, and a Cotton Wad Kit (Salivette, Sarstedt, Germany). The reason for employing two saliva collection techniques is explained in Chapter 3 and Chapter 4, and the methodology for saliva collection is detailed in Chapter 3 and Chapter 4. Specific instructions, as well as a dot-point summary of these instructions, for both passive dribble and cotton wad saliva collection methods, including the equipment required, were provided to participants (see Appendix 11 and Appendix 12 respectively; and see Appendix 13 for the saliva pathology request form). Irrespective of the saliva collection method undertaken, participants were instructed to keep the samples refrigerated. Participants were offered $10 to
deliver their samples to a local Healthscope Pathology Ltd. collection centre. Samples were then couriered to the pathology laboratory.

Five participants (1 early-HD, 4 pre-HD) were required to re-collect their saliva samples due to an inability to produce enough saliva through attempts at passive dribble, missing more than one saliva collection time, erroneously discarding their samples, a query of possible pregnancy, or their original samples could not be analysed because of unsatisfactory saliva quality. Because sleep and stress are susceptible to variability, and there is the potential for variability in sleep and stress to affect cortisol concentrations, PSQI and PSS questionnaires were sent for recompletion at the time of saliva recollection. Responses obtained from these recompleted questionnaires were used for data analysis.

2.3.3. The analysis and assaying of salivary cortisol in this research project.

The analytical assay used for salivary cortisol is detailed in Chapter 3 and Chapter 4. Further to the explanation provided, the Cobas Assay Kit (Roche Diagnostics, Mannheim, Germany) indicates a detection range of 0.5–1750 nmol/L. Automated immunoassays are considered reliable assessment tools for salivary cortisol measurement (Chiu, Collier, Clark, & Wynn-Edwards, 2003; van Aken, Romijn, Miltenburg, & Lentjes, 2003).

Only single samples were tested for each participant for each time point. The inter-assay coefficient of variation was recorded as 12.5% at the level of 8nmol/L and 3.7% at the level of
94nmol/L. The Roche Cobas automated cortisol assay offers a good analytical performance at low concentration ranges (Vogeser, Durner, Seliger, & Auernhammer, 2006).

2.3.4. Blood sampling.

A small one-off blood sample was collected at the beginning of the participant visit for the purpose of measuring other potential biomarkers. Results from analyses of the bloods are not reported in this thesis.

2.4. Study protocol

The study protocol is outlined in Chapter 3 and Chapter 4, and displayed in Table 2.4 (see page 74). Participant testing took roughly 3–4 hours in total. To ensure the study visit protocol was followed, the researcher completed a Visit Activities Log for each participant (See Appendix 14). Participants then undertook saliva collection off-site at their own location within 2 weeks of the study visit.
Chapter 2 – Methods

Table 2.4.

Study protocol indicating the order of activities to be undertaken at the study visit

<table>
<thead>
<tr>
<th>Order</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Participant greeted</td>
</tr>
<tr>
<td>2</td>
<td>Obtain consent</td>
</tr>
<tr>
<td>3</td>
<td>Collect blood draw</td>
</tr>
<tr>
<td>4</td>
<td>Administer UHDRS – Motor scale</td>
</tr>
<tr>
<td>5</td>
<td>Administer psychiatric assessment and cognitive test battery (see Table 2.2.)</td>
</tr>
<tr>
<td>6</td>
<td>Explain saliva collection and cortisol testing procedure to participant. Provide participant with saliva collection kit and identify the most convenient collection centre. Ensure participant understands saliva collection instructions</td>
</tr>
<tr>
<td>7</td>
<td>Arrange follow-up phone call on a pre-arranged day to remind participant about saliva collection and delivery to closest collection centre</td>
</tr>
<tr>
<td>8</td>
<td>Conclude the visit. Answer any questions the participant might have and note queries to be followed up. Provide participant payment</td>
</tr>
<tr>
<td>9</td>
<td>Score test battery</td>
</tr>
<tr>
<td>10</td>
<td>Store papers/forms/questionnaires in appropriate files and enter data</td>
</tr>
</tbody>
</table>

2.5. Statistical analyses

The statistical analyses undertaken are detailed in Chapter 3 and Chapter 4.
CHAPTER THREE

– STUDY 1 –

CORTISOL AND DEPRESSION IN PRE-DIAGNOSED AND EARLY STAGE HUNTINGTON’S DISEASE

This chapter constitutes a manuscript submitted for publication to the journal Psychoneuroendocrinology on 29th March 2012 as:


The presentation of the manuscript reflects the guidelines required by the journal Psychoneuroendocrinology. The format of the manuscript at the time of submission has been retained, and therefore is not consistent with the presentation and section numbering of the wider thesis. If citations used in this manuscript were not used elsewhere in Chapter 1, Chapter 2, or Chapter 5, then they are not included in the list of Thesis references.
Huntington’s disease (HD) patients can display a range of neuropsychiatric and cognitive signs many years before a clinician is confident that an individual with the HD CAG expansion is manifesting the diagnostic HD motor signs. Therefore, the disease process in HD is starting well in advance of a diagnosis being made. Linking pathophysiological biomarkers to the early clinical signs is a key focus of HD research, and of neurodegenerative and neuropsychiatric research more widely. Cortisol levels are a functional indicator of hypothalamic-pituitary-adrenal (HPA) axis integrity, and past research has indicated that cortisol is abnormally high in pre-HD, early-HD, and in the moderate–advanced stages of HD. However, no one study has measured cortisol levels at multiple time points over a 24-hour period across the different stages of the disease, nor has there been a thorough investigation of whether abnormal cortisol levels are associated with the neuropsychiatric or cognitive signs of HD. Depression is a neuropsychiatric feature that is common in HD. It can present many years before HD motor signs begin, and it is highly likely that depression in HD has an organic aetiology since the unique presentation of a depressed mood in HD cannot be completely attributed to psychosocial stressors. In many non-HD conditions that display signs of depression which are phenotypically similar to HD, including major depressive disorder, a link between the disease phenotype and HPA axis dysfunction been established. In particular, depression seems to be associated with hypercortisolism in the morning, around the time of waking. Study 1 of this thesis investigates whether depressed mood, as assessed by the Inventory of Depressive Symptomatology – Self-report (IDS–SR), is related to abnormal salivary cortisol levels, with a particular focus on the morning time point in pre-HD and early-HD compared to HD CAG normal controls.
Chapter 3 – Study 1

DECLARATION FOR THESIS CHAPTER 3

Declaration by candidate

In the case of Chapter 3, the nature and extent of my contribution to the work was the following:

<table>
<thead>
<tr>
<th>Nature of contribution</th>
<th>Extent of contribution (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recipient of research funding from the School of Psychology and Psychiatry, Monash University, design and conduct of the study, manage participant recruitment, data analysis, and writing the manuscript for publication.</td>
<td>80%</td>
</tr>
</tbody>
</table>

The following co-authors contributed to the work:

<table>
<thead>
<tr>
<th>Name</th>
<th>Nature of contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Phyllis Chua</td>
<td>A recipient of the 2007 Pfizer Neuroscience Research Grant, assisted with the design of the study and clinical assessment of participants.</td>
</tr>
<tr>
<td>Dr. Andrew Churchyard</td>
<td>Assisted with participant recruitment and the clinical assessment of participants.</td>
</tr>
<tr>
<td>Assoc. Prof. Anthony J. Hannan</td>
<td>A recipient of the 2007 Pfizer Neuroscience Research Grant and assisted with the design of the study.</td>
</tr>
<tr>
<td>Dr. Georgia Lowndes</td>
<td>Assisted with drafting of the manuscript.</td>
</tr>
<tr>
<td>Dr. Terence Y. Pang</td>
<td>Assisted with the design of the study and provided advice for the cortisol collection methodology.</td>
</tr>
<tr>
<td>Prof. Ed Chiu</td>
<td>Assisted with the clinical assessment of participants.</td>
</tr>
<tr>
<td>Prof. Julie C. Stout</td>
<td>Oversaw the design of the study, statistical analyses, interpretation of the results, and drafting and editing of the manuscript.</td>
</tr>
</tbody>
</table>

Candidate’s Signature  | Date:
Declaration by co-authors

The undersigned hereby certify that:

(1) the above declaration correctly reflects the nature and extent of the candidate’s contribution to this work, and the nature of the contribution of each of the co-authors.

(2) they meet the criteria for authorship in that they have participated in the conception, execution, or interpretation, of at least that part of the publication in their field of expertise;

(3) they take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;

(4) there are no other authors of the publication according to these criteria;

(5) potential conflicts of interest have been disclosed to (a) granting bodies, (b) the editor or publisher of journals or other publications, and (c) the head of the responsible academic unit; and

(6) the original data are stored at the following location(s) and will be held for at least five years from the date indicated below:

<table>
<thead>
<tr>
<th>Location(s)</th>
<th>School of Psychology and Psychiatry, Monash University, Clayton Campus.</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Author</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Phyllis Chua</td>
<td>2/5/12</td>
</tr>
<tr>
<td>Dr. Andrew Churchyard</td>
<td>30/4/12</td>
</tr>
<tr>
<td>Assoc. Prof. Anthony J. Hannan</td>
<td>2/5/12</td>
</tr>
<tr>
<td>Dr. Georgia Lowndes</td>
<td>18/04/12</td>
</tr>
<tr>
<td>Dr. Terence Y. Pang</td>
<td>2/5/12</td>
</tr>
<tr>
<td>Prof. Ed Chiu</td>
<td>19/4/2012</td>
</tr>
<tr>
<td>Prof. Julie C. Stout</td>
<td>2/5/2012</td>
</tr>
</tbody>
</table>
Title/Running title: Cortisol and depression in pre-diagnosed and early stage Huntington’s disease

Authors:
Christopher A. Shirbin (BASc [Hons])\textsuperscript{1}, Phyllis Chua (MBBS)\textsuperscript{123}, Andrew Churchyard (MBBS, PhD)\textsuperscript{124}, Georgia Lowndes\textsuperscript{1} (MPsych, PhD), Anthony J. Hannan (PhD)\textsuperscript{5}, Terence Y. Pang (PhD)\textsuperscript{5}, Edmond Chiu\textsuperscript{123} (MBBS, PhD), and Julie C. Stout (PhD)\textsuperscript{1*}

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SUMMARY

Hypothalamic-pituitary-adrenal (HPA) axis dysfunction and depression have both been shown to occur in Huntington’s disease (HD) gene carriers prior to diagnosis (pre-HD) and in diagnosed HD patients. However, the relationship between HPA axis dysfunction and the severity of depressive symptomatology in pre-HD and early-HD has not been systematically examined, despite morning hypercortisolism being a characteristic feature of some subtypes of idiopathic depression. The aim of this study was to investigate whether HPA axis function is related to levels of depression in pre-HD and early-HD. To assess HPA axis function we obtained salivary cortisol concentrations from 20 controls, 20 pre-HD, and 17 early-HD participants at four time-points over a 24 hour period. Depression symptoms were assessed using the Inventory of Depressive Symptomatology – Self-Report. Of the participants who were found not to be depressed, the early-HD group had significantly lower morning cortisol levels relative to pre-HD and controls. In contrast, the early-HD group with at least mild or greater levels of depression symptoms had a comparable cortisol concentration to pre-HD and controls. The results suggest that early-HD may be associated with hypocortisolism. However when depressed, a hyperactive HPA axis response may still be induced in early-HD and lead to cortisol levels that are similar to pre-HD and controls. Our study reveals that cortisol levels in HD may be modified by the presence or absence of depressive symptomatology. Depression may be an important factor for understanding how the HPA axis is affected in HD, particularly in the morning.

Keywords: Huntington’s disease; Hypothalamic-pituitary-adrenal axis; Cortisol; Saliva; Depression; Stress.
INTRODUCTION

Huntington’s Disease (HD) is an autosomal dominant neurodegenerative disease resulting from an unstable CAG expansion in the HTT gene (The Huntington's Disease Collaborative Research Group, 1993). The condition is characterized by a progressive manifestation of motor disability, cognitive impairment, and psychiatric disturbance. Depression is one of the earliest clinical signs of HD (Duff et al., 2007). One study reported that more than 30% of HD gene carriers initially presented for clinical attention with symptoms of depression in the absence of any neurological motor signs (Shiwach, 1994). Depression in HD is thought to reflect both environmental and endogenous aetiologies (Shiwach, 1994). The high rate of clinical depression in HD, particularly with closer proximity to motor onset, has not been completely explained by the knowledge of a gene-positive status, awareness of emerging neurological symptoms, or familial factors (Almqvist et al., 2003; Julien et al., 2007; van Duijn et al., 2008). It is likely that there is an organic component underlying HD depression.

In depression outside of the context of HD, a key pathological characteristics is hypothalamic-pituitary-adrenal (HPA) axis dysregulation (Gold et al., 1988). The HPA axis is the main physiological regulator of the body’s stress response. HPA axis activity involves the release of corticotropin-releasing hormone (CRH) from the hypothalamus, which stimulates adrenocorticotropic hormone (ACTH) release from the pituitary gland. This in turn triggers the production and secretion of cortisol from the adrenal cortex. A negative feedback system then suppresses CRH and ACTH release. Cortisol is subject to circadian variation (Weitzman et al., 1971) with a marked spike in ACTH and cortisol on average 30 minutes after waking. This cortisol awakening response can be interpreted as reflecting the production capacity of
the adrenal gland (Pruessner et al., 1997). Depression is commonly associated with HPA axis hyperactivity, which is most apparent shortly after waking (Sachar et al., 1973). Abnormal morning cortisol is often considered a reliable physiological marker of depression (Bhagwagar et al., 2005).

With regard to HD, HPA hyperactivity has been identified in transgenic HD mice (Björkqvist et al., 2006), as well as in human HD (Aziz et al., 2009; Björkqvist et al., 2006; Heuser et al., 1991; Kurlan et al., 1988). Elevated cortisol has been recorded in morning blood samples of diagnosed HD patients compared to controls (Leblhuber et al., 1995; Saleh et al., 2009), and in morning saliva samples of pre-diagnosed HD (pre-HD) compared to diagnosed HD patients and controls (van Duijn et al., 2010). Only one study has undertaken 24-hour HPA axis profiling in human HD, which showed abnormally high morning and afternoon cortisol in the plasma of early-stage HD patients (Aziz et al., 2009).

Despite several papers suggesting that depression in HD could be associated with abnormal HPA functioning (Björkqvist et al., 2006; for review, see Petersén and Björkqvist, 2006), and a broad acceptance that cortisol secretion is commonly altered in both HD and depression, there has been little systematic investigation of the relationship between HPA axis function and depression in HD. In two HD studies (each \( n < 20 \)), circulating cortisol levels were not associated with a prior diagnosis of major depression (Heuser et al., 1991) or current depression ratings (Aziz et al., 2009). These studies, however, did not report how the severity of depressive symptomatology changed with diurnal variation in cortisol, or specifically with morning cortisol levels. In addition, despite assessing depression, van Duijn et al. (2010) did not report examining the relationships between depression and HPA axis activity. One
investigation has found a positive correlation between afternoon cerebrospinal fluid CRH levels and a rating of depression severity in early HD patients who met the diagnostic criteria for depressive disorder (major depression or dysthymia) (Kurlan et al., 1988). However, neither the 24-hour cortisol profile nor morning cortisol levels were reported.

The aim of the current study was to examine how depression in pre-HD and early-HD relates to the 24-hour cortisol profile, and in particular to morning cortisol concentrations. We hypothesised that both cortisol levels and depressive symptomatology would be highest in early-HD compared to pre-HD, and lowest in controls. Furthermore we hypothesised that depression would act as a modifier of cortisol levels in pre-HD and early-HD. Given the findings that morning time is sensitive to the relationship between HPA axis dysfunction and depressed mood (Bhagwagar et al., 2005), we predicted that depression effects in HD would be more evident in morning cortisol levels.

PATIENTS AND METHODS

Participants

Fifty-nine participants (19 early-HD, 20 pre-HD, 20 controls) met the eligibility criteria and were enrolled in the study (see Table 1). Recruitment was conducted through the Monash University HD Participant Registry, St. George’s Health Service HD clinic, a private neurology outpatient list, and the Huntington’s Victoria newsletter. Participants in the early-HD group had all been diagnosed within the past 5 years. Participants in the pre-HD group had their gene status confirmed positive by DNA testing but did not meet criteria for HD
diagnosis. Controls were either absent of family history for HD or confirmed by DNA test as not having the HD gene. Exclusion criteria included: less than 18 years of age; a history of an endocrine or neurological disorder (other than HD); alcohol or recreational drug abuse within the previous year, learning or intellectual disability; current night shift work; pregnancy, lactation, or use of pharmaceutical contraceptives. Informed consent was obtained from all participants. The study was approved by the St. Vincent’s Hospital Human Research and Ethics Committee, and Monash University Human Research Ethics Committee.

In early-HD and pre-HD, CAG repeat length was used to calculate a burden of pathology score (age x [CAG-35.5]) (Penney et al., 1997). Gender and the proportion of smokers were similar across the groups. Pre-HD were significantly younger than the control and early-HD ($p < 0.001$), and had a lower disease burden score than early-HD ($p < 0.001$). Anti-depressant medication use differed between the three groups ($p < 0.001$).

**Neuropsychiatric assessment**

To facilitate comparisons with previous studies, we used both a diagnostic screening measure of depression (Mood disorders subscale from the Primary Care Evaluation of Mental Disorders (PRIME-MD) (Spitzer et al., 1994)) and a measure of depression symptom severity (the Inventory of Depressive Symptomatology – Self-report (IDS–SR) (Rush et al., 1996)). The PRIME-MD is a 26-item self-completed yes/no questionnaire, which guides further probe questions administered by the examiner to obtain a diagnostic label. The IDS–SR is a 30 item self-rated questionnaire that assesses depression symptom severity. Item scores are summed and the total can be converted to a depression severity rating from 0 (None) – 4 (Very severe).
The IDS–SR was selected because it assesses depressive features which are common in HD, and it de-emphasises motor features which can confound depression assessment in neurological patients. Due to a small sample size, we collapsed IDS–SR total scores into two categories of depression symptom severity: non-depressed (IDS–SR total <14, no or minimal symptoms) and depressed (IDS–SR total ≥14, at least mild symptoms) (Rush et al., 1996).

Stress and sleep quality were assessed as potential mediators of cortisol levels. We assessed stress using the Perceived Stress Scale (PSS), a 14-item self-rated questionnaire about stressors in the previous month (Cohen et al., 1983). We assessed sleep quality using the Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989), a 19-item self-report about different components of sleep and fatigue. Bed partner or roommate items were not administered.

A similar proportion of early-HD and pre-HD participants were classified as depressed according to the PRIME-MD. Early-HD had a higher IDS–SR total score than controls \( (p = 0.015) \), however early-HD and pre-HD did not differ significantly. When participants were dichotomised according to IDS–SR depression symptom severity, the proportion of early-HD depressed (i.e., at least mild depressive symptomatology) was greater than pre-HD. The IDS–SR was selected as the primary measure of depression for further analysis in this study based on its ability to differentiate between the proportions of depressed participants in the diagnostic groups. In comparison to controls, early-HD reported statistically significant worse sleep quality \( (p = 0.017) \), and more perceived stress \( (p = 0.053) \) (see Table 2).
Chapter 3 – Study 1 (Manuscript)

Study Protocol

All participants were assessed using the motor exam component of the Unified Huntington’s Disease Rating Scale (UHDRS) (Huntington Study Group, 1996) by a neurologist (AC) or psychiatrist (PC, EC) specialising in HD. In addition to the depression, stress, and sleep questionnaires administered at the study visit, participants also had a blood draw and underwent cognitive testing, results of which are not presented here.

At the completion of testing, participants were provided with written and verbal instructions on how to collect their own saliva. Saliva was collected within two weeks of the study visit by the participant passively dribbling 3-5mL into a collection tube at four time points across a 24 hour period at Day 1: 11pm; Day 2: 8am (within 30 minutes after waking), 4pm, 11pm. Participants were instructed to fast from food and drink, except water, for an hour before collection and overnight before the morning collection, not to brush their teeth or apply make-up before sample collection, and avoid alcohol for 12 hours prior. All participants using inhalants, topical applications, or eye drops known to contain steroids (2 early-HD, 1 pre-HD, 4 controls) ceased use of these 24 hours prior to beginning saliva collection. Ten participants (8 early-HD, 1 pre-HD, 1 control) experienced difficulties with the passive dribble collection method and were instead instructed to chew on cotton wads (‘Salivette’, Sarstedt, Nümbrecht, Germany), which were later centrifuged to extract the saliva. Salivary cortisol concentrations obtained from both methods have been shown to be comparable (Poll et al., 2007; Shirtcliff et al., 2001).
All saliva was untreated and cortisol concentrations were analysed by Healthscope Functional Pathology Ltd. (Melbourne, Australia) using a competitive electrochemiluminescence immunoassay method on the automated Roche Cobas 3 601 Cobas analyser (Roche Diagnostics, Mannheim, Germany). A significant Spearman’s correlation coefficient between the two 11pm cortisol samples, \( r = 0.47, p < 0.001 \), indicated salivary cortisol concentration consistency between participants. Two early-HD participants provided no saliva samples and thus were excluded from cortisol analyses.

**Statistical analysis**

First, to compare the cortisol profile of participants in this study with other studies investigating HPA axis activity in HD that have not included depression as a moderating factor, we used cortisol levels at each of the four time points as dependent measures in a two-way multivariate analysis of covariance (MANCOVA) to reveal the effects of diagnostic group (early-HD, pre-HD, control) on 24 hour cortisol levels. Age was included as a covariate. To then examine our hypotheses about depression and cortisol levels, we added IDS–SR depression status (depressed or non-depressed) and its interaction with diagnostic group into the factorial model. Group differences were identified by the comparison of estimated marginal means. Parameter coefficients (\( \beta \)) were examined to determine whether there was a unique relationship between cortisol levels and individual predictor variables. Post-hoc analyses were undertaken, where necessary to further explore the nature of group relationships. Partial eta-squared (\( \eta^2 \)) was used as a measure of statistical effect size.
Statistical analyses were performed using the Statistical Package for Social Sciences v19.0 (SPSS Inc, Chicago, Illinois).

Given the potential confound of group age on cortisol levels (Van Cauter et al., 1996), we computed a bias-corrected and accelerated bootstrap re-estimation of a multi-variable regression model (1 000 replications) to estimate group 8am cortisol level under three different age conditions. Cortisol levels at 8am were chosen since morning cortisol levels show the greatest variability. Estimated 95% confidence intervals for the difference between diagnostic group with unrestricted and restricted age ranges indicated that controlling for age did not affect individual group cortisol levels. Age was therefore included as a covariate in all group analyses.

RESULTS

Cortisol levels across the four collection times

The 24-hour cortisol profile for all diagnostic groups followed the expected circadian pattern of highest cortisol in the morning, lower levels in the afternoon, and lowest cortisol in the late evening. The first MANCOVA, without IDS–SR depression status as a moderating factor, revealed no main effect of group on cortisol level, $F(4, 110) = 1.27, p = 0.27$. However, there was a trend for 8am cortisol to vary by diagnostic group, $F(2, 53) = 2.53, p = 0.089$ (Partial $\eta^2 = 0.087$). Specifically, pre-HD had a higher morning cortisol than early-HD ($p = 0.086$). We found no other statistically significant differences between groups, though we note there was a
Chapter 3 – Study 1 (Manuscript)

relationship between pre-HD and higher 4pm cortisol levels ($\beta = 4.64, p = 0.036$) (Partial $\eta^2 = 0.08$), and pre-HD cortisol levels tended to be consistently higher than early-HD at each collection time (see Figure 1). Older age was associated with higher cortisol levels across all groups at every collection time: Day 1: 11pm, $F(1, 53) = 3.61, p = 0.063$; Day 2: 8am, $F(1, 53) = 4.69, p = 0.035$; 4pm, $F(1, 53) = 4.08, p = 0.048$; 11pm, $F(1, 53) = 3.61, p = 0.063$.

Two pre-HD participants collected their 4pm sample outside of a 2-hour allowable deviation window. We retained these participants, given that this study already had a small sample size, and imputed the mean cortisol concentration for pre-HD at 4pm (11.22 nmol/L) for these two values. To test whether this approach affected the group main effect on 4pm cortisol levels, we removed the two pre-HD cases from an analysis of covariance (ANCOVA) for 4pm cortisol and found that it did not impact on the result.

**Depression moderates 8am cortisol in early-HD**

The second MANCOVA, including IDS–SR depression status as a moderating factor, also did not reveal an overall main effect of group, $F(8, 94) = 1.05, p = 0.40$. Nor was there a main effect of depression status on cortisol levels, $F(4, 47) = 0.85, p = 0.50$ or an overall group by depression status interaction effect on cortisol levels, $F(8, 94) = 0.85, p = 0.56$. The main effect of diagnostic group on 8am cortisol was no longer present, $F(2, 50) = 1.99, p = 0.15$. However, parameter estimates at the 8am collection time revealed an association between early-HD and lower cortisol ($\beta = -10.64, p = 0.043$) (Partial $\eta^2 = 0.08$). Group ANCOVA at the 8am collection time for non-depressed participants only, indicated a significant difference
between diagnostic group cortisol levels, $F(2, 38) = 4.65, p = 0.016$ (Partial $\eta^2 = 0.2$).

Specifically, early-HD 8am cortisol was significantly lower than pre-HD ($p = 0.015$) and there was also a trend for lower early-HD 8am cortisol in comparison to controls ($p = 0.094$) (see Figure 2a). We did not observe a group 8am cortisol difference for depressed participants (see Figure 2b).

Importantly, the relationship between 8am cortisol and the early-HD group differed depending on whether early-HD participants were depressed or not depressed (see Figure 3). Specifically, there was a statistically significant association between 8am cortisol and the interaction of early-HD and depression status ($\beta = 21.06, p = 0.047$) (Partial $\eta^2 = 0.077$). Directly comparing 8am cortisol between early-HD depression categories revealed a trend for higher cortisol in depressed early-HD participants, $F(1, 14) = 4.03, p = 0.064$ (Partial $\eta^2 = 0.22$). An interaction between depression status and being in the pre-HD or control group was not related to cortisol levels at any collection time.

**Stress and other variables do not mediate the relationship between depression and 8am cortisol in early-HD**

We computed a two-step hierarchical standard regression to examine the role of stress, using PSS Total scores, in the relationship between depression status and 8am cortisol in early-HD. Age was entered in the first step, with depression and stress entered together in the second step. Step 1 explained a significant proportion of 8am cortisol, $F(1, 15) = 5.30, p = 0.036$, thereby highlighting the impact of age. With the addition of depression and stress in Step 2,
the overall model was still significant accounting for 47% of the variance in 8am cortisol, $F(3, 13) = 3.83, p = 0.036$ (Adjusted $R^2 = 0.35$). Standardised beta values ($b$) for Step 2 indicated that higher depression level was associated with lower 8am cortisol ($b = -0.50, p = 0.042$). In contrast, the stress measure did not account for any independent variation in cortisol ($b = -0.23, p = 0.32$). Stress was therefore not considered to be a significant mediator in the relationship between depression and 8am cortisol in early-HD. Older age was also associated with higher cortisol in Step 2 ($b = 0.47, p = 0.038$). Adding gender, anti-depressant medication use, and sleep quality to the regression model did not explain any further variability in 8am cortisol, $F(6, 10) = 2.24, p = 0.13$.

DISCUSSION

Our study indicates that depression is associated with morning cortisol levels in early-HD. Specifically, non-depressed early-HD patients had significantly lower cortisol in the morning compared to non-depressed pre-HD and control groups. Surprisingly, morning cortisol in early-HD patients with depression was higher than non-depressed early-HD, and was similar to depressed pre-HD and control groups. Our findings are in contrast with previous reports of elevated cortisol in early-HD compared to pre-HD. It is therefore possible that in previous research demonstrating heightened cortisol in HD, the sample may have been at least mildly depressed. This is plausible given the high rate of depression in HD (Paulsen et al., 2005). Just four out of the eight studies investigating HPA axis functioning in HD have included a measure of depression (Aziz et al., 2009; Heuser et al., 1991; Kurlan et al., 1988; van Duijn et al., 2010). Of these, two included diagnostic measures only which do not capture the level of depression severity or the difference in depression levels between diagnostic groups (Heuser et
When we examined the cortisol levels of all participants ignoring depression as a moderating factor, we found that cortisol was higher in pre-HD compared to early-HD in the morning. This is similar to van Duijn et al. (2010), who did not examine depression, and who reported higher morning cortisol in pre-HD compared to diagnosed HD. When we did examine depression as a moderating factor, pre-HD and control had a relatively similar cortisol profile whether depressed or not depressed, yielding no significant differences between these groups. The findings of our study indicated that depression was a moderating factor of morning cortisol levels for early-HD. Our study design is the first to report the specific effect of depression and the cortisol profile in pre-HD and early-HD by utilising both a diagnostic and rating measure of depression, as well as accounting for the diurnal variation of cortisol.

Low morning cortisol in non-depressed early-HD patients is a novel finding and suggests that the early stage of HD may be associated with a circadian cortisol profile which is indicative of hypocortisolism. Hypocortisolism is a common consequence of prolonged exposure to physical and emotional stress, such as in post-traumatic stress disorder (PTSD) (for review, see Raison and Miller, 2003). Extended periods of heightened cortisol can lead to hypocortisolism because of lowered glucocorticoid production, exaggerated negative feedback from glucocorticoid receptor hypersensitivity, or inefficient glucocorticoid receptor signal functioning. As a result, patients with depression can display both HPA axis hyperactivity and hypoactivity depending on the specific pattern and severity of symptoms (Bremmer et al., 2007; Penninx et al., 2007; Wardenaar et al., 2011). In the context of this study, hypercortisolism preceding hypocortisolism could explain the previously postulated theories about the change in HPA axis activity through HD progression (van Duijn et al., 2010).
Beginning in the pre-HD stage, there are several factors that could increase cortisol levels. Family and psychosocial stress, abnormally heightened perceived stress (Downing et al., 2011), and possibly even inflammatory responses to neuroanatomical damage which may precede motor symptoms by 10 years (Aylward et al., 2004) each have the potential to raise HPA axis activity. Prolonged exposure to heightened cortisol throughout the pre-HD phase might eventually lead to the suppression of HPA axis activity in early-HD patients by the time motor symptoms start and physical deterioration ensues.

In early-HD, adding the burden of a depressed mood to an already chronically disturbed HPA axis might then induce an unregulated stress response so that heightened cortisol becomes re-evident, making the cortisol profile of depressed early-HD appear normal or similar to pre-HD and controls. This is seen outside of HD, where depressed patients with histories of chronic abuse and/or PTSD who have low basal cortisol levels can still demonstrate exaggerated HPA axis responses to endogenous endocrinological events or external psychosocial stressors because of a dysregulated feedback system (Bremner et al., 2003; Heim et al., 2000). A history of hypercortisolism may even be a risk factor for the onset of depression (for review, see Pariante and Lightman, 2008). These differences in cortisol levels are evident especially around the time of waking because this time point is sensitive to the relationship between mood and HPA axis function (Bhagwagar et al., 2005). Such relationships are harder to detect when sampling afternoon and evening cortisol levels, which are inherently low.

The high prevalence of depression in HD (Paulsen et al., 2005) and the view that HD depression may carry a unique phenotype (Craufurd et al., 2001), make this study very relevant to further investigations of mood symptoms in HD, the clinical management of HD,
and the drug development pathway. Neuropathology across HPA axis structures, including the hippocampus which is a localised negative feedback site, has been identified in human HD (Kassubek et al., 2004; Kremer et al., 1991; Kremer et al., 1990; Rosas et al., 2003; Spargo et al., 1993) and mouse-model HD (Björkqvist et al., 2006; Gil et al., 2005; Lazic et al., 2004; Simpson et al., 2011). Yet the functional impact of these abnormalities on HPA axis activity is yet to be investigated in HD. To localise the point of HPA axis dysfunction in HD and better understand its association with depression, future research could apply mood measures to 24-hour HPA axis challenge tests in HD, by administering exogenous CRH, ACTH, and dexamethasone to stress HPA axis negative feedback regulation. To explore the impact of stress levels through the pre-HD and early-HD stage on the relationship between cortisol levels and depression, a follow-up longitudinal investigation should track emotional stress and pathophysiological stress, by sampling inflammatory markers (Dalrymple et al., 2007) across disease progression. In our study perceived stress scores did not contribute to the relationship between depression status and 8am cortisol in early-HD. Other affective domains might better account for this relationship, such as the chronicity of stress, anxiety, or long-term sleep disturbance which we did not measure.

Effective pharmacological treatment of mood is another potential explanation for attenuated morning cortisol (Nikisch et al., 2005; Scharnholz et al., 2010) in the non-depressed early-HD group, and could be examined within a larger future study. We did not exclude participation based on mood medication, although participants on medications were required to have a stable drug regimen. Several early-HD participants were taking anti-depressants, neuroleptics, and anti-epilepsy medications. The impact of mood medications on HPA axis functioning in
HD has been previously investigated (Saleh et al., 2009), although again depression levels were not considered.

A small sample size made it difficult to examine the interaction between parameters of depression and other variables such as sleep quality, gender, and mood medication use. In addition, the pre-HD group had a low disease burden score, suggesting that they were, as a group, relatively far from disease onset. A larger study with a broader range of participant CAG lengths could help determine how depression is related to HPA axis functioning on the continuum from early in the pre-HD stage to post-diagnosed HD.

This study found that depression constitutes a significant modifier of morning cortisol levels which can distinguish between pre-HD and early-HD. Morning cortisol levels could potentially be a useful biomarker for the study of HPA axis activity through the earliest stages of HD. Future studies will be required to confirm this and to identify the precise nature of HPA axis pathology as the disease progresses.
CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

The authors thank the participants for giving generously of their time. We also thank Dr. John Taffe for statistical advice. Finally we thank Ms. Angela Komiti for assisting with a portion of the data collection. The research was supported by a Pfizer Neuroscience Research Grant and funding from the School of Psychology and Psychiatry, Monash University. Neither of these institutions were involved in the design, conduct, or publication of this study.
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TABLES

All tables with table title and legend have been uploaded online. The saved file name refers to the table cited in the text.
FIGURE CAPTIONS

Figures have been uploaded online and the saved file name refers to the figure captions below:

**Figure 1** Salivary cortisol concentrations for all participants.
Data are presented as mean cortisol concentrations with standard error bars.
† $p < 0.1$ (Significance testing was undertaken via MANCOVA, covarying for age, and revealed a trend for a 8am group cortisol difference).

**Figure 2** Salivary cortisol concentrations for a) IDS–SR Non-depressed participants only, b) IDS–SR Depressed participants only.
Data are presented as mean cortisol concentrations with standard error bars.
* $p < 0.05$ (Significance testing was undertaken via ANCOVA, covarying for age, at the 8am collection time and revealed a significant 8am cortisol difference between diagnostic groups in non-depressed participants).

**Figure 3** Association between 8am mean cortisol level and the interaction of diagnostic group and IDS–SR depression status.
Data are presented as mean cortisol concentrations at the 8am collection time.
A significant relationship between 8am cortisol and depression status was found in the early-HD group only ($p < 0.05$). Depression status did not statistically significantly affect 8am cortisol levels in pre-HD or controls.
### Table 1: Demographics and clinical information.

<table>
<thead>
<tr>
<th></th>
<th>Early-HD (E)</th>
<th>Pre-HD (P)</th>
<th>Control (C)</th>
<th>p value</th>
<th>Group comparison(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of participants</strong></td>
<td>19</td>
<td>20</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Demographic information</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male n (%)</td>
<td>10 (52.6%)</td>
<td>10 (50.0%)</td>
<td>9 (45.0%)</td>
<td>0.89 n.s.</td>
<td></td>
</tr>
<tr>
<td>Age (year)(^b)</td>
<td>Mean (SE)</td>
<td>58.0 (2.3)</td>
<td>42.5 (2.8)</td>
<td>&lt;0.001 P &lt; C, E***</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>41.8-72.3</td>
<td>19.2-66.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Clinical information</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease burden Mean (SE)</td>
<td>401.3 (14.5)</td>
<td>269.3 (22.2)</td>
<td>n/a</td>
<td>&lt;0.001 E &gt; P***</td>
<td></td>
</tr>
<tr>
<td>UHDRS Total Motor Score Mean (SE)</td>
<td>30.1 (3.3)</td>
<td>3.6 (1.0)</td>
<td>2.6 (0.6)</td>
<td>&lt;0.001 E &gt; C, P***</td>
<td></td>
</tr>
<tr>
<td>Anti-depressants(^c) n (%)</td>
<td>13 (68.4%)</td>
<td>7 (35.0%)</td>
<td>1 (5.0%)</td>
<td>&lt;0.001 E &gt; C, P*</td>
<td></td>
</tr>
<tr>
<td>Current smoker n (%)</td>
<td>3 (15.8%)</td>
<td>4 (20.0%)</td>
<td>2 (10.0%)</td>
<td>0.75 n.s.</td>
<td></td>
</tr>
</tbody>
</table>
Note: The identification of a group difference was based on a one-way analysis of variance or chi-squared tests, with Fischer’s exact test applied where necessary to cell counts less than five. Tests were two-tailed.

\textsuperscript{a}To investigate which groups differed significantly, post-hoc group comparisons were undertaken using Bonferroni adjustment (for ANOVA) or standardised residuals (for chi-squared tests) where appropriate. Tests were two-tailed.

\textsuperscript{b}Age at time of assessment.

\textsuperscript{c}Anti-depressant medications included tricyclics, selective serotonin-reuptake inhibitors (SSRIs), serotonin and noradrenaline reuptake inhibitors (SNRIs).

\( \dagger p < 0.1, * p < 0.05, *** p < 0.001. \)

n/a: Not applicable. n.s.: No significant difference.
### Table 2 Neuropsychiatric assessment scores.

<table>
<thead>
<tr>
<th></th>
<th>Early-HD (E, n = 19)</th>
<th>Pre-HD (P, n = 20)</th>
<th>Control (C, n = 20)</th>
<th>p value</th>
<th>Group comparison^a</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PRIME-MD</strong>^b</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depressed n (%)</td>
<td>5 (26.3%)</td>
<td>5 (25.0%)</td>
<td>0 (0%)</td>
<td>0.023</td>
<td>C &lt; E, P^†</td>
</tr>
<tr>
<td>IDS–SR Total</td>
<td>Mean (SE)</td>
<td>15.68 (2.59)</td>
<td>11.85 (2.54)</td>
<td>0.025</td>
<td>E &gt; C*</td>
</tr>
<tr>
<td>Depressed n (%)</td>
<td>9 (47.4%)</td>
<td>5 (25.0%)</td>
<td>3 (15.0%)</td>
<td>0.038</td>
<td>n.s.</td>
</tr>
<tr>
<td>PSS Total</td>
<td>Mean (SE)</td>
<td>34.5 (1.8)</td>
<td>32.9 (1.8)</td>
<td>0.024</td>
<td>E &gt; C^†</td>
</tr>
<tr>
<td>PSQI Global</td>
<td>Mean (SE)</td>
<td>7.8 (1.2)</td>
<td>5.1 (0.6)</td>
<td>4.5 (0.6)</td>
<td>0.007 E &gt; C*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>E &gt; P^†</td>
</tr>
</tbody>
</table>

Note: The identification of a group difference was based on a one-way analysis of variance or chi-squared tests, with Fischer's exact test applied where necessary to cell counts less than five. Tests were one-tailed.

^aTo investigate which groups differed significantly, post-hoc group comparisons were undertaken using Bonferroni adjustment (for ANOVA) or standardised residuals (for chi-squared tests) where appropriate. Tests were two-tailed.
bPRIME-MD depression includes identification of Major depressive disorder; Partial remission of major depressive disorder; Minor depressive disorder; Dysthymia; Depressive disorder due to physical disorder, medications, or other drug.

c‘Depressed’ = IDS–SR Total ≥14, reporting at least a mild level of depressive symptom severity.

† p < 0.1, * p < 0.05.

n.s.: No significant difference.
**Figure 1** Salivary cortisol concentrations for all participants.
Figure 2 Salivary cortisol concentrations for a) IDS–SR Non-depressed participants only, b) IDS–SR Depressed participants only.
Figure 3 Association between 8am mean cortisol level and the interaction of diagnostic group and IDS–SR depression status.
CHAPTER FOUR

– STUDY 2 –

THE RELATIONSHIP BETWEEN CORTISOL AND VERBAL MEMORY IN THE EARLY STAGES OF HUNTINGTON’S DISEASE

This chapter constitutes a manuscript submitted for publication to the Journal of Neurology on 19th May 2012 as:


The presentation of the manuscript reflects the guidelines required by the Journal of Neurology. The format of the manuscript at the time of submission has been retained, and therefore is not consistent with the presentation and section numbering of the wider thesis. If citations used in this manuscript were not used elsewhere in Chapter 1, Chapter 2, or Chapter 5, then they are not included in the list of Thesis references.
Study 1 indicated that morning cortisol levels in early-HD are moderated by mood state, so that the presence or absence of a depressed mood is related to either high or low morning cortisol levels respectively. If abnormal cortisol levels are related to mood in HD, it is possible that HPA axis dysfunction is associated with other clinical signs too. Cognitive difficulties have been detected around the same time as psychiatric signs and symptoms in HD, and the aetiological basis of these cognitive manifestations are also poorly understood. Cognition is a broad concept, and there are a number of cognitive domains which could be investigated in the context of cortisol measurement at the early stages of HD. Study 2 will focus on verbal learning and memory function. Verbal learning and memory impairments are well documented through the pre-HD phase, and these difficulties progress as motor signs become more pronounced and patients transition to a diagnosed HD phase. Verbal learning and memory deficits are associated with heightened cortisol in a number of neurodegenerative, neuropsychiatric, and medical conditions that display phenotypic similarities to HD, such as Alzheimer’s disease, post-traumatic stress disorder, and depressive disorder. In addition, toxically high levels of cortisol are thought to cause pathological damage to the hippocampus, which subserves learning and memory function. Study 2 investigates whether salivary cortisol levels are associated with performance on a verbal learning and memory task – the California Verbal Learning Test–Second Edition (CVLT–II). The same sample used in Study 1 will be used in Study 2. However, in Study 2 participants will be stratified in two ways: the first is by diagnostic group (i.e., early-HD, pre-HD, control), and the second is by a measure of motor sign severity (Total Motor Score). The relationship between CVLT–II performance and cortisol levels will be analysed for each of the two participant classification methods separately.
Declaration by candidate

In the case of Chapter 4, the nature and extent of my contribution to the work was the following:

<table>
<thead>
<tr>
<th>Nature of contribution</th>
<th>Extent of contribution (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recipient of research funding from the School of Psychology and Psychiatry, Monash University, design and conduct of the study, manage participant recruitment, data analysis, and writing the manuscript for publication.</td>
<td>80%</td>
</tr>
</tbody>
</table>

The following co-authors contributed to the work:

<table>
<thead>
<tr>
<th>Name</th>
<th>Nature of contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Phyllis Chua</td>
<td>A recipient of the 2007 Pfizer Neuroscience Research Grant, assisted with the design of the study and clinical assessment of participants.</td>
</tr>
<tr>
<td>Dr. Andrew Churchyard</td>
<td>Assisted with participant recruitment and the clinical assessment of participants.</td>
</tr>
<tr>
<td>Assoc. Prof. Anthony J. Hannan</td>
<td>A recipient of the 2007 Pfizer Neuroscience Research Grant and assisted with the design of the study.</td>
</tr>
<tr>
<td>Dr. Georgia Lowndes</td>
<td>Assisted with drafting of the manuscript.</td>
</tr>
<tr>
<td>Prof. Julie C. Stout</td>
<td>Oversaw the design of the study, statistical analyses, interpretation of the results, and drafting and editing of the manuscript.</td>
</tr>
</tbody>
</table>

Candidate’s Signature

Date:
Declaration by co-authors

The undersigned hereby certify that:

(1) the above declaration correctly reflects the nature and extent of the candidate’s contribution to this work, and the nature of the contribution of each of the co-authors.

(2) they meet the criteria for authorship in that they have participated in the conception, execution, or interpretation, of at least that part of the publication in their field of expertise;

(3) they take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;

(4) there are no other authors of the publication according to these criteria;

(5) potential conflicts of interest have been disclosed to (a) granting bodies, (b) the editor or publisher of journals or other publications, and (c) the head of the responsible academic unit; and

(6) the original data are stored at the following location(s) and will be held for at least five years from the date indicated below:

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Title: The relationship between cortisol and verbal memory in the early stages of Huntington’s disease

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Abstract

Hypothalamic pituitary adrenal (HPA) axis hyperactivity has been linked to learning and memory difficulties in a range of neurodegenerative and neuropsychiatric conditions. In Huntington’s disease (HD), both declines in learning and memory and HPA axis dysfunction are present early in the disease. However, the relationship between specific learning and memory deficits and HPA axis functioning in HD has not been examined. The aim of this study was to investigate cortisol levels in relation to verbal learning and memory in pre-diagnosed (pre-HD) and patients in the early stages of diagnosed HD (early-HD). Cortisol concentration was assayed in saliva samples from 57 participants (17 early-HD, 20 pre-HD, and 20 controls) at four time-points across a 24-hour period. Verbal memory was assessed using the California Verbal Learning Test–Second Edition (CVLT–II). We examined cortisol levels and verbal memory function in relation to diagnostic group (control, pre-HD, early-HD), and in a separate set of analyses combining pre-HD and early-HD (and excluding controls) we also examined cortisol and verbal memory performance in relation to the severity of HD-related motor signs. Of these two classification approaches, HD motor sign severity was more strongly associated with high evening cortisol levels and both reduced information encoding and memory retrieval. Separately, there was also a trend of higher cortisol levels in pre-HD. The findings suggest hypercortisolism and the underlying pathological changes may begin many years before a clinical diagnosis is made, but the memory decline associated with HPA axis disturbance can only be detected once motor signs become pronounced.

Keywords: Huntington’s disease; HPA axis; Cortisol; Saliva; Learning and memory
Introduction

Huntington’s disease (HD) is an autosomal dominant neurodegenerative condition caused by an expansion in the number of CAG repeats in the \textit{HTT} gene [1]. Clinically HD manifests as a motor disorder, in conjunction with psychiatric disturbances and a cognitive impairment. The cognitive decline associated with HD is one of the earliest signs of disease onset, yet the pathophysiology underlying this change is not well understood. Hypothalamic-pituitary-adrenal (HPA) axis activity has been discussed as a prospective biomarker system that may provide insights into factors underlying the HD phenotype [2]. The HPA axis is a neuroendocrinological system that regulates circulating cortisol concentration in response to psychological and physical stress. It is implicated in a number of physiological pathways and functions including the sleep-wake cycle, mood, cognition, energy storage and metabolism, digestion, sexual behaviour, and the immune system; many of which are disturbed in HD.

HPA axis abnormalities and heightened cortisol levels have been identified in HD [3-7]. There is evidence of HPA axis dysfunction early in the disease process with heightened cortisol concentrations recorded in \textit{pre-HD} CAG expanded individuals who have not yet received a motor-based diagnosis [8], and in newly diagnosed \textit{early-HD} patients [9].

Dysregulated HPA axis hyperactivity and poor learning and memory performance occur in neuropsychiatric and medical conditions that exhibit similar clinical signs to those seen in HD. These conditions include major depressive disorder [10], mild dementia of the Alzheimer’s type [11], chronic Cushing’s syndrome and Cushing’s disease [12], post-traumatic stress disorder [13], and mild cognitive impairment [14]. Elevated cortisol levels have also been associated with reduced learning and memory abilities in normal aging [15]. There is a
particular interest in the relationship between HPA axis abnormalities and learning and memory, compared to other cognitive domains, due to the critical role of the hippocampus in declarative memory formation [16] and evidence demonstrating that the hippocampus can be damaged by prolonged HPA axis hyperactivity [17]. These relationships are relevant to HD because there is evidence of structural hippocampal reduction in HD humans [18, 19] and in transgenic murine models of the disease [20]. This includes the R6/2 HD mouse [21] which is the same transgenic mouse that displays abnormally elevated glucocorticoid levels and pathological changes across the HPA axis [3].

Verbal learning and memory performance has been proposed as a clinical correlate of biomarker changes in the early stages of HD [22, 23]. Deficits have been identified in pre-HD [24], some 9-15 years before predicted clinical onset [22, 25, 26]. The deterioration in verbal learning and memory function in HD appears to be associated with increasing HD-related motor signs in both pre-HD and HD [22-24, 27]. The verbal learning and memory profile in HD is unique compared to other neurodegenerative dementias [28, 29]. In particular, in HD there are reductions in verbal encoding and total memory storage capacity [24, 28-30], and in the amount of information that can be retrieved following short-term and long-term delay [24, 29, 30]. Furthermore, information retrieval is often inconsistent across learning trials with intrusion and perseveration errors [28-30]. Whilst recognition memory (the ability to accurate discriminate learned and stored information from new information) might also be affected in pre-HD [26], a clear deficit may only emerge 2-5 years after a clinical motor-based diagnosis is made [24, 28, 29].
Despite suggestions that verbal learning and memory tasks are particularly sensitive to heightened cortisol levels [10], the association between HPA axis activity and verbal learning and memory ability has not been examined in HD. In fact within HD there has been no investigation of specific cognitive functions that could be detrimentally affected by chronic high levels of circulating cortisol. Only one study investigating HPA axis function in HD has examined a cognitive measure in relation to cortisol levels [31]. This study used the Mini-Mental Status Examination (MMSE), a brief screening tool for global cognitive impairment that conflates a broad range of cognitive functions into a single score. Test item difficulty is relatively low, and thus the MMSE has limited sensitivity for the detection of subtle or specific relationships between elevated levels of HPA axis and particular cognitive capacities in HD. Not surprisingly, Markianos et al [31] did not identify an association between a low MMSE score and heightened cortisol concentration in HD.

The aim of this study was to identify whether there is an association between cortisol levels and verbal learning and memory in the early stages of HD. In the absence of previous literature indicating whether diagnostic status or severity of motor signs might be most relevant to this relationship, we conducted two different sets of analyses with participants stratified in two ways: i) by diagnostic group (early-HD, pre-HD, and control); and ii) according to their United Huntington’s Disease Rating Scale (UHDRS) Total Motor Score (TMS) [32]. The former is the clinical diagnostic label applied by a specialist HD clinician taking into account the genetic status and collective presentation of clinical signs and symptoms. The latter is a clinical measure of disease progression based on a range of HD-related motor signs which is used as a proxy of clinical progression and can be applied across both pre-HD and early-HD [22, 23, 27]. We hypothesised that poorer verbal learning and
memory performances would be associated with higher cortisol levels in participants with a more advanced disease state as defined both by clinical diagnostic group, and by TMS.

**Materials and Methods**

**Participants**

Fifty-nine individuals (19 early-HD, 20 pre-HD, and 20 HD CAG normal controls) participated in the study. Participants were recruited through the Monash University HD Participant Registry, the St George’s Health Service HD clinic, a private neurology outpatient list, and the Huntington’s Victoria newsletter. Early-HD participants had been diagnosed within the past 5 years. Pre-HD participants were confirmed as having the HD CAG expansion by DNA testing, but they did not meet criteria for HD diagnosis. Controls were verified as having neither the HD CAG expansion or a family history of HD. Potential participants were excluded if they were under 18 years of age; had a history of an endocrine disease or neuropsychiatric syndrome other than HD; experienced alcohol or recreational drug abuse within the previous year; had a learning or intellectual disability; undertook current night shift work; were pregnant, lactating, or using pharmaceutical contraceptives. All participants provided informed consent.

Burden of pathology score was calculated according to the formula (age x [CAG-35.5]) [33]. Maximum level of education was classified according to the International Standard Classification of Education (ISCED) – 1997 [34]. We estimated verbal IQ (VIQ) using the Wechsler Test of Adult Reading (WTAR) [35] to ensure that VIQ ability did not confound
comparisons of memory performance between participants. There were no significant differences in sex, level of education, smoking status, or intellectual functioning between diagnostic groups. Pre-HD participants were significantly younger than controls and early-HD ($p < 0.001$), and had a significantly lower estimate of TMS ($p < 0.001$) and genetic disease burden ($p < 0.001$) than early-HD. Demographic and disease-related information is presented in Table 1.

Study protocol

The study visit occurred over one session. The UHDRS was performed at the start of the study visit by a neurologist (AC) or psychiatrist (PC, Prof. E Chiu) with specialist training in HD. TMS was obtained by summing the total of 31 items from the UHDRS motor scale, which are scored 0-4 denoting increasing severity of a given sign, resulting in a maximum score of 124. Before administering the neuropsychological assessment of learning and memory, a psychiatric assessment of depression, stress, and sleep quality was included in the study protocol because these are factors which are known to be associated with HPA axis function and changes in cortisol levels. The Inventory of Depressive Symptomatology – Self-report (IDS–SR) was used to provide a measure of depression severity on a scale of 0-84, based on responses to 30 self-rated items [36]. We assessed stress using the Perceived Stress Scale (PSS), a 14-item self-rated questionnaire about the degree of life stress over the past month [37]. The Pittsburgh Sleep Quality Index (PSQI), a 19-item self-report questionnaire, was used to assess sleep quality in the past month across different components of sleep and fatigue [38]. Bed partner or roommate items were excluded from the PSQI. A one-way analysis of variance (two-tailed) revealed that early-HD, in comparison to controls, reported a
higher level of depression symptomatology ($p = 0.025$), worse sleep quality ($p = 0.017$), and more perceived stress ($p = 0.053$). Because the maximum depression severity was in the moderate range according to IDS–SR criteria, and due to reduced statistical power because of a small sample size, IDRS-SR data was not deemed sensitive enough to examine any further in the statistical analyses. PSS – Total score and PSQI – Global score were significantly correlated, $r = 0.57$, $p < 0.001$.

Following the psychiatric and neuropsychological assessments, participants were provided with written and verbal instructions for using a salivary cortisol collection kit and were required to complete sample collection within two weeks of the study visit. The study protocol was approved by the St Vincent’s Hospital Human Research and Ethics Committee, and Monash University Human Research Ethics Committee. The data used for this report were collected as a part of a larger study.

Assessment of learning and memory

The California Verbal Learning Test–Second Edition (CVLT–II) [39] is a measure of auditory-verbal word learning and memory. It was administered according to standardised instructions. The CVLT–II comprises a list of 16 words (belonging to four semantic categories) read over five free recall trials (Immediate free recall – Trial 1-5). An interference list is then presented for recall (Interference – Immediate free recall), after which participants are asked to remember the original list both freely (Short delay free recall) and with the provision of semantic category cues (Short delay cued recall). Following a 20-minute delay, the free (Long delay free recall) and cued (Long delay cued recall) recall of the original list is
administered. The recognition memory trial was last and required participants to identify the original 16 words from a list of 64 words read by the researcher (Long delay Yes/No recognition).

To measure the construct of learning and memory in this study, we examined encoding, retrieval, and storage, which comprise core components of the hippocampal-dependent declarative memory system [16]. Total immediate recall (encoding) was calculated from the number of words correctly recalled over Trial 1-5 (possible range 0-80). Short- and long-delay free recall were summed to give total delayed free recall (retrieval) (possible range 0-32), and recognition discrimination (storage) was obtained by subtracting false positive responses from the number of hits (possible range 0-16). We used raw scores for all statistical analyses of learning and memory performance and entered age as a covariate. As expected, early-HD performance was significantly poorer than pre-HD and controls on all CVLT–II scores. In contrast, pre-HD did not perform significantly below controls on any CVLT–II measure (Table 2). The recognition discrimination memory score is not distributed normally across the population (see CVLT–II manual) [39] nor was it in our sample. We tried analysing the data using ranks rather than actual scores to normalise the distribution of recognition discrimination scores, but found that this did not significantly alter the effect of group and so we used the original unranked data for all subsequent analyses.

Cortisol collection and measurement

Participants collected their saliva by passively dribbling 3-5mL at four time points across a 24-hour period at Day 1: 2300hr; Day 2: 0800hr (waking), 1600hr, 2300hr. Participants were
instructed to avoid alcohol for 12 hours prior to collecting saliva, and to fast from food and drink, except water, for an hour before each collection and overnight before the morning collection. They were also asked not to brush their teeth or apply make-up before saliva collection. The use of any inhalants, topical applications, or eye drops known to contain steroids was ceased 24 hours prior to beginning saliva collection. Ten participants (8 early-HD, 1 pre-HD, 1 control) could not complete saliva collection via the passive dribble collection method and were instead instructed to chew on cotton wads (Salivette, Sarstedt, Nümbrecht, Germany), which were later centrifuged to extract the saliva. Comparison studies indicate that salivary cortisol concentrations obtained from both methods are analogous [40, 41].

Untreated salivary cortisol concentrations were analysed by Healthscope Functional Pathology, Ltd (Melbourne, Australia) using a competitive electrochemiluminescence immunoassay method on the automated Roche Cobas 3 601 Cobas analyser (Roche Diagnostics, Mannheim, Germany). A statistically significant Spearman’s correlation coefficient between the two 2300hr samples, \( r = 0.47, p < 0.001 \) affirmed salivary cortisol stability within participants. Saliva samples were unavailable for two early-HD participants and as a result salivary cortisol concentrations were analysed for 57 participants (17 early-HD, 20 pre-HD, 20 controls). Two pre-HD participants collected their 1600hr sample outside of a 2-hour window and so the mean cortisol concentration for pre-HD at 1600hr (11.22 nmol/L) was imputed. This was considered to be an acceptable practice because removing these two pre-HD cases from the analysis did not statistically alter the group main effect on cortisol at the 1600hr collection time.
Statistical analysis

To compare the 24 hour cortisol profiles in our groups with those reported in other studies, we first compared cortisol levels of the three diagnostic groups across the four saliva collection times using multiple analysis of covariance (MANCOVA), covarying for age. The study hypotheses were then tested by using each CVLT–II score as a dependent measure in separate three-step hierarchical standard regression equations, which allowed us to progressively build a model for predicting CVLT–II performance whilst analysing the individual association between CVLT–II scores and independent predictor variables added to the model at each step as detailed below.

Education and age tend to be associated with verbal learning and memory performance but they were not the focus of the study. Thus, we entered education levels and age in Step 1 of the regression model to allow us to partial out any nuisance variability in CVLT–II scores prior to examining variables of interest. Then, in Step 2 we entered the main effect of disease state (either diagnostic group or TMS) and Day 1: 2300hr salivary cortisol concentration. To reduce the likelihood of a Type I error, only salivary cortisol concentrations from the Day 1: 2300hr collection time were used in regression analyses. We used 2300hr cortisol levels because the literature suggests that late night cortisol concentration is sensitive to abnormal HPA axis function [42], and elevated evening cortisol levels are related to reduced cognitive performance [43]. An interaction term between disease state and cortisol concentration was added and tested in Step 3. From the results, we examined the change in $R^2$ ($\Delta R^2$) at each step to determine whether the independent variables in that step of the model contributed to a significant additional portion of the variance in the CVLT–II score. Significant standardised
regression coefficients ($b$) were interpreted as showing that an independent variable accounted for a portion of the variance in the learning and memory score independently of other variables in that step. Semi-partial correlations ($sr$) were computed to describe the unique relationship between the CVLT–II score and each independent variable with the effect of all other independent variables removed from that predictor. The overall $R$ for the equation indicated whether the model as a whole accounted for a significant proportion of the variance in each of the three memory measures. Regression models involving TMS were computed only for participants in the pre-HD and early-HD groups ($n = 37$). All tests were two-tailed. Partial eta-squared ($\eta^2$) was used as a measure of statistical effect size for the overall model. Statistical analyses were performed using the Statistical Package for Social Sciences v19.0 (SPSS Inc., Chicago, Illinois).

When the interaction term added in Step 3 of the regression model was statistically significantly associated with CVLT–II performance, or had a statistical trend for an association with CVLT–II performance, a graph was used to illustrate the effect that cortisol concentration (x-axis) at different disease states (for either diagnostic group or TMS) had on CVLT–II performance (y-axis). In order to show the effect of this association using a single graph across all participants analysed in the interaction, cortisol concentration was converted from a continuous variable to a discrete ordinal variable. The x-axis therefore presents the sample cortisol concentration mean ($\bar{x}$) ± 1 standard deviation (SD) for all participants analysed in the interaction. An interaction of statistical interest between cortisol levels and disease state occurs when the lines of association between CVLT–II performance and cortisol concentration are not parallel across the different disease states. Because a linear graph was
used to illustrate the effect of the interaction, the line of association between CVLT–II performance and cortisol concentration has the potential to mathematically represent a negative CVLT–II score or cortisol concentration depending on the size and direction of the relationship, even though a negative value for these variables is not literally possible.

**Results**

Twenty-four hour group salivary cortisol profile

All groups displayed the expected 24-hour cortisol profile of high morning cortisol, lower afternoon levels, and nadir in the evening. There was no overall main effect of diagnostic status on salivary cortisol concentration, \(F(4, 110) = 1.27, p = 0.27\). However at the 0800hr collection time there was a non-significant trend for cortisol difference between diagnostic groups \(F(2, 53) = 2.53, p = 0.089\) (Partial \(\eta^2 = 0.087\)). More specifically, pre-HD cortisol levels tended to be higher than early-HD (\(p\) for the paired comparison of the pre-HD and HD groups = 0.086). In addition, pre-HD tended to display higher cortisol than early-HD at all other collection time points (Fig. 1).

Relationship between verbal learning and recall and evening cortisol levels

Using the three groups (control, pre-HD, early HD) in the regression model (Table 3), the overall \(R\) statistical model accounted for a significant proportion of the performance on CVLT–II measures of *total immediate recall*, \(F(7, 49) = 7.87, p < 0.001\), (Partial \(\eta^2 = 0.53\),
and total delayed free recall, $F(7, 49) = 3.83, p = 0.002$, (Partial $\eta^2 = 0.35$). Examination of individual predictors indicated that being in the early-HD group was independently associated with lower scores on both of these CVLT–II measures. Only total delayed free recall from the CVLT–II was significantly associated with an interaction between diagnostic group and Day 1: 11pm salivary cortisol concentration ($p = 0.045$), indicating that a higher 2300hr salivary cortisol concentration in early-HD was associated with poorer delayed recall compared to control and pre-HD (Fig. 2). The addition of this interaction term did not account for significantly more variance in the model, possibly due to the non-significant pre-HD by cortisol interaction added in the same step of the analyses.

When using UHDRS TMS as a clinical indicator of HD severity in the regression analyses (rather than the grouping measure; Table 4) overall $R$ for the multiple regression models were also statistically significant for total immediate recall, $F(5, 31) = 8.05, p < 0.001$, (Partial $\eta^2 = 0.57$), and total delayed free recall, $F(5, 31) = 4.97, p = 0.002$, (Partial $\eta^2 = 0.45$). High TMS and elevated 2300hr salivary cortisol concentration were both independently associated with lower scores on these CVLT–II measures. An association between total immediate recall and the interaction of TMS and cortisol concentration indicated that poorer verbal learning ability was significantly and independently associated with higher evening cortisol levels and more pronounced HD motor signs ($p = 0.024$) (Fig. 3a). Adding the interaction between TMS and cortisol concentration significantly increased the amount of variance accounted for by the model from 49% (Adjusted $R^2 = 0.42$) to 57% (Adjusted $R^2 = 0.50$), $F_{\text{change}}(1, 31) = 5.63, p = 0.024$. A similar non-significant trend was seen for lower total delayed free recall performance in participants with elevated 2300hr cortisol concentration and a higher TMS ($p$
= 0.072) (Fig. 3b), which increased the amount of explained variance in total delayed free recall from 38% (Adjusted $R^2 = 0.31$) to 45% (Adjusted $R^2 = 0.37$), $F_{\text{change}}(1, 31) = 3.47, p = 0.072$.

TMS is a better predictor of verbal learning and memory performance than diagnostic group

A step-wise multiple regression analysis was used to assess which of the two methods for characterising HD, either diagnostic group or TMS, best accounted for CVLT–II score in participants with the HD CAG expansion ($n = 37$). Age and 2300hr cortisol concentration were entered as constant independent variables in the first step, HD diagnostic status and TMS were entered in Step 2. TMS was the better predictor of both total immediate recall, ($b = -0.57$, $sr = -0.52$, $p < 0.001$) and total delayed free recall, ($b = -0.56$, $sr = -0.56$, $p < 0.001$).

Neither disease state variable strongly predicted recognition discrimination.

Exploring stress and sleep as possible mediators of the association between cortisol and learning and memory in HD

Our small sample size precluded an examination of whether stress (PSS – Total score) or sleep (PSQI – Global score) were significant mediators of the relationships between learning and memory and cortisol levels. Instead the association between PSS and PSQI and the individual predictors of interest in the regression model (i.e., diagnostic group, TMS, Day 1: 2300hr cortisol concentration, and CVLT–II scores) were explored using separate descriptive analysis of variance (for diagnostic groups) or correlation (for TMS, 2300hr cortisol concentration, and CVLT–II scores), controlling for age where appropriate.
For perceived stress, early-HD had higher levels than controls, $F(2, 56) = 3.21, p = 0.048$, and higher levels of stress were associated with higher TMS, $r = 0.32, p = 0.014$. Stress was significantly and negatively related to Day 1: 2300hr salivary cortisol concentration, $r = -0.27, p = 0.041$. There was a significant correlation between high perceived stress and low total immediate recall, $r = -0.27, p = 0.041$, and a trend between high PSS and low total delayed free recall, $r = -0.25, p = 0.060$, but PSS was not significantly related to recognition discrimination, $r = -0.16, p = 0.22$.

For sleep quality, early-HD had worse sleep quality compared to both pre-HD and control, $F(2, 56) = 4.72, p = 0.013$. Poorer sleep quality was also associated with higher TMS, $r = 0.47, p < 0.001$. There was no association between PSQI and Day 1: 2300hr salivary cortisol levels. Worse sleep quality was, however, significantly associated with reduced performance on CVLT–II total immediate recall, $r = -0.28, p = 0.036$. A similar non-significant trend was seen for total delayed free recall, $r = -0.23, p = 0.08$, and recognition discrimination, $r = -0.23, p = 0.08$.

**Discussion**

This is the first study to show evidence of a relationship between verbal learning and memory performance and HPA axis function in the early stages of HD. A pattern of reduced verbal encoding ability and poorer delayed memory retrieval was associated with higher evening cortisol concentration in participants with a high TMS score and in participants belonging to the early-HD group. Although no causal relationship can be discerned in this study, our findings suggest that dysregulation of cortisol concentrations may be involved in the decline
of verbal learning and memory recall ability in HD. We did not find relations between recognition memory performance and cortisol levels. This may have been because the CVLT–II lacked sensitivity due to a restricted range and the skewed distribution of recognition discriminability scores, or instead verbal memory storage might simply not be affected by cortisol abnormalities in the early stages of HD. In considering the moderating effect of a pathophysiological biomarker, these results extend the previous literature into learning and memory changes in HD, which up until now has only described verbal learning and memory impairments as a main effect of diagnostic status [24] and of HD-motor sign severity [22, 23, 27].

Of the two modes used to classify disease state (i.e., diagnostic group and TMS), a higher TMS showed the strongest relationship to higher cortisol levels and poorer learning and memory performance. There are two potential explanations for this. The first is that HD motor features might be directly associated with HPA dysfunction. In this case, HPA axis abnormalities only affect verbal learning and memory ability once motor signs become clinically evident. The other possibility is that the diagnostic classifications of participants may have been too broad, unlike the motor sign severity range afforded by the UHDRS motor scale TMS. Categorising HD CAG expanded individuals as either not diagnosed (i.e., pre-HD) or diagnosed (i.e., early-HD) may be too imprecise or too broad, particularly for the pre-HD group, thus reducing power and in turn masking the relationship between cortisol and memory. The pre-HD participants in our sample had relatively low disease burdens and therefore many were not at an immediate risk of overt disease onset. This was highlighted by the non-significant difference in CVLT–II performance between pre-HD and controls, which is contrary to the literature that reports a deterioration in learning and memory 9-15 years prior
to motor onset in gene-positive patients [22, 25, 26]. Sub-categorisation of diagnostic groups might have been more effective, for example near versus far from predicted diagnosis. Our small sample size prevented such analyses.

The verbal learning and memory deficits identified in our study may be related to hippocampal dysfunction due to abnormally high cortisol levels. We observed reduced encoding and reduced retrieval which is similar to the profile of a number of other disorders that also display abnormal hippocampal morphology associated with dysregulated HPA axis activity, such as Alzheimer’s disease [44] and major depressive disorder [43]. In our study, the highest cortisol levels were not recorded by the furthest progressed HD groups. Instead pre-HD displayed the highest cortisol recording, although these differences were not statistically significant and were not related to cognitive performance. Therefore HPA axis hyperactivity may begin in the pre-HD stage with a prolonged period of hypercortisolism damaging the hippocampus [45]. Functional learning and memory deficits might only become evident after transitioning to the early-HD stage and unequivocal HD motor signs are displayed. Imaging and pathological investigations are required to further explore hippocampal changes in human HD.

The growing body of evidence identifying HPA axis dysfunction in HD raises the cortisol neurotoxicity hypothesis. According to this theory, the hippocampus, which is responsible for negative feedback control of HPA axis activity in conjunction with the hypothalamus and pituitary gland, induces a perpetuating cycle of dysregulated heightened cortisol levels and ongoing hippocampal degeneration once it becomes damaged by chronically high cortisol levels [46]. Stress may exacerbate this toxic effect, although our study was too small to definitively address this issue. We did find suggestive correlations showing that heightened
stress was present in HD expansion carriers, and was related to evening salivary cortisol levels, as well as learning and memory performance. This is consistent with previous studies in other diseases which have indicated associations between prolonged stress, high cortisol, hippocampal damage, and learning and memory deficits [47].

Our preliminary descriptive results indicate that poor sleep could also have been a factor in the poor memory performance of early-HD. In the wider population, night time HPA axis hyperactivity can disrupt sleep quality and as a result cause memory deficits that impair encoding of information into the memory store [48]. Sleep disturbance in HD patients has been shown to be associated with both depression and low cognitive performance [49]. A small sample size in our study prevented sleep from being assessed as a mediator in the statistical analysis of cortisol concentration, disease group, and learning and memory performance. Future larger studies could examine whether disturbed sleep quality in the early stages of HD is linked to abnormal cortisol levels and lowered learning and memory ability.

The high prevalence of depression in HD [50] means that future studies should further examine the relationship between depression and cognitive performance in the context of HPA axis integrity in HD. In addition, impairments in other domains of cognition could have had an impact on verbal learning and memory performance in this study. Attentional ability, processing speed, working memory, and executive functioning have all been implicated in the neuropsychological profile of HD [24]. Whilst it was outside the scope of our analyses to assess the effect of these on learning and memory performance and HPA axis activity, they should be a factor for consideration when interpreting the findings of this paper. Finally, the diagnostic categorisation applied to gene-positive participants (i.e., pre-HD or early-HD) was
based on the subjective clinical opinion of the HD specialist. This may have contributed to the finding that diagnostic status was a less powerful stratification approach than TMS rating.

In conclusion, the ability to encode verbal information and then retrieve that information from memory was poorer in participants with high evening salivary cortisol concentrations and more pronounced motor signs. HD motor sign severity is more sensitive to the relationship between cortisol levels and learning and memory than diagnostic status. The findings suggest that the functional impact of HPA hyperactivity on memory performance might only occur once HD-motor signs are relatively advanced. This study was the first to report the utility of cortisol levels as a biomarker of cognitive decline in HD. The results need to be investigated further in a larger sample, allowing more detailed consideration for the impact of stress, sleep quality, and hippocampal integrity on learning and memory in Huntington’s disease.
Conflict of interest

The authors declare that they have no conflict of interest.

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References


Figure captions

Figures have been uploaded online and the saved file name refers to the figure captions below:

**Fig. 1** Salivary cortisol concentrations for all participants by diagnostic group
Mean cortisol concentrations with standard error bars. Significance testing was undertaken via MANCOVA, covarying for age
† $p < 0.1$.

**Fig. 2** Association between CVLT–II Total delayed free recall score and the interaction of diagnostic group and Day 1: 11pm cortisol concentration

**Fig. 3** Association between (a) CVLT–II Total immediate recall score, and (b) CVLT–II Total delayed free recall score and the interaction of TMS and Day 1: 11pm cortisol concentration.
To display the effect of TMS on the interaction term, TMS was stratified as high (mean TMS + 1 SD), medium (mean TMS), and low TMS (mean TMS - 1 SD)
Tables

All tables have been uploaded online, and files contain a table caption (title) and legend explaining the components of the table. The saved file name refers to the table cited in the text.
Fig. 1 Salivary cortisol concentrations for all participants by diagnostic group
Fig. 2 Association between CVLT–II Total delayed free recall score and the interaction of diagnostic group and Day 1: 11pm cortisol concentration
Fig. 3 Association between (a) CVLT–II Total immediate recall score, and (b) CVLT–II Total delayed free recall score and the interaction of Day 1: 11pm cortisol concentration and TMS.
Table 1 Demographics and clinical information

<table>
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<tr>
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</tr>
<tr>
<td><strong>Demographic information</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 (52.6%)</td>
<td>10 (50.0%)</td>
<td>9 (45.0%)</td>
<td>0.89</td>
<td>n.s.</td>
</tr>
<tr>
<td>Age (year)</td>
<td>Mean (SE)</td>
<td>Range</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>58.0 (2.3)</td>
<td>41.8-72.3</td>
<td>57.0 (2.7)</td>
<td>&lt;0.001</td>
<td>P &lt; C, E***</td>
</tr>
<tr>
<td>Education – ISCED</td>
<td>Mean (SE)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.58 (0.3)</td>
<td>3.90 (0.2)</td>
<td>0.93 (0.2)</td>
<td>0.12</td>
<td>n.s.</td>
</tr>
<tr>
<td>Estimated VIQ</td>
<td>Mean (SE)</td>
<td>Range: Min-Max</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>105.89 (8.52)</td>
<td>92-118</td>
<td>106.35 (8.79)</td>
<td>90-118</td>
<td>n/s</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>110.8 (6.61)</td>
<td>94-119</td>
<td></td>
</tr>
<tr>
<td><strong>Clinical information</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease burden</td>
<td>Mean (SE)</td>
<td></td>
<td>n/a</td>
<td>&lt;0.001</td>
<td>E &gt; P***</td>
</tr>
<tr>
<td></td>
<td>401.3 (14.5)</td>
<td>269.3 (22.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UHDRS Total Motor Score</td>
<td>Mean (SE)</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
<td>E &gt; C, P***</td>
</tr>
<tr>
<td></td>
<td>30.1 (3.3)</td>
<td>3.6 (1.0)</td>
<td>2.6 (0.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-depressants*</td>
<td>n (%)</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
<td>E &gt; C, P*</td>
</tr>
<tr>
<td></td>
<td>13 (68.4%)</td>
<td>7 (35.0%)</td>
<td>1 (5.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>n (%)</td>
<td></td>
<td></td>
<td>0.75</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>3 (15.8%)</td>
<td>4 (20.0%)</td>
<td>2 (10.0%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The identification of a group difference was based on a one-way analysis of variance or chi-squared tests, with Fischer’s exact test applied where necessary to cell counts less than five. Tests were two-tailed.
To investigate which groups differed significantly, post-hoc group comparisons were undertaken using Bonferroni adjustment (for ANOVA) or standardised residuals (for chi-squared tests) where appropriate. Tests were two-tailed.

Anti-depressant medications included tricyclics, selective serotonin-reuptake inhibitors (SSRIs), serotonin and noradrenaline reuptake inhibitors (SNRIs)

†$p < 0.1$, *$p < 0.05$, ***$p < 0.001$

n/a: Not applicable

n.s.: No significant difference
Chapter 4 – Study 2 (Manuscript)

Table 2 Mean (and Standard deviation) of raw scores for CVLT–II performance by diagnostic group

<table>
<thead>
<tr>
<th></th>
<th>Early-HD n = 19</th>
<th>Pre-HD n = 20</th>
<th>Control n = 20</th>
<th>p value</th>
<th>Group comparison&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total immediate recall</td>
<td>33.95 (12.21)</td>
<td>53.05 (9.96)</td>
<td>57.00 (8.46)</td>
<td>0.000***</td>
<td>HD &lt; P, C***</td>
</tr>
<tr>
<td>Total delayed free recall</td>
<td>14.21 (7.47)</td>
<td>22.30 (6.66)</td>
<td>24.05 (4.57)</td>
<td>0.000***</td>
<td>HD &lt; P, C***</td>
</tr>
<tr>
<td>Recognition discrimination</td>
<td>8.89 (5.16)</td>
<td>11.40 (5.09)</td>
<td>13.75 (2.12)</td>
<td>0.004**</td>
<td>HD &lt; C**</td>
</tr>
</tbody>
</table>

Analysis of covariance of CVLT–II variables with post-hoc pairwise comparisons were completed using raw scores. Age was entered as a covariate. Tests were two-tailed.

<sup>a</sup>To investigate which groups differed significantly, post-hoc group comparisons were undertaken using Bonferroni adjustment (for analysis of variance) or standardised residuals (for chi-squared tests) where appropriate. Tests were two-tailed.

† p < 0.1, ** p < 0.01, *** p < 0.001
### Table 3 Hierarchical standard regression analysis for diagnostic status in comparison to controls and Day 1: 2300hr salivary cortisol concentration correlates of CVLT–II performance (n = 57)

<table>
<thead>
<tr>
<th>CVLT–II measure</th>
<th>Step 1</th>
<th>Step 2</th>
<th>Step 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age</td>
<td>Education</td>
<td>Diagnostic status</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>sr</td>
<td>Pre-HD</td>
</tr>
<tr>
<td>Total immediate recall</td>
<td>0.00</td>
<td>0.00</td>
<td>-0.09</td>
</tr>
<tr>
<td>Total delayed free recall</td>
<td>0.14</td>
<td>0.11</td>
<td>0.00</td>
</tr>
<tr>
<td>Recognition discrimination</td>
<td>-0.05</td>
<td>-0.04</td>
<td>-0.25</td>
</tr>
</tbody>
</table>

Standard regression coefficients (b) and semi-partial correlations (sr) represent values at Step 3 of the hierarchical regression (i.e., the whole model including all independent variables).

*a In comparison to the control group

* p < 0.05, ** p < 0.01, *** p < 0.001

† p < 0.1
Table 4 Hierarchical standard regression analysis for UHDRS TMS and Day 1: 2300hr salivary cortisol concentration correlates of CVLT–II performance (n = 37)

<table>
<thead>
<tr>
<th>CVLT–II measure</th>
<th>Step 1</th>
<th></th>
<th></th>
<th>Step 2</th>
<th></th>
<th></th>
<th>Step 3</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age</td>
<td>Education</td>
<td>b</td>
<td>sr</td>
<td>UHDRS TMS</td>
<td>Salivary cortisol</td>
<td>UHDRS TMS</td>
<td>x Cortisol</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Day 1: 2300hr</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Δ R²</td>
<td></td>
<td></td>
<td>Δ R²</td>
<td></td>
<td>Δ R²</td>
</tr>
<tr>
<td>Total immediate recall</td>
<td>0.03</td>
<td>0.03</td>
<td>0.20</td>
<td>0.20</td>
<td>-0.78***</td>
<td>-0.40*</td>
<td>-0.31</td>
<td>0.31**</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.62</td>
<td>0.34*</td>
<td>0.78***</td>
</tr>
<tr>
<td>Total delayed free recall</td>
<td>0.25</td>
<td>0.21</td>
<td>0.18</td>
<td>0.17</td>
<td>-0.79***</td>
<td>-0.43*</td>
<td>-0.33</td>
<td>0.34**</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.63</td>
<td>0.28</td>
<td>0.79***</td>
</tr>
<tr>
<td>Recognition discrimination</td>
<td>0.02</td>
<td>0.02</td>
<td>0.18</td>
<td>0.17</td>
<td>-0.31</td>
<td>-0.25</td>
<td>-0.18</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.24</td>
<td>0.04</td>
<td>0.25†</td>
</tr>
</tbody>
</table>

Standard regression coefficients (b) and semi-partial correlations (sr) represent values at Step 3 of the hierarchical regression (i.e., the whole model including all independent variables)

* p < 0.05, ** p < 0.01, *** p < 0.001
† p < 0.1
CHAPTER FIVE

– GENERAL DISCUSSION –
Chapter 5 – General discussion

This project has established evidence that cortisol concentration is associated with depression levels and memory performance in the early stages of Huntington’s disease (HD). Ultimately the aim of the project, as stated in Chapter 1 of this thesis, was to make a contribution to HD biomarker research by extending the limited knowledge about HPA axis function in the early stages of HD and its relationship to the early clinical signs of the disease. Cortisol is a biological marker of HPA axis integrity, and therefore an association between anomalous cortisol levels (whether these are abnormally high or low) and a depressed mood or poor memory ability could reveal new insights into the HD degenerative process, as well as the relationship between HD pathophysiology and the manifestation of clinical signs. The outcome of the investigations also has the potential to benefit future research and the development of therapeutic interventions. Previous literature has identified evidence of cortisol dysregulation, HPA axis dysfunction, and HPA axis pathology in human HD and HD mouse models. However there has been no thorough focus on how these changes are related to clinical signs of psychiatric disturbance and cognitive functioning in HD, most specifically in the early phase of the disease process. Results from two studies in this thesis showed that high morning cortisol concentration is associated with more severe depression, and high evening cortisol concentration is associated with poorer learning and memory performance in early-HD. Cortisol dysfunction may, however, begin in pre-HD, before a person with the HD CAG expansion manifests clinical signs of the disease and before a diagnosis is made. That is, there was evidence of hypercortisolism in pre-HD, but an association between abnormal cortisol levels and clinical signs of HD only manifested in early-HD. After briefly summarising Study 1 and Study 2, the General Discussion will describe how addressing three key methodological
issues has made it possible to considerably advance the understanding of the HPA axis in HD. The research findings will then be interpreted in the context of what they indicate about HD progression and the clinical signs of HD. Whilst this was not a study into the utility of cortisol as a biomarker per se, this chapter will discuss the benefit of using cortisol as a biomarker in HD once its relationship with the clinical signs of HD is understood. A comment will then be made about the methodological and analytical limitations of this study. Finally, there are a number of suggestions for future research studies in order to further develop the findings of this thesis.

5.1. Summary of Study 1: “Cortisol and depression in pre-diagnosed and early stage Huntington’s disease”

Study 1 (see Chapter 3) investigated whether there is a relationship between HPA axis function and depression in pre-HD and early-HD, compared to controls. The first set of analyses undertaken was an examination of the group effect (early-HD, pre-HD, control) on cortisol concentration. This was done as a preliminary step to put this study in the context of previous HD HPA axis studies where a comparison of group HPA axis hormone levels have been the primary research focus. Cortisol concentrations were assessed at four time points over a 24-hour period in each participant, and based on past literature it was hypothesised that cortisol levels measured at each of the four time points would be higher in early-HD than pre-HD, and lowest in controls. The data indicated that the salivary cortisol concentration profile did not significantly differ between participant groups. There was, however, a statistical trend for higher salivary cortisol concentration at 8am (within half an hour of waking) in pre-HD compared to early-HD. At every other collection point pre-HD also had higher levels of
circulating cortisol, but these differences were not statistically significant. Next, we examined the severity of depression symptoms using the Inventory of Depressive Symptomatology – Self-rated (IDS–SR) to investigate whether cortisol levels are associated with depression in HD. Using IDS–SR scores and published cut-offs, participants were dichotomised as being either depressed or non-depressed. To be consistent with evidence from depression research, which suggests that morning cortisol levels are relatively sensitive markers of depression, the analyses particularly focussed on the 8am cortisol concentration. The results produced somewhat unexpected findings by showing that cortisol levels, particularly in the morning, were moderated by the presence of depression. Specifically, the non-depressed early-HD group displayed a statistically lower 8am cortisol level compared to non-depressed pre-HD and controls. This group difference was not observed for the depressed early-HD group (i.e., there was no statistically significant difference in morning cortisol levels between depressed early-HD, pre-HD, and control). In addition, the depressed early-HD had higher morning 8am cortisol levels than non-depressed early-HD. Cortisol levels in pre-HD and controls, on the other hand, were not significantly moderated by the presence of depression. The interaction between group cortisol levels and depression state did not appear to be mediated by perceived stress levels, sleep quality, anti-depressant medication use, or gender.

5.2. Summary of Study 2: “The relationship between cortisol and verbal memory in the early stages of Huntington’s disease”

The second manuscript (see Chapter 4) investigated whether there is a relationship between HPA axis function and the verbal learning and memory profile in pre-HD and early-HD. In two separate sets of analyses participants were stratified according to their diagnostic status
(i.e., early-HD, pre-HD, control), or their UHDRS Total Motor Score (TMS) rating. The relationship between cortisol levels and learning and memory performance was analysed for each stratification method separately. We used both stratification methods because both diagnostic status and motor sign severity have been used as indicators of disease progression in previous assessments of learning and memory ability in HD (Stout et al., 2007). The hypotheses in Study 2 focussed on 11pm cortisol because of reports in the literature that elevated evening cortisol concentration is associated with cognitive difficulties (Gomez et al., 2006; G. Li et al., 2006). Specifically, we hypothesised that poor performance on the California Verbal Learning Test – Second Edition (CVLT–II) would be associated with higher cortisol levels in more severely affected participants, whether stratified by group (in which case the most affected participants were early-HD) or TMS (in which case the most affected participants had a high TMS). The results of this study were less clear than Study 1 because the statistical strength of the relationship between CVLT–II performance and evening cortisol concentration differed depending on whether participants were stratified by group or TMS. Specifically, the ability to encode verbal information (as measured by CVLT–II – Total immediate recall score) was significantly lower in HD CAG expanded participants with high evening cortisol concentrations and a higher TMS. However, this relationship was not seen for the early-HD group (i.e., when participant stratification by group was used in the analysis instead of TMS). With regard to free and unaided retrieval of learned information (as measured by CVLT–II – Total delayed free recall score), performance was significantly lower in participants with high evening cortisol who were in the early-HD group. When TMS was used in the analysis, instead of participant group, there was a statistical trend for a similar relationship of low memory retrieval ability in participants with high evening cortisol concentrations and a higher TMS. Lastly, the relationship between verbal information storage
ability (CVLT–II – *Recognition discrimination*) and cortisol concentration was not statistically significant for either participant stratification method. However, the results did follow a similar pattern to the relationship established in the analyses of information encoding and memory retrieval ability. Overall, a pattern emerged that suggested there was an association of heightened cortisol and learning and memory difficulties in HD. The findings generally supported the hypothesised pattern of results. An unexpected finding, which could explain the results of the regression analyses that are outlined above, was that for evening salivary cortisol concentration, TMS rating was more strongly associated with CVLT–II performance than the diagnostic group stratification method. Stress and sleep quality were not examined as mediators in the interaction relationships because of insufficient statistical power, due to the small sample size and large number of variables already included in the regression models. Instead, preliminary descriptive analyses were used to explore which biological and clinical variables stress and sleep quality were related to. The results revealed that higher levels of stress and poorer sleep quality were related to high evening cortisol concentration, a higher TMS, being in the early-HD group, and lower CVLT–II scores.

5.3. Findings of the thesis in the context of past literature: Addressing the gap in HD

HPA axis research

To appreciate the contribution that this research project has made to the understanding of the HPA axis in HD, the gap in HD research that previously existed will be highlighted by three pieces of evidence, which together justify the rationale for undertaking this project (see Figure 5.1., page 159). First was the growing body of evidence indicating that HPA axis dysregulation is a feature of HD across the disease spectrum. In particular, abnormal cortisol
levels are present in both pre-HD and in early-HD (Aziz et al., 2009; Kurlan et al., 1988; van Duijn et al., 2010). Secondly, depression and learning/memory deficits are widely accepted to be amongst the earliest HD signs detected in those with the HD CAG expansion, in some cases these signs appear up to 9–15 years before motor symptom onset (Duff et al., 2007; Kingma et al., 2008; Lemiere et al., 2004; Robins Wahlin et al., 2007; Shiwach, 1994; Solomon et al., 2007; Stout et al., 2011). Third, there is parallel evidence between HD and several medical and neuropsychiatric disorders that display similar psychiatric and cognitive signs to HD, in the context of HPA axis dysfunction, and in the context of pathological changes to the HPA axis, which includes research implicating the hippocampus. In reference to this last point, a combination of hypercortisolism, depressed mood, low memory ability, and reduced volume and impaired functional integrity of the hippocampus have been identified in major depressive disorder, mild cognitive impairment, post-traumatic stress disorder, and Alzheimer’s disease (Csernansky et al., 2006; Gomez et al., 2006; Gomez et al., 2009; Hinkelmann et al., 2009; Lind et al., 2007; Sapolsky, 2000; Sheline et al., 1996).
Chapter 5 – General discussion

HPA abnormalities have been reported in HD

Is HPA axis dysfunction associated with depression, and learning and memory in the early stages of HD?

Depression and learning and memory difficulties are early clinical signs of HD onset

Non-HD conditions display similar psychiatric and cognitive signs to HD in the context of HPA axis dysregulation and pathology

Figure 5.1. The rationale for investigating the association between HPA axis dysfunction and psychiatric and cognitive symptomatology in the early stages of HD.

Essentially, there has been no previous systematic investigation of the association between HPA axis function and the earliest clinical signs of HD. In the small amount of HD HPA axis literature that does exist there have been methodological limitations and inconsistencies. The gap in the literature provided an opportunity for this project to add new insights into the pathophysiology of HD. In order to fill this gap and build on the findings of previous literature, three key methodological issues were addressed in the design of this research project. These were: 1) a precise assessment of depression, and learning and memory ability, for the purpose of examining their relationship to HPA axis function; 2) the collection of cortisol samples at four time points over a 24-hour period to take into account circadian fluctuations in HPA axis activity; and 3) the examination of two stages of the disease (pre-HD
and early-HD) to account for the progressive nature of HD and potential changes across these different stages. The way in which each of these three areas helped contribute to the HD HPA axis literature will now be discussed in turn.

5.3.1. Measuring specific clinical signs can help explain the reason behind abnormal cortisol levels in HD and the aetiology contributing to HPA axis dysfunction.

Previous HD HPA axis studies have not focussed on understanding the association between HPA axis function and the clinical phenotype of HD. Instead they have primarily described HPA axis hormone levels at different stages of HD (i.e., pre-HD or diagnosed HD) in comparison to hormone levels of HD CAG normal controls. Where psychiatric and cognitive HD signs and symptoms have been examined, the assessment measures used were either not targeted to a specific cognitive or psychiatric domain (e.g. the MMSE, which is a screening tool for global cognitive impairment; Markianos et al., 2007), or were not sensitive to the severity of HD-specific clinical signs (e.g. the DSM or CIDI, which are diagnostic measures only; Heuser et al., 1991; van Duijn et al., 2010). Until the current project, the relationship between clinical signs of HD and HPA axis function had not been comprehensively investigated in previously published research. In the current research project, a neuropsychiatric and neuropsychological assessment battery assessed the presence of specific psychiatric and cognitive disturbances, as well as the severity of these signs. Study 1 assessed the psychiatric disorder of depression, and Study 2 assessed the cognitive domain of learning and memory ability.
In terms of how measuring specific clinical signs can help explain cortisol regulation in HD, the use of a specific measure of depression severity (the IDS–SR) in Study 1 revealed that depression moderates morning cortisol levels in early-HD. Specifically early-HD participants with high levels of depression had significantly higher morning cortisol concentrations than non-depressed early-HD participants. Depression itself, and the psychiatric confounds that come with being depressed, should therefore be a factor for consideration when interpreting all previous HD HPA axis research. Previous research has generally reported that cortisol levels are higher in diagnosed HD participants compared to controls (Aziz et al., 2009; Björkqvist et al., 2006; Heuser et al., 1991; Kurlan et al., 1988; Leblhuber et al., 1995; Saleh et al., 2009). It is possible that HD participants in these studies who have high cortisol levels were in fact depressed. Only van Duijn et al. (2010) reported that cortisol concentration in HD was not dissimilar to normal controls and instead pre-HD had the highest cortisol levels. In the case of van Duijn et al., the HD participants were potentially euthymic or normal in mood because they displayed a similar morning cortisol profile to the non-depressed early-HD participants in Study 1 of this thesis. Coincidentally, the number of depressed participants in the study by van Duijn et al. was very low, which increases the likelihood that majority of the participants were in fact not depressed. Given that non-depressed early-HD participants have low cortisol levels, future HD HPA axis studies could use these participants as the benchmark against which the impact of any mood disturbance on the HPA axis can be measured. Depression is, however, hard to quantify in HD with the current scales that are available, largely because of its unique phenotype (Craufurd et al., 2001). This has been discussed previously in this thesis (see Section 1.2.1.). Kurlan et al. (1988) used a combination of a diagnostic screening tool and a mood rating scale, similar to Study 1 in this thesis. Kurlan et al. is the only published study aside from Study 1 in this thesis that has detected an association between high cortisol and
depression symptoms in HD patients. Until a HD-specific depression tool is developed, a two-pronged method using a diagnostic tool and a depression severity rating scale might be the most effective way to assess depression in HD HPA axis studies.

Measuring clinical signs can also provide an insight into the aetiology of cortisol disturbance. With reference to Study 2, the CVLT–II does more than assess verbal memory ability and, for that matter, the state of individual components of the memory system. It also speaks to the pathophysiological problem underlying the memory impairment, because word list learning tasks are a neuropsychological indicator of hippocampal function (Lezak et al., 2004; Strauss et al., 2006). Using the CVLT–II in Study 2 revealed that difficulties encoding information and then retrieving that information from the memory store are specific cognitive functions associated with high cortisol in HD. Information encoding and memory retrieval are also functions subserved by the hippocampus (Lupien et al., 1998; Sapolsky, 2000; Sheline et al., 1996; Starkman et al., 1992). Hippocampal volume and function have previously been reported to be affected in human HD (Rosas et al., 2003; Spargo et al., 1993) and mouse models of HD (Fedele et al., 2011; Gil et al., 2005; Lazic et al., 2004; Simpson et al., 2011). Since information encoding and memory retrieval are signs consistently identified in pre-HD and early-HD (Hamilton et al., 1999; Lemiere et al., 2004; Lundervold et al., 1994; Massman et al., 1990; Rosenberg et al., 1995), using the CVLT–II in Study 2 tied in cortisol concentration, and by default HPA axis integrity, to the potential link between learning and memory changes and hippocampal morphology in the early stages of HD progression.
5.3.2. Different clinical signs of HD may be individually associated with cortisol dysregulation at different times of the day.

The second methodological issue in the HD HPA axis literature that this project addressed was the irregularity of cortisol collection times across previous studies. In previous research there is a particular disregard for fluctuations in the circadian cycle. To date Aziz et al. (2009) is the only HD study that has measured cortisol at multiple time points over a 24-hour period. The current project is the first to extend on the work of Aziz et al. by sampling cortisol concentration at numerous times over a 24-hour period as well as undertaking a sensitive assessment of depression and memory. The results showed that the two classes of HD signs were related to cortisol concentration at different time points in the 24-hour circadian cortisol cycle. Consistent with the literature from non-HD disorders, high morning cortisol concentration (which could be taken as an guide of the cortisol awakening response and the production capacity of the HPA axis (Pruessner et al., 1997)) was related to a depressed mood (Bhagwagar et al., 2005; Sachar et al., 1973), and high evening cortisol was a marker of poor learning and memory performance (Gomez et al., 2006; Karlamangla, Singer, Chodosh, McEwen, & Seeman, 2005; G. Li et al., 2006). Therefore, when investigating the relationship between clinical signs and HPA axis activity, HPA axis hormones should be assessed at numerous time points throughout the cycle.

On the topic of cortisol collection, cortisol sampling techniques are inconsistent across previous HD HPA axis studies. In the current project, salivary cortisol was chosen as an indicator of HPA functioning because it reflects the free and active circulating cortisol concentration. Salivary cortisol measurement is a convenient, well-validated, and approved
first step to take when examining the integrity of the HPA axis in any condition (Carroll et al., 2008; Viardot et al., 2005). Van Duijn et al. (2010) also used salivary collection to measure cortisol levels. It would be beneficial for HD HPA axis research to keep sampling methods consistent across studies. However, in saying that, future studies should choose sampling techniques carefully depending on the research question they are seeking to answer as well as the capabilities and limitations at hand.

5.3.3. The relationship between cortisol levels and clinical signs of HD changes as people with the HD CAG expansion progress through early stages of the disease.

Despite the fact that HD is, by nature, a condition of progressive change, only three out of the eight studies on the topic of HPA axis function in HD have included a sample that spanned a range of disease stages (i.e., normal, pre-HD, HD; Björkqvist et al., 2006; Markianos et al., 2007; van Duijn et al., 2010). Often the disease severity of participants has been poorly defined, in that it is not specified whether a HD participant is early or advanced in the progression of HD. A poor demarcation between those who are early or late in the disease process makes it hard to interpret whether HPA axis abnormalities are a characteristic of pre-HD, early-HD, or late-stage HD. The understanding of HPA axis function in the early stages of HD is especially limited because the early stages of HD onset has not always been a focus in previously published research.

To investigate the association between cortisol concentration and clinical signs through HD progression, the current research project recruited participants from pre-HD and early-HD. Sampling participants from two distinct stages of the disease helped to gauge a rudimentary
timeline of change in HPA axis function. This timeline revealed that pre-HD displayed the highest cortisol concentrations, which is consistent with the findings of van Duijn et al. (2010) but contrary to the original hypothesis of HD patients having the highest cortisol concentrations. Given that pre-HD had a low disease burden score and were therefore far from expected disease onset, the results suggest that cortisol dysregulation begins many years before a diagnosis of HD is made. Study 1 and Study 2 then report that cortisol concentration is associated with depression and poor memory ability in early-HD but not pre-HD. Therefore, depression and learning and memory are only related to cortisol levels in people with the HD CAG expansion once they have progressed beyond the pre-HD stage and are clinically categorised as early-HD.

The transition from pre-HD to early-HD appeared to mark a shift in the 24-hour cortisol profile, causing morning and evening cortisol to vary independently of each other. Specifically, 8am cortisol was diminished in early-HD compared to pre-HD, whereas 11pm cortisol did not undergo the same relative decline. Even within early-HD, cortisol levels at different stages of the disease process were related to different clinical signs. The relationship between cortisol concentration and depression severity was strong in early-HD, compared to the relationship between cortisol concentration and learning and memory performance in early-HD, which was not as clear. The link between cortisol concentration and memory performance strengthened when TMS was used as the participant stratification method in the statistical analyses instead of group. Therefore, an association between cortisol and learning and memory difficulties may only emerge when motor symptoms become severely pronounced and the disease is more advanced.
As an additional note, Study 2 revealed that there is a potential for the relationship between cortisol and clinical signs in HD to differ depending on the way that the disease severity of people with the HD CAG expansion is measured and stratified (i.e., according to their diagnostic status versus UHDRS TMS rating). A similar scenario has been reported in Alzheimer’s disease, whereby hypercortisolism was predominantly related to a quantifiable measure of disease progression (e.g., cognitive state) rather than non-specific guides of disease state (e.g., illness duration, or age), which do not directly reflect the severity of clinical signs (Weiner, Vobach, Olsson, Svetlik, & Risser, 1997). A suggestion to be drawn from these findings is that all future HD HPA axis research studies should include a quantitative measure of HD disease progression in addition to recruiting a participant sample that is representative of a range of diagnostically labelled HD stages.

5.4. Integrating and interpreting the findings

This research project has made an important contribution to the HD HPA axis literature and, more widely, to the HD biomarker literature. The HD HPA axis literature has been extended by the collective findings of Study 1 and Study 2, which show that the cortisol profile of people with the HD CAG expansion changes as participants transition from pre-HD to early-HD. Study 1 and Study 2 also provide evidence that cortisol levels are associated with clinical signs of depression and lowered memory ability in early-HD. The intent of this research project was not to establish the cause of HPA axis disturbance in people with the HD CAG expansion. However, interpreting the findings of Study 1 and Study 2, and integrating them with previous literature about HPA axis dysfunction in both HD and non-HD conditions could speak to the underlying biochemical and physiological changes in the HPA axis through the
progression of HD, as well as the timeline of these changes with regards to the onset of clinical signs.

5.4.1. A theory about the progressive deterioration of the HPA axis in HD and the cortisol toxicity hypothesis.

Existing HD literature raises the notion that the initial decline of HPA axis function in HD begins in the hypothalamus. Aziz et al. (2009) proposed that HPA axis negative feedback is impaired by disturbed hypothalamic glucocorticoid receptor (GR) function. We know that there is hypothalamic pathology in both pre-HD and diagnosed HD (Kassubek et al., 2004; Kremer et al., 1991; Kremer et al., 1990; Petersén et al., 2005; Politis et al., 2008; Soneson et al., 2010). Therefore, pathological evidence alone suggests that cortisol hypersecretion might begin at the level of the hypothalamus very early in the HD disease process. The hypothalamus could also impact on the clinical presentation of HD, because it regulates mood and body homeostasis. A depressed mood, heightened perceived stress, and disrupted sleep in HD are early clinical signs of disease onset in people with the HD CAG expansion, and this could coincide with hypothalamic pathology. Findings from the current research project help tie together the existing pathological and clinical literature about the initial changes to the HPA axis in HD. Whilst the hypothalamus was not directly assessed in Study 1 or Study 2, a combination of pathological measures and clinical assessments revealed results that are consistent with previous evidence supporting the progressive decline in hypothalamic functioning in HD. The results demonstrated cortisol dysregulation in pre-HD and then showed that the clinical sign of depression associated with cortisol dysregulation subsequently manifests in early-HD. The development of depression is highly relevant to the proposition
Chapter 5 – General discussion

that the hypothalamus is disturbed early in HD. Theoretically this makes sense because HPA axis abnormalities, specifically elevated morning cortisol, are a chronic marker of depression (Vreeburg et al., 2009) and so the development of this association is a long term process that probably occurs through pre-HD but only manifests a clinical association with depression post-diagnosis. Whatever the reason for this phenomenon, the point where mood disturbances emerge is when the ongoing deterioration in HPA axis integrity begins.

Mood irregularities, in particular a depressed mood, have the potential to stress an already dysfunctional system and induce ongoing episodes of hypercortisolism, especially in the morning. The negative feedback loop continues to fail because GR, which are supposed to control elevated glucocorticoids, are not operating effectively. A process of chronic hypercortisolism then commences. Not only do the psychiatric symptoms exacerbate, but a physical phenotype – which is reflective of a hypothalamic disturbance – also appears. In human HD, this can manifest as poor sleep, irritability, cognitive deficits, sexual dysfunction, and peripheral weight loss despite adequate nutrition. In transgenic mouse models, physical symptoms of abnormal fat deposits, muscular atrophy, reduced bone density, and insulin resistance are seen (Aziz et al., 2007; Björkqvist et al., 2006; Petersén & Björkqvist, 2006). By the time HD CAG expanded patients are at the early phase of HD, van Duijn et al. (2010) suggests that the HPA axis is exhausted or over regulated as a consequence of chronic hypercortisolism. Cortisol production capacity is therefore low and early-HD display a profile of hypocortisolism which is most clearly seen in the morning. However, in depressed early-HD patients, HPA axis hyperactivity can still be induced against a background of hypocortisolism because of problems regulating the HPA axis’ response to a stressed or
Chapter 5 – General discussion

depressed state. This effect has been well documented in neuropsychiatric conditions of chronic stress such as post-traumatic stress disorder (PTSD; Bremner et al., 2003; Heim et al., 2000), and it could potentially have occurred in the early-HD participants involved in Study 1 of this thesis.

Problems with the HPA axis are unlikely to stop at this point in the disease, and instead they may compound as HD progresses. This is because the longer that the HPA axis is dysregulated, the greater the risk of damage to neuroanatomical sites that are susceptible to the effects of prolonged excessive cortisol levels. The findings of this research project suggest that as HD progresses, HPA axis deterioration will eventually involve the hippocampus. As briefly discussed in Chapter 1 (Introduction), the hippocampus is particularly vulnerable to HPA axis hyperactivity. In non-HD conditions there is clear evidence of hippocampal atrophy as a result of chronic and unregulated levels of heightened cortisol (de Leon et al., 1988; Sapolsky, 2000; Sapolsky, Krey, & McEwen, 1985; Sapolsky, Uno, Rebert, & Finch, 1990; Sheline et al., 1996; Starkman et al., 1992). It is possible that changes in the hippocampus in HD (Rosas et al., 2003; Spargo et al., 1993) are also due to the effects of cortisol damage. There are two implications of hippocampal damage in HD. The first is that impaired hippocampal function can affect information encoding and memory recall. Study 2 revealed that poor learning and memory performance was associated with high evening cortisol concentrations (11 pm), which is when mineralocorticoid receptors (MR) should be active. It makes sense that MR, which are principally expressed in the hippocampus, would not be functioning effectively if the hippocampus was degenerating. Therefore, after a long duration of hypercortisolism throughout the pre-HD phase, learning and memory impairments might only become evident as a result of hippocampal damage later on in early-HD, once an advanced motor disorder has
ensued. Early-HD participants in Study 2 were only 5 years post-diagnosis at most. In more progressed HD, a stronger association between abnormal HPA axis activity and learning and memory decline might manifest more clearly.

The second hypothetical implication of hippocampal damage in HD is that changes to MR number and function would disrupt the fundamental regulation of baseline cortisol concentration. This is in addition to the lost control of elevated peak secretion periods due to disturbed hypothalamic GR function. In this scenario, cortisol levels would be high in the afternoon and late evening, when cortisol concentrations are typically low, and there would be additional unregulated spikes in the morning cortisol awakening response (CAR) when a depressive mood disturbance is present. These ongoing effects throughout the circadian cycle continue to promote the toxic degradation of the hippocampus and potentially other structures involved in regulating the HPA axis. This progressive and self-perpetuating process is effectively the cortisol toxicity hypothesis discussed by Swaab et al. (1994). Thus, the cortisol toxicity hypothesis should be considered as a possible explanation for psychiatric, cognitive, and neuropathological changes in HD. Effectively, the pre-HD phase is a prodromal stage of symptomatic HD similar to mild cognitive impairment (MCI; Lind et al., 2007; Lupien et al., 1998), or the prodromal phase in psychoses (Garner et al., 2005). During the prodrome the course of a disease can be altered by a number of endogenous physiological processes (de Leon et al., 1988; Sapolsky, 1985) and the psychiatric state of the patient (Stein-Behrens, Mattson, Chang, Yeh, & Sapolsky, 1994) – both of which are affected in HD. Once the patient transitions to the diagnosed HD phase the structural and functional integrity of the HPA axis is
damaged and permanently dysregulated, which perpetuates its own demise and is accompanied by psychiatric and cognitive clinical signs.

5.4.2. Stress and sleep are other factors to consider in the relationship between cortisol concentration and clinical signs in HD.

In this study, high perceived stress was reported by early-HD participants. Other studies have shown that high stress levels raise high cortisol levels even further, and therefore can propagate the cycle of hypercortisolism and its toxic effect on hippocampal degredation (Sapolsky, 1996). It is important to consider several types of stressors in HD; these include psychosocial stressors, anxiety, and physiological stress at the biochemical level. These types of stress are intertwined with HPA axis responses, the cortisol neurotoxicity hypothesis, and clinical signs of HD. The potential impact of each of these three stressors will be briefly mentioned in turn. Firstly, psychosocial stress in HD families can be very high because of the care and management of affected family members, uncertainty about one’s own gene status, the stress of undergoing genetic testing, fear of symptom onset, and other socioeconomic factors (Wexler, 1979). Each of these factors change across time and each would have an effect on HPA axis function. Anxiety is closely aligned with psychological and physiological aspects of stress, and therefore should be considered in HD HPA axis research because it is significantly associated with high cortisol levels (Sachar et al., 1973; Vreeburg et al., 2009; E. A. Young, Abelson, & Cameron, 2004). Whilst comorbid anxiety comprises a large component of depression in HD (Craufurd et al., 2001; J. C. Thompson et al., 2002), anxiety may affect the HPA axis in a different way to isolated depression and therefore should be assessed separately. Additionally, it is important to examine the inter-relationship between
psychosocial stress or anxiety and HD cognitive signs. The ability to deal with stress, and manage its impact, may be diminished for people with the HD CAG expansion who are more cognitively impaired, which further promotes a maladaptive physiological response to stress. Finally, biochemical inflammatory markers in HD are reflective of internal physiological stress. Physiological stress is a very topical research area in HD and there are many questions about whether markers of physiological stress are present centrally or peripherally; are they a cause or a reaction to neuroanatomical changes which may begin as early as childhood; are they a response to the presence of the abnormal protein huntingtin; and what can be done to reduce them (Aylward, 2011; Björkqvist, 2011; Leavitt, 2011; Tabrizi, 2011)?

Sleep is another aspect of HD that should be taken into account when investigating the HPA axis. The inextricable link between sleep and the circadian profile of the HPA axis in HD, the functional role of the hypothalamus in the sleep, and the inter-relationship between stress and sleep are relevant to the findings of this thesis. In the current research study, poor sleep quality was identified in both early-HD and pre-HD according to the criterion set by the PSQI authors (Buysse et al., 1989). Research validates the clinical observation that sleep disturbance is a common concern reported by almost 90% of the HD patient group (Taylor & Bramble, 1997). It can affect the quality of life of HD patients and adds to the caregiver burden of sleep partners (Gupta, Ankush, Sue, & Kathleen, 2010). Signs of sleep disturbance in pre-HD and diagnosed HD includes frequent waking, early waking, insomnia, daytime fatigue and somnolence, increased sleep latency, REM sleep disturbance, and excessive nocturnal activity unrelated to the HD motor signs (Arnulf et al., 2008; Stephen et al., 2005; Taylor & Bramble, 1997; Wiegand et al., 1991). There is an alarming link between sleep quality, poor cognition, and mood in HD (Aziz et al., 2010; Shannon & Moore, 2001). There is likely to be a
biological component underlying poor sleep quality in HD. A small amount of research in this area has proposed that molecular and neuropathological abnormalities could explain circadian sleep behaviour in HD (Morton et al., 2005; Wiegand et al., 1991), but the involvement of the HPA axis with sleep quality in HD has not yet been investigated.

5.5. Cortisol is a useful biomarker of HD progression and a pathophysiological correlate of the clinical signs of HD, but more research is required before it can be used in the development of a therapeutic intervention

Part of the reason why HD is complex is because of the complex relationship between neuropathology, physiological function, and the development of psychiatric and cognitive signs in relation to motor signs. The thesis attempts to try and unravel a small part of this complexity in HD by using cortisol as a biomarker of HD progression and a correlate of clinical signs. Study 1 and Study 2 also emphasise the importance of including measures of psychiatric and cognitive signs in HD biomarker research, not only because of the high prevalence of these signs in HD but due to their bi-directional influence on the HPA axis. For example, hypothalamic pathology could contribute to a depressed mood in HD, and then depression could induce hypercortisolism by stressing a dysfunctional HPA axis negative feedback loop in early-HD. With the findings of Study 1 and Study 2 in mind, Figure 5.2a. and 5.2b. (see page 175) summarise the interplay between HPA axis function and HD clinical markers. Cortisol concentration is high through pre-HD due to HPA axis dysfunction. In Figure 5.2a, psychiatric signs emerge in the prodrome phase before motor signs are diagnosed. Depending on whether patients become depressed post-diagnosis or whether mood remains euthymic, cortisol levels may either increase or decrease respectively. Figure 5.2b. shows that
the association between cortisol concentration and learning and memory performance manifests later than the association between cortisol concentration and depression. Unlike depression severity, the severity of learning and memory difficulties will continue to worsen irrespective of the shift in cortisol concentration because hippocampal damage has already been sustained through pre-HD due to chronic exposure to harmful levels of circulating cortisol.

Neuropathological change in HD is complex and whilst hippocampal integrity was not directly measured in this project, a theoretical interpretation was made based on evidence from the literature and evidence from Study 2 of this thesis that suggest a large amount of neuropathological damage has already occurred by the time motor signs are severe enough to diagnose HD. Cortisol could therefore be used as a biomarker to detect HD-related changes before motor signs emerge. A key outcome of this research project is the potential for the nature of the relationship between cortisol and HD clinical signs to help differentiate between pre-HD and early-HD.
Chapter 5 – General discussion

Figure 5.2. A model proposing how cortisol levels are related to (a) depression, and (b) learning and memory ability as the disease state progresses from pre-HD to early-HD. In 5.2a., the dashed red line reflects the depression severity of depressed early-HD. The accompanying dashed blue line is the rise in cortisol levels in early-HD as a result of being depressed. In 5.2b., the dashed blue line is still shown to illustrate the two potential courses of cortisol concentration in early-HD.
As to whether the HPA axis itself should be a target for therapeutic intervention ... not yet. The mounting evidence that hypercortisolism begins in pre-HD suggests that HPA axis dysfunction needs to be corrected early on. However, the HPA axis is an extremely complex physiological system with myriad factors that it affects and is, in turn, affected by. We do not yet understand all of these factors in HD. At the moment, cortisol and HPA axis function are potentially good biomarkers of HD progression and good pathophysiological correlates of the clinical signs of HD. They fit the criteria of a biomarker as specified by Henley et al. (2005), but more research is required and future studies should heed the learnings and limitations from this thesis before cortisol or other aspects of the HPA axis are involved in the development of therapeutic interventions.

5.6. Limitations

Limitations have already been noted in the two manuscripts submitted for publication (see Chapter 3 and Chapter 4). The main limitation of the project was the small sample size. This restricted the statistical options available to analyse the data. It prevented the analysis of each of the four cortisol concentrations with measures of depression (Study 1) and learning and memory (Study 2). Taking Study 2 as an example, 24 separate analyses would have been required to analyse the relationship between the three CVLT–II scores, two systems of classifying participants, and four cortisol concentrations. In a small sample size, such as the one in this research project, performing so many analyses inflates the Type I error rate (the probability of finding a statistically significant result when one does not actually exist, i.e., a false positive result) to an unacceptably high level. To prevent the risk of a Type I error, the cortisol concentration from only one collection time was analysed in each of the two studies,
and the collection time used was chosen based on information available in the literature. Furthermore, the small sample size, relative to the number of variables used in the analyses, restricted the ability to assess covariate variables such as stress, sleep, and medication use in the relationships established in Study 1 and Study 2. The small sample size also limited statistical power. Despite this, Study 1 and Study 2 revealed several statistically significant relationships with at least medium effect sizes (as determined by a medium effect size equating to Partial $\eta^2 = 0.06 \leq \eta^2 < 0.14$), and several additional relationships showed statistical trends towards significance. The final issue with having a small sample size was the risk that some participant characteristics would lack natural variability. For example, the disease burden score of pre-HD participants was homogeneous and low. Despite best intentions to sample participants across the disease progression, pre-HD who are relatively close to HD diagnosis are at a difficult point in their lives and can therefore be hard to recruit. Future studies should aim to sample this population as a part of recruiting a larger overall sample size.

An additional methodological issue in the current project was the need to employ two different saliva collection methods. Although the initial plan was to sample saliva by passive dribble, cotton wads were introduced for some participants who had difficulties with passive saliva collection. Xerostomia (dry mouth due to a lack of salivation) has been associated with HD (Wood et al., 2008), though it was not originally expected to be a problem for pre-HD and early-HD participants in this study. Before employing cotton wads, Healthscope Functional Pathology Ltd. (Melbourne, Australia) was consulted about whether there would be a difference in the cortisol concentration assayed if saliva was collected by passive dribble or cotton swabs. The initial advice was that using two different saliva collection techniques...
would not be an issue. The two manuscripts cite literature to support this advice (Poll et al., 2007; Shirtcliff, Granger, Schwartz, & Curran, 2001). However, some conflicting evidence was uncovered that suggests there may be a discrepancy between cortisol levels obtained from these two collection methods (Strazdins et al., 2005). Future studies need to carefully account for this issue and also the potential impact medications could have on xerostomia.

5.7. Recommendations for future research

Aside from the obvious need to address the limitations identified within the thesis, several additional suggestions for future prospective studies have been proposed in the two manuscripts submitted for publication (see Chapter 3 and Chapter 4). Including these recommendations in ongoing large-scale, multi-site, longitudinal research studies such as PREDICT-HD, TRACK-HD, COHORT, and IMAGE-HD would be a very effective data-collection tool for understanding the HPA axis and its association with clinical signs in HD. Three of these suggested recommendations are elaborated below.

Firstly, this project was not a longitudinal study. Longitudinal studies are needed to describe progressive changes in the HPA axis in HD and how these changes are associated with the progression of clinical HD signs. Ideally, such studies would comprise an ongoing repeated-measures design in which 24-hour cortisol levels, as well as psychiatric and cognitive functioning, are assessed across several years encompassing both pre-HD and early-HD participants. Including HPA axis challenge tests, which are described in Chapter 1 (Introduction), would also help to more precisely determine the part of the HPA axis system that is dysfunctional in HD.
Secondly, to relate HPA axis changes to gross morphological changes, imaging techniques should be used to examine the pathological changes in the HPA axis. Imaging studies into other disorders have found this to be useful. For example, Garner, et al. (2005) found pituitary swelling in prodromal schizophrenics was related to the onset of first episode psychosis. In HD, there has been some imaging work done on the structure and function of the hypothalamus (Kassubek et al., 2004; Politis et al., 2008; Soneson et al., 2010), and one study has briefly commented on hippocampal volume (Rosas et al., 2003). However there has been no reported thorough structural and functional imaging of the pituitary gland, adrenal gland, or hippocampus in living pre-HD or HD humans. Brain imaging would also help translate the HD mouse study by Grote et al. (2005), who used SSRI medication to rescue hippocampal neurogenesis and improving depressive and cognitive behaviour, to humans. Understanding morphological changes of the HPA axis in HD at the gross neuroanatomical level would help guide subsequent research into the molecular neuropathology of pre-HD and early-HD.

Finally, this research project focussed exclusively on the clinical signs of depression, and learning and memory ability, despite a board range of clinical manifestations that are seen in HD. Other aspects of psychiatric functioning and cognition could have been involved in the relationships established by Study 1 and Study 2. A focus on the association between HPA axis function and clinical signs of depression and memory was guided by the literature, and by the need to limit the breadth of the project appropriate to a thesis with very limited funding. Other psychiatric and neuropsychological facets were considered outside of the scope of the research project. However, to take the findings in this thesis to a broader clinical context, cognitive domains of processing speed, attentional ability, working memory, language ability,
visual processing, and executive functioning, as well as the psychiatric domain of anxiety (as already discussed), should also be considered.

5.8. Conclusions

The overarching purpose of this research project was to extend the limited understanding of the HPA axis in HD. Up until now, the research has predominantly only described HPA axis hormone levels in HD, without specifically assessing the involvement of HD clinical signs. This thesis considerably extends the state of this knowledge to reveal evidence of a unique and complicated link between HPA axis functioning, as indicated by cortisol levels, and the psychiatric and cognitive signs in pre-HD and early-HD. The findings indicate that cortisol levels are high in pre-HD and therefore there is evidence, consistent with previous literature, that HPA axis dysfunction may begin many years before someone with the HD CAG expansion is clinically diagnosed with HD. After chronic HPA axis malfunctioning and long-term exposure to hypercortisolism the clinical signs of HD can manifest. There is a relationship between cortisol dysregulation and depression, and between cortisol dysregulation learning and memory in HD, but this association is only evident in early-HD; not pre-HD. These relationships help distinguish between pre-HD and early-HD. This thesis provides an empirical base from which it is possible to advance the current understanding of the neuropathological mechanisms underlying cortisol abnormalities and the development of clinical signs in HD. There are limitations in the study, and future research is needed to evolve our understanding of the integrity of the HPA axis in HD. The relationship between clinical signs of HD and cortisol provides a new insight into the neurobiology of HD which might redefine how HD is diagnosed and how the disease is monitored and managed.
– Thesis references –
The list of Thesis references contains citations used in Chapter 1 (Introduction), Chapter 2 (Methods), and Chapter 5 (General discussion) of this thesis. Not all citations used in the respective manuscripts presented in Chapter 3 (Study 1) and Chapter 4 (Study 2) are included in the list of Thesis references. Instead, the manuscripts presented in Chapter 3 and Chapter 4 contain their own reference list.


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