The utility of multidisciplinary rehabilitation as a treatment strategy for circadian rhythm and sleep disturbances in premanifest Huntington’s Disease

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The Utility of Multidisciplinary Rehabilitation as a Treatment Strategy for Circadian Rhythm and Sleep Disturbances in Premanifest Huntington’s Disease

This thesis presented for the degree of 
Doctor of Philosophy

Danielle Megan Bartlett

School of Medical and Health Sciences

Edith Cowan University

2018

Supervisors:

Professor Mel Ziman

Dr Travis Cruickshank
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Abstract

**Background:** Huntington’s disease (HD) is a rare, neurodegenerative disease caused by an expanded cytosine-adenine-guanine (CAG) sequence in the Huntingtin gene, resulting in the production of an aberrant protein, mutant huntingtin (mHTT). The mHTT protein exhibits a toxic loss and gain in function, leading to degeneration of neurons in the brain. Consequently, the classic triad of motor, cognitive and mood features of the disease develop. Among the earliest features of HD are circadian rhythm and sleep disturbances. These anomalies present many years prior to formal clinical diagnosis of HD and, while it has been postulated that these disturbances arise as a result of hypothalamic pathology, the neurobiological mechanisms underpinning these sleep disturbances have not yet been robustly investigated.

The hypothalamus of the brain contains several key nuclei that are essential in maintaining circadian rhythm and sleep/wake cycles. Hypothalamic pathology and dysregulation of neuroendocrine factors that mediate the sleep/wake cycle have been reported in HD, as early as the premanifest stage. It is not known however if hypothalamic pathology precedes neuroendocrine dysregulation. Identification of mechanisms underpinning sleep and circadian rhythm disturbances will enable the development of therapeutic strategies aimed at mitigating sleep and circadian rhythm anomalies. To date, no therapies exist to combat pathological changes in sleep architecture and circadian rhythm.

Evidence from mouse models of HD shows that the circadian rhythm and sleep-wake cycle are amenable to environmental interventions, including exercise, bright light therapy and temporally scheduled feeding. Furthermore, previous studies in HD of multidisciplinary rehabilitation- a construct of exercise and cognitive training, along with social interaction have been shown to increase grey matter volume in the caudate tail and dorsolateral prefrontal cortex in manifest HD, with accompanying improvements in verbal learning and memory. It is postulated that this intervention paradigm could also improve sleep outcomes in HD. Studies
in Parkinson’s disease have shown that multidisciplinary rehabilitation improves sleep quality, however, the effects of multidisciplinary rehabilitation on circadian rhythm and sleep outcomes have not yet been investigated in HD and particularly not in premanifest HD when the effects of intervention would be most beneficial.

**Aims:** The initial aim of this thesis was to determine, through a review of the literature, the potential neurobiological mechanisms associated with circadian rhythm and sleep disturbances in individuals with premanifest HD. This was used to inform the next study, which was to determine whether hypothalamic pathology was associated with circadian rhythm and habitual sleep disturbances in individuals with premanifest HD. The next aim was to then determine if nine-months of multidisciplinary rehabilitation could impact on circadian rhythm and habitual sleep outcomes and associated hypothalamic volume in individuals with premanifest HD. The aim of the final study was to explore the effects of a nine-month multidisciplinary rehabilitation program on sleep architecture in individuals with premanifest HD.

**Methods:** For the study presented in Chapter 3 (aim 2), 32 individuals with premanifest HD and 29 healthy age- and gender-matched controls underwent magnetic resonance imaging scans to evaluate hypothalamic volume. Circadian rhythm and habitual sleep were assessed via measurement of morning and evening cortisol and melatonin levels, wrist-worn actigraphy, the Consensus Sleep Diary and sleep questionnaires. Information on mood, physical activity levels and body composition were also collected.

In the study presented in Chapter 4 (aim 3) 18 individuals with HD (ten premanifest and eight prodromal) undertook a nine-month multidisciplinary rehabilitation intervention (intervention group) and were compared to a community sample of 11 individuals with premanifest HD receiving standard care (control group). Hypothalamic volume, blood-based BDNF, salivary cortisol and melatonin concentrations, subjective sleep quality, daytime somnolence, habitual
sleep-wake patterns and stress, anxiety and depression symptomatology were all evaluated prior to and following the intervention. Sixteen of these individuals also underwent polysomnography and sleep-dependent memory consolidation prior to and following the nine-month intervention to assess sleep architecture and sleep-dependent memory consolidation (Chapters 4 and 5).

**Results:** Here in Chapter 2, a review of the literature revealed the hypothalamus as a potential modulator of circadian rhythm and sleep disturbances in HD. Chapter 3 shows that hypothalamic grey matter volume in premanifest HD individuals is reduced compared to age- and gender-matched healthy controls. We also observed reduced sleep quality and an increased number of awakenings in premanifest HD individuals compared to healthy controls. Contrary to expectation, there were no strong associations between sleep outcomes and hypothalamic volume. There were, however, differences in the associations between hypothalamic volume and neuroendocrine factors in premanifest HD individuals compared to healthy controls.

Following nine months of multidisciplinary rehabilitation, a reduced rate of loss of grey matter volume in the hypothalamus was observed in the premanifest HD intervention group compared to the premanifest HD standard care group (Chapter 4). This was accompanied by a maintenance of brain-derived neurotrophic factor (BDNF) levels in the intervention compared to the control group. No robust changes were observed in the release of circadian-regulated hormones or in habitual sleep outcomes; however, exploratory data revealed changes in sleep architecture, particularly in REM percentage and latency, following the nine-month intervention (Chapter 5).

**Conclusion:** Data presented in this thesis suggests that, although hypothalamic volume is reduced in individuals with premanifest HD, circadian rhythm is maintained, perhaps via neural compensation. Moreover, we provide, for the first time, preliminary data suggesting that
multidisciplinary rehabilitation is useful in reducing the loss of volume of the hypothalamus and, while no robust effects on circadian rhythm were observed, improvements in sleep architecture were observed in individuals with premanifest HD. Further randomised controlled studies in a larger cohort of individuals with premanifest and manifest HD are required to confirm and extend on these preliminary findings.
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First and foremost, I would like to thank my supervisors, Mel and Travis. Thank you for believing in me and for your ongoing support, guidance and friendship over the years. You have taught me many invaluable lessons and provided me with the opportunity to accomplish such a great achievement and for that, I am sincerely thankful.

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This thesis is submitted as a series of papers.

As a result of the work performed for this thesis, one paper has been published, one paper has been accepted for publication and two papers are ready for submission for publication:

**Neuroendocrine and Neurotrophic Signaling in Huntington’s Disease: Implications for Pathogenic Mechanisms and Treatment Strategies**

Danielle M. Bartlett, Travis M. Cruickshank, Anthony J. Hannan, Peter R. Eastwood, Alpar S. Lazar, Mel R. Ziman.

Published: Neuroscience and Biobehavioral Reviews, 2016, Volume 71, Pages 444-454.

**Mechanisms Associated with Sleep Disturbances in Premanifest Huntington’s Disease**


*Danielle M. Bartlett and Juan F. Dominguez D contributed equally to the manuscript

Published: Neurobiology of Sleep and Circadian Rhythms

**Multidisciplinary Rehabilitation Reduces Hypothalamic Grey Matter Volume Loss in Individuals with Premanifest Huntington’s Disease: An Exploratory Study**

Danielle Bartlett, Juan Dominguez, Alpar Lazar, Catarina Kordsachia, Tim Rankin, Brian Power, Amit Lampit, Peter Eastwood, Travis Cruickshank, Mel Ziman.
The Effects of Multidisciplinary Rehabilitation on Sleep and Sleep-Dependent Memory Consolidation Outcomes in Individuals with Premanifest Huntington’s Disease: An Exploratory Study

Danielle Bartlett, Juan F. Dominguez D, Kathleen Maddison, Robert Newton, Peter Eastwood, Alpar Lazar, Govinda Poudel, Mel Ziman, Travis Cruickshank.
**Statement of Contribution Towards Publications**

While this thesis was part of a larger research program, the project presented herein was devised by me in collaboration with my supervisors specifically for this thesis. For the article presented in Chapter 2, I conceptualised, wrote and edited the manuscript with the assistance of the co-authors. For the article presented in Chapter 3, and for which I am equal first author, I contributed to the study design. I collected all the samples and I performed all the biochemical measures and analyses of results for all salivary samples. I also collected and analysed all of the actigraphy and sleep, mood and physical activity questionnaires. Dr Juan Dominguez, who is equal first author on this manuscript, was responsible for analysing brain images and running association analyses between hypothalamic volume and cortisol, melatonin and sleep outcomes. For the study presented in Chapters 4, I contributed to the study design, I collected all the blood samples and I performed all the biochemical analyses of salivary and blood samples. I collected and analysed all of the sleep, mood and physical activity questionnaires and I played a key role in the delivery of the cognitive training component of the nine-month intervention. For the study presented in Chapter 5, I conducted the cognitive training component of the nine-month intervention, I collected data for memory consolidation analysis and I completed the statistical analyses of the sleep and memory consolidation data. I wrote all manuscripts presented in this thesis and edited each manuscript with assistance from co-authors.
# Table of Contents

Use of Thesis........................................................................................................ii
Declaration............................................................................................................ iii
Abstract ................................................................................................................ iv
Acknowledgements............................................................................................... viii
Statement of Contribution Towards Publications .............................................. xi
List of Figures ......................................................................................................... xv
List of Tables .......................................................................................................... xvi
List of Abbreviations .............................................................................................. xvii

Chapter 1 ................................................................................................................. 1

General Introduction .............................................................................................. 1

1.1 Overview .......................................................................................................... 1
1.2 The Genetics of Huntington’s Disease ........................................................... 1
1.3 Features of Huntington’s Disease ................................................................ 2
1.4 The Hypothalamus ........................................................................................ 4
1.5 The Role of Sleep .......................................................................................... 6
1.6 The Regulation of the Circadian Rhythm and Sleep/Wake Cycle .............. 8
1.7 The Circadian Regulation of Cortisol and Melatonin ............................... 14
1.8 Circadian Rhythm and Sleep Disturbances in HD .................................. 19
1.9 The Pathological Effects of Sleep and Circadian Rhythm Disturbances in Huntington’s Disease .............................................................................. 20
1.10 Potential Pathological Mechanisms Underpinning Sleep Disturbances in Huntington’s Disease ...................................................................................... 22
1.11 Therapies for Huntington’s Disease ............................................................. 22
1.12 Multidisciplinary Rehabilitation as a Non-Pharmaceutical Strategy for Sleep and Circadian Rhythm Disturbances .................................................. 25
1.13 Theoretical Framework .............................................................................. 27
1.14 Hypothesis and Aims .................................................................................. 29

Chapter 2 ................................................................................................................. 30

Neuroendocrine and Neurotrophic Signaling in Huntington’s Disease: Implications for Pathogenic Mechanisms and Treatment Strategies .......................... 30

2.1 Abstract .......................................................................................................... 31
2.2 Introduction .................................................................................................... 31
2.3 Normal Function of the HPA-Axis and the SCN ......................................... 34
2.4 Stress and the Role of Cortisol .................................................................... 37
2.5 Pathological Effects of Chronic Glucocorticoid Release ............................ 38
List of Figures

Figure 1.1. Organisation of the hypothalamus.................................................................5
Figure 1.2. The neurological circuitry of the circadian system...........................................9
Figure 1.3. The components of the ascending arousal system involved in the maintenance of
wakefulness.........................................................................................................................12
Figure 1.4. The hypothalamic-pituitary-adrenal (HPA) axis..................................................16
Figure 1.5. The neural pathway of melatonin release............................................................18
Figure 2.1. The interrelated consequences of HPA-axis and circadian rhythm disruption on
BDNF expression and sleep and the implications on neurobiology....................................35
Figure 3.1. Hypothalamic volume in its association with cortisol and melatonin output........61
Figure S3.2. Hypothalamus mask (in red). Slice labels are displayed on top. L, left.............75
Figure S3.3. Volume measurements for three participants scanned twice at each site.............76
Figure S3.4. Average within and between sites differences across volume measurements...77
Figure S3.5. VBM analysis of striatal grey matter volume....................................................78
Figure 4.1. Effects of nine months of multidisciplinary therapy on hypothalamic grey matter
(GM) volume. .........................................................................................................................93
List of Tables

Table 1.1. Frequencies, waveforms and characteristics of stages of sleep.................................10
Table 2.1. Summary of HD pathologies relevant to this review article..................................33
Table 3.1. Demographic and clinical characteristics of premanifest Huntington’s disease and healthy control participants.................................................................63
Table 3.2. Cortisol and melatonin values, subjective and objective sleep outcomes and stress, anxiety and depression questionnaire scores for premanifest Huntington’s disease and healthy control participants.................................................................65
Table 3.3. Association analyses between hypothalamic volume and CAPs, cortisol and melatonin.................................................................................................................................67
Table 4.1. Demographic and clinical characteristics of premanifest plus prodromal HD intervention group and control group at baseline .................................................................92
Table 4.2. Results from voxel-based morphometry analysis.........................................................93
Table 4.3. Mean salivary cortisol and melatonin concentrations, subjective sleep outcomes and affective symptomatology outcomes in the premanifest HD intervention and control groups.................................................................................................................................95
Table S4.4. Demographic and clinical characteristics of premanifest HD and prodromal HD intervention groups and premanifest HD control group at baseline ..................................104
Table S4.5. Mean salivary cortisol and melatonin concentrations, subjective sleep outcomes .................................................................................................................................105
Table S4.6. Results from voxel-based morphometry analysis.........................................................106
Table 5.1. Demographic and clinical characteristics of premanifest HD participants (n=16).........................................................................................................................................................113
Table 5.2. Sleep and memory consolidation outcomes following a nine-month multidisciplinary rehabilitation intervention (n=16).................................................................................................114
**List of Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AUC&lt;sub&gt;G&lt;/sub&gt;</td>
<td>Area under the curve with respect to ground</td>
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<td>BDNF</td>
<td>Brain-derived neurotrophic factor</td>
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<td>BMI</td>
<td>Body mass index</td>
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<td>CAG</td>
<td>Cytosine-adenine-guanine</td>
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<tr>
<td>CAPs</td>
<td>Cytosine-adenine-guanine age product score</td>
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<tr>
<td>CSD</td>
<td>Consensus Sleep Diary</td>
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<tr>
<td>DARTEL</td>
<td>Diffeomorphic Anatomical Registration through Exponentiated Lie</td>
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<tr>
<td>DBS</td>
<td>Disease burden score</td>
</tr>
<tr>
<td>EEG</td>
<td>Electroencephalography</td>
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<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
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<tr>
<td>ESS</td>
<td>Epworth Sleepiness Scale</td>
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<tr>
<td>HADS</td>
<td>Hospital anxiety and depression scale</td>
</tr>
<tr>
<td>HD</td>
<td>Huntington’s disease</td>
</tr>
<tr>
<td>HPA</td>
<td>Hypothalamic-pituitary-adrenal</td>
</tr>
<tr>
<td>MET</td>
<td>Metabolic equivalents</td>
</tr>
<tr>
<td>MNI</td>
<td>Montreal Neurological Institute</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>NREM</td>
<td>Non-rapid eye movement</td>
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<tr>
<td>PET</td>
<td>Positron emission tomography</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>PLM</td>
<td>Periodic limb movement</td>
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<tr>
<td>PQSI</td>
<td>Pittsburgh Sleep Quality Index</td>
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<tr>
<td>PSG</td>
<td>Polysomnography</td>
</tr>
<tr>
<td>PSS</td>
<td>Perceived Stress Scale</td>
</tr>
<tr>
<td>REM</td>
<td>Rapid eye movement</td>
</tr>
<tr>
<td>RMR</td>
<td>Resting metabolic rate</td>
</tr>
<tr>
<td>SCN</td>
<td>Suprachiasmatic nucleus</td>
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<tr>
<td>SOL</td>
<td>Sleep onset latency</td>
</tr>
<tr>
<td>SWS</td>
<td>Slow wave sleep</td>
</tr>
<tr>
<td>TIB</td>
<td>Time in bed</td>
</tr>
<tr>
<td>TST</td>
<td>Total sleep time</td>
</tr>
<tr>
<td>UHDRS-TMS</td>
<td>Unified Huntington’s Disease Rating Scale-Total Motor Score</td>
</tr>
<tr>
<td>WASO</td>
<td>Wake after sleep onset</td>
</tr>
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Chapter 1

General Introduction

1.1 Overview

Huntington’s disease (HD) is a rare, progressive neurodegenerative disorder affecting 2.71 per 100,000 people worldwide (Pringsheim et al., 2012). The classic triad of features by which the disease is clinically diagnosed include motor impairment, cognitive dysfunction and mood disturbances (Bates et al., 2015). However, it is becoming increasingly evident that sleep and circadian rhythm disturbances are common and debilitating features that present early in the disease process and progressively worsen as the disease course lengthens (Lazar et al., 2015; Morton, 2013; Morton et al., 2005). Sleep disturbances have the potential to exacerbate the severity of clinical features of the disease and hasten neurodegeneration (Musiek & Holtzman, 2016; Wulff, Gatti, Wettstein, & Foster, 2010). It is therefore pivotal to understand the mechanisms underpinning sleep and circadian rhythm disturbances in HD in order to develop suitable therapeutic strategies.

1.2 The Genetics of Huntington’s Disease

George Huntington was the first to officially describe the inheritance pattern of HD in his first and only publication (Huntington, 1872). The account of the inheritance pattern of “hereditary chorea”, as it was termed, was vividly described as autosomal dominant. Despite this description, it was not until 1993 that the causative gene region for HD was identified on chromosome 4p16.3 (The Huntington's Disease Collaborative Research Group, 1993). It was discovered that the mutant variant of the $HTT$ gene, termed mutant huntingtin ($mHTT$), contained an expanded cytosine-adenine-guanine (CAG) repeat sequence in exon 1, translating to an aberrant protein. Identification of the disease causing gene has allowed for genetic testing to determine if an individual will indeed develop HD based on the number of CAG repeats (CAGn). For example, an individual possessing less than 36 CAG repeats will not develop HD,
whereas an individual with greater than 40 CAG repeats will show near complete penetrance and will develop HD within their lifespan (Kremer et al., 1994). Individuals with 36 to 39 CAG repeats exhibit incomplete penetrance and may or may not develop HD later in life (Brinkman, Mezei, Theilmann, Almqvist, & Hayden, 1997; Rubinsztein et al., 1996). Although individuals with 27 to 35 CAG repeats are themselves unaffected, genetic anticipation can result in these individuals passing in excess of 35 CAG repeats on to their offspring and consequently, these offspring will be at risk of developing HD (Ranen et al., 1995).

A relationship has been identified between the number of CAG repeats and the age at onset of HD, with the number of CAG repeats accounting for 47% to 72% of variability in the age at onset (Andrew et al., 1993; Brinkman et al., 1997; Craufurd & Dodge, 1993; Lee et al., 2012; Ranen et al., 1995; Rosenblatt et al., 2001). Of the remainder of the variability in age of onset, environmental factors have been suggested to account for 60%, with several studies, including those on HD gene positive monozygotic twins, identifying lifestyle as a key factor (Bonner-Jackson et al., 2013; Trembath et al., 2010; Wexler & The US–Venezuela Collaborative Research Project, 2004). Identification of the disease-causing gene has also allowed for the development of the CAG-age product score (CAP score) quotient (Zhang et al., 2011), which is a product of the individual’s CAG repeat number and age and allows for classification of individuals based on estimated proximity to diagnosis of manifest disease, indicated by onset of motor features.

1.3 Features of Huntington’s Disease

The classic triad of clinical HD features include motor, cognitive and mood disturbances. HD gene carriers typically transition from asymptomatic, to displaying subtle clinical signs (premanifest HD), to overt progressive extrapyramidal motor signs of HD (manifest HD) (Bates et al., 2015). Although cognitive and mood features typically arise during the premanifest stage, diagnosis of manifest disease is based on the presence of unmistakable
extrapyramidal motor signs identified by a qualified clinician using the Unified Huntington’s Disease Rating Scale (UHDRS) (The Huntington Study Group, 1996).

Progressive neuronal dysfunction and death are thought to give rise to the clinical features of HD. The exact cause of neuronal degeneration in HD is not known; however, a myriad of cellular processes have been implicated. The huntingtin protein is vital in transcription regulation, protein trafficking, vesicle transport, post-synaptic signalling and apoptosis (Gil & Rego, 2008). Expression of the mutated huntingtin protein results in a loss of normal function, as well as a potential gain in toxic function, including excitotoxicity, mitochondrial dysfunction, metabolic impairment, oxidative stress and autophagy (Guedes-Dias et al., 2016; Schapira, Olanow, Greenamyre, & Bezard, 2014). The loss of normal function and toxic gain in function of the protein is thought to lead to the early, selective loss of GABAergic medium spiny neurons that is observed in the striatum of HD gene-positive individuals, with greater loss of cortical neurons and interneurons occurring as the disease progresses (Aylward et al., 1997; de la Monte, Vonsattel, & Richardson, 1988; Kim et al., 2014; Tabrizi et al., 2012; Thieben et al., 2002; Thu et al., 2010). These microscopic changes are evident at the macroscopic level using magnetic resonance imaging (MRI). Neuroimaging studies have shown significant levels of atrophy of the caudate and putamen, areas which play vital roles in learning and memory, planning and the regulation of voluntary motor movements, in individuals with premanifest HD (Domínguez D et al., 2013; Georgiou-Karistianis et al., 2013; Tabrizi et al., 2011; van den Bogaard et al., 2011). Several studies have reported the rates of caudate and putamen atrophy to be 1.1% to 2.4% and 2.3% per year, respectively, in premanifest HD individuals compared to healthy controls (Aylward et al., 2000; Hobbs et al., 2009; Tabrizi et al., 2011; Weir, Sturrock, & Leavitt, 2011).

These neuropathological changes give rise to the motor, cognitive and mood features observed in HD. Motor disturbances in HD manifest as involuntary movements, such as chorea, and loss
of control of voluntary movements, leading to problems with balance, mobility, speech and swallowing. In some individuals however, cognitive and mood disturbances occur prior to the onset of overt motor features (Roos, 2010). Individuals with HD have a predominant reduction in capacity for memory storage, encoding of verbal learning, cognitive flexibility, processing speed, planning and problem solving and attention, as well as impairments in social cognition, which arise as early as nine to fifteen years before clinical diagnosis of HD (during the premanifest stages) and worsen as the disease progresses (Eddy & Rickards, 2015; Lemiere, Decruyenaere, Evers-Kiebooms, Vandenbussche, & Dom, 2004; Lundervold, Reinvang, & Lundervold, 1994; Massman, Delis, Butters, Levin, & Salmon, 1990; Robins Wahlin, Lundin, & Dear, 2007; Rosenberg, Sorensen, & Christensen, 1995; Stout et al., 2011). In addition, premanifest HD individuals also experience neuropsychiatric changes, such as anxiety, depression, irritability and apathy. Beyond the classic triad of clinical features, it is becoming increasingly evident that individuals with HD suffer from circadian rhythm and sleep disturbances, presumably as result of hypothalamic degeneration (Petersén et al., 2005; Politis et al., 2008; Soneson et al., 2010).

1.4 The Hypothalamus

The hypothalamus is located below the thalamus within the diencephalon of the brain. It is bound by the optic chiasm in the anterior, the optic tracts laterally and the mammillary body posteriorly (Figure 1.1). The hypothalamus contains distinct nuclei arranged in three longitudinally oriented regions: the preoptic area, the tuberal hypothalamus and the posterior hypothalamus (Saper & Lowell, 2014). Each of these areas contains specific nuclei that mediate endocrine, autonomic and behavioural functions.
Figure 1.1. Organisation of the hypothalamus.

The hypothalamus contains distinct nuclei that are vital in endocrine, autonomic and behavioural functions, including circadian rhythm, sleep/wake regulation, thermoregulation, metabolism, feeding and sexual behaviour (image adapted from Kelly (2016)).

The preoptic area of the hypothalamus contains the suprachiasmatic nucleus (SCN) and the ventrolateral and median preoptic nuclei (Saper & Lowell, 2014). The SCN and ventrolateral preoptic nucleus are responsible for the neural regulation of the circadian rhythm and sleep/wake cycle, respectively, while the median preoptic nucleus regulates body fluid electrolyte balance and temperature though the release of vasopressin (antidiuretic hormone; ADH) (Boulant, 2000).

The tuberal hypothalamus contains the paraventricular nucleus (PVN), lateral hypothalamic area, dorsomedial and ventromedial nuclei, supraoptic nucleus and arcuate nucleus. The PVN contains sub-divisions of parvocellular and magnocellular neurons. Parvocellular neurons express corticotropin-releasing factor (CRF), which initiates the cascade leading to the release of cortisol (see Section 1.7.1), while magnocellular neurons secrete oxytocin or vasopressin. The dorsomedial nucleus is also responsible for regulating the circadian rhythm, while also
functioning alongside the lateral hypothalamic area, ventromedial nucleus and arcuate nucleus to regulate food intake and body weight through the actions of neuropeptide Y (Ahima & Antwi, 2008).

The posterior hypothalamus consists of the posterior hypothalamic nucleus and the tuberomammillary and supramammillary bodies. The posterior hypothalamic nucleus maintains wakefulness through the release of orexin following innervation by the SCN (Abrahamson, Leak, & Moore, 2001). The tubero- and supramammillary bodies are also involved in the maintenance of wakefulness, predominantly through histaminergic and glutamatergic innervation of the cerebral cortex, basal forebrain and hippocampus, promoting arousal (Blandina, Munari, Provensi, & Passani, 2012; Pedersen et al., 2017; Saper & Lowell, 2014).

Given its multi-faceted role in the regulation of circadian rhythm and the sleep/wake cycle, pathological changes in the hypothalamus have been suggested to drive the disruption of the circadian rhythm and sleep/wake changes in individuals with HD. Emerging evidence suggests that detectable changes in the hypothalamus (Politis et al., 2008; Soneson et al., 2010) and circadian rhythm and sleep disturbances arise prior to the manifestation of motor, cognitive and mood disturbances. Changes in circadian rhythm and the sleep/wake cycle have been purported to exacerbate the clinical severity of the disease and accelerate neurodegeneration (Musiek & Holtzman, 2016; Wulff et al., 2010).

1.5 The Role of Sleep

A lack of consensus exists on the function of sleep (Krueger, Frank, Wisor, & Roy, 2016). However, sleep is theorised to play a pivotal role in several physiological processes, including memory consolidation, neural development and plasticity, repletion of energy and clearance of
toxins and cellular waste products (Krueger et al., 2016; Schmidt, 2014; Telzer, Goldenberg, Fuligni, Lieberman, & Gálvan, 2015; Tononi & Cirelli, 2014).

Sleep has been suggested to facilitate learning and memory, particularly procedural memories such as skill acquisition, via modulation of long term potentiation (Hobson & Pace-Schott, 2002; Stickgold, LaTanya, & Hobson, 2000). For example, improvements following training on a motor sequence task, motor adaptation task and visual texture discrimination task have been observed in individuals following a night of sleep, but not following a wake period of equal duration (Fischer, Hallschmid, Elsner, & Born, 2002; Huber, Ghilardi, Massimini, & Tononi, 2004; Walker, Brakefield, Morgan, Hobson, & Stickgold, 2002). Furthermore, individuals with partial or total sleep deprivation did not experience the same improvements, indicating that sleep is essential for the reinforcement of memories.

Sleep has been reported to be a vital mediator of the clearance of metabolites from the brain. Clearance of metabolites from the brain during sleep has been demonstrated to be defective in Alzheimer’s disease mouse models, suggesting an involvement of sleep in the maintenance of metabolic homeostasis by removal of potential neurotoxins (Xie et al., 2013).

Disruption of sleep has considerable consequences, both short- and long-term. In the short-term, sleep disruption results in increased responsivity to stress, mood disturbances, emotional distress and deficits in cognition and memory. Long-term adverse effects of sleep disruption include metabolic syndrome, hypertension and cardiovascular disease and dyslipidaemia (Cedernaes, Schioth, & Benedict, 2015; McCoy & Strecker, 2011; Medic, Wille, & Hemels, 2017; Meisinger, Heier, Löwel, Schneider, & Döring, 2007). Given that sleep quality and architecture have been shown to be perturbed in HD, it is possible that sleep disturbances could exacerbate the cognitive, motor and mood features of the disease. Furthermore, disturbances in sleep may impair metabolite removal and energy repletion processes in the brain, further
contributing to neurodegenerative processes in HD. It is therefore necessary to understand the pathological mechanisms underpinning these changes in order to develop therapies aimed at ameliorating these changes to reduce the impact of the disease.

1.6 Regulation of the Circadian Rhythm and Sleep/Wake Cycle

1.6.1 The Regulation of the Circadian Rhythm

The hypothalamus contains several key nuclei that are central to regulating the circadian rhythm and sleep/wake cycles (Figure 1.2). In particular, situated directly above the optic chiasm is the master circadian oscillator, the SCN, which is responsible for synchronising the circadian rhythm of endogenous clocks existing in all cells of the body (Moore, 2013; Moore & Eichler, 1972). The SCN is entrained to the light/dark cycle by receiving photic stimuli from the retinohypothalamic tract. This information is then relayed from the SCN to the dorsal and ventral subparaventricular zones. The neurons of the dorsal and ventral subparaventricular zones provide outputs to the dorsomedial nucleus of the hypothalamus, which integrates these inputs and projects information via excitatory and inhibitory neurotransmitters to the ventrolateral preoptic nucleus to promote sleep, to the lateral hypothalamus to induce wakefulness and regulate feeding patterns and to the paraventricular nucleus to induce the cascades leading to the release of cortisol and melatonin (Moruzzi & Magoun, 1949; Saper, Scammell, & Lu, 2005; Sherin, Shiromani, McCarley, & Saper, 1996; Starzl, Taylor, & Magoun, 1951).
Figure 1.2. The neurological circuitry of the circadian system.

The SCN of the hypothalamus controls various behavioural and physiological processes, including sleep and wakefulness, corticosteroid release and thermoregulation, through indirect neuronal connections with other key hypothalamic nuclei (image from Saper et al., 2005).

1.6.2 The Stages of Sleep

Sleep is classified into two stages: non-rapid eye movement (NREM) sleep and REM sleep. NREM is further categorised as stages N1, N2 and N3, characterised by specific cortical neuron oscillations, of which the resultant electrophysiology can be measured using electroencephalography (EEG). These stages can be distinguished from one another by EEG waveforms (see Table 1.1), as well as other physiological phenomena, such as eye and muscle movements, measured using electrooculography and electromyography, respectively (Berry et al., 2015; Rechtschaffen & Kales, 1968).
Stage N1 is characterised by drowsiness and the onset of sleep. This then transitions into the light sleep stage, N2, which is characterised by EEG signatures known as spindles and k-complexes. The exact physiological roles for these spindles and k-complexes are not yet understood, however, they are thought to be involved in sleep-dependent memory consolidation, particularly motor memory, and the maintenance of sleep (Clemens, Fabo, & Halasz, 2005; Schabus et al., 2004). Stage N3, also known as slow wave sleep (SWS), is the deepest stage of NREM sleep, and is thought to modulate memory consolidation and formation. This is generally followed by REM sleep which, as the name suggests, is characterised by rapid movement of the eyes, as well as muscle atonia and an EEG tracing similar to that of wake and is indicative of dreaming (Peigneux, Urbain, & Schmitz, 2012). REM sleep is purported to play a role in the reorganisation and consolidation memory, particularly emotional memory (Genzel, Spoormaker, Konrad, & Dresler, 2015; W. Li, Ma, Yang, & Gan, 2017; Peever & Fuller, 2017).

**Table 1.1.** Frequencies, waveforms and characteristics of stages of sleep.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Frequency</th>
<th>Waveform</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>3-7 Hz</td>
<td>Theta waves. The transition from wake to sleep. Little or no body movement, slowing of the breathing rate, slow oscillating eye movements. 5-10% of TST.</td>
<td></td>
</tr>
<tr>
<td>N2</td>
<td>11-16 Hz</td>
<td>Sigma waves with occasional spindles and k-complexes. The light stage of sleep. Body temperature and heart rate decrease, cessation of eye movements. 45-55% of TST.</td>
<td></td>
</tr>
<tr>
<td>N3</td>
<td>0.5-2 Hz</td>
<td>Delta waves. The deepest sleep stage, also known as slow wave sleep. 20-25% of TST.</td>
<td></td>
</tr>
<tr>
<td>REM</td>
<td>15-30 Hz</td>
<td>Desynchronised waves close to that of wake (low amplitude, mixed frequency). Accompanied by eye movements and muscle atonia. 20-25% of TST.</td>
<td></td>
</tr>
</tbody>
</table>

N1= stage 1 of non-rapid eye movement sleep (NREM), N2= stage 2 of NREM, N3= stage 3 of NREM, REM= rapid eye movement, Hz= Hertz, TST= total sleep time. Images adapted from Virk and Kotecha (2016).
Under normal conditions, individuals will transition through these sleep stages five times per night, with each cycle lasting 90 to 110 minutes. During normal sleep, 75% to 80% of the total sleep time is spent in NREM, with the remaining 20% to 25% of total sleep time spent in REM sleep (Berry et al., 2015). Deprivation of the quantity of each sleep stage, perhaps due to multiple awakenings, alcohol or caffeine consumption or the use of opioid-based drugs, will result in an increase in the percentage of that sleep stage over the following night, also known as “rebound” (Garcia & Salloum, 2015). For example, a reduction in the quantity of REM sleep will result in REM rebound the following night, such that the percentage of the total sleep time spent in REM will increase, indicating the presence of a homeostatic sleep drive to regulate the duration of each sleep stage (Achermann & Borbély, 2003; Schwartz & Roth, 2008).

1.6.3 The Regulation of Wakefulness

Sleep/wake cycles are tightly regulated by the secretion of excitatory and inhibitory neurotransmitters from several brain regions, resulting in the “switching” between wake and sleep states (Schwartz & Roth, 2008). Wakefulness is maintained by an ascending arousal system that originates in the rostral pons and continues through the reticular formation of the midbrain and is facilitated by monoamine neurotransmitters, including noradrenaline (NA) and dopamine (DA), as well as acetylcholine (Ach) and orexin (ORX). The maintenance of wakefulness involves two main pathways: an ascending pathway to the thalamus and an ascending pathway that bypasses the thalamus, activating the lateral hypothalamus and basal forebrain (Figure 1.3) (Saper et al., 2005).
Figure 1.3. The components of the ascending arousal system involved in the maintenance of wakefulness.

The yellow pathway depicts the ascending pathway to the thalamus and the red pathway indicates the ascending pathway that bypasses the thalamus, activating the hypothalamus and basal forebrain. Both pathways activate the cerebral cortex, maintaining wakefulness (image from Saper et al., 2005).

The ascending pathway to the thalamus originates in the pedunculopontine and laterodorsal tegmental nuclei and is driven by acetylcholine. These nuclei provide inputs to the thalamic-relay nuclei and reticular nucleus of the thalamus. Input from the pedunculopontine and laterodorsal tegmental nuclei to the reticular nucleus acts as a gateway mechanism that blocks neural transmission between the thalamic relay nuclei and the cerebral cortex; allowing the innervation of the cerebral cortex by the thalamic relay nuclei induces a state of wakefulness (McCormick, 1989; Saper et al., 2005). Maintenance of cortical arousal is also thought to be partly regulated by the intralaminar and midline nuclei (Krout, Belzer, & Loewy, 2002).
The second pathway, which bypasses the thalamus, is initiated by release of a number of neurotransmitters in the upper brainstem and caudal hypothalamus, including the locus coeruleus (noradrenergic), dorsal and median raphe nuclei (serotonergic), ventral periaqueduct (dopaminergic) and tuberomammillary (histaminergic) neurons (Saper, 1985; Saper, Chou, & Scammell, 2001). Lateral hypothalamic neurons containing melanin-concentrating hormone or orexin and cholinergic or gamma-aminobutyric acid (GABA) basal forebrain neurons enhance the input of the upper brainstem and caudal hypothalamic neurons to the cerebral cortex (Saper et al., 2005).

1.6.4 The Regulation of Sleep

The promotion of sleep originates at the ventrolateral preoptic nucleus. Neurons of the ventrolateral preoptic nucleus project to key nuclei that are responsible for arousal in the hypothalamus and brainstem (Sherin et al., 1996). These neurons contain the inhibitory neurotransmitters GABA and galanin. Activation of these neurons results in inhibition of the ascending arousal system, thereby promoting sleep (Gaus, Strecker, Tate, Parker, & Saper, 2002; Sherin, Elmquist, Torrealba, & Saper, 1998; Szymusiak, Alam, Steininger, & McGinty, 1998).

Historical studies into the regulation of REM sleep implicated the switching between inhibition of cholinergic and serotonergic neurons of the raphe nuclei, with the noradrenergic neurons of the locus coeruleus of the pons (Hobson, McCarley, & Wyzinski, 1975; Pace-Schott & Hobson, 2002). However, emerging evidence suggests that regulation of REM sleep is controlled by GABAergic and glutamatergic neurons of the brain stem (Boissard, Fort, Gervasoni, Barbagli, & Luppi, 2003; Sapin et al., 2009; Verret, Fort, Gervasoni, Leger, & Luppi, 2006), which project to the basal forebrain to regulate cortical neuron oscillations and to the medulla and spinal cord to regulate atonia during REM sleep (J. Lu, Sherman, Devor, & Saper, 2006), as well as by neurons outside the brainstem, particularly those of the anterior and lateral
hypothalamus (Clement et al., 2012; Jego et al., 2013; Ju. Lu et al., 2002; Sapin et al., 2010; Weber et al., 2018).

In general, sleep itself is thought to be regulated by homeostatic and circadian drives (Achermann & Borbély, 2003; Saper et al., 2005). The homeostatic drive is believed to be initiated as a result of prolonged wakefulness, where the accumulation of a substrate, for example adenosine, results in an increased drive to sleep (Radulovacki, Virus, Djuricic-Nedelson, & Green, 1984; Strecker et al., 2000). The circadian sleep drive is regulated by an autoregulatory transcription-translation feedback loop that originates within the SCN. This molecular clock relies on the heterodimerisation of the transcription factors clock circadian regulator (CLOCK) and brain and muscle Arnt-like 1 (BMAL1) in order to activate period circadian regulator 1 (PERIOD), Cryptochrome Circadian Clock 1 (CRYPTOCHROME) and Nuclear Receptor Subfamily 1 Group D Member 1 (REV-ERBα) expression (Gekakis et al., 1998). These factors work in positive and negative feedback loops to produce the clock machinery of the cell. The SCN innervates the dorsomedial nucleus both directly and indirectly, which, via GABAAergic neuronal projections, innervates the VLPO and lateral hypothalamic area to control the sleep/wake cycle (Aston-Jones, Chen, Zhu, & Oshinsky, 2001; Chou et al., 2002; Chou et al., 2003; Deurveilher & Semba, 2005; Yoshida, McCormack, España, Crocker, & Scammell, 2006). On a 24-hour basis, the functional output of the SCN is reset by light stimuli transmitted from the retina, via the retino-hypothalamic tract, to the SCN during daylight hours and melatonin secretion from the pineal gland during the night.

**1.7 The Circadian Regulation of Cortisol and Melatonin**

The circadian release of cortisol and melatonin is mediated by the connection between the SCN and paraventricular nucleus. The circadian rhythm generated at the SCN is transmitted to the paraventricular nucleus via projections of the subparaventricular zone and dorsomedial nucleus.
of the hypothalamus, which then initiates the cascades leading to the release of cortisol and melatonin.

1.7.1 The Regulation of Cortisol Release

The PVN releases corticotrophin releasing factor into the hypophyseal portal system, which comes into direct contact with the anterior of the pituitary gland, stimulating the corticotropes to release adrenocorticotropic hormone (Figure 1.4). Once in the systemic circulation, this hormone stimulates the release of cortisol from the adrenal cortex. The physiological effects of cortisol include increasing blood pressure and glucose uptake, maintaining blood calcium levels, metabolising fat and decreasing inflammation (Golden, Wand, Malhotra, Kamel, & Horton, 2011). In the brain, cortisol has been shown to impact on memory, particularly long-term and primed burst potentiation, by interacting with mineralocorticoid and glucocorticoid receptors in a dose-dependent manner (D. M. Diamond, Bennett, Fleshner, & Rose, 1992; McEwen, 2007; Pavlides, Kimura, Magarinos, & McEwen, 1994; Pavlides, Watanabe, Magarin, & McEwen, 1995). For example, long-term potentiation occurs efficiently in the presence of low levels of cortisol, however high levels of cortisol have been reported to impair long-term potentiation (and therefore impact on memory formation and retrieval) (Joëls, 2006). Cortisol also binds to glucocorticoid receptors in the paraventricular nucleus and pituitary, inhibiting further release of corticotropin releasing factor and adrenocorticotropic hormone in a negative feedback loop (Hyman, 2009; Keller-Wood & Dallman, 1984).
Figure 1.4. The hypothalamic-pituitary-adrenal (HPA) axis.

Structures of the HPA axis include the hypothalamus, pituitary and adrenal glands. The paraventricular nuclei release corticotrophin releasing factor, which stimulates the release of adrenocorticotropic hormone from the anterior pituitary. Adrenocorticotropic hormone then stimulates the release of glucocorticoids (cortisol) from the cortex of the adrenal glands, which regulates responses to stress in both the central and peripheral systems. Glucocorticoids provide a negative feedback loop, inhibiting further release of corticotrophin releasing factor and adrenocorticotropic hormone. This axis becomes dysfunctional in premanifest HD, leading to alterations in cortisol release (image from Hyman, 2009).

There is a large circadian variation in blood cortisol concentrations between individuals, however, the phenomenon known as the cortisol awakening response (CAR) is relatively stable intra-individually (Clow, Thorn, Evans, & Hucklebridge, 2004; Stone et al., 2001). The CAR occurs within the first 30 minutes following awakening and is represented by a 75% surge in cortisol release (Pruessner et al., 1997). While the role of the CAR is not yet known, it is
thought to be a biochemical response to awakening that facilitates the activation of memory to allow individuals to orient themselves in anticipation of the day ahead (Fries, Dettenborn, & Kirschbaum, 2009). Following this spike in cortisol, concentrations then gradually decrease across the day, reaching a nadir around midnight, which coincides with the rise in melatonin levels (Brzezinski, 1997; Chan & Debono, 2010).

### 1.7.2 Regulation of Melatonin Release

The regulation of melatonin release involves the neural projections of the paraventricular nucleus to the intermediolateral cell column (of the spinal cord). This then provides input to the superior cervical ganglion, which then directly innervates the pinealocytes to synthesise melatonin from tryptophan molecules according to the following pathway (Axelrod & Weissbach, 1960; Coon et al., 1995; Rath et al., 2016):

\[
\text{Tryptophan} \xrightarrow{TPH} \text{Hydroxytryptophan} \xrightarrow{DDC} \text{Serotonin} \xrightarrow{AANAT} \text{N-Acetylserotonin} \xrightarrow{ASMT} \text{Melatonin}
\]

The release of melatonin from the pineal gland is thought to stabilise and reinforce the circadian rhythm in all cells of the body, in addition to initiating the thermoregulatory cascade. Melatonin regulates body temperature via the activation of MT₁ or MT₂ receptors to induce vasoconstriction or vasodilation, respectively, in the vascular beds of the extremities. This differentially effects the flow of blood to ensure the optimal internal body temperature to promote the onset of sleep (Brzezinski, 1997; Cajochen, Kräuchi, & Wirz-Justice, 2003; Cook, Sauder, & Ray, 2010; Krauchi, Cajochen, Werth, & Wirz-Justice, 1999, 2000).
Figure 1.5. The neural pathway of melatonin release.

Melatonin is secreted via a multi-step pathway beginning at the SCN, which is synchronised to the day/night cycle by photic stimuli received via the retinohypothalamic tract. The SCN then projects to the dorsomedial nucleus, which controls the release of melatonin via projections to the intermediolateral cell column. The Intermediolateral cell column innervates the superior cervical ganglion, which directly stimulates the pineal gland to synthesise and release melatonin (image from Cardinali and Pevet, 1998).

Ultimately, the SCN and other key nuclei of the hypothalamus are responsible for the sleep/wake cycle and circadian regulation of cortisol and melatonin release. It is plausible that hypothalamic pathology in HD could affect these nuclei, influencing the regulation of sleep, circadian rhythm and neuroendocrine output.
1.8 Circadian Rhythm and Sleep Disturbances in HD

Circadian rhythm and sleep disturbances have been reported in both HD mouse models and humans (Kudo et al., 2011; Lazar et al., 2015; Morton, 2013). These circadian changes appear to commence during the premanifest stages of HD and worsen as the course of the disease lengthens. In particular, early changes in the circadian regulation of cortisol and melatonin and a sleep phase delay have been noted in individuals with premanifest HD (Aziz, Anguelova, Marinus, Lammers, & Roos, 2010; Aziz, Pijl, Frölich, Schroder-van der Elst, et al., 2009; Aziz, Pijl, Frölich, van der Graaf, et al., 2009; Kalliologia et al., 2014; van Duijn et al., 2010).

The CAR is a characteristic of the circadian rhythm of cortisol (Clow, Hucklebridge, Stalder, Evans, & Thorn, 2010; Clow et al., 2004). Due to its stability across days, the CAR can be used to measure changes in cortisol circadian regulation. Compared to mutation-negative healthy controls (from HD families), premanifest HD individuals exhibit increased salivary cortisol within the hour following awakening when measuring the CAR (van Duijn et al., 2010). Furthermore, Hubers et al. (2015) reported an increase in the total salivary cortisol output during the CAR, using area under the curve with respect to increase (AUCi) analysis in individuals with premanifest compared to manifest HD, indicating perturbations in cortisol release in premanifest HD individuals.

Alterations in melatonin secretion have also been reported in HD. Recent work by Kalliologia et al. (2014) described a decrease in levels and a greater temporal spread in melatonin, indicating a temporal shift in melatonin release in HD. Given that melatonin is released in a circadian manner and modulated by the SCN, this shift in melatonin release could indicate a disruption of the circadian rhythm. Furthermore, as melatonin is vital in promoting the onset of sleep, the disruption of melatonin release could result in delayed sleep onset, a feature that has been observed in HD (Lewy, Ahmed, Jackson, & Sack, 1992).
In addition to the dysregulation of circadian-regulated hormone, altered rest-activity profiles have also been reported in HD. Studies in the R6/2 HD mouse model have revealed altered night-day activity ratios that worsen as the disease progresses (Kudo et al., 2011; Maywood et al., 2010; Morton et al., 2005). Furthermore, dysregulation of clock gene expression has been reported, particularly within the SCN, in HD mouse models (Morton et al., 2005). While it is not possible to directly measure the functioning of the SCN, it is believed that the circadian rhythm dysfunction present in mouse models is recapitulated in humans with HD. For example, similar to that in HD mouse models, altered night-day activity has been reported in individuals with manifest HD (Morton et al., 2005), which has also been reported to be accompanied by altered sleep-wake timing (Hurelbrink, Lewis, & Barker, 2005), potentially leading to the sleep disturbances observed in HD.

Recent findings by Lazar and colleagues (2015) show that sleep disturbances are an early feature of HD. In particular, premanifest HD individuals are known to experience sleep fragmentation, indicated by an increase in the number of awakenings throughout the night. These sleep disturbances worsen as the disease progresses and include reduced sleep efficiency, excessive daytime sleepiness, increased sleep onset latency, reduced slow wave sleep and REM sleep, increased REM latency, increased sleep spindles and delayed awakenings (Arnulf et al., 2008; Piano et al., 2015; Piano et al., 2017). Given that disturbances in circadian rhythm and sleep arise during the premanifest stage of HD and worsen as the disease progresses, identification of potential mechanisms and early treatments aimed at reducing or ameliorating circadian rhythm and habitual sleep disturbances are warranted.

1.9 The Pathological Effects of Sleep and Circadian Rhythm Disturbances in Huntington’s Disease

Sleep disturbances have been reported to impair cognitive function and can induce psychological features, such as depression, anxiety and stress (Ferrie et al., 2011; Harrison &
Horne, 2000; Kribbs & Dinges, 1994). Studies in animal models have shown that sleep deficits can impact on hippocampal function, leading to impairments in consolidation of memory (Prince & Abel, 2013; Tartar et al., 2006). This is thought to occur due to disruption of synaptic plasticity (long-term potentiation) and reduced N-Methyl-D-Aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor signalling, as well as fluctuations in glutamate (Campbell, Guinan, & Horowitz, 2002; Disbrow & Ruth, 1984; McDermott et al., 2003; Ravassard et al., 2009). Furthermore, sleep deficits have been shown to negatively impact executive functions associated with the prefrontal cortex, although the molecular events leading to this phenomenon are yet to be confirmed (Blagrove, Alexander, & Horne, 1995; Boonstra, Stins, Daffertshofer, & Beek, 2007; Dahl, 1996; Harrison & Horne, 1997, 1998; Horne, 1988; Lo et al., 2012; Muzur, Pace-Schott, & Hobson, 2002). Furthermore, disrupted or restricted sleep leads to increased activity of the HPA axis (Buckley & Schatzberg, 2005; Meerlo, Sgoifo, & Suchecki, 2008) and increased sympathetic activation demonstrated by increases in heart rate and blood pressure (Zhong et al., 2005).

Morton et al (2005) reported that premanifest HD individuals show a disrupted circadian profile, which worsens as the disease progresses. Disruption of the circadian rhythm can result in altered release of cortisol and melatonin and decreased sleep quality and can exacerbate impairments in cognition, including attention and executive function, glucose metabolism, motor control and mood (Briançon-Marjollet et al., 2015; Ju, Lucey, & Holtzman, 2014; Scullin & Bliwise, 2015; Videnovic, Lazar, Barker, & Overeem, 2014; Wirz-Justice et al., 1981; Wulff et al., 2010). Ultimately, alterations in circadian rhythm are likely to potentiate pathological mechanisms that are already present in HD individuals.
1.10 Potential Pathological Mechanisms Underpinning Sleep Disturbances in Huntington’s Disease

Several studies have reported hypothalamic pathology in HD as early as 10 years prior to estimated clinical onset (Politis et al., 2008; Soneson et al., 2010). Significant hypothalamic nuclear atrophy and hypothalamic neuronal loss have also been reported in HD mouse models (Björkqvist et al., 2006; Du et al., 2012; Du & Pang, 2015) and in patients (Douaud et al., 2006; Gabery et al., 2010; Kassubek et al., 2004; Kremer, Roos, Dingjan, Marani, & Bots, 1990; Soneson et al., 2010; Timmers, Swaab, van de Nes, & Kremer, 1996). These findings suggest that hypothalamic pathology is a key feature of HD. Post-mortem investigations reveal significant neuronal loss in the nucleus tuberalis lateralis and lateral hypothalamus (Kremer et al., 1991; Kremer et al., 1990; Petersén & Björkqvist, 2006; Petersén et al., 2005; Timmers et al., 1996; Vogt, 1952). Furthermore, a significant loss of orexin-releasing neurons in the lateral hypothalamus has been reported in HD (Petersén et al., 2005). Hypothalamic atrophy has also been reported in individuals with manifest and premanifest HD using voxel based morphometry (VBM) (Douaud et al., 2006; Kassubek et al., 2004; Soneson et al., 2010).

Considering the importance of the hypothalamic nuclei in maintaining circadian rhythm and other behavioural processes, it is plausible that changes in the hypothalamus alters the circadian release of neuroendocrine factors, particularly cortisol and melatonin, adversely impacting on sleep/wake behaviour.

1.11 Therapies for Huntington’s Disease

Despite intense efforts, there are currently no proven disease modifying strategies for HD. Many promising pharmaceutical candidates are currently under investigation (Frank, 2014), however many of these candidates will fail due to futility and tolerability issues. Successful pharmaceutical candidates may also take up to a decade to become available to HD sufferers (Bates, Tabrizi, & Jones, 2014). There is, therefore, a fundamental need to identify non-
pharmaceutical strategies that can positively impact on the clinical and pathological progression of HD. Lifestyle strategies such as multidisciplinary rehabilitation, which encompass exercise and cognitive training, appear to be a particularly promising therapeutic approach that is safe and can be trialled immediately in premanifest HD individuals, prior to severe neuronal loss and manifestation of clinical features.

Long-standing evidence shows that lifestyle factors, such as sedentary behaviour, poor education and excessive calorie intake, impact on disease onset, symptomatology and progression (Bonner-Jackson et al., 2013; López-Sendón et al., 2011; Trembath et al., 2010; Wexler & The US–Venezuela Collaborative Research Project, 2004). Sleep disturbances may be an environmental modifier that contributes to disease related pathology (Musiek, 2015). Emerging evidence suggests that treatment of circadian rhythm and sleep disturbances may lead to improvements in symptoms of HD (H.-B. Wang et al., 2018; H.-B. Wang et al., 2017).

A study by Cuesta and colleagues demonstrated that behavioural manipulation prevents the emergence of circadian abnormalities in HD animal models (Cuesta, Aungier, & Morton, 2014). This study explored the individual and synchronistic effects of bright light therapy and free or restricted wheel running, two behavioural interventions known to modulate the circadian output of the SCN, on circadian behaviour in the R6/2 mouse model, a transgenic model of HD with a rapidly progressive phenotype. Results from this study suggest that incremental exposure to light and exercise, together and in unison, have the potential to entrain the circadian rhythm in HD.

Further evidence from other clinical and healthy populations also supports the use of non-pharmaceutical interventions, such as exercise and cognitive training, for treating circadian rhythm and sleep disturbances in HD. Several studies have reported that exercise favourably modulates circadian rhythm and improves sleep outcomes in healthy populations (Edwards,
Exercise has also been reported to improve sleep in other neurodegenerative populations, including Parkinson’s disease and Alzheimer’s disease, as well as individuals with sleep disorders such as insomnia (Nascimento et al., 2014; Passos et al., 2011; Reid et al., 2010).

Frazitta et al. (2015) reported an improvement in sleep quality, motor symptoms and daytime somnolence using the Parkinson’s Disease Sleep Scale after a four week intensive multidisciplinary rehabilitation intervention encompassing stretching, functional training and aerobic exercise. In another study by Silva-Batista et al. (2017), twelve weeks of resistance training was found to significantly improve Pittsburgh Sleep Quality Index values in Parkinson’s disease patients, indicating an improvement in sleep quality. Finally, Nascimento et al. (2014), reported an attenuation of sleep disturbances in Parkinson’s and Alzheimer’s disease patients following six months of aerobic and resistance training exercise.

In older adults with insomnia, improvements in sleep quality have been observed following an eight-week computerised cognitive training intervention (Haimov & Shatil, 2013). Additionally, Diamond and colleagues (2015) reported improvements in subjective sleep quality following seven weeks of the Healthy Brain Ageing Cognitive Training (HBA-CT) program in older adults. Taken together, these results suggest that cognitive training can improve subjective sleep quality in older populations with and without insomnia.

Despite many studies documenting the positive effects of environmental interventions on circadian rhythm and sleep outcomes in HD mouse models and subjective sleep outcomes in other clinical populations, no studies to date have assessed the effects of these therapies on circadian rhythm and subjective or objective sleep outcomes in HD patients.
1.12 Multidisciplinary Rehabilitation as a Non-Pharmaceutical Strategy for Sleep and Circadian Rhythm Disturbances

Emerging evidence indicates that multidisciplinary therapy is feasible, well tolerated and associated with positive clinical and physiological benefits in HD (Cruickshank et al., 2015; Piira et al., 2013; Thompson et al., 2013; Veenhuizen et al., 2011; Zinzi et al., 2007).

Zinzi et al. (2007) were the first to document significant improvements in motor performance and activities of daily living in individuals with manifest HD after an eighteen-month individualised intervention comprising three-weekly intensive cognitive, physical, respiratory and vocational exercises up to three times per year. Subsequently, a study by Veenhuizen et al. (2011) reported that eighteen months of individualised outpatient multidisciplinary care provided by a specialised multidisciplinary team was perceived as beneficial and appreciated by the twenty individuals with manifest HD that participated in the study, as well as their carers and the health care workers involved. Following this, Piira et al. (2013) demonstrated that intensive (five days per week for eight hours, for three weeks, three times per year) inpatient multidisciplinary rehabilitation, comprising physiotherapy, occupational therapy, speech therapy, resistance exercises and hydrotherapy, improved balance, gait and physical quality of life, as well as reduced symptoms of depression and anxiety in individuals with manifest HD. This data was reinforced by research from our team indicating that nine-months of multidisciplinary rehabilitation, comprising once-weekly supervised group aerobic and resistance training sessions in an exercise clinic, fortnightly occupational therapy sessions and an individualised home-based exercise program, reduces deterioration of motor and postural stability, significantly improves strength and fat-free mass and, to a smaller extent, improves cognition, depression and quality of life (Thompson et al., 2013).

More recently, data from our team has demonstrated, for the first time, that multidisciplinary therapy can increase grey matter volume in the dorsolateral prefrontal cortex and the right
caudate of patients with manifest HD, and that such increases are strongly associated with a preserved performance in verbal learning and memory (Cruickshank et al., 2015). In addition to studies in individuals with HD, lifestyle and multidisciplinary rehabilitation interventions have been reported to positively impact on pathological disease burden and clinical outcomes in other neurodegenerative diseases, such as Parkinson’s disease (Ellis et al., 2008; Frazzitta, Bertotti, et al., 2013; 2015; Trend, Kaye, Gage, Owen, & Wade, 2002; van der Marck, Bloem, et al., 2013; van der Marck, Munneke, et al., 2013; Wade et al., 2003) and multiple sclerosis (Asano, Raszewski, & Finlayson, 2014; Khan, Turner-Stokes, Ng, & Kilpatrick, 2007; Rietberg, van Wegen, Eyssen, Kwakkel, & the MS study group, 2014; Salhofer-Polanyi et al., 2013). Taken together, these studies suggest that multidisciplinary rehabilitation paradigms are feasible, well-tolerated and have the potential to improve the clinical aspects of HD. It remains to be determined, however, whether multidisciplinary rehabilitation positively impacts on circadian rhythm and sleep outcomes in HD.
1.13 Theoretical Framework

Sleep and circadian rhythm disturbances are common features of HD that arise during the premanifest stage of the disease and worsen as the disease progresses. Emerging evidence from animal models and investigations in other neurodegenerative populations shows that circadian rhythm and sleep disturbances contribute to age-and disease-related atrophy of the brain and exacerbates disease features. Despite this knowledge of the impact of circadian rhythm and sleep disruption on disease progression and symptomatology, there is a paucity of data investigating the mechanisms underpinning circadian rhythm and sleep disturbances in HD. Furthermore, no treatments exist to combat circadian rhythm and sleep disturbances in HD.

The circadian rhythm and sleep/wake cycle are tightly regulated by the interaction between key nuclei in the hypothalamus. These nuclei also mediate the release of neuroendocrine factors, particularly cortisol and melatonin, which further mediate the sleep/wake cycle. As the release of cortisol and melatonin are regulated by the circadian rhythm, changes in the temporal release of these hormones can indicate changes in the circadian rhythm. Hypothalamic pathology and disruption of the circadian release of neuroendocrine factors has been reported in premanifest HD. It is not known however, if this is the contributing pathological factor for the reported disturbances in circadian rhythm and sleep observed in HD.

The regulation of the circadian rhythm and sleep/wake cycle is coordinated by the SCN, which responds to environmental cues, known as zeitgebers. Physical activity and cognitive training are known zeitgebers that act to entrain the circadian rhythm and sleep/wake cycle. The combined effects of physical exercise and cognitive training, in the form of multidisciplinary rehabilitation, have been shown to be beneficial and feasible in HD, as well as in other neurodegenerative populations, such as Parkinson’s disease and Alzheimer’s disease. Furthermore, multidisciplinary rehabilitation has been reported to improve subjective sleep
quality in patients with Parkinson’s disease. The effects of multidisciplinary rehabilitation on circadian rhythm and sleep outcomes is yet to be investigated in HD.

Therefore, the overall aim of this thesis was to investigate the neurobiological mechanisms underpinning circadian rhythm and sleep disturbances in premanifest HD, with particular focus on the hypothalamus. This thesis also aimed to investigate, for the first time, the effects of multidisciplinary rehabilitation on measures of circadian rhythmicity, as well as subjective and objective sleep in individuals with premanifest HD. The studies presented in this thesis focussed on individuals with premanifest HD, the stage of the disease when an intervention would be most beneficial, as at this stage the disease can be ameliorated more effectively prior to severe, irreversible neuronal loss.
1.14 Hypothesis and Aims

The overarching hypothesis of this thesis is that multidisciplinary rehabilitation improves circadian rhythm and sleep outcomes by preserving hypothalamic volume in premanifest HD.

Aim 1:

To perform a thorough literature investigation of the potential neurobiological mechanisms associated with circadian rhythm and sleep architecture in individuals with Huntington’s disease.

Aim 2:

To determine the extent to which hypothalamic volume is associated with circadian rhythm and habitual sleep outcomes in individuals with premanifest Huntington’s disease.

Aim 3:

To assess the effects of a nine-month multidisciplinary therapy program on measures of circadian rhythm and habitual sleep and associated hypothalamic volume in individuals with premanifest Huntington’s disease.

Aim 4:

To explore the effects of a nine-month multidisciplinary therapy program on sleep architecture in individuals with premanifest Huntington’s disease.
Chapter 2

Neuroendocrine and Neurotrophic Signaling in Huntington’s Disease: Implications for Pathogenic Mechanisms and Treatment Strategies

Review Article

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Keywords: sleep, circadian rhythm, suprachiasmatic nucleus (SCN), hypothalamus, hypothalamic-pituitary-adrenal (HPA) axis, brain-derived neurotrophic factor (BDNF)
2.1 Abstract

Huntington’s disease (HD) is a fatal neurodegenerative disease caused by an extended polyglutamine tract in the huntingtin protein. Circadian, sleep and hypothalamic-pituitary-adrenal (HPA) axis disturbances are observed in HD as early as 15 years before clinical disease onset. Disturbances in these key processes result in increased cortisol and altered melatonin release which may negatively impact on brain-derived neurotrophic factor (BDNF) expression and contribute to documented neuropathological and clinical disease features. This review describes the normal interactions between neurotrophic factors, the HPA-axis and circadian rhythm, as indicated by levels of BDNF, cortisol and melatonin, and the alterations in these intricately balanced networks in HD. We also discuss the implications of these alterations on the neurobiology of HD and the potential to result in hypothalamic, circadian, and sleep pathologies. Measurable alterations in these pathways provide targets that, if treated early, may reduce degeneration of brain structures. We therefore focus here on the means by which multidisciplinary therapy could be utilised as a non-pharmaceutical approach to restore the balance of these pathways.

2.2 Introduction

Huntington’s disease (HD) is a fatal autosomal dominant neurodegenerative disease caused by an expanded cytosine-adenine-guanine (CAG) repeat sequence in exon 1 of the Huntingtin gene (HTT) (The Huntington's Disease Collaborative Research Group, 1993). This expanded sequence encodes a mutant version of the protein, huntingtin (mHTT), which is associated with ubiquitous molecular and cellular anomalies, widespread neuronal dysfunction and cell loss (Kim et al., 2014) and the presentation of motor and non-motor features, including progressive impairments in motor control, cognitive function and mood (Tabrizi et al., 2013). Evidence also indicates that individuals suffer from sleep disturbances (Arnulf et al., 2008; Hansotia, Wall, & Berendes, 1985; Lazar et al., 2015; Morton, 2013; Piano et al., 2015; Wiegand, Moller,
Schreiber, Lauer, & Krieg, 1991), autonomic abnormalities (e.g. hyperhidrosis, micturition disturbances, swallowing difficulties, sexual dysfunction, altered heart rate variability) (Andrich et al., 2002; Kobal et al., 2010) and metabolic irregularities (Browne et al., 1997; Mazziotta et al., 1987), with some non-motor features, such as cognitive and sleep abnormalities, emerging years before the onset of motor signs (Lazar et al., 2015; Tabrizi et al., 2011).

Although the pathophysiology underlying the development and progression of these clinical features is complex, the accompanying alterations in neuroendocrine signalling, including cortisol (Aziz, Pijl, Frölich, van der Graaf, et al., 2009; Hubers et al., 2015) and melatonin (Aziz, Pijl, Frölich, Schroder-van der Elst, et al., 2009; Kalliolia et al., 2014) release, and changes in circadian rhythmicity (Aziz et al., 2010; Morton et al., 2005) suggest that the activity of the hypothalamic-pituitary-adrenal (HPA) axis and the suprachiasmatic nucleus (SCN) are impaired in HD (see Table 2.1 for a summary of pathologies relevant to this review). Neuropathological changes including volume loss, the loss of orexin-releasing neurons and decreased protein levels of vasoactive intestinal peptide (VIP) and arginine vasopressin (AVP) in the hypothalamus support this supposition (Petersén et al., 2005; Politis et al., 2008; Soneson et al., 2010).
### Table 2.1. Summary of HD pathologies relevant to this review article

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<tr>
<th>Pathologies in HD</th>
<th>Evidence</th>
<th>References</th>
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<tr>
<td><strong>HPA-axis and SCN pathologies</strong>&lt;br&gt;Premanifest HD:&lt;br&gt;<strong>Hypothalamic degeneration, pituitary and adrenal pathologies</strong>&lt;br&gt;<strong>Loss of orexin-releasing neurons</strong></td>
<td>Mouse models:&lt;br&gt;<strong>Hypothalamic degeneration, pituitary and adrenal pathologies</strong>&lt;br&gt;<strong>Loss of orexin-releasing neurons</strong></td>
<td>(Björkqvist et al., 2006; Du et al., 2012; Petersén et al., 2005; Sathasivam et al., 1999)</td>
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<td><strong>Manifest HD:</strong>&lt;br&gt;<strong>Hypothalamic volume loss (shown by voxel-based morphometry)</strong></td>
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<td><strong>Premanifest HD:</strong>&lt;br&gt;<strong>Hypothalamic volume loss (shown by voxel-based morphometry)</strong>&lt;br&gt;<strong>Loss of neurons in the nucleus tuberalis lateralis (NTL) and lateral hypothalamus (at post-mortem)</strong>&lt;br&gt;<strong>Loss of orexin-releasing neurons (at post-mortem)</strong>&lt;br&gt;<strong>Decreased protein levels of vasoactive intestinal peptide (VIP) and arginine vasopressin (AVP) (at post-mortem)</strong></td>
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<td>(Aziz et al., 2008; Douaud et al., 2006; Gabery et al., 2010; Kassubek et al., 2004; Kremer et al., 1991; 1990; van Wamelen et al., 2013)</td>
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<td><strong>Increased cortisol levels</strong>&lt;br&gt;<strong>Premanifest HD:</strong>&lt;br&gt;<strong>Increases in the cortisol awakening response (CAR)</strong>&lt;br&gt;<strong>Increased daily cortisol output</strong></td>
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<td>(Hubers et al., 2015; van Duijn et al., 2010)</td>
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<td><strong>Decreased BDNF</strong>&lt;br&gt;Mouse models:&lt;br&gt;<strong>Decreased brain BDNF levels</strong></td>
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<td>(Pang, Stam, Nithianantharajah, Howard, &amp; Hannan, 2006; Zuccato et al., 2005)</td>
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<td><strong>Manifest HD:</strong>&lt;br&gt;<strong>Reduction of between 53-82% of BDNF in the caudate and putamen (at post-mortem)</strong></td>
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<td>(Ferrer, Goutan, Marin, Rey, &amp; Ribalta, 2000)</td>
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<td><strong>Circadian rhythm disturbances</strong>&lt;br&gt;Mouse models:&lt;br&gt;<strong>Breakdown in rest-activity cycle</strong>&lt;br&gt;<strong>Dysregulation of circadian gene expression, particularly in the SCN</strong>&lt;br&gt;<strong>Reduced circadian rhythm of spontaneous electrical activity in SCN neurons</strong></td>
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<td>(Kudo et al., 2011; Maywood et al., 2010; Morton et al., 2005)</td>
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<td><strong>Manifest HD:</strong>&lt;br&gt;<strong>Disturbances in rest-activity profiles</strong>&lt;br&gt;<strong>Abnormal day-night ratios</strong>&lt;br&gt;<strong>Altered sleep-wake timing</strong></td>
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<td>(Hurelbrink et al., 2005; Morton et al., 2005)</td>
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<td><strong>Alterations in melatonin release</strong>&lt;br&gt;<strong>Premanifest HD:</strong>&lt;br&gt;<strong>Temporal spread of melatonin rise</strong></td>
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<td>(Kalliolia et al., 2014)</td>
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<td><strong>Manifest HD:</strong>&lt;br&gt;<strong>Decreased mean and acrophase melatonin levels</strong>&lt;br&gt;<strong>Temporal spread of melatonin rise</strong>&lt;br&gt;<strong>Delayed rise phase</strong></td>
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<td>(Aziz, Pijl, Frölich, Schroder-van der Elst, et al., 2009; Kalliolia et al., 2014)</td>
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<td><strong>Sleep disturbances</strong>&lt;br&gt;<strong>Premanifest HD:</strong>&lt;br&gt;<strong>Fragmented sleep profile</strong>&lt;br&gt;<strong>Decreased theta power during REM sleep</strong></td>
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<td>(Lazar et al., 2015)</td>
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<td><strong>Manifest HD:</strong>&lt;br&gt;<strong>Insomnia</strong>&lt;br&gt;<strong>Decreased REM</strong>&lt;br&gt;<strong>Decreased slow wave sleep (SWS)</strong>&lt;br&gt;<strong>Decreased sleep efficiency</strong>&lt;br&gt;<strong>Advanced sleep phase</strong>&lt;br&gt;<strong>Frequent nocturnal awakenings</strong>&lt;br&gt;<strong>Increased periodic leg movements (PLMs)</strong></td>
<td></td>
<td>(Arnulf et al., 2008; Hansotia et al., 1985; Morton, 2013; Morton et al., 2005; Neutel et al., 2015; Piano et al., 2015; Wiegand, Möller, et al., 1991)</td>
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Premanifest HD= individuals carrying the gene for Huntington’s disease who do not yet display overt motor signs, however may exhibit mild cognitive decline and mood disturbances; Manifest HD= individuals carrying the Huntington’s disease gene who display overt motor signs of disease, cognitive decline and mood disturbances; HD= Huntington’s disease; HPA-axis= hypothalamic-pituitary-adrenal axis; BDNF= brain-derived neurotrophic factor; SCN= suprachiasmatic nucleus; REM= rapid eye movement.
The HPA-axis is central to neuroendocrine signalling. Indeed, an intricate balance exists between neuroendocrine signalling and expression of neurotrophic factors, particularly brain-derived neurotrophic factor (BDNF) (Issa, Wilson, Terry Jr, & Pillai, 2010; Smith, Makino, Kvetnansky, & Post, 1995). In this review, we present for the first time the biological impact of HPA-axis dysfunction on circadian rhythm, neuroendocrine signalling, and neurotrophic factor support in HD (for a diagrammatic view, see Figure 2.1). We also draw on existing evidence in animal models and patients with HD and other disorders to review non-pharmaceutical treatment strategies, particularly multidisciplinary therapy, exercise, cognitive therapy and social interaction, which may positively impact on HPA-axis dysfunction and potential downstream mechanisms and thereby delay disease onset in individuals with premanifest HD. Since candidate pharmaceutical treatment strategies for HD have been reviewed recently (Ross et al., 2014), here we detail non-pharmaceutical multidisciplinary approaches as they have been reported to exert beneficial effects on HPA-axis function, circadian rhythm and BDNF, are of minimal cost and can be implemented throughout life with few side effects.

2.3 Normal Function of the HPA-Axis and the SCN

The structures of the HPA-axis, including the hypothalamus, pituitary and adrenal glands, function in a tightly regulated manner to control responses to physiological and psychological stress, autonomic and immune functions and sleep-wake behaviour through the release of hormones, such as cortisol, in a circadian manner (Steiger, 2002; Ulrich-Lai & Herman, 2009; Webster Marketon & Glaser, 2008).
Figure 2.1. The interrelated consequences of HPA-axis and circadian rhythm disruption on BDNF expression and sleep and the implications on neurobiology.

Disruption of the HPA-axis and circadian rhythmicity as a result of hypothalamic dysfunction facilitates alterations in cortisol release and disrupted sleep architecture. Conversely, disrupted sleep can lead to increased cortisol and negatively affect the circadian rhythm. Increased cortisol can also disrupt sleep architecture and the circadian rhythm. Circadian rhythm abnormalities and increased cortisol can negatively affect the release of BDNF and this, in turn, can induce cognitive deficits, disrupt sleep architecture and reduce neuronal support which can facilitate the loss of brain volume. Multidisciplinary therapy has the potential to favourably affect this mechanism at several levels. HPA axis= hypothalamic-pituitary-adrenal axis; SCN= suprachiasmatic nucleus; NTL= nucleus tuberalis lateralis; REM= rapid eye movement; SWS= slow wave sleep; PLMs= periodic leg movements; BDNF= brain-derived neurotrophic factor.

The paraventricular nucleus (PVN) releases corticotrophin releasing factor (CRF) into the hypophyseal portal system, where it stimulates the release of adrenocorticotropic hormone
(ACTH) from the corticotropes of the anterior pituitary (Rivier & Vale, 1983). ACTH is released into the systemic circulation and stimulates the adrenal cortex to release glucocorticoids, such as corticosterone in mice and cortisol in humans, which regulate responses to stress in central and peripheral systems. These glucocorticoids then provide negative feedback, inhibiting further release of CRF and ACTH by binding to glucocorticoid receptors (GRs) at the PVN and pituitary level, inhibiting further HPA-axis activation via glucocorticoid response elements (GREs) (Keller-Wood & Dallman, 1984).

Glucocorticoid release is subject to inputs from other brain regions, particularly the amygdala, stria terminalis and hippocampus, which are all regions fundamentally involved in emotional regulation and memory (Hauger & Dautzenberg, 2000). However, the basal circadian release of glucocorticoids is facilitated by the connection between the PVN and SCN.

The SCN is located in the anterior hypothalamus and functions as the central circadian clock that is the principle site of circadian rhythm coordination in mammals (Nishino, Koizumi, & Brooks, 1976). The SCN receives information from the retina and other brain regions and synchronises the circadian rhythms of the organism emerging at cellular, physiological and behavioural levels to various zeitgebers, the most important of which is ambient light. Synchronization is mediated through neural and humoral signals. On a molecular level the circadian rhythm in mammals is based on an autoregulatory transcriptional-translational feedback mechanism involving CLOCK and BMAL1 transcription factors and PERIOD (PER1, 2 and 3) and CRYPTOCHROME (CRY 1 and 2) core clock genes (Gekakis et al., 1998). This molecular clock regulates a considerable proportion of the human genome. Importantly, through its connections with the PVN and mediation of the HPA-axis, the SCN controls daily variations in melatonin and cortisol release which are involved, amongst other things, in sleep-wake behaviour and autonomic arousal regulation.
More specifically, activity of the SCN is synchronised to the environmental light-dark cycle directly through the retinal-hypothalamic tract and indirectly through the retinogeniculate pathways and conveys this information to other hypothalamic nuclei, the reticular formation and the pineal gland, coordinating the diurnal activities of these brain regions (Brzezinski, 1997). Melatonin coordinates circadian rhythms in response to the day-night cycle and initiates the thermoregulatory cascade, decreasing core body temperature to induce sleepiness (Brzezinski, 1997; Krauchi et al., 2000). The circadian variation of core body temperature is also associated with the internal structure of sleep, particularly with the circadian rhythm of REM (Dijk & Czeisler, 1995).

2.4 Stress and the Role of Cortisol

Cortisol secretion follows a circadian rhythm in individuals with normal sleep-wake cycles. Within the first 30 minutes of awakening, cortisol levels increase by up to 75% (Pruessner et al., 1997). Cortisol levels then tend to plateau and around midnight reach their nadir. There is large variation in circadian cortisol levels between individuals, however morning cortisol levels are relatively stable intra-individually, allowing for measurement of the cortisol awakening response (CAR), which serves as an indication of HPA-axis function and circadian rhythmicity (Stone et al., 2001).

In addition to its natural circadian rhythm, cortisol is released in response to physiological and psychological stress (Staufenbiel, Penninx, Spijker, Elzinga, & van Rossum, 2013). Stress has many contributing factors and occurs when environmental demands surpass the individual’s coping abilities (Fink, 2010). The response to stress, particularly adaptation, varies among individuals and is influenced by an individual’s resilience (Russo, Murrough, Han, Charney, & Nestler, 2012). The biological processes that occur in order to allow the individual to adapt to environmental stressors are collectively termed allostasis and involve the release of cortisol
and adrenalin among many other chemical mediators (Juster, McEwen, & Lupien, 2010; Sterling & Eyer, 1988).

Cortisol acts to maintain blood pressure, mobilise energy resources and decrease inflammation (Fraser et al., 1999; Peckett, Wright, & Riddell, 2011; Petrovsky, McNair, & Harrison, 1998). Although individuals have the ability to adapt to these biological effects of cortisol, excessive or insufficient activation of the HPA-axis can contribute to maladaptive consequences, leading to pathology (McEwen & Stellar, 1993).

2.5 Pathological Effects of Chronic Glucocorticoid Release

Significant increases in the CAR and daily cortisol output have been documented in premanifest HD (preHD) compared to healthy controls and manifest HD (Hubers et al., 2015; Shirbin et al., 2013; van Duijn et al., 2010), which implies disruption of the circadian rhythm and therefore of the HPA-axis (Aziz, Pijl, Frölich, van der Graaf, et al., 2009; Politis et al., 2008).

Disruption of the HPA-axis leads to altered circadian release of cortisol (Saper et al., 2005). Severe alterations in glucocorticoids have been shown to exacerbate excitotoxic processes in neurons, predominantly those of the hippocampus (Crochemore et al., 2005; Sapolsky, Packan, & Vale, 1988; Sorrells, Munhoz, Manley, Yen, & Sapolsky, 2014; Stein-Behrens, Mattson, Chang, Yeh, & Sapolsky, 1994). Chronic exposure to glucocorticoids decreases neurogenesis, arborisation of dendrites and density of synapses in the hippocampus and prefrontal cortex (PFC) (Crochemore et al., 2005; Tata, Marciano, & Anderson, 2006) and results in abnormalities of the caudate, putamen and amygdala in animal models (Delgado y Palacios, Verhoye, Henningsen, Wiborg, & Van der Linden, 2014). Chronic stress has been shown to modulate the onset and progression of disease features in the R6/1 HD mouse model (Mo, Pang, et al., 2014; Mo, Renoir, & Hannan, 2014). Higher glucocorticoid levels, such as those
seen in post-traumatic stress disorder (PTSD), have also been linked to a loss of volume in the PFC and striatum and associated impairments in cognitive function and sleep homoeostasis, which suggests that exacerbated cortisol levels, such as those observed in preHD, may accelerate the onset and progression of disease features, particularly cognitive and mood disturbances (Sapolsky, 1999; Staufenbiel et al., 2013). Chronically elevated cortisol levels have also been associated with reduced levels of BDNF in wild-type and schizophrenia rodent models and in humans in other clinical populations, including schizophrenia and major depressive disorder (Issa et al., 2010; Smith, Makino, Kvetňanský, & Post, 1995). This reduction in BDNF could further exacerbate loss of volume in striatal and extra-striatal structures and further disrupt melatonin release and HPA-axis function. Such an association between chronic cortisol release and reduced BDNF requires further investigation in preHD for treatment strategies.

2.6 Effects of Glucocorticoids on BDNF

BDNF is essential for survival, differentiation and outgrowth of neurons in the central and peripheral nervous systems and protects neurons from excitotoxin-induced degeneration (Bemelmans et al., 1999; Husson et al., 2005). BDNF is synthesised in cortical neurons and delivered to the striatum via axonal transport of vesicles (Altar et al., 1997; L. J. Goodman et al., 1996). BDNF deficits have been documented in cell lines expressing mHTT and in brains of HD mouse models and patients at post-mortem (Ferrer et al., 2000; Gauthier et al., 2004; Zuccato et al., 2001; Zuccato et al., 2005). Analyses of post-mortem brain tissue of four HD subjects indicated regional BDNF deficits of between 53% and 82% in the caudate and putamen compared to age-matched controls (Ferrer et al., 2000; Gauthier et al., 2004), suggesting that volume loss in these regions may, at least in part, be mediated by a lack of neurotrophic factor support.
Significantly elevated glucocorticoids have been reported to decrease the expression of BDNF in animal models and other clinical populations (Issa et al., 2010; Smith, Makino, Kvetňanský, et al., 1995). Chronic stress in rodents induced by repeated restraint results in a negative correlation between plasma glucocorticoid levels and hippocampal BDNF mRNA expression (Murakami, Imbe, Morikawa, Kubo, & Senba, 2005; Smith, Makino, Kvetnansky, et al., 1995). Furthermore, exogenous administration of glucocorticoids is associated with a transient, dose-dependent reduction in BDNF mRNA and protein in the hippocampus of adrenalectomized (ADX) rodent models (Hansson et al., 2006; Schaaf, de Jong, de Kloet, & Vreugdenhil, 1998). However, five days of oral corticosterone treatment in the R6/1 HD mouse model did not significantly affect hippocampal BDNF expression, emphasising the need to characterise the effects of chronic elevated stress on BDNF levels in HD animal models and patients. It is conceivable that elevated glucocorticoid levels, as observed in preHD, contribute to decreased BDNF expression, thereby potentiating neuronal dysfunction and cell loss in cortical and subcortical brain structures.

In addition to supporting normal neuronal functioning, BDNF is thought to be integral in the homeostatic regulation of sleep (Datta, Knapp, Koul-Tiwari, & Barnes, 2015). Alterations in BDNF signalling as a result of irregular cortisol regulation may potentiate sleep deficits, which are evident early in HD. The interplay between glucocorticoids, neurotrophic factor support and sleep are not well understood but are likely to be important considerations in better understanding the interaction of the HPA-axis and circadian rhythm disruption as features of HD.

2.7 Pathologies of the HPA-Axis and SCN in HD

mHTT causes progressive neuronal dysfunction and cell loss in striatal and extra-striatal regions, including the hypothalamic nuclei (H. Li, Li, Johnston, Shelbourne, & Li, 2000; Tabrizi et al., 2011). Studies in mouse models report significant degeneration of the
hypothalamus, as well as pituitary and adrenal pathologies (Björkqvist et al., 2006; Du et al., 2012; Petersén & Björkqvist, 2006; Sathasivam et al., 1999). Post-mortem and structural imaging studies in HD mutation carriers have reported volume loss in the hypothalamus, with significant hypothalamic nuclear atrophy, neuronal loss (particularly that of the nucleus tuberalis lateralis (NTL) and lateral hypothalamus (Kremer et al., 1991; Kremer et al., 1990)) and microglial activation (Douaud et al., 2006; Gabery et al., 2010; Kassubek et al., 2004; Politis et al., 2008). Post-mortem studies have also reported loss of orexin-releasing neurons, responsible for innervating the SCN, in the hypothalamus in HD (Aziz et al., 2008; Petersén et al., 2005). Loss of this neuronal population is thought to contribute to circadian rhythm disturbances, HPA-axis dysfunction and subsequent alterations in cortisol release (Petersén et al., 2005). Moreover, HPA-axis dysregulation has been proposed as a contributing factor to comorbid depression in neurodegenerative diseases, including HD (for a comprehensive review, see Du & Pang, 2015).

Hypothalamic atrophy has also been reported in preHD individuals using voxel-based morphometry (VBM) (Soneson et al., 2010). This may, at least in part, explain the observed alterations in cortisol release in preHD. A recent study, however, reported no hypothalamic volume loss in preHD individuals at a 12 month follow-up scan (Gabery et al., 2015). These conflicting findings reinforce the need to better characterise hypothalamic and other regional volumetric changes in preHD, and determine whether such changes mediate or contribute to circadian rhythm disturbances and clinical features in HD.

**2.8 Circadian Rhythm Disruption in HD**

Circadian rhythmicity is progressively disrupted in HD, suggesting a possible bi-directional relationship with the neurodegenerative disease process (for a review see Videnovic et al., 2014). Support for this notion comes from transgenic animal models of HD, such as in the R6/2, R6/1 and BACHD mice, which have shown that circadian disruption precedes the
presentation of disease features (Kudo et al., 2011; Maywood et al., 2010; Morton et al., 2005).
Furthermore, humans with manifest HD also display circadian rhythm abnormalities, with disturbances in rest-activity profiles and abnormal day-night ratios, as well as alterations in sleep-wake timing and melatonin and cortisol profiles (Hurelbrink et al., 2005; Morton et al., 2005; Videnovic et al., 2014). The neurobiology underlying these changes has yet to be clarified.

Several studies point to alterations in the SCN as being central to circadian disruptions in HD. For example, in the R6/2 mouse model of HD the rhythmic transcription of core clock genes in the SCN and other brain regions is disrupted in vivo, but then rescued when assessed in in vitro explants, suggesting that circadian deficits are due to alterations of the intrinsic circuitry of the SCN (Maywood et al., 2010; Morton et al., 2005). This is supported by a reduced circadian rhythm in spontaneous electrical activity in SCN neurons in BACHD transgenic mice (Kudo et al., 2011). Histopathological studies reveal reduced protein levels of VIP and AVP within the SCN of HD patients at post-mortem (van Wamelen et al., 2013). In transgenic animal models of HD, the decrease in VIP levels is associated with circadian disruption (Fahrenkrug, Popovic, Georg, Brundin, & Hannibal, 2007). Recent evidence from Alzheimer’s disease (AD) patients indicates that the number of VIP-expressing SCN neurons in the post-mortem brain correlates with circadian rhythm amplitude of motor activity (J. L. Wang et al., 2015).

Disruption of circadian rhythmicity has the potential to affect a broad range of molecular, cellular and physiological processes, most noticeably sleep (Archer et al., 2014; Lazar et al., 2015). Disturbances in sleep are known to have multiple negative consequences on human physiology, including neuronal dysfunction and loss of brain volume (Joo et al., 2013), metabolic disturbances (Schmid, Hallenschmid, & Schultes, 2015), immune dysregulation (Irwin, 2002), impaired cardiovascular function (Zhong et al., 2005), cognitive impairments (Lo et al., 2012) and mood disturbances (Dinges et al., 1997). Sleep disturbance can, by itself, cause
further disruption to circadian rhythmicity (Moller-Levet et al., 2013). It is highly likely that sleep and circadian disturbances are interrelated in HD.

2.9 Melatonin and Sleep Disturbance in HD

Melatonin promotes the onset of sleep by inducing the thermoregulatory cascade (Krauchi et al., 2000). Significant decreases in mean and acrophase (times of peak rhythm) melatonin levels have been reported in manifest HD, with trends towards decreased melatonin levels in preHD (Kalliolia et al., 2014). A temporal shift in melatonin release has also been documented in HD mutation carriers, which could explain documented sleep disturbances (Kalliolia et al., 2014). The morning rise phase of melatonin has also been shown to be delayed in HD individuals (Aziz, Pijl, Frölich, Schroder-van der Elst, et al., 2009), which provides a mechanism underlying the delayed sleep-wake timing reported to occur in these patients (Aziz et al., 2010; A. O. Goodman, Morton, & Barker, 2010). The precise mechanism responsible for the decrease or delayed melatonin levels observed in HD is unclear, but could potentially be attributed to the progressive neuronal dysfunction in the SCN (Kalliolia et al., 2014; van Wamelen et al., 2013).

Disrupted or restricted sleep leads to increased activity of the HPA-axis (Meerlo et al., 2008). Acute sleep deprivation is associated with increased sympathetic activation, reflected by increases in heart rate and blood pressure (Zhong et al., 2005) and has been described as a chronic stressor that can elevate glucocorticoids and exacerbate disease pathways, such as neuronal dysfunction and degeneration (Leproult, Copinschi, Buxton, & Van Cauter, 1997; McEwen, 2006). It is also important to note that disturbances in sleep, particularly disruption of slow wave sleep and decreased sleep duration, result in declines in cognitive and motor performance, as well as altered mood (Ferrie et al., 2011; Mander et al., 2015; Mander, Rao, Lu, Saletin, Ancoli-Israel, et al., 2013; Mander, Rao, Lu, Saletin, Lindquist, et al., 2013).
Studies in animal models have shown that sleep deficits can negatively affect hippocampal function, and can lead to impaired synaptic plasticity (long-term potentiation) and changes in N-Methyl-D-Aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor signalling and fluctuations in levels of glutamate, a ligand for these receptors (Kopp, Longordo, Nicholson, & Lüthi, 2006; Ravassard et al., 2009). Sleep has been reported to promote the formation of branch-specific dendritic spines following motor learning in mice, with disruption of non-REM (NREM) sleep preventing the formation of branch-specific dendritic spines (Yang et al., 2014). Furthermore, sleep has been shown to drive clearance of neurotoxic waste from the central nervous system in mice and disruption of sleep could potentially facilitate accumulation of these substances (Xie et al., 2013). Sleep deficits have also been shown to negatively affect executive functions associated with the PFC, such as working memory and lateral thinking, likely due to the differential activation of adenosine receptors and disruption of synaptic homeostasis (Florian, Vecsey, Halassa, Haydon, & Abel, 2011; Harrison & Horne, 1998; Lo et al., 2012). Loss of volume of the frontal, temporal and parietal cortices are associated with sleep deficits (Sexton, Storsve, Walhovd, Johansen-Berg, & Fjell, 2014) and could also affect executive functions associated with these regions.

Sleep disturbances have been reported in manifest HD, with several studies reporting insomnia, decreased REM, slow wave sleep and sleep efficiency, advanced sleep phase, frequent nocturnal awakenings and increased periodic leg movements (PLMs) (Arnulf et al., 2008; Hansotia et al., 1985; Morton et al., 2005; Neutel et al., 2015; Piano et al., 2015; Wiegand, Möller, et al., 1991). Furthermore, a recent study by Lazar et al (2015) showed that sleep disruption is evident in preHD, characterised by a fragmented sleep profile and a decrease in theta power during REM sleep (Lazar et al., 2015). These features were associated with disease burden score. It is notable that sleep deficits seem to appear at a time when cognitive
Impairments also start to emerge, indicating a potential relationship between these disease features.

Sleep deficits are of particular interest in HD, as cognitive functions associated with both the hippocampus and PFC are affected in this population before disease onset (Tabrizi et al., 2011), and can be negatively impacted by sleep disturbances, as well as increased glucocorticoids (Belenoff, Gross, Yager, & Schatzberg, 2001; Blagrove et al., 1995; Oei, Everaerd, Elzinga, van Well, & Bermond, 2006). Ultimately, alterations in circadian rhythm marked by changes in the molecular clock and facilitated by hypothalamic and SCN pathologies, could result in changes in melatonin release and increased cortisol levels, with resultant sleep disturbances, which are likely to potentiate neurodegeneration and associated changes in cognitive and motor deficits and mood disturbances in HD.

2.10 Environmental Enrichment: A Comprehensive Non-Pharmaceutical Strategy to Reduce the Impact of Circadian Rhythm Disturbances and HPA-Axis Dysfunction in HD

Several non-pharmaceutical strategies have been employed to ameliorate circadian and HPA-axis dysfunctions in mouse models of HD, including bright light and behavioural therapy (Cuesta et al., 2014) and environmental enrichment (EE). EE is an experimental approach reported to change intrinsic and behavioural rest-activity circadian rhythmicity and glucocorticoid release and has been widely studied in transgenic and drug-induced AD, Parkinson’s disease (PD) and HD mouse models (reviewed in Nithianantharajah & Hannan, 2006). EE employs exercise, cognitive and sensory stimulation to promote neurogenesis and improve cognitive and behavioural function, motor features and overall pathological processes underpinning these clinical features (Hannan, 2014; Lazic et al., 2006; Nithianantharajah & Hannan, 2006).
The first demonstration that EE could be beneficial in a genetic animal model involved HD mice (van Dellen, Blakemore, Deacon, York, & Hannan, 2000) and demonstrated that EE could delay disease onset and progression displayed by improved motor function and preserved peristriatal brain structures. Ensuing investigations revealed that EE has cognitive and body composition benefits in R6/2 (Hockly et al., 2002) and N171 HD mice (Schilling et al., 2004), while also ameliorating cognitive deficits (Nithianantharajah, Barkus, Murphy, & Hannan, 2008) and affective (depressive-like) abnormalities in R6/1 mice (Pang, Du, Zajac, Howard, & Hannan, 2009; Renoir et al., 2013). Considering the more rapid disease progression of the R6/2 model compared to R6/1, this demonstrates that EE is effective in both rapid and more prolonged disease progression phenotypes.

EE has been reported to increase the length of neuronal dendrites in the dorsomedial nucleus of the hypothalamus, which is thought to play a role in the circadian control of sleep and waking behaviours (Chou et al., 2003), and alter stress reactivity in outbred rats (Francis, Diorio, Plotsky, & Meaney, 2002; Mitra & Sapolsky, 2012), and in the female R6/1 mouse model, EE modulates HPA-axis activity (Du et al., 2012). Furthermore, circadian rhythm disturbances have also been ameliorated through bright light therapy and exercise in the R6/2 mouse model (Cuesta et al., 2014). This demonstrates that environmental interventions have the potential to modulate functions of the HPA-axis and the SCN in mouse models of HD and warrants further investigation into whether this can be recapitulated in the human HD population.

Moreover, EE in HD mice has been shown to rescue BDNF protein levels in the striatum and hippocampus (Spires et al., 2004), with associated delays in disease onset, including a reduction in cognitive decline (Nithianantharajah et al., 2008). These studies suggest that modulation of HPA-axis function and circadian rhythm facilitated by EE may, at least in part, rescue BDNF levels, ultimately contributing to neuroprotection and neurogenesis (Mattson, 2012).
Evidence suggests that physical activity in itself can be beneficial in delaying the progression of HD in mouse models. Pang et al. (2006) demonstrated that voluntary wheel running delayed onset of rear paw clasping, a feature of HD in mouse models, ameliorated cognitive deficits and also normalised rearing behaviour (Pang et al., 2006). Additionally, wheel running from a juvenile age (4 weeks) delayed onset of rear paw clasping and of deficits in motor coordination and rescued locomotor activity and exploratory behaviour (van Dellen, Cordery, Spires, Blakemore, & Hannan, 2008). It has been suggested that some of the behavioural improvements resulting from voluntary physical activity are modulated by the upregulation of monoamines, such as serotonin, dopamine and nor-adrenaline, across several brain regions (Renoir, Chevarin, Lanfumey, & Hannan, 2011). Interestingly, wheel running is associated with sex-dependent increases in BDNF expression, with only female HD mice exhibiting increases in BDNF following physical activity alone and male HD mice showing increases in BDNF only following EE (Zajac et al., 2010). This indicates that voluntary physical activity can up-regulate key molecules that modulate cognitive and behavioural changes in mouse models of HD in a sex-dependent manner.

Studies in several animal disease models have demonstrated the importance of social interaction in mediating the benefits of EE. For example, co-housed AD APP/PS1 mouse models exhibit amelioration of memory deficits facilitated by increased BDNF-dependent neurogenesis in the hippocampus (Hsiao, Hung, Chen, & Gean, 2014). Transgenic HD sheep exhibit circadian abnormalities when housed only with other HD flock, with circadian abnormalities absent in those HD gene positive sheep housed with wild-type sheep (Morton et al., 2014). Although physical activity and co-housing, when assessed alone, can produce positive outcomes in mouse models of HD, greater beneficial effects are observed when used in conjunction with other components of EE. Such findings demonstrate that environmental
interventions have a positive impact on disease processes in animal models of HD and warrant further investigation into the translation of these programs into the human HD population.

2.11 Effects of Multidisciplinary Therapy on Brain Volume and Potential Biomarkers of HD in Humans

Preclinical studies show that EE has positive effects on the pathological and clinical course of HD. However, translation of EE from the laboratory to the clinic has proven difficult due to the strict parameters of the experimental model, such as diet and housing conditions. Several research teams have, nevertheless, begun to address some of these translational gaps using multidisciplinary therapy; a complex, interdisciplinary therapeutic approach comprising physical activity, cognitive stimulation and social interaction.

Studies evaluating the utility of multidisciplinary therapy have documented significant changes in grey matter volume, as well as improvements in memory, processing speed, balance and gait, mood and quality of life in patients with manifest HD (Cruickshank et al., 2015; Piira et al., 2013; Thompson et al., 2013; Veenhuizen et al., 2011). Recent data from our research programme has shown, in particular, that multidisciplinary therapy increases grey matter volume in the caudate tail and dorsolateral PFC in patients with manifest HD (Cruickshank et al., 2015). This therapy has also been reported to improve cognitive function, quality of life and depressive symptoms in patients with mild AD and cognitive impairment without dementia (Santos et al., 2015) and in PD, multidisciplinary therapy has been reported to improve motor performance, dyskinesias, balance and gait and slow disease progression (Frazzitta, Abbruzzese, et al., 2013; Frazzitta et al., 2012; Frazzitta, Bertotti, et al., 2013). The molecular mechanisms driving these neural and clinical changes are yet to be investigated. Several lines of evidence suggest, however, that multidisciplinary therapy may restore normal HPA-axis function, circadian rhythmicity and basal BDNF levels, promoting neural and clinical benefits in HD.
EE is capable of restoring normal HPA-axis function, circadian rhythmicity and basal BDNF levels in HD mouse models (Cuesta et al., 2014; Du et al., 2012; Zajac et al., 2010), with significant delays in peristriatal degeneration and cognitive and motor decline (Nithianantharajah et al., 2008; Pang et al., 2006; van Dellen et al., 2008), which could be facilitated by restoration of the HPA-axis and circadian rhythmicity and increases in BDNF levels. While these positive molecular changes are yet to be reported in patients with HD, evidence from other diseases suggests that multidisciplinary therapy may impact on the neuropathological and clinical course of the disease in a similar fashion to EE. For example, in PD multidisciplinary therapy has been reported to increase serum BDNF levels and lessen clinical burden in the early stages of the disease (Frazzitta, Maestri, Bertotti, et al., 2015; Frazzitta et al., 2014).

The effects of multidisciplinary therapy on HPA-axis function, stress reactivity and circadian rhythmicity are yet to be investigated in any disease population. Furthermore, the effects of this therapy on BDNF in HD are also yet to be reported. However, the benefits of EE on the HPA-axis, BDNF levels and circadian rhythmicity in HD mouse models, both before disease features appear and following onset, the increase in serum BDNF in PD patients and the increase in brain volume in manifest HD patients following multidisciplinary therapy, highlight the importance of assessing the effects of this therapy on HPA-axis function, circadian rhythmicity and BDNF levels in HD. It is conceivable that multidisciplinary therapy could regulate the HPA-axis, and possibly circadian rhythm, and increase BDNF levels, resulting in the positive brain changes observed in HD individuals following this therapy. Rationale for this can be seen in other populations when the effects of each of the components of multidisciplinary therapy are evaluated individually.

Physical activity, cognitive training and social interaction have a range of benefits on striatal and extra-striatal brain structures, HPA-axis function, circadian rhythm and BDNF levels.
Higher physical activity levels are associated with increased hippocampal and PFC volume, accompanying improvements in memory in healthy older adults (Colcombe et al., 2006; Erickson et al., 2011) and increased BDNF levels (de Melo Coelho et al., 2014; Pereira et al., 2013). The latter could be mediated by the continual induction and eventual down-regulation of the stress response due to acute, transient increases in cortisol following exercise (Luger et al., 1987), leading to an adaptive stress response. The regulation of circadian rhythmicity and melatonin levels by exercise, indicated by shifts in onset and increases in peak melatonin release (Buxton, Lee, L'Hermite-Baleriaux, Turek, & Van Cauter, 2003; Miyazaki, Hashimoto, Masubuchi, Honma, & Honma, 2001), could also facilitate an increase in BDNF and regulation of the HPA-axis, and lead to improvements in brain volume and associated functions.

Cognitive training has also been documented to increase grey matter volume in the cortex in regions involved in episodic memory in individuals with subjective memory impairment, a common risk factor for AD (Engvig et al., 2014). Furthermore, cognitive training has been shown to reduce stress-related symptoms and improve sleep onset latency and efficiency in individuals with stress-related exhaustion (Gavelin, Boraxbekk, Stenlund, Järvholm, & Neely, 2015) and in older adults with insomnia (Haimov & Shatil, 2013), respectively. The regulation of stress symptoms and improved sleep efficiency could facilitate an increase in BDNF, which has been reported following cognitive training in individuals with PD (Angelucci et al., 2015).

Lastly, social interaction has been shown to increase whole brain volume, with associated improvements in visual attention and verbal learning (Mortimer et al., 2012). Social interaction attenuates the cortisol response to stressful stimuli, likely through coping or resiliency mechanisms (Kirschbaum, Klauer, Filipp, & Hellhammer, 1995), which is likely to facilitate the increase in BDNF observed in AD mouse models following social interaction (Hsiao et al., 2014).
These findings collectively indicate that lifestyle interventions could favourably impact on clinical and pathological aspects of HD. Indeed, evidence from animal models and human studies indicate that multidisciplinary therapy has significant potential to treat many of the clinical consequences of HPA-axis and circadian rhythm disturbances in HD. Such an approach may even have the potential to reduce the rate and/or forestall neuropathological changes that occur in individuals with preHD.

2.12 Conclusion

HD individuals exhibit a wide spectrum of clinical features indicative of degeneration in striatal and extra-striatal structures, including the hypothalamus. Hypothalamic pathologies are likely to result in HPA-axis dysfunction and circadian rhythm dysregulation, features which have been reported in HD mouse models and gene-positive individuals. The consequent increase in glucocorticoids and dysregulation of melatonin and sleep patterns are associated with decreased BDNF levels and have the potential to contribute to, or even exacerbate, disease processes. Much is still to be understood about the interaction between glucocorticoids, BDNF and sleep. However, emerging evidence of potential strategies to ameliorate negative downstream consequences of these interactions, suggest a positive role for multidisciplinary therapy. Preliminary studies using such strategies have demonstrated favourable effects on HPA-axis and circadian rhythm disturbances in animal models of HD, as well as other clinical populations, however the effects of multidisciplinary therapy on HPA-axis dysfunction, circadian rhythmicity and BDNF levels in HD gene-positive individuals are yet to be investigated.

2.13 Acknowledgements

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Chapter 3

Mechanisms Associated with Sleep Disturbances in Premanifest Huntington’s Disease

Original Article

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3.1 Abstract

**Objective:** Pathological changes within the hypothalamus have been proposed to mediate circadian rhythm and habitual sleep disturbances in individuals with Huntington’s disease (HD). However, investigations examining the relationships between hypothalamic volume and circadian rhythm and habitual sleep in individuals with HD are sparse. This study aimed to comprehensively evaluate the relationships between hypothalamic pathology and circadian rhythm and habitual sleep disturbances in individuals with premanifest HD.

**Methods:** Thirty-two individuals with premanifest HD and twenty-nine healthy age- and gender-matched controls participated in this dual-site, cross-sectional study. Magnetic resonance imaging scans were performed to evaluate hypothalamic volume. Circadian rhythm and habitual sleep were assessed via measurement of morning and evening cortisol and melatonin levels, wrist-worn actigraphy, the Consensus Sleep Diary and sleep questionnaires. Information on mood, physical activity levels and body composition were also collected.

**Results:** Compared to healthy controls, individuals with premanifest HD displayed significantly reduced grey matter volume in the hypothalamus, decreased habitual sleep efficiency and increased awakenings; however, no alterations in morning cortisol or evening melatonin release were noted in individuals with premanifest HD. While differences in the associations between hypothalamic volume and cortisol and melatonin output existed in individuals with premanifest HD compared to healthy controls, no consistent associations were observed between hypothalamic volume and circadian rhythm or habitual sleep outcomes.

**Conclusion:** While significant differences in associations between hypothalamic volume and cortisol and melatonin existed between individuals with premanifest HD and healthy controls, no differences in circadian markers were observed between the groups. This suggests that circadian regulation is maintained despite hypothalamic pathology, perhaps via neural compensation. Longitudinal studies are required to further understand the relationships
between the hypothalamus and circadian rhythm and habitual sleep disturbances in HD as the disease course lengthens.

3.2 Introduction

Circadian rhythm and habitual sleep disturbances are common features of Huntington’s disease (HD) that occur early in the disease course and exacerbate impairments in cognitive function, metabolism, hormone regulation, mood and quality of life (Aziz et al., 2010; Briançon-Marjollet et al., 2015; Lazar et al., 2015; Morton et al., 2005).

A number of studies have reported dysregulation of markers of circadian rhythm and habitual sleep-wake outcomes in individuals with HD. In particular, increased morning cortisol output has been reported in individuals with premanifest HD (Hubers et al., 2015; van Duijn et al., 2010). Reduced mean and acrophase concentrations and a temporal spread of melatonin release, indicating a potential phase shift in melatonin, have been reported in individuals with premanifest HD (Kalliolia et al., 2014). Furthermore, studies have reported disruption of the sleep-wake cycle and delayed sleep phase in individuals with HD (Aziz et al., 2010; Morton et al., 2005). Despite these findings, the neurobiological origin of circadian rhythm and habitual sleep-wake disturbances in individuals with HD has been poorly investigated and warrants further exploration.

Pathological changes within the hypothalamus have been proposed to disrupt circadian rhythm and sleep in individuals with HD (Aziz et al., 2010; Morton et al., 2005). Hypothalamic nuclei, particularly the suprachiasmatic nucleus, are known to be integrally involved in the regulation of circadian markers such cortisol and melatonin, as well as sleep (Saper et al., 2005; Steiger, 2002). Studies have reported grey matter volume loss and microglial activation within the hypothalamus and circadian rhythm and sleep disturbances that arise concomitantly in individuals with premanifest HD (Lazar et al., 2015; Morton et al., 2005; Politis et al., 2008;
Soneson et al., 2010). Despite these findings, only one study has examined the potential link between neural pathology and circadian rhythm and sleep disturbances in individuals with HD (Baker et al., 2016). Baker et al (2016) reported an association between subjective sleep disturbances and neural pathology in HD. However, authors did not use formalised sleep questionnaires to examine sleep and did not evaluate markers of circadian rhythm relative to neural pathology. There is, therefore, a need for subsequent research to investigate more closely the potential relationships between hypothalamic pathology and circadian rhythm and sleep disturbances in HD.

The purpose of this study was to evaluate whether hypothalamic pathology is associated with the dysregulation of biological and clinical markers of circadian rhythm, particularly cortisol, melatonin and sleep-wake timing, and habitual sleep in individuals with premanifest HD. We hypothesized that hypothalamic pathology would be associated with dysregulation of circadian rhythm and habitual sleep, as evidenced by significant alterations in cortisol and melatonin release and habitual sleep-wake cycles in individuals with premanifest HD.

### 3.3 Materials and Methods

#### 3.3.1 Participants

Thirty-five premanifest HD individuals and 31 age- and gender-matched healthy controls were recruited from existing databases, HD clinics and media advertisements in Perth and Melbourne. Inclusion criteria for premanifest HD individuals were a cytosine-adenine-guanine (CAG) repeat length ≥ 40 and a diagnostic confidence score < 2 on the Unified Huntington’s Disease Rating Scale Total Motor Score (UHDRS-TMS) (Reilmann, Leavitt, & Ross, 2014). Exclusion criteria for all participants were, presence of known musculoskeletal, metabolic, endocrine, cardiovascular or sleep disorders, recent or long-standing substance abuse, shift work other neurological conditions and, for healthy controls, a family history of HD. Five
participants withdrew from the study or contributed incomplete data and were excluded from analyses, leading to a total of 32 premanifest HD and 29 healthy individuals.

All aspects of the study were conducted in accordance with the Declaration of Helsinki. Ethical approval was granted by the Edith Cowan University and Monash University Human Research Ethics Committees. All participants provided written informed consent.

3.3.2 Study Procedures

Testing procedures included 3T MRI scans for hypothalamic imaging, saliva sampling to quantify morning cortisol and evening melatonin, wrist-worn actigraphy for monitoring sleep, a sleep diary for monitoring habitual sleep patterns and questionnaires for monitoring sleep quality, psychological stress, physical activity, anxiety and depression.

3.3.3 Acquisition and Pre-Processing of MRI Data

T1-weighted structural images of the brain were obtained from each participant in Perth or Melbourne using a GE Healthcare Discovery and a Siemens Skyra 3T MRI scanner, respectively. In Perth, images were acquired with a 24-channel head coil using an IR-SPGR sequence (TA = 9 m 59 s, TR = 3 s, TE = Min, TI = 400 ms, flip angle = 11°, field of view = 256 mm, image matrix = 256 x 256, 1 mm3 isotropic voxels). In Melbourne, acquisition took place with a 32-channel head coil and an MP-RAGE sequence (TA = 9 m 14 s, TR = 2.3 s, TE = 2.96 ms, TI = 900 ms, flip angle = 9°, field of view = 256 mm, image matrix = 256 x 256). Images were acquired consistently across both sites according to the Alzheimer’s Disease Neuroimaging Initiative protocols for multi-site imaging (Jack et al., 2008). Pre-processing of images was conducted according to the SPM12 pipeline (supplementary data, section 3.10).

3.3.4 Measurement of Biological Markers of Circadian Rhythm

Salivary cortisol and melatonin have previously been shown to be useful measures of circadian rhythm (Dickmeis, 2009; Voultsios, Kennaway, & Dawson, 1997). Participants were given
written and verbal instructions to collect saliva samples by passive drool into polypropylene collection tubes (SSI Bio) at the same time on two consecutive days for determination of morning cortisol and evening melatonin concentrations. Participants collected saliva samples at four time points in the morning (15, 30, 45 and 60 minutes following awakening) for morning cortisol analysis and four time points in the evening at one hour intervals from two hours before their usual bedtime (T1) until one hour after their usual bedtime (T4) for melatonin analysis. Saliva samples were collected according to criteria from previous studies (van Duijn et al., 2010; Voultsios et al., 1997) to avoid contamination of samples (see supplementary files). Based on these criteria, a questionnaire was devised to monitor participant compliance. Saliva samples were stored at -80°C until analysis using commercially available salivary cortisol and melatonin ELISA kits (Salimetrics, USA) according to the manufacturer’s instructions.

3.3.5 Measures of Habitual Sleep-Wake Parameters

Actigraphy

At the commencement of the study, a convenience sample of individuals with premanifest HD and healthy controls were given the opportunity to undertake habitual sleep monitoring using actigraphy. Of the original cohort, 19 premanifest HD and 24 healthy individuals underwent home-based actigraphy sleep measurement for seven consecutive nights to assess habitual sleep-wake patterns. Actigraphy was recorded at 30 Hz using wrist-worn GT3X+ ActiGraph monitors (ActiGraph, USA) on the non-dominant wrist. The start and end time of sleep periods were recorded using the Consensus Sleep Diary (Carney et al., 2012).

Wrist-based actigraphy has been used previously to assess circadian rhythm via analysis of habitual sleep-wake patterns in individuals with HD and in other populations (Ancoli-Israel, Cole, et al., 2003; Morton et al., 2005). Therefore, here we similarly used ActiGraph monitors, which were initialised, downloaded and analysed using the ActiLife software (version 6.8). Data were scored with a low frequency extension filter in 60 second epochs as awake or sleep
according to the Cole-Kripke algorithm (Cole, Kripke, Gruen, Mullaney, & Gillin, 1992). Total sleep time, sleep onset latency, wake after sleep onset, number of awakenings and sleep efficiency outcomes were calculated for each night and then averaged across the seven nights to obtain single values for use in subsequent analyses.

Consensus Sleep Diary

Habitual sleep-wake timing was evaluated using the Consensus Sleep Diary (Carney et al., 2012). Participants were required to document time in bed, time of awakening, time to sleep onset, number of awakenings and sleep quality for seven nights in combination with actigraphy assessment.

Subjective Sleep Quality and Daytime Somnolence

Sleep quality was assessed in all participants using the Pittsburgh Sleep Quality Index (PSQI) (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989). Daytime somnolence was measured using the Epworth Sleepiness Scale (ESS) (Johns, 1991).

3.3.6 Stress, Anxiety and Depression Symptomatology

The Perceived Stress Scale (Cohen, Kamarck, & Mermelstein, 1983) was used to measure psychological stress in the previous month. The Perceived Stress Scale has been demonstrated to be a valid measure of perceived psychological stress in individuals with HD (Downing et al., 2011). Symptoms of anxiety and depression were measured using the Hospital Anxiety and Depression Scale (HADS). This scale has been previously demonstrated to be valid and reliable in HD (De Souza, Jones, & Rickards, 2010).

3.3.7 Physical Activity

Physical activity levels were recorded prior to and during saliva sampling days using the Minnesota Leisure Time Physical Activity Questionnaire. Metabolic equivalents (METs) were calculated using recorded physical activity levels, the Compendium of Physical Activities
database and estimated resting metabolic rate (RMR) (Ainsworth et al., 2011). RMR was calculated using the Harris-Benedict formula (see supplementary file) (Harris & Benedict, 1918).

3.3.8 Statistical Analysis

Hypothalamic Volume

A hypothalamus mask from the WFU Pick Atlas (http://fmri.wfubmc.edu/software/pickatlas), dilated by 3mm, was used to restrict analysis to this area (Breen et al., 2016). Grey matter images from individuals with premanifest HD and healthy controls were compared voxel-wise using a two-sample t-test to evaluate group differences in the hypothalamus. Gender, site and age were included as covariates of no interest. Next, we investigated group differences in the association between hypothalamic volume and cortisol and/or melatonin output in individuals with premanifest HD compared to healthy controls, using a categorical by continuous covariate interaction model. The model included group regressors, one for each group, and regressors modelling change in cortisol or melatonin output, one for each group. The following contrasts were used to model the group by cortisol/melatonin output interaction effect: [0 0 1 -1] and [0 0 -1 1]. Gender, site, age, PSS, ESS, and PSQI were included as covariates of no interest. In premanifest HD, we adjusted also for CAP score.

We also evaluated the relationship between hypothalamic volume and disease status (i.e. CAP score). Given the exploratory nature of this study and our a priori interest in the hypothalamus, the threshold for statistical significance for all analyses was set at $\alpha = 0.05$.

Salivary Cortisol and Melatonin

Missing data points from saliva sampling (removed due to suspected blood contamination) were imputed by calculating the average of the previous and subsequent values in the curve (n= 2 of 504, 0.40%), unless the missing value was the first time point in each curve (n= 3 of
504, 0.60%), in which case the group average was imputed to avoid removing the participant from analyses and maintain sample size (van Duijn et al., 2010). Area under the curve with respect to ground (AUCG) was calculated using the trapezoid rule for morning cortisol and evening melatonin output on the two consecutive days (Dijk et al., 2012; van Duijn et al., 2010). Normality assumptions for all variables were tested using a Shapiro-Wilk test. Between-group differences were examined using a t-test for continuous variables and a two sample proportion test for categorical variables. Spearman correlation coefficient was calculated to assess relationships between cortisol AUCG, melatonin AUCG and Perceived Stress Scale, PSQI and Consensus Sleep Diary scores. Statistical significance was set at p ≤ 0.05. Statistical analyses were performed using STATA version 9.1.

3.4 Results

3.4.1 Participant Demographics and Clinical Characteristics

There were no significant differences for age or gender between premanifest HD patients and healthy controls (p = 0.472 and 0.283, respectively; Table 3.1).

3.4.2 Hypothalamic Volume

A significant decrease in grey matter volume in the anterior-superior region of the left side of the hypothalamus was observed in individuals with premanifest HD compared to healthy controls (peak voxel at MNI -9 1 -4; t = 2.38; k = 22; see, Figure 3.1A). We also found a significant negative association in premanifest HD between grey matter volume bilaterally in the anterior-superior and anterior-inferior regions of the hypothalamus and CAP score (r = -0.42; see Table 3.3 and Figure 3.1B).
Figure 3.1. Hypothalamic volume in its association with cortisol and melatonin output.

Results from voxel-based morphometry analyses showing overlap between maps of (A) the hypothalamic volume group difference, (B) association between hypothalamus loss and CAPs in premanifest HD, (C) group by cortisol output interaction and (D) group by melatonin output interaction. (E) shows the map of group by cortisol output interaction effect on hypothalamic volume (as per voxel-wise analysis) and a scatterplot illustrating the interaction and (F) shows the map of group by melatonin output interaction effect on hypothalamic grey matter volume and scatterplot illustrating the interaction. The data points in the scatterplot correspond to the average intensity across all voxels within the area exhibiting a significant interaction effect for each participant. Slice labels are displayed on top. Cross-slices are shown. Crosshairs in (A), (B), (C), and (D) are centred at MNI -9 1 -5. L= left; R= right.
3.4.3 Biological Markers of Circadian Rhythm

No significant differences were observed between individuals with premanifest HD and healthy control participants in the amplitude of morning cortisol or evening melatonin output at any of the time points sampled (p > 0.05; Table 3.2). Furthermore, no differences were observed in total morning cortisol (p = 0.357) or evening melatonin (p = 0.219) output when subject to area under the curve analysis with respect to ground (AUCG; Table 3.2).

3.4.4 Habitual Sleep Outcomes

Actigraphy

Premanifest HD individuals exhibited a decreased sleep efficiency compared to healthy controls (p = 0.012). A significant increase in the number of awakenings (p = 0.039) and time spent awake after sleep onset (p = 0.028) were also observed in premanifest HD participants compared to healthy controls (Table 3.2).
<table>
<thead>
<tr>
<th></th>
<th>Premanifest HD (n=32)</th>
<th>Healthy Controls (n=29)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic Characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, mean ± SD</td>
<td>44.5 ± 11.4</td>
<td>44.3 ± 10.8</td>
<td>0.472</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>11 (34.4)</td>
<td>8 (27.6)</td>
<td>0.283</td>
</tr>
<tr>
<td><strong>Clinical Characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAGn, mean ± SD</td>
<td>42.8 ± 2.8</td>
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<td>N/A</td>
</tr>
<tr>
<td>Estimated age of onset, median (IQR)</td>
<td>47.4 (44.9-55.7)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Diagnostic Confidence Level, mean ± SD</td>
<td>0.38 ± 0.70</td>
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<td>N/A</td>
</tr>
<tr>
<td>UHDRS-TMS, mean ± SD</td>
<td>4.35 ± 7.24</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Disease burden score, mean ± SD</td>
<td>305.0 ± 76.8</td>
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<td>N/A</td>
</tr>
<tr>
<td>CAPs, mean ± SD</td>
<td>0.89 ± 0.18</td>
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<td>N/A</td>
</tr>
<tr>
<td>BMI, mean ± SD</td>
<td>26.47 ± 4.5</td>
<td>26.75 ± 5.9</td>
<td>0.417</td>
</tr>
<tr>
<td>Smoker, n (%)</td>
<td>6 (18.8)</td>
<td>0 (0.0)</td>
<td>0.007*</td>
</tr>
<tr>
<td>High alcohol consumption, n (%)**</td>
<td>5 (15.6)</td>
<td>6 (20.7)</td>
<td>0.303</td>
</tr>
<tr>
<td>Psychotropic medication, n (%)</td>
<td>3 (9.4)</td>
<td>5 (17.2)</td>
<td>0.181</td>
</tr>
<tr>
<td>Monthly METs, mean ± SD</td>
<td>3278.0 ± 6272.5</td>
<td>7481.2 ± 9584.5</td>
<td>0.022*</td>
</tr>
<tr>
<td>METs (previous day), mean ± SD</td>
<td>168 ± 359.2</td>
<td>85.1 ± 211.8</td>
<td>0.141</td>
</tr>
</tbody>
</table>

Group differences were analysed using t-tests for continuous variables and two sample proportion tests for categorical variables. Disease burden score was calculated using the formula: age x (CAGn – 35.5). CAPs was calculated using the formula: (age x (CAGn - 33.66))/432.3326. *Results are significant at p < 0.05. **High alcohol consumption equates to in excess of 14 alcoholic drinks per week. HD = Huntington’s disease; CAGn = number of cytosine-adenine-guanine repeats; UHDRS-TMS = Unified Huntington’s Disease Rating Scale-Total Motor Score; CAPs = scaled CAG age product score; BMI = body mass index; MET= metabolic equivalents; METs (previous day) refers to the METs calculated for the day prior to saliva sampling; N/A = not applicable.
Consensus Sleep Diary

No significant differences were observed in self-report sleep outcomes using the Consensus Sleep Diary (Table 3.2).

Subjective Sleep Outcomes

There were no significant differences between premanifest HD individuals and healthy controls in global PSQI score (p = 0.134). The average PSQI score in the premanifest HD group fell above the cut-off of 5, suggesting a disruption in sleep quality. Although healthy controls scored significantly higher than premanifest HD individuals on the ESS, scores for both groups remained within the normative range (< 10) (Johns, 1991).

3.4.5 Stress, Anxiety and Depression Questionnaires

No significant differences were observed in stress (p = 0.062), anxiety (p = 0.164) or depression (p = 0.427) symptomatology between premanifest HD and healthy individuals (Table 3.2). Furthermore, values on the Perceived Stress Scale and HADS were considered below threshold, indicating no clinically meaningful stress, anxiety or depressive symptomatology in the premanifest HD and healthy control groups.

3.4.6 Physical Activity

The premanifest HD group reported significantly less monthly physical activity (METs) than healthy controls (p = 0.022; Table 3.1). Physical activity on the day prior to saliva sampling did not differ between the groups, negating any acute effects of physical activity on hormone levels. Furthermore, there was no association between reported physical activity levels and measures of sleep, cortisol and melatonin output and hypothalamic volume.
Table 3.2. Cortisol and melatonin values, subjective and objective sleep outcomes and stress, anxiety and depression questionnaire scores for premanifest Huntington’s disease and healthy control participants.

<table>
<thead>
<tr>
<th></th>
<th>Premanifest HD</th>
<th>Healthy Controls</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cortisol nmol/L (mean ± SD)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time of awakening (hh:mm)</td>
<td>6:06 ± 1:01</td>
<td>6:21 ± 0:45</td>
<td>0.141</td>
</tr>
<tr>
<td>Time of sample relative to awakening:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+15 mins</td>
<td>11.08 ± 4.40</td>
<td>11.28 ± 3.32</td>
<td>0.421</td>
</tr>
<tr>
<td>+30 mins</td>
<td>13.84 ± 4.59</td>
<td>13.42 ± 4.29</td>
<td>0.357</td>
</tr>
<tr>
<td>+45 mins</td>
<td>13.69 ± 3.56</td>
<td>13.30 ± 4.41</td>
<td>0.352</td>
</tr>
<tr>
<td>+60 mins</td>
<td>12.26 ± 3.47</td>
<td>11.62 ± 3.72</td>
<td>0.244</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;G&lt;/sub&gt;</td>
<td>38.17 ± 11.30</td>
<td>39.20 ± 10.61</td>
<td>0.357</td>
</tr>
<tr>
<td><strong>Melatonin (pg/mL)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reported usual bedtime (hh:mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-2 hrs</td>
<td>10.87 ± 7.68</td>
<td>10.02 ± 6.19</td>
<td>0.319</td>
</tr>
<tr>
<td>-1 hr</td>
<td>12.16 ± 7.07</td>
<td>13.71 ± 7.84</td>
<td>0.210</td>
</tr>
<tr>
<td>+0 hrs</td>
<td>17.10 ± 9.30</td>
<td>19.44 ± 11.10</td>
<td>0.187</td>
</tr>
<tr>
<td>+1 hr</td>
<td>19.42 ± 12.02</td>
<td>22.07 ± 11.11</td>
<td>0.188</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;G&lt;/sub&gt;</td>
<td>49.20 ± 25.88</td>
<td>44.41 ± 21.77</td>
<td>0.219</td>
</tr>
<tr>
<td>Actigraphy measures:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total time in bed (min)</td>
<td>464.28 ± 46.73</td>
<td>458.58 ± 43.10</td>
<td>0.680</td>
</tr>
<tr>
<td>Total sleep time (min)</td>
<td>403.17 ± 45.20</td>
<td>415.12 ± 40.25</td>
<td>0.365</td>
</tr>
<tr>
<td>Sleep onset latency (min)</td>
<td>7.10 ± 6.80</td>
<td>4.39 ± 3.77</td>
<td>0.106</td>
</tr>
<tr>
<td>Wake after sleep onset (min)</td>
<td>54.02 ± 25.97</td>
<td>39.07 ± 16.87</td>
<td>0.028*</td>
</tr>
<tr>
<td>Number of awakenings</td>
<td>18.47 ± 6.72</td>
<td>14.55 ± 5.33</td>
<td>0.039*</td>
</tr>
<tr>
<td>Average duration of awakenings (min)</td>
<td>2.87 ± 0.64</td>
<td>2.75 ± 0.87</td>
<td>0.625</td>
</tr>
<tr>
<td>Sleep efficiency (%)</td>
<td>86.98 ± 5.42</td>
<td>90.68 ± 3.75</td>
<td>0.012*</td>
</tr>
<tr>
<td>PSQI global score</td>
<td>5.76 ± 3.02</td>
<td>4.97 ± 2.44</td>
<td>0.134</td>
</tr>
<tr>
<td>Epworth Sleepiness Scale Score</td>
<td>4.69 ± 3.61</td>
<td>6.76 ± 3.37</td>
<td>0.025</td>
</tr>
<tr>
<td>Consensus Sleep Diary:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total time in bed (min)</td>
<td>430.42 ± 61.33</td>
<td>434.84 ± 68.48</td>
<td>0.372</td>
</tr>
<tr>
<td>Total sleep time (min)</td>
<td>385.82 ± 53.90</td>
<td>387.92 ± 74.77</td>
<td>0.453</td>
</tr>
<tr>
<td>Sleep onset latency (min)</td>
<td>16.65 ± 19.90</td>
<td>14.35 ± 13.92</td>
<td>0.303</td>
</tr>
<tr>
<td>Wake after sleep onset (min)</td>
<td>19.04 ± 25.37</td>
<td>16.93 ± 22.89</td>
<td>0.412</td>
</tr>
<tr>
<td>Sleep efficiency</td>
<td>90.52 ± 5.17</td>
<td>89.32 ± 9.96</td>
<td>0.487</td>
</tr>
<tr>
<td>Number of awakenings</td>
<td>1.25 ± 1.47</td>
<td>1.75 ± 1.03</td>
<td>0.057</td>
</tr>
<tr>
<td>Average duration of awakenings (min)</td>
<td>10.18 ± 23.46</td>
<td>8.97 ± 11.11</td>
<td>0.059</td>
</tr>
<tr>
<td>Restorative quality of sleep</td>
<td>3.75 ± 0.81</td>
<td>3.80 ± 0.92</td>
<td>0.411</td>
</tr>
<tr>
<td>Perceived Stress Scale</td>
<td>17.12 ± 6.72</td>
<td>19.86 ± 6.85</td>
<td>0.062</td>
</tr>
<tr>
<td>HADS:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total score</td>
<td>7.54 ± 5.30</td>
<td>8.38 ± 5.69</td>
<td>0.276</td>
</tr>
<tr>
<td>Anxiety</td>
<td>5.06 ± 3.48</td>
<td>6.00 ± 3.97</td>
<td>0.164</td>
</tr>
<tr>
<td>Depression</td>
<td>2.49 ± 2.52</td>
<td>2.38 ± 2.14</td>
<td>0.427</td>
</tr>
</tbody>
</table>

Data are reported as mean and standard deviation, unless stated otherwise. Group differences were analysed using t-tests. AUC<sub>G</sub> was calculated using the trapezoid rule for morning cortisol and evening melatonin output using the curve generated from the average cortisol values and the average melatonin values, respectively. Sleep efficiency was calculated using the formula: (total sleep time/total time in bed) x 100. PSQI score greater than 5 indicates poor sleepers. The Consensus Sleep Diary - restorative quality of sleep item is a Likert scale ranging from 1 (not restorative) to 5 (very restorative). Higher Perceived Stress Scale scores indicate greater stress. HADS sub-scores greater than 7 indicate clinically relevant anxiety and depression symptomatology. *Results are significant at p < 0.05.

HD= Huntington’s disease; AUC<sub>G</sub>= area under the curve with respect to ground; PSQI= Pittsburgh Sleep Quality Index; HADS= Hospital Anxiety and Depression Scale.
A significant negative association was revealed by voxel-wise analysis between grey matter volume in the left hypothalamus and morning cortisol output in the premanifest HD group. This association was found to be moderate ($r = -0.39$), indicating that lesser grey matter volume in the left side of the hypothalamus is associated with greater morning cortisol output. No significant association was observed in the healthy control group (Figure 3.1E and Table 3.3). This group difference in the association between hypothalamic volume and morning cortisol output was statistically significant, as shown by interaction analysis (Figure 3.1C, Figure 3.1E and Table 3.3). The group by cortisol output interaction encompassed two separate areas – first, the region spanning the posterior hypothalamus and the superior tuberal hypothalamus and second, the anterior-inferior hypothalamic region.

A negative relationship between grey matter volume in the right side of the hypothalamus and morning cortisol output was seen in healthy controls, but not in premanifest HD participants, and the group by cortisol output interaction was significant (Table 3.3). However, the association between right hypothalamic grey matter volume and morning cortisol output in healthy controls was weak ($r = -0.06$), indicating a non-existent relationship in this case.

Grey matter volume in the left side of the hypothalamus was positively associated with evening melatonin output in healthy controls ($r = 0.36$), but not in premanifest HD individuals (Figure 3.1F and Table 3.3). This group difference in the correlation between hypothalamic volume and evening melatonin output was statistically significant (Figure 3.1D, Figure 3.1F and Table 3.3). The group by melatonin output interaction encompassed the region extending from the superior tuberal hypothalamus to the anterior-inferior hypothalamus.
Table 3.3. Association analyses between hypothalamic volume and CAPs, cortisol and melatonin

<table>
<thead>
<tr>
<th></th>
<th>Side</th>
<th>k</th>
<th>Peak voxel</th>
<th>t score</th>
<th>MNI coordinates</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CAPs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative association</td>
<td>B</td>
<td>204</td>
<td></td>
<td>3.16</td>
<td>-3 2 -7</td>
</tr>
<tr>
<td>with CAPs in</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>premanifest HD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cortisol</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative association</td>
<td>L</td>
<td>161</td>
<td></td>
<td>2.54</td>
<td>-10 1 -4</td>
</tr>
<tr>
<td>with cortisol output</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>in premanifest HD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group by cortisol</td>
<td>L</td>
<td>186</td>
<td></td>
<td>2.32</td>
<td>-10 1 -4</td>
</tr>
<tr>
<td>output interaction</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Melatonin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive association</td>
<td>L</td>
<td>161</td>
<td></td>
<td>2.67</td>
<td>-10 1 -6</td>
</tr>
<tr>
<td>with melatonin output</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>in healthy controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group by melatonin</td>
<td>L</td>
<td>201</td>
<td></td>
<td>2.41</td>
<td>-6 2 -8</td>
</tr>
<tr>
<td>output interaction</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Group differences were analysed using two-sample t-tests.
*Results are significant at $p < 0.05$, uncorrected.
$k =$ No. of voxels; MNI = Montreal Neurological Institute; HD = Huntington’s disease; B = bilateral; L = left.

3.4.8 Associations Between Hypothalamic Volume and Habitual Sleep Outcomes

No reliable pattern of associations was observed between hypothalamic volume and measures of subjective sleep quality, sleep onset latency, number of awakenings, sleep efficiency or perceived stress in the premanifest HD or healthy cohorts.

3.5 Discussion

Hypothalamic pathology and disturbances in circadian rhythm and sleep arise during the premanifest stages of HD. Despite the central role of the hypothalamus in mediating the circadian rhythm and sleep-wake timing, only one study has attempted to discern the possible relationship between hypothalamic pathology and sleep disturbances in individuals with HD (Baker et al., 2016). In the absence of robust data, the aim of this study was to examine the potential relationship between hypothalamic pathology and circadian rhythm and habitual sleep disturbances in individuals with premanifest HD.
Consistent with previous findings (Soneson et al., 2010), significantly reduced grey matter volume was observed in the hypothalamus of individuals with premanifest HD compared to healthy controls. Using normative parcellations of the hypothalamus that rely on visible anatomic landmarks in MR images (Makris et al., 2013), this reduced grey matter volume can be located to the anterior-superior region of the hypothalamus, a region comprising the paraventricular nucleus (PVN), which mediates the release of cortisol and melatonin. Reduced hypothalamic volume was found to be associated with CAP score, suggesting a relationship between estimated time to disease onset and loss of hypothalamic grey matter volume. Interestingly, degeneration within the hypothalamus was leftward biased. The reason for this is unknown. Studies have reported a leftward-biased pattern of grey matter atrophy in the striatum in individuals with manifest HD (Minkova et al., 2018; Mühlau et al., 2007), however, this has not been reported in the striatum or the hypothalamus in individuals with premanifest HD. Minkova et al. (2018) postulated that the earliest pathological changes in the hypothalamus occur on the left side and become more apparent as individuals approach clinical onset, however additional research is needed to support this supposition.

The pathological mechanisms responsible for reduced hypothalamic volume in individuals with HD are not yet understood. Evidence from post-mortem investigations and studies in mouse models provides insight into potential mechanisms by which hypothalamic changes could occur, as well as mechanisms by which these changes could impact on circadian rhythm and habitual sleep outcomes. For example, neuronal inclusions of mutant huntingtin in suprachiasmatic nucleus (SCN) tissue at post-mortem (Aziz et al., 2008), may directly mediate changes in the functioning of the SCN. Such neuronal inclusions of mutant huntingtin within the SCN could reduce the number of vasoactive intestinal polypeptide and arginine vasopressin expressing neurons, which are crucial in the regulation of SCN activity (Aton, Colwell, Harmar, Waschek, & Herzog, 2005; Hofman & Swaab, 1994), as well as post-transcriptional
changes in these neuropeptides, which have been reported in HD (van Wamelen et al., 2013). These changes, together with a loss of orexin-releasing neurons in the lateral hypothalamus which has been reported in individuals with HD at post-mortem and in mouse models of HD (Aziz et al., 2008; Petersén et al., 2005), could result in impaired functioning of the hypothalamic nuclei and lead to the disruption of the circadian rhythm and sleep-wake cycle that has been reported in individuals with HD and in HD mouse models (Kudo et al., 2011; Loh, Kudo, Truong, Wu, & Colwell, 2013; Morton, 2013; Morton et al., 2005). These potential mechanisms are complex and interrelated and require further investigation.

The reduction in hypothalamic grey matter volume in individuals with premanifest HD was significantly associated with morning cortisol release. This relationship was not observed in healthy controls. Conversely, the association between hypothalamic volume and evening melatonin concentrations observed in healthy controls was absent in individuals with premanifest HD. Both the group by cortisol output and the group by melatonin output interaction effect on hypothalamic grey matter volume occurred across regions encompassing the SCN and the PVN. Despite significantly reduced habitual sleep efficiency and an increase in the number of awakenings and time spent awake after sleep onset in individuals with premanifest HD, which aligns with previous reports of sleep disturbances in premanifest HD (Lazar et al., 2015), hypothalamic volume was not significantly associated with habitual sleep outcomes. The lack of consistent associations between hypothalamic volume and habitual sleep outcomes was unexpected, especially considering the known role of the hypothalamus in regulating the sleep-wake cycle via the SCN and its connections with the ventrolateral preoptic nucleus and the lateral area within the hypothalamus (Bartlett et al., 2016; Saper et al., 2005).

While inconsistent relationships were observed between hypothalamic volume and habitual sleep outcomes, further studies should assess the impact of reduced hypothalamic volume on changes in the underlying sleep electroencephalogram, which is reported to be altered in HD.
mouse models and in individuals with HD (Fisher et al., 2013; Fisher et al., 2016; Kantor, Szabo, Varga, Cuesta, & Morton, 2013; Lazar et al., 2015; Piano et al., 2017). Such analyses would allow further characterisation of the impact of hypothalamic changes on sleep outcomes in HD.

Despite observing differences in the relationships between hypothalamic grey matter volume and cortisol and melatonin in the premanifest HD cohort compared to healthy controls, no differences in morning cortisol or evening melatonin release were observed between the two groups, which is contradictory to previous reports (Kalliolia et al., 2014; van Duijn et al., 2010). It is important to note that a large proportion of our premanifest HD cohort were females (65.6%). This is of relevance as sex-specific differences in circadian rhythm dysfunction have been reported in HD mouse models (Kuljis et al., 2016). In particular, female HD mice exhibit less severe or delayed changes in activity levels and behavioural fragmentation compared to male HD mice (Kuljis et al., 2016). Therefore, the lack of differences in cortisol and melatonin release between individuals with premanifest HD and healthy controls may be related to sex-specific effects.

Conceivably, the right side of the hypothalamus, or indeed other structures involved in the release of cortisol and melatonin, such as the pituitary or pineal glands, may be able to compensate for the reduced hypothalamic volume during the premanifest stages of the disease and thereby maintain normal regulation of cortisol and melatonin release. This is supported by emerging evidence indicating the presence of compensatory neural functions in individuals with HD (Gregory et al., 2017; Scheller, Minkova, Leitner, & Kloppel, 2014). This theory nevertheless requires further validation in larger longitudinal studies.

This study is not without limitations. Firstly, current imaging methods are not yet sensitive enough to capture the individual nuclei within the hypothalamus; however, parcellation
approaches that rely on anatomic landmarks visible on MR images (e.g. Makris et al., 2013) allow the distinction of the different hypothalamic regions, which affords some insight into which structures may be affected. Secondly, this study evaluated cortisol and melatonin regulation using saliva sampling, which is known to be more variable than blood sampling. However, salivary cortisol and melatonin sampling was preferred to blood sampling as previous studies indicate that blood sampling elevates cortisol levels (Weckesser et al., 2014). Thirdly, this study included a large proportion of females with HD, which may have influenced our ability to find significant differences in cortisol/melatonin release between individuals with premanifest HD and healthy controls. Discrepancies in cortisol and melatonin findings between this study and others may also reflect differences in measurement protocols (i.e., frequency of sampling time points), patient characteristics or seasonal differences (Kallioli et al., 2014; Stoathard et al., 2017; van Duijn et al., 2010). A lack of concordance also existed between the habitual sleep measures, potentially due to differences in reporting methods (i.e. subjective versus objective). No evidence of elevated stress, anxiety or depression was noted, indicating that mood disorders did not impact on markers of circadian rhythm or habitual sleep. Future studies should assess the relationship between hypothalamic pathology and sleep architecture in individuals with premanifest HD using polysomnography.

In summary, our findings show that individuals with premanifest HD exhibit leftward biased hypothalamic pathology that is differentially associated with markers of circadian rhythm, but not consistently associated with habitual sleep-wake deficits, when compared to healthy controls. However, the lack of differences in concentrations of markers of circadian rhythm between the two groups suggests the possibility of neural compensation, facilitated by the right hemisphere of the hypothalamus or by other brain structures involved in the circadian and sleep cycles, as a mechanism involved in maintaining the regulation of the circadian rhythm and habitual sleep-wake function in individuals with premanifest HD. Larger, longitudinal studies
are required to further investigate the role of hypothalamic pathology in circadian rhythm and habitual sleep-wake disturbances in HD as the disease course lengthens.

3.6 Acknowledgements

We would like to acknowledge the assistance of Ms Linda Hoult, Mr Timothy Rankin, Dr Catarina Kordsachia and Professor Brian Power.

3.7 Sources of Funding

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3.8 Conflicts of Interest

None.

3.9 Author Contributions

DMB, MZ and TMC conceptualised and ran the study. JFDD analysed brain imaging data. AR assisted with statistical analyses. PZ assisted with sample collection and interpretation of data. KWF assisted with collection, analysis and interpretation of brain imaging data. RN and JAS assisted with actigraphy data collection and analysis. AJH, PRE and ASL contributed to the design of the study and interpretation of data. DMB, JFDD, MZ and TMC wrote the manuscript. All authors contributed to the writing and revision of the manuscript.
3.10 Supplementary Data

Acquisition of MRI Data

Scans were obtained at two locations: 49 participants were scanned at Perth Radiological Clinic using a 3T GE Healthcare Discovery MR750w MRI scanner with a 24-channel head coil using an IR-SPGR sequence (TA = 9 m 59 s, TR = 3 s, TE = Min, TI = 400 ms, flip angle = 11˚, field of view = 256 mm, image matrix = 256 x 256, 1 mm3 isotropic voxels); 12 participants were scanned at Monash Biomedical Imaging in Melbourne using a 3T Siemens Skyra MRI scanner with a 32-channel head coil using an MP-RAGE sequence (TA = 9 m 14 s, TR = 2.3 s, TE = 2.96 ms, TI = 900 ms, flip angle = 9˚, field of view = 256 mm, image matrix = 256 x 256).

Pre-Processing of MRI Data

Images were pre-processed according to a SPM12 pipeline (http://www.fil.ion.ucl.ac.uk/spm) running on MATLAB R2015a (MathWorks Inc., USA). Segmentation of the T1-weighted images into grey and white matter tissue was performed (Ashburner & Friston, 2005) and then registered through Diffeomorphic Anatomical Registration through Exponentiated Lie algebra (DARTEL) (Ashburner, 2007). The resulting study-specific template was registered to Montreal Neurological Institute (MNI) space. The normalisation procedure included modulating the grey matter tissue probability maps by the Jacobian determinants of the deformation field. As the analysis focused on the hypothalamus, the normalised grey matter images were smoothed with a 6 mm full width at the half maximum Gaussian kernel, which is more appropriate for small structures (Salmond et al., 2002).

MRI Site Consistency Analysis

To ensure consistency of MR imaging across scanning sites, we evaluated a range of morphometric and volumetric measurements across three participants scanned twice at both
sites. First, we obtained an estimate of individual hypothalamic volume per participant from images pre-processed according to the voxel-based morphometry pipeline described above. The estimate of hypothalamic volume corresponded to the average intensity across all voxels in a hypothalamus mask (from the WFU Pick Atlas—http://fmri.wfubmc.edu/software/pickatlas—dilated by 3 mm) (Breen et al., 2016). In addition, grey and white matter volumes were also calculated (in mm3) with SPM12’s Tissue Volumes tool form the output of the Segment routine. Volumes for caudate, putamen, pallidum, brain stem, thalamus and hippocampus were also obtained (in mm3) after segmentation with FMRIB’s Integrated Registration and Segmentation Tool from the raw T1-weighted images (Patenaude, Smith, Kennedy, & Jenkinson, 2011). All segmentations were visually inspected to ensure their accuracy.

As a further quality assurance procedure, whole-brain, voxel-based morphometry analysis was carried out to ascertain baseline group differences in grey matter volume between premanifest Huntington’s disease and healthy control participants. Spatial pre-processing was the same as before except that images were smoothed with an 8 mm full width at the half maximum (FWHM) Gaussian kernel.

Three participants were scanned twice in Perth and Melbourne (i.e., we obtained two structural images from each participant at each site). Parcellation of the T1-weighted images into grey and white matter tissue was performed with the Segment routine in SPM12 (Ashburner & Friston, 2005). Grey and white matter volume was then calculated with SPM12’s Tissue Volumes tool. We then used voxel-based morphometry to extract a measure of grey matter volume from the hypothalamus. Grey and white matter tissue images resulting from the Segment routine were co-registered through DARTEL (Ashburner, 2007). The resulting study-specific template was registered to MNI space. The normalisation procedure included
modulating the grey matter tissue probability maps by the Jacobian determinants of the deformation field. A hypothalamus mask from the WFU Pick Atlas (http://fmri.wfubmc.edu/software/pickatlas) was used (dilated by 3mm; Figure S1) to obtain an estimate of individual hypothalamic volume per participant (the average intensity across all voxels in the mask) (Breen et al., 2016). Grey matter volume in the region of interest was calculated using the FMRIB Software Library (FSL) tool “fslstats” within FSL version 5.0.9 (www.fmrib.ox.ac.uk).

Segmentation of other subcortical structures (comprising caudate, putamen, pallidum, brain stem, thalamus and hippocampus) was performed with FMRIB’s Integrated Registration and Segmentation Tool (FIRST) from the raw T1-weighted images. All segmentations were visually inspected to ensure their accuracy.

We then calculated, for all measurements (whole-brain grey and white matter, hypothalamus, caudate, putamen, pallidum, brain stem, thalamus and hippocampus), the average percent absolute difference within each participant across both sites as well as the average percent

*Figure S3.2. Hypothalamus mask (in red). Slice labels are displayed on top. L, left*
absolute difference across participants between both sites. Measurements as well as within and between site differences are displayed in Figures S3.3 and S3.4.

Results show a high level of consistency across within and between site measurements of all structures. In addition, discrepancies between within and between site differences did not reveal a consistent pattern (i.e., between site differences were not consistently larger than within site differences or vice versa).

Figure S3.3. Volume measurements for three participants scanned twice at each site.

Shades of blue indicate scans from Perth and shades of orange denote scans from Melbourne. Lighter shades (of blue or orange) represent first scan, darker shades designate second scan. Measurements from subjects 1, 2 or 3 are grouped across plots A-D as indicated in plot D. Measurements in plot D correspond to arbitrary VBM units. GM, grey matter; WM, white matter.
Whole-Brain, Voxel-Based Morphometry Analysis

Whole-brain, voxel wise statistical analysis was carried out to ascertain baseline group differences in grey matter volume between the 33 premanifest HD and 29 control participants. The premanifest HD sample in this analysis included one extra participant not included in the main paper as they contributed incomplete sleep data (results were almost identical including or excluding the extra participant. Here we report the full sample). Spatial pre-processing followed the same SPM12 pipeline as all other voxel-based morphometry analyses in this paper (except for the smoothing kernel used = FWHM 8 mm). Images from premanifest HD and controls were then contrasted using a two sample t-test and cluster correction at FWE p = 0.05 (with a cluster defining threshold p = 0.001 and a voxel extent threshold k = 20). Sex, site and age were included as covariates of no interest.

Results revealed significant grey matter loss in the striatum in premanifest HD compared to controls (Figure S3.5). This is as expected for individuals at this stage of the disease, consistent with previous studies (i.e., Tabrizi et al., 2009). The well-known caudo-rostral and dorso-ventral gradient of striatal neurodegeneration is apparent (Douaud et al., 2006; Kassubek et al., 2004).
Figure S3.5. VBM analysis of striatal grey matter volume

Results from VBM analysis showing statistically significant grey matter loss in the striatum in premanifest HD compared to controls. Peak activation voxel on the right, MNI 13 -5 19, t = 6.22; and on the left, MNI -11 -5 19, t = 5.95. Slice labels are displayed on top. L, left

Salivary Cortisol and Melatonin Collection and Analysis

To avoid contamination of saliva samples, participants were asked to refrain from consuming alcohol 12 hours prior and avoid eating, drinking (with the exception of water) and brushing their teeth one hour prior to sampling. For melatonin sampling, participants were also instructed to wear sunglasses and remain in a dim lit room during the three-hour sampling timeframe to avoid suppressive effects of light on melatonin rise. Based on these criteria, a questionnaire was devised to monitor participant compliance. Saliva samples were transported on dry ice from Melbourne to Perth and were stored at -80°C until analysis at Edith Cowan University, Western Australia. Salivary cortisol and melatonin concentrations were then measured in duplicate using salivary cortisol and melatonin ELISA kits (Salimetrics) according to
manufacturer’s instructions. The limit of detection of the cortisol and melatonin ELISAs was reported to be 70 pg/mL and 1.37 pg/mL, respectively.

**Physical Activity**

Metabolic equivalents (MET) were calculated using recorded physical activity levels, the Compendium of Physical Activities database and estimated resting metabolic rate (RMR) with the following formula:

Corrected MET value = MET value (from Compendium code) \times 3.5 \text{ ml.kg/Harris-Benedict}

RMR was calculated using the Harris-Benedict equation seen below:

\[
\text{Male} = 66.4730 + 5.0033 (\text{Height cm}) + 13.7516 (\text{Weight kg}) - 6.7550 (\text{Age yr})
\]

\[
\text{Female} = 655.0955 + 1.8496 (\text{Height cm}) + 9.5634 (\text{Weight kg}) - 4.6756 (\text{Age yr})
\]

**Statistical Analyses**

**Hypothalamic Volume**

The hypothalamus mask (dilated by 3 mm) from the WFU Pick Atlas was used to restrict analysis to this area (Breen et al., 2016). Results are reported in MNI space with a voxel size of 1 mm isotropic and displayed on the ch256 anatomical template available as part of MRIcroGL (http://www.mccauslandcenter.sc.edu/mricrogl/). To illustrate the group by continuous covariate interactions in relevant scatterplots and to estimate the strength of the relationship we used the MarsBaR SPM12 tool box (Brett, Anton, Valabregue, & Poline, 2002) to extract parameter estimates from relevant contrasts (grey matter average of all significant voxels in the relevant contrast).
The interaction model used to assess differences in the association between hypothalamic volume and cortisol/melatonin output in individuals with premanifest HD compared to healthy controls included group regressors, one for each group, and regressors modelling cortisol or melatonin, one for each group. Gender, site, age and Perceived Stress Scale, ESS and PSQI scores were included as covariates of no interest. In premanifest HD, we adjusted also for CAG Age Product score (CAPs). Covariates of interest in the above models were mean centred across both groups. Furthermore, to determine whether hypothalamic volume was associated with measures of subjective sleep quality (PSQI), behavioural sleep/wake data (Consensus Sleep Diary; sleep onset latency, number of awakenings, sleep efficiency) and stress (Perceived Stress Scale), we used a categorical by continuous covariate interaction model, adjusting for evening melatonin, gender and age, as well as CAPs in premanifest HD. Although measurements across sites were found to be consistent and whole-brain differences between premanifest HD and controls were as expected, we also included site as a covariate of no interest in all statistical analyses to remove any residual site effects.

Contrasts modelled positive and negative associations between each group and cortisol or melatonin ([0 0 1 0], [0 0 -1 0], [0 0 0 1], and [0 0 0 -1]) as well as group differences in these associations ([0 0 1 -1] and [0 0 -1 1]); that is, the group by cortisol/melatonin output interaction effect.

Salivary Cortisol and Melatonin

The intra- and inter-assay coefficients of variation were below 10% for all cortisol and melatonin ELISAs. One sample was removed due to suspected blood contamination. Samples were taken on two consecutive days, with each time point on day 1 correlating significantly with its counterpart on day 2 (r = 0.891, p < 0.001). Area under the curve with respect to
ground (AUCG) was calculated using the trapezoid rule for morning cortisol and evening melatonin output on the two consecutive days (Dijk et al., 2012; van Duijn et al., 2010).
Chapter 4

Multidisciplinary Rehabilitation Reduces Hypothalamic Grey Matter Volume Loss in Individuals with Premanifest Huntington’s Disease: An Exploratory Study

Original Article

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4.1 Abstract

Objective: Hypothalamic pathology has been reported in Huntington’s disease (HD) a decade prior to predicted clinical onset and has been proposed to contribute to circadian rhythm and habitual sleep disturbances. Currently, no therapies exist to combat hypothalamic changes, nor circadian rhythm and habitual sleep disturbances in HD. Therefore, we aimed to assess whether multidisciplinary rehabilitation could reduce the loss of hypothalamic volume and improve circadian rhythm and habitual sleep outcomes in individuals with premanifest HD.

Methods: Eighteen individuals with HD (ten premanifest and eight prodromal) undertook a nine-month multidisciplinary rehabilitation intervention (intervention group) and were compared to a community sample of eleven individuals with premanifest HD receiving standard care (control group). Hypothalamic volume, blood-based BDNF, salivary cortisol and melatonin concentrations, subjective sleep quality, daytime somnolence, habitual sleep-wake patterns and stress, anxiety and depression symptomatology were evaluated.

Results: A significant decrease in hypothalamic grey matter volume loss was observed in the intervention group compared to the control group. This was accompanied by a maintenance of BDNF levels in the intervention group, whereas BDNF levels in the control group decreased significantly. Daytime somnolence and anxiety and depression symptomatology were also significantly reduced in the intervention group relative to controls. Both the intervention and control groups exhibited decreases in cortisol and melatonin concentrations following the nine-month study period.

Conclusion: This study provides preliminary evidence that multidisciplinary rehabilitation can significantly reduce the rate of hypothalamic volume loss, maintain peripheral BDNF levels and improve sleep and mood outcomes in premanifest HD, but does not have a robust effect
on markers of circadian rhythm. Larger, randomised controlled trials are required to confirm these findings.

4.2 Introduction

Hypothalamic pathology, particularly grey matter volume loss and microglial activation, has been reported as early as a decade prior to clinical manifestation of Huntington’s disease (HD) (Politis et al., 2008; Soneson et al., 2010). Degeneration within the hypothalamus, particularly the suprachiasmatic nucleus (SCN), which is responsible for generating the circadian rhythm, is believed to underpin circadian rhythm and habitual sleep disturbances in individuals with HD (Aziz et al., 2010; Bartlett et al., 2016; Moore, 1995; Morton et al., 2005; Saper et al., 2005).

Disturbances in circadian rhythm and sleep are documented in HD mouse models, as well as in humans (Kudo et al., 2011; Lazar et al., 2015; Morton, 2013). Circadian changes appear to commence during the premanifest stages of HD and worsen as the course of the disease lengthens. In particular, early changes in the circadian regulation of cortisol and melatonin and a sleep phase delay have been noted in individuals with premanifest HD (Aziz et al., 2010; Aziz, Pijl, Frölich, Schroder-van der Elst, et al., 2009; Aziz, Pijl, Frölich, van der Graaf, et al., 2009; Kalliolia et al., 2014; van Duijn et al., 2010). Furthermore, studies in the R6/2 HD mouse model have revealed altered night-day activity ratios and this altered night-day activity is recapitulated in individuals with manifest HD (Morton et al., 2005). These alterations in circadian rhythm could potentially mediate sleep deficits that have been reported in HD (Lazar et al., 2015). Lazar et al (2015) reported early changes in sleep architecture, particularly increased sleep fragmentation, prior to clinical onset of HD. Given that the onset of circadian rhythm and disturbances occur during the premanifest phase of HD, early treatments aimed at reducing or ameliorating degeneration of the hypothalamus or targeting circadian rhythm and habitual sleep disturbances are warranted.
Evidence from animal models and other clinical populations suggests that interventions comprising exercise have the potential to improve circadian rhythm and sleep outcomes (Cuesta et al., 2014; Nascimento et al., 2014), although it is not known if these improvements are mediated by changes in the hypothalamus. In patients with HD, our team has previously shown that multidisciplinary rehabilitation, involving exercise and cognitive training, enhances brain volume in the striatum and prefrontal cortex and improves cognition and motor function (Cruickshank et al., 2015; Thompson et al., 2013). The exact mechanism by which multidisciplinary rehabilitation improves brain volume in these areas is not yet known. A possibility is that multidisciplinary rehabilitation exerts its effects on the brain by upregulating levels of brain derived neurotrophic factor (BDNF), since BDNF is vital for neurogenesis following environmental enrichment in animal models (Rossi et al., 2006). This is supported by studies in HD mouse models, in which BDNF levels were rescued following environmental enrichment, as well as by research into other neurodegenerative and clinical populations, whereby increases in BDNF levels have been reported following intervention paradigms comprising an exercise component (Cotman & Berchtold, 2002; Frazzitta et al., 2014; Spires et al., 2004).

In the present study, we aimed to examine the effects of a nine-month multidisciplinary rehabilitation intervention on BDNF levels, hypothalamic volume, circadian rhythm, and habitual sleep outcomes in individuals with premanifest HD. In line with findings suggesting an increase in BDNF levels following multimodal exercise paradigms (Nascimento et al., 2014) and with the proposed role of the hypothalamus in circadian rhythm disturbances in HD (Aziz et al., 2008; Bartlett et al., 2016), we hypothesised that multidisciplinary rehabilitation would increase BDNF levels, attenuate hypothalamic volume loss and improve circadian rhythm and habitual sleep outcomes in individuals with premanifest HD.
4.3 Materials and Methods

4.3.1 Study Design

The present investigation was a controlled exploratory study on the effects of nine months of multidisciplinary rehabilitation on hypothalamic volume, blood-based BDNF, markers of circadian rhythm and habitual sleep-wake outcomes in individuals with premanifest HD. Subjective sleep quality, daytime somnolence and stress, anxiety and depression symptomatology were also examined. Study participants were allocated to receive nine months of either multidisciplinary rehabilitation (intervention group) or standard care (control group). The length of the intervention was informed by our previous work (Cruickshank et al., 2015; Thompson et al., 2013).

4.3.2 Study Approval and Patient Consent

All aspects of the study were conducted in accordance with the declaration of Helsinki. Ethical approval for study procedures was granted by the North Metropolitan Area Mental Health Service (2009_16), Edith Cowan University (13145), Monash University (CF15/117-201500058) and Deakin University (2015-052) human research ethics committees at Perth and Melbourne study sites. All participants provided written informed consent.

4.3.3 Participants

Twenty-seven premanifest HD and eight prodromal HD individuals were recruited in Perth and Melbourne through ENROLL-HD, existing study databases, clinicians and HD community organisations. Inclusion criteria were as follows: 1) Unified Huntington’s Disease Rating Scale (UHDRS) Diagnostic Confidence Level (DCL) of ≤ 2 and 2) a UHDRS Total Motor Score (UHDRS-TMS) of < 5 for premanifest or > 5 for prodromal HD (Reilmann et al., 2014). Exclusion criteria included: 1) concomitant neurological, cardiovascular, musculoskeletal, endocrine, metabolic or sleep disorders, 2) shift work, 3) recent or ongoing substance abuse, and 4) the inability to understand written and verbal English.
4.3.4 Multidisciplinary Rehabilitation Intervention

The multidisciplinary rehabilitation intervention was designed and implemented by an experienced team of clinical exercise physiologists, strength and conditioning experts, cognitive training specialists and neuroscientists. The intervention was fully supervised and consisted of autoregulated periodised aerobic and resistance training, computerised cognitive training, dual task training and social events. Periodised aerobic and resistance training was performed twice weekly for one hour (thirty minutes for each mode of exercise) and comprised resistance, endurance and high-intensity interval training (Harries, Lubans, & Callister, 2015; Jimenez & Paz, 2011). Supervised computerised cognitive training was informed by a meta-analysis and by previous investigations (Lampit, Ebster, & Valenzuela, 2014; Lampit, Hallock, Moss, et al., 2014; Lampit, Hallock, Suo, Naismith, & Valenzuela, 2015; Lampit, Hallock, & Valenzuela, 2014). Computerised cognitive training was performed three times weekly for one hour using NeuroNation (Synaptikon, Berlin, Germany) and Captain’s Log MindPower Builder (BrainTrain Inc., Richmond, VA) software (30 minutes each program) and targeted working memory, visual scanning, processing speed, attention, planning, problem solving and task switching cognitive domains. Cognitive-motor interference training was performed once weekly for one hour and consisted of combined aerobic and resistance training and cognitive exercises (Fritz, Cheek, & Nichols-Larsen, 2015; Yogev-Seligmann, Giladi, Brozgol, & Hausdorff, 2012). Intervention sessions were conducted in small groups (2-4 participants per session) and social events were organised every twelve weeks to encourage social engagement, which is thought to be a zeitgeber that acts to modulate the circadian rhythm (Foster et al., 2013).

4.3.5 MRI Data Acquisition and Pre-processing

T1-weighted structural images of the brain were obtained from each participant in Perth and Melbourne using a GE Healthcare Discovery or a Siemens Skyra 3T MRI scanner, respectively.
In Perth, images were acquired with a 24-channel head coil using an IR-SPGR sequence (TA = 9 m 59 s, TR = 3 s, TE = Min, TI = 400 ms, flip angle = 11°, field of view = 256 mm, image matrix = 256 x 256, 1 mm3 isotropic voxels). In Melbourne, acquisition took place with a 32-channel head coil and an MP-RAGE sequence (TA = 9 m 14 s, TR = 2.3 s, TE = 2.96 ms, TI = 900 ms, flip angle = 9°, field of view = 256 mm, image matrix = 256 x 256). Images were acquired using consistent methodology across both sites according to the Alzheimer’s Disease Neuroimaging Initiative protocols for multi-site imaging (Jack et al., 2008). Three participants also underwent additional scans at both sites to ensure reproducibility. Image pre-processing was conducted according to Breen et al. (2016) using a hypothalamus mask from the WFU Pick Atlas (http://fmri.wfubmc.edu/software/pickatlas), dilated by 3mm to restrict analysis to this area and for displaying results. Results are reported in MNI space with a voxel size of 1 mm isotropic and displayed on the ch256 template. We also used the MarsBaR SPM12 tool box (Brett et al., 2002) to extract parameter estimates from relevant contrasts (average of all significant voxels in the relevant contrast).

4.3.6 Blood-based BDNF Analysis

Blood was collected from participants via venepuncture into serum gel separator tubes (Vacuette, Greiner Bio-one) one hour following awakening. Blood was collected at the same time prior to and following the study period to minimise the potential effects of circadian variation on BDNF levels (Piccinni et al., 2008). The blood was left to clot for at least 30 minutes and then centrifuged at 1800 x g for 10 minutes. Serum was then aliquoted and stored at -80°C until analysis in duplicate using BDNF Emax ELISA kits (Promega, Madison, WI) according to the manufacturer’s instructions (Ciammola et al., 2007).

4.3.7 Salivary Cortisol and Melatonin Analysis

Saliva samples were collected by participants in their own homes. Participants were instructed to passively drool into four separate polypropylene collection tubes (SSI Bio) at four time
points in the morning at 15, 30, 45 and 60 minutes following awakening for morning cortisol analysis and at four time points across the evening at one hour intervals from two hours before their usual bedtime (T1) until one hour after their usual bedtime (T4) for melatonin analysis (van Duijn et al., 2010; Voultsios et al., 1997). To avoid contamination of samples, participants were instructed to refrain from consuming alcohol 12 hours prior and to avoid eating, drinking (with the exception of water) and brushing their teeth within the hour prior to sampling (van Duijn et al., 2010). For melatonin sampling, participants were also instructed to wear sunglasses and to remain in a dimly lit room during the three-hour sampling timeframe to avoid suppressive effects of light on melatonin rise (Voultsios et al., 1997). Based on these criteria, a questionnaire was devised to monitor participant compliance. Saliva samples were stored at -80°C until analysis in duplicate using salivary cortisol and melatonin ELISA kits (Salimetrics, USA) according to the manufacturer’s instructions.

**4.3.8 Subjective Sleep Assessments**

Sleep questionnaires were used to measure habitual sleep/wake timing, sleep quality and daytime somnolence, as used previously (Bartlett et al., 2019 [Chapter 3]). Habitual sleep wake timing was measured using the Consensus Sleep Diary (CSD) (Carney et al., 2012), which was devised to standardise the measurement of habitual sleep parameters. Subjective sleep quality was assessed using the Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989). The PSQI comprises seven components relating to subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleep medication and daytime dysfunction. Scores are summated to provide a global PSQI score, of which a score of greater than five indicates poor sleep quality. Daytime somnolence was assessed using the Epworth Sleepiness Scale, with a score of greater than 10 indicating excessive daytime sleepiness (Johns, 1991). The PSQI and Epworth Sleepiness Scale have been used previously in HD research (Aziz et al., 2010; Lazar et al., 2015).
4.3.9 Mood Measures

Psychological stress over the previous month was measured using the 14-item Perceived Stress Scale (Cohen et al., 1983) and anxiety and depression symptomatology were measured using the Hospital Anxiety and Depression Scale (HADS) (Zigmond & Snaith, 1983). Both scales are deemed valid for use in HD research (De Souza et al., 2010; Downing et al., 2011).

4.3.10 Statistical Analysis

Data from the premanifest (prior to onset of motor signs) and prodromal HD (slight, but no overt, motor signs) intervention groups were combined. This was based on preliminary analyses that showed no differences between any of the primary or secondary outcome measures at baseline between the premanifest and prodromal intervention groups (data on groups separated can be viewed in supplementary files). Data from the combined intervention group was compared to the premanifest HD community sample. To control for clinical differences, UHDRS-TMS was included as a covariate in analyses.

Missing saliva sampling data points were imputed to avoid removing the participant from analyses and to maintain sample size (van Duijn et al., 2010). Area under the curve with respect to ground (AUCₖ) was calculated using the trapezoid rule for morning cortisol and evening melatonin output (Dijk et al., 2012; van Duijn et al., 2010).

Shapiro-Wilk tests were used to test normality assumptions. Baseline group differences were assessed using independent t-tests and Mann-Whitney U tests. Within-group and between-group differences were assessed using a mixed model analysis of variance (ANOVA), with age, gender, CAG repeat and baseline values of the variable of interest included as covariates.

To assess voxel-wise differences in hypothalamic volume change between baseline and nine-month follow-up between intervention and control groups, grey matter Jacobian change images from each respective group were compared using a two-sample t-test, with sex and age included
as covariates of no interest. Using the same grey matter Jacobian change images, we also investigated group differences in the association between change in hypothalamic volume and change in cortisol and/or melatonin output in the intervention compared to the control group, using a categorical by continuous covariate interaction model (see supplementary data for details of this model). Given the exploratory nature of this study and our a priori interest in the hypothalamus, all results are reported at $\alpha = 0.05$.

4.4 Results

4.4.1 Participant Demographic and Clinical Characteristics

Two participants withdrew from the study prior to baseline testing, two withdrew following baseline testing and a further two participants did not provide complete data at follow-up. Ten premanifest HD and eight prodromal HD participants underwent a nine-month multidisciplinary rehabilitation intervention (intervention group). Eleven premanifest HD participants receiving standard care served as a reference group for this study (control group).

Demographic and clinical characteristics for the intervention and control groups are presented in Table 4.1. Age differed significantly between the intervention group and the control group, but no significant differences were observed in gender, CAG repeat number, disease burden score, CAP score, body mass index (BMI), smoking status, alcohol consumption or use of psychotropic medication between the groups. No participants reported changes in the use of sleep medications using the PSQI at baseline or following the nine-month study period. Higher UHDRS-TMS and diagnostic confidence level scores were observed in the intervention group compared to the control group (Table 4.1 and Supplementary Table S4.4) due to the inclusion of individuals with prodromal HD in the intervention group.
Table 4.1. Demographic and clinical characteristics of premanifest plus prodromal HD intervention group and control group at baseline

<table>
<thead>
<tr>
<th></th>
<th>Intervention group (n=18)</th>
<th>Control Group (n=11)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic Characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, mean ± SD</td>
<td>40.89 ± 11.73</td>
<td>50.55 ± 9.49</td>
<td>0.029*</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>6 (33)</td>
<td>4 (36)</td>
<td>0.868</td>
</tr>
<tr>
<td><strong>Clinical Characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAGn, mean ± SD</td>
<td>43.67 ± 3.28</td>
<td>41.91 ± 2.02</td>
<td>0.123</td>
</tr>
<tr>
<td>DCL, mean ± SD</td>
<td>0.67 ± 0.84</td>
<td>0.00 ± 0.00</td>
<td>0.000*</td>
</tr>
<tr>
<td>UHDDS-TMS, mean ± SD</td>
<td>7.56 ± 8.56</td>
<td>0.09 ± 0.30</td>
<td>0.000*</td>
</tr>
<tr>
<td>Disease burden score, mean ± SD</td>
<td>309.52 ± 91.87</td>
<td>309.36 ± 61.51</td>
<td>0.996</td>
</tr>
<tr>
<td>CAPs, mean ± SD</td>
<td>0.89 ± 0.22</td>
<td>0.93 ± 0.13</td>
<td>0.598</td>
</tr>
<tr>
<td>BMI, mean ± SD</td>
<td>26.60 ± 3.85</td>
<td>25.29 ± 1.99</td>
<td>0.303</td>
</tr>
<tr>
<td>Smoker, n (%)</td>
<td>6 (33)</td>
<td>1 (9)</td>
<td>0.172</td>
</tr>
<tr>
<td>High alcohol consumption, n (%)</td>
<td>2 (11)</td>
<td>0 (0)</td>
<td>0.394</td>
</tr>
<tr>
<td>Psychotropic medication, n (%)</td>
<td>2 (11)</td>
<td>3 (27)</td>
<td>0.264</td>
</tr>
</tbody>
</table>

*Values are significant at p ≤ 0.05.
CAGn= cytosine-adenine-guanine repeat number; DCL= diagnostic confidence level; UHDDS-TMS= Unified Huntington’s Disease Rating Scale- Total Motor Score; CAPs= CAG-age product score; BMI= body mass index.

4.4.2 Hypothalamic Volume

Voxel-wise analysis revealed significantly less right hypothalamic grey matter volume loss in the intervention group compared to the control group following the nine-month study period (Table 4.2 and Figure 4.1). The observed differences corresponded to a large effect size (Cohen’s d = 1.1, computed from associated parameter estimates) and suggest that multidisciplinary rehabilitation slows the rate of grey matter loss in the right hypothalamus.
Table 4.2. Results from voxel-based morphometry analysis.

<table>
<thead>
<tr>
<th>Group Effect</th>
<th>Side</th>
<th>No. of voxels</th>
<th>T-score</th>
<th>p-value</th>
<th>MNI Coordinates (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group vs. intervention group</td>
<td>R</td>
<td>321</td>
<td>2.90</td>
<td>0.004</td>
<td>5       2    -7</td>
</tr>
<tr>
<td>Group x Cortisol Interaction Effect</td>
<td>Control group vs. intervention group</td>
<td>R</td>
<td>305</td>
<td>2.47</td>
<td>0.012</td>
</tr>
<tr>
<td>Group x Melatonin Interaction Effect</td>
<td>Control group vs. intervention group</td>
<td>R</td>
<td>86</td>
<td>2.36</td>
<td>0.014</td>
</tr>
</tbody>
</table>

Results are significant at $p < 0.05$, uncorrected.
$k =$ No. of voxels; MNI = Montreal Neurological Institute; R = right.

Figure 4.1. Effects of nine months of multidisciplinary therapy on hypothalamic grey matter (GM) volume.

(A) Group effect on GM volume and bar graphs illustrating the effect. Values in the bar graphs correspond to the mean volume change in each group (after averaging across voxels in each participant). (B) Group by cortisol output change interaction effect on GM volume (control group outliers excluded from the analysis are in cross-hatching). (C) Group by melatonin output change interaction effect on GM volume. Scatterplots in (B) and (C) illustrate the respective interaction effects (a.u., arbitrary units). Heat maps correspond to T-scores thresholded at $p < 0.05$, uncorrected. The maps are displayed at $x = \sim 8$ (MNI) and are scaled to the peak T-score for the relevant analysis (see Table 4.2).
4.4.3 Blood-Based BDNF Analysis

Serum BDNF did not significantly differ at baseline between the intervention and control groups, however, when comparing the two groups at follow-up, a significant difference in BDNF levels was observed ($p = 0.030$; Table 4.3 and Supplementary Table S4.5). Post-hoc analysis revealed a significant decrease in serum BDNF levels in the control group ($p = 0.008$), while serum BDNF levels were maintained in the intervention group over the study period ($p = 0.869$), suggesting that multidisciplinary rehabilitation facilitates the maintenance of serum BDNF levels in premanifest HD.
### Table 4.3: Mean salivary cortisol and melatonin concentrations, subjective sleep outcomes and affective symptomatology outcomes in the premanifest HD intervention and control groups.

<table>
<thead>
<tr>
<th></th>
<th>Within-Group Differences</th>
<th>Between-Group Differences</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intervention group (n=18)</td>
<td>Control group (n=11)</td>
</tr>
<tr>
<td></td>
<td>Baseline</td>
<td>Follow-Up</td>
</tr>
<tr>
<td>Serum BDNF (pg/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>27634.53 ± 27587.55</td>
<td>5870.90</td>
<td>4969.02</td>
</tr>
<tr>
<td>Cortisol (nmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Awakening (hh:mm)</td>
<td>06:16 ± 01:01</td>
<td>06:11 ± 00:52</td>
</tr>
<tr>
<td>AUC_t</td>
<td>40.37 ± 9.69</td>
<td>33.86 ± 9.50</td>
</tr>
<tr>
<td>Melatonin (pg/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bedtime (hh:mm)</td>
<td>22:02 ± 00:41</td>
<td>22:03 ± 00:37</td>
</tr>
<tr>
<td>AUC_t</td>
<td>55.48 ± 32.00</td>
<td>36.34 ± 31.03</td>
</tr>
<tr>
<td>CSD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total time in bed (min)</td>
<td>430.59 ± 57.26</td>
<td>422.50 ± 48.46</td>
</tr>
<tr>
<td>Total sleep time (min)</td>
<td>383.83 ± 55.45</td>
<td>379.69 ± 46.27</td>
</tr>
<tr>
<td>Sleep onset latency (min)</td>
<td>23.20 ± 26.12</td>
<td>20.75 ± 23.05</td>
</tr>
<tr>
<td>WASO (min)</td>
<td>22.00 ± 28.25</td>
<td>11.91 ± 20.30</td>
</tr>
<tr>
<td>Number of awakenings</td>
<td>1.72 ± 1.48</td>
<td>1.31 ± 1.12</td>
</tr>
<tr>
<td>Sleep efficiency (%)</td>
<td>89.48 ± 9.29</td>
<td>90.31 ± 9.20</td>
</tr>
<tr>
<td>Restorative quality of sleep</td>
<td>3.63 ± 0.76</td>
<td>3.70 ± 0.88</td>
</tr>
<tr>
<td>PSQI Global Score</td>
<td>6.00 ± 2.94</td>
<td>5.06 ± 3.15</td>
</tr>
<tr>
<td>Epworth Sleepiness Scale</td>
<td>5.76 ± 3.31</td>
<td>4.71 ± 3.02</td>
</tr>
</tbody>
</table>

*Significant at p < 0.05.*
Data are presented as mean ± standard deviation. Baseline differences between groups were assessed using independent t-tests for parametric and Mann-Whitney U tests for non-parametric data. Mixed model analysis of variance (ANOVA) was used to test within-group and between-group differences at follow-up. Partial η², 0.01, 0.06 and 0.14 are defined as small, medium and large effects, respectively.

*Values are significant at p < 0.05

CSD= Consensus Sleep Diary; WASO= wake after sleep onset; PSQI= Pittsburgh Sleep Quality Index; HADS= Hospital Anxiety and Depression Scale.

<table>
<thead>
<tr>
<th></th>
<th>Perceived Stress Scale</th>
<th>HADS total score</th>
<th>Anxiety</th>
<th>Depression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>18.35 ± 7.57</td>
<td>9.00 ± 5.30</td>
<td>6.22 ± 3.35</td>
<td>2.78 ± 2.69</td>
</tr>
<tr>
<td></td>
<td>15.29 ± 8.32</td>
<td>6.17 ± 3.75</td>
<td>4.94 ± 3.08</td>
<td>1.22 ± 1.11</td>
</tr>
<tr>
<td></td>
<td>0.134</td>
<td>0.004*</td>
<td>0.034*</td>
<td>0.009*</td>
</tr>
<tr>
<td></td>
<td>0.385</td>
<td>0.616</td>
<td>0.398</td>
<td>0.758</td>
</tr>
<tr>
<td></td>
<td>16.18 ± 4.49</td>
<td>5.55 ± 3.59</td>
<td>3.73 ± 2.65</td>
<td>1.82 ± 1.66</td>
</tr>
<tr>
<td></td>
<td>16.18 ± 6.32</td>
<td>6.00 ± 3.87</td>
<td>4.82 ± 3.19</td>
<td>1.18 ± 1.47</td>
</tr>
<tr>
<td></td>
<td>1.000</td>
<td>0.654</td>
<td>0.221</td>
<td>0.161</td>
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<tr>
<td></td>
<td>0.000</td>
<td>0.121</td>
<td>0.372</td>
<td>0.408</td>
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<tr>
<td></td>
<td>0.400</td>
<td>0.068</td>
<td>0.046*</td>
<td>0.519</td>
</tr>
<tr>
<td></td>
<td>0.205</td>
<td>0.300</td>
<td>0.263</td>
<td>0.122</td>
</tr>
<tr>
<td></td>
<td>0.060</td>
<td>0.089</td>
<td>0.077</td>
<td>0.028</td>
</tr>
<tr>
<td></td>
<td>0.249</td>
<td></td>
<td></td>
<td>0.426</td>
</tr>
</tbody>
</table>
4.4.4 Salivary Cortisol and Melatonin Analysis

Cortisol and melatonin measures did not significantly differ at baseline between the intervention and control groups. Moreover, both the intervention and control groups exhibited a significant decrease in cortisol AUCG following the study period \((p < 0.05; \text{Table 4.3 and Supplementary Table S4.5})\) and the magnitude of change was not different between the groups. Following the nine-month study period, the intervention group exhibited a decrease in melatonin levels at all time points and in total melatonin output \((p < 0.05; \text{AUCG, Table 4.3})\). Melatonin levels in the control group also significantly decreased within the hour leading up to usual bedtime, at usual bedtime and the hour following the usual bedtime, with a trend towards a decrease in total melatonin output. Comparison of the two groups following the nine-month study period revealed no differences in cortisol or melatonin concentrations at any of the sampling time points, suggesting that the multidisciplinary rehabilitation program does not have a robust effect on morning cortisol or evening melatonin release.

4.4.5 Subjective Sleep Assessments

At baseline, the intervention group exhibited a significantly higher number of awakenings (identified using the CSD) than the control group. When comparing the groups following the nine-month study period, a significantly later awakening time and increased total time in bed and total sleep time was observed in the control group compared to the intervention group. Within group analyses revealed that sleep timing, as measured using the CSD, did not significantly change over the nine-month study period in the intervention group, however the total time in bed and total sleep time significantly increased in the control group (Table 4.3). No other differences were observed in CSD outcomes or subjective sleep quality (PSQI) within the intervention or control group following the nine-month study period.

Daytime somnolence significantly decreased in the intervention group, but did not differ in the control group (Table 4.3). No other differences were observed in habitual sleep/wake timing
(CSD), subjective sleep quality (PSQI scores) or daytime somnolence (ESS scores) at baseline or follow-up between any of the groups (Table 4.3 and Supplementary Table S4.5). These results suggest that multidisciplinary rehabilitation does not significantly alter subjective sleep quality, but does reduce daytime somnolence and facilitates the maintenance of sleep/wake timing.

4.4.6 Affective Symptoms

There were no significant differences between groups at baseline for depression and perceived stress outcomes (Table 4.3 and Supplementary Table S4.5). Anxiety symptomatology was significantly higher in the intervention group compared to the control group at baseline; however, it is important to note that anxiety and depression scores on the HADS were subthreshold (< 8) for both groups, indicating no clinically meaningful anxiety symptomatology for both groups (Zigmond & Snaith, 1983). Following the intervention period, a significant decrease in anxiety and depressive symptomology, but not perceived stress, was observed in the intervention group, but not the control group.

4.4.7 Correlative Results Between Change in Hypothalamic Volume and Change in Cortisol and Melatonin Output

A significant interaction effect was observed between group (intervention or control) and change in cortisol output (Table 4.2 and Supplementary Table 4.6). Parameter estimates for this interaction were used to generate a scatterplot with fitted lines for each group. Visual inspection revealed a positive slope for the relationship between change in hypothalamus volume and change in cortisol output in the control group (Figure 4.1B). Associated correlation coefficients in the control group, computed from parameter estimates extracted from the relevant areas, were $r = -0.73$, in the right, and $r = -0.57$, in the left. A group by cortisol output change interaction effect on hypothalamus grey matter volume loss was also observed.
bilaterally (right, k = 368, t = 2.54, p = 0.01, MNI = 7 0 -12; left, k = 29, t = 2.11, p = 0.024, MNI = -9 -8 -4; Figure 4.1B).

A group by melatonin output change interaction effect on grey matter volume loss was observed in the right hypothalamus when comparing the intervention group to the control group (Figure 4.1C, Table 4.2 and Supplementary Table S4.6). Parameter estimates revealed the association between nine-month right hypothalamus grey matter volume loss and change in evening melatonin output was positive in the intervention group (r = 0.47) and negative in the control group (r = -0.51).

These results suggest a shift towards a positive association between right hypothalamic grey matter volume and evening melatonin output in the intervention group. Interestingly, the location of this particular finding was in the basal hypothalamus, a region that contains the suprachiasmatic nucleus.

**4.5 Discussion**

Pathological disturbances in circadian rhythmicity and sleep are debilitating features of HD, thought to occur primarily due to degeneration within the hypothalamus. To date, no proven therapies exist to treat circadian rhythm and habitual sleep disturbances in individuals with HD. Here we show, for the first time, that multidisciplinary rehabilitation significantly attenuates grey matter volume loss within the hypothalamus, coinciding to a small extent with changes in cortisol and melatonin and reduced symptoms of daytime somnolence, anxiety and depression in individuals with premanifest HD.

Studies by our team and others show that individuals with premanifest and prodromal HD have significantly reduced grey matter volume in the hypothalamus as early as ten years prior to predicted clinical disease onset (Bartlett et al., 2016; Bartlett et al., 2019 [Chapters 2 and 3]; Soneson et al., 2010). Results from the present study indicate that multidisciplinary
rehabilitation significantly reduces hypothalamic grey matter volume loss, suggesting that multidisciplinary rehabilitation may act to preserve the hypothalamus. To the authors’ knowledge, this is the first study to document a significant reduction in grey matter volume loss in the hypothalamus following multidisciplinary rehabilitation in individuals with HD. This promising finding builds upon previous work by our team, where multidisciplinary rehabilitation was found to significantly increase grey matter volume in the caudate and dorsolateral prefrontal cortex (Cruickshank et al., 2015). Together, these findings provide compelling evidence that adds to the growing body of preclinical and clinical evidence suggesting that lifestyle approaches, particularly environmental enrichment and multidisciplinary rehabilitation, have neuroprotective effects capable of preserving grey matter in brain structures vulnerable to neurodegeneration in HD (Lazic et al., 2006; Nithianantharajah & Hannan, 2006; Spires et al., 2004).

The biological mechanisms mediating the attenuation of hypothalamic volume loss after multidisciplinary therapy are not known. However, it is possible that preservation of BDNF levels observed in the intervention group may have contributed. BDNF plays a crucial role in the protection and plasticity of neurons (Bemelmans et al., 1999). Our results demonstrate that nine months of multidisciplinary rehabilitation attenuates the loss of serum BDNF concentrations and this coincides with a reduction in the loss of hypothalamic grey matter volume. Studies using animal models have shown that BDNF crosses the blood-brain barrier (Pan, Banks, Fasold, Bluth, & Kastin, 1998; Pan, Banks, & Kastin, 1998; Pan & Kastin, 1999), however this is yet to be confirmed in humans. This, together with evidence showing that BDNF is required for neurogenesis (Bekinschtein, Oomen, Saksida, & Bussey, 2011; Rossi et al., 2006), suggests that maintenance of serum BDNF levels as a result of regular exercise and cognitive training could contribute to the support and preservation of the hypothalamus.
Significant attenuation of grey matter volume loss in the hypothalamus following multidisciplinary rehabilitation was positively associated with changes in cortisol and melatonin release; however, contrary to our expectations, this was not associated with improvements in habitual sleep quality following multidisciplinary rehabilitation, as has been reported in Parkinson’s and Alzheimer’s disease (Nascimento et al., 2014). We did, however, observe reduced daytime somnolence in the group receiving the multidisciplinary rehabilitation intervention, which was accompanied by a reduction in anxiety and depression symptomatology, though it should be noted that affective symptomology in this population was subthreshold. Given the known role of the hypothalamus in the modulation of sleep (Saper et al., 2005), we expected attenuation of hypothalamic volume loss to positively coincide with habitual sleep outcomes. However, accumulating evidence indicates that multiple brain structures are responsible for regulating the sleep/wake cycle, including the thalamus and locus coeruleus (Fuller, Gooley, & Saper, 2006; Saper et al., 2005). While beneficial for the hypothalamus, multidisciplinary rehabilitation may not have widespread positive effects on other structures necessary for the regulation of circadian rhythm and sleep. Therefore, future studies should assess the effects of multidisciplinary rehabilitation on other structures regulating the sleep/wake cycle and perhaps use objective measures to assess the effects of multidisciplinary rehabilitation on sleep architecture rather than the subjective measures used here.

A number of limitations are associated with this study. The small sample size and non-randomised structure of the trial limits the generalisations that can be made to the wider HD community. Nevertheless, this study provides proof-of-concept data on the neuroprotective effects of multidisciplinary rehabilitation for the hypothalamus, which can be used to inform
sample size estimations for future randomised controlled trials. It is also important to note that this study used sleep questionnaires to evaluate habitual sleep, which are often prone to subjective bias. Future studies should assess the effects of multidisciplinary rehabilitation on habitual sleep outcomes, as well as sleep architecture using polysomnography in a laboratory setting in individuals with premanifest HD.

In summary, this study provides novel, preliminary evidence that multidisciplinary rehabilitation reduces hypothalamic grey matter volume loss, possibly due to preservation of basal serum BDNF levels, and decreases daytime somnolence in individuals with premanifest HD. Larger randomised controlled trials are nevertheless required to confirm these preliminary findings, as well as further explore the effects of multidisciplinary rehabilitation on circadian rhythm and habitual sleep outcomes in individuals with premanifest HD.
4.6 Supplementary Data

**MRI Data Pre-Processing**

Voxel-based morphometry (Ashburner & Friston, 2000) was performed in SPM12 (v6225, http://www.fil.ion.ucl.ac.uk/spm) following the same longitudinal VBM spatial pre-processing protocol as in Eshaghi et al. (2014). First, the pairwise longitudinal registration toolbox was used to register baseline and follow-up images to a within-subject mid-point average to avoid asymmetric treatment of time-points. Next, a Jacobian change rate map was generated for each participant that encodes the contraction or expansion of each voxel between time points. The Segment routine was then used to extract grey matter, white matter and cerebrospinal fluid probability maps from each of the mid-point average images (Ashburner & Friston, 2005; Streitbürger et al., 2014). The resulting grey and white matter maps of all participants were non-linearly transformed to a customized template in standard MNI space using a diffeomorphic registration algorithm [DARTEL; (Ashburner, 2007)]. Following from this, grey matter Jacobian change images were computed by multiplying the grey matter probability map by the Jacobian change rate map for each of the participants in native space. Flow fields from the DARTEL step above were used to normalize the grey matter Jacobian change images to MNI space with Gaussian smoothing. As the analysis focused on the hypothalamus, the normalised grey matter images were smoothed with a 6 mm full width at the half maximum (FWHM) Gaussian kernel, which is more appropriate for small structures (Salmond et al., 2002). No modulation was applied as this may induce multiplicative noise related to inter-subject variability of brain shape (Eshaghi et al., 2014). In addition to the grey matter Jacobian change images, we also generated grey matter Jacobian determinant images for each time point. The aim of this was to compare the associations between changes in hypothalamus volume and changes in cortisol, melatonin and sleep measures following the nine-month study period.
**Statistical Analysis**

The continuous covariate interaction model used to assess the differences in associations between hypothalamic volume and cortisol/melatonin between the intervention and control groups included group regressors, one for each group, and regressors modelling change in cortisol or melatonin output, one for each group. Contrasts modelled the interaction between group and change in cortisol or melatonin output ([0 0 1 -1] and [0 0 -1 1]). Two-sample t-tests were used to evaluate the statistical significance of these contrasts. Covariates of interest in the above models (i.e., cortisol and melatonin) were mean centred across both groups. Sex and age were included as covariates of no interest.

**Table S4.4.** Demographic and clinical characteristics of premanifest HD and prodromal HD intervention groups and premanifest HD control group at baseline

<table>
<thead>
<tr>
<th></th>
<th>Premanifest HD Intervention Group (n=10)</th>
<th>Prodromal HD Intervention Group (n=8)</th>
<th>Premanifest HD Control Group (n=11)</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic Characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, mean ± SD</td>
<td>37.2 ± 8.7</td>
<td>45.5 ± 13.9</td>
<td>50.5 ± 9.5</td>
<td>0.027*</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>4 (40.0)</td>
<td>2 (25.0)</td>
<td>4 (36.4)</td>
<td>0.790</td>
</tr>
<tr>
<td><strong>Clinical Characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAGn, mean ± SD</td>
<td>43.6 ± 2.9</td>
<td>43.8 ± 4.0</td>
<td>41.9 ± 2.0</td>
<td>0.402</td>
</tr>
<tr>
<td>DCL, mean ± SD</td>
<td>0.1 ± 0.3</td>
<td>1.4 ± 0.7</td>
<td>0.0 ± 0.0</td>
<td>0.000††</td>
</tr>
<tr>
<td>UHDRS-TMS, mean ± SD</td>
<td>1.3 ± 1.8</td>
<td>15.4 ± 6.9</td>
<td>0.1 ± 0.3</td>
<td>0.000††</td>
</tr>
<tr>
<td>Disease burden score, mean ± SD</td>
<td>290.35 ± 82.6</td>
<td>333.5 ± 102.7</td>
<td>309.4 ± 61.5</td>
<td>0.545</td>
</tr>
<tr>
<td>CAPs, mean ± SD</td>
<td>0.83 ± 0.21</td>
<td>1.00 ± 0.20</td>
<td>0.93 ± 0.13</td>
<td>0.306</td>
</tr>
<tr>
<td>BMI, mean ± SD</td>
<td>27.6 ± 3.9</td>
<td>25.4 ± 3.7</td>
<td>25.3 ± 2.0</td>
<td>0.221</td>
</tr>
<tr>
<td>Smoker, n (%)</td>
<td>2 (20)</td>
<td>1 (12.5)</td>
<td>1 (9.1)</td>
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</tr>
<tr>
<td>High alcohol consumption, n (%)</td>
<td>2 (20)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.217</td>
</tr>
<tr>
<td>Psychotropic medication, n (%)</td>
<td>2 (20)</td>
<td>0 (0)</td>
<td>3 (27.3)</td>
<td>0.287</td>
</tr>
</tbody>
</table>

Values are significant at \( p \leq 0.05 \).

*Significant differences between premanifest intervention and premanifest control groups

†Significant differences between premanifest intervention and prodromal intervention groups

CAGn= cytosine-adenine-guanine repeat number; DCL= diagnostic confidence level; UHDRS-TMS= Unified Huntington’s Disease Rating Scale- Total Motor Score; CAPs= CAG-age product score; BMI= body mass index.
Table S4.5. Mean salivary cortisol and melatonin concentrations, subjective sleep outcomes and affective symptomatology outcomes in premanifest HD intervention and control groups.

<table>
<thead>
<tr>
<th></th>
<th>Premanifest HD Intervention group (n=10)</th>
<th>Prodromal HD Intervention Group (n=8)</th>
<th>Premanifest HD Control group (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Follow-Up</td>
<td>Baseline</td>
</tr>
<tr>
<td><strong>BDNF</strong></td>
<td>26976.62 ± 6213.40</td>
<td>28694.70 ± 4569.96</td>
<td>28456.92 ± 5716.52</td>
</tr>
<tr>
<td><strong>Cortisol (nmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Awakening (hh:mm)</td>
<td>6:20 ± 0:58</td>
<td>6:18 ± 0:52</td>
<td>6:12 ± 0:01:09</td>
</tr>
<tr>
<td>Time of sample relative to awakening:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+15 mins</td>
<td>11.28 ± 3.84</td>
<td>9.05 ± 4.17</td>
<td>11.63 ± 4.05</td>
</tr>
<tr>
<td>+30 mins</td>
<td>13.41 ± 3.97</td>
<td>11.35 ± 5.08</td>
<td>15.69 ± 4.87</td>
</tr>
<tr>
<td>+60 mins</td>
<td>11.96 ± 2.52</td>
<td>10.38 ± 2.80</td>
<td>13.14 ± 3.67</td>
</tr>
<tr>
<td><strong>AUC_G</strong></td>
<td>38.59 ± 8.42</td>
<td>32.78 ± 10.46</td>
<td>42.61 ± 11.26</td>
</tr>
<tr>
<td><strong>Melatonin (pg/mL)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bedtime (hh:mm)</td>
<td>21:50 ± 0:45</td>
<td>22:00 ± 0:43</td>
<td>22:20 ± 0:30</td>
</tr>
<tr>
<td>Time of sample relative to bedtime:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-1 hr</td>
<td>12.89 ± 4.77</td>
<td>6.54 ± 5.33</td>
<td>19.63 ± 16.12</td>
</tr>
<tr>
<td>+0 hrs</td>
<td>20.78 ± 9.51</td>
<td>12.27 ± 6.83</td>
<td>24.66 ± 17.25</td>
</tr>
<tr>
<td>+1 hr</td>
<td>24.48 ± 15.29</td>
<td>13.73 ± 8.68</td>
<td>27.43 ± 19.65</td>
</tr>
<tr>
<td><strong>AUC_G</strong></td>
<td>51.45 ± 19.57</td>
<td>28.73 ± 15.44</td>
<td>60.53 ± 44.07</td>
</tr>
<tr>
<td><strong>CSD</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total time in bed (min)</td>
<td>430.59 ± 57.26</td>
<td>422.50 ± 48.46</td>
<td>415.00 ± 84.63</td>
</tr>
<tr>
<td>Total sleep time (min)</td>
<td>383.83 ± 55.45</td>
<td>379.69 ± 46.27</td>
<td>370.67 ± 73.28</td>
</tr>
<tr>
<td>Sleep onset latency (min)</td>
<td>23.20 ± 26.12</td>
<td>20.75 ± 23.05</td>
<td>18.75 ± 21.67</td>
</tr>
<tr>
<td>Wake after sleep onset (min)</td>
<td>22.00 ± 28.25</td>
<td>11.91 ± 20.30</td>
<td>25.00 ± 38.47</td>
</tr>
<tr>
<td>Number of awakenings</td>
<td>1.72 ± 1.48*</td>
<td>1.31 ± 1.12</td>
<td>1.00 ± 0.95</td>
</tr>
<tr>
<td>Sleep efficiency (%)</td>
<td>89.48 ± 9.29</td>
<td>90.31 ± 9.20</td>
<td>89.89 ± 9.25</td>
</tr>
<tr>
<td>Restorative quality of sleep</td>
<td>3.63 ± 0.76</td>
<td>3.70 ± 0.88</td>
<td>3.50 ± 0.84</td>
</tr>
<tr>
<td>PSQI Global Score</td>
<td>6.00 ± 2.94</td>
<td>5.06 ± 3.15</td>
<td>5.14 ± 2.85</td>
</tr>
<tr>
<td>Epworth Sleepiness Scale</td>
<td>5.76 ± 3.31</td>
<td>4.71 ± 3.02</td>
<td>5.29 ± 3.64</td>
</tr>
<tr>
<td>HADS total score</td>
<td>8.90 ± 5.82</td>
<td>6.10 ± 4.41</td>
<td>9.13 ± 4.97</td>
</tr>
<tr>
<td>Anxiety</td>
<td>6.30 ± 3.71</td>
<td>5.20 ± 3.68</td>
<td>6.13 ± 3.09</td>
</tr>
<tr>
<td>Depression</td>
<td>2.60 ± 2.72</td>
<td>1.90 ± 0.99</td>
<td>3.00 ± 2.83</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard deviation. Baseline differences between groups were assessed using independent t-tests for parametric and Mann-Whitney U tests for non-parametric data. Within group differences at follow-up were assessed using paired t-tests for parametric and Wilcoxon Signed Rank tests for non-parametric data. Analysis of covariance (ANCOVA) was used for between-group analyses at follow-up. Non-parametric data were log-transformed prior to ANCOVA analysis. Values are significant at p ≤ 0.05.

*Significant differences compared to baseline values

†Significant differences compared to premanifest HD control group baseline values

‡Significant differences compared to prodromal HD intervention group baseline values

§Significant differences compared to premanifest HD control group follow-up values

||Significant differences compared to prodromal HD intervention group follow-up values

CSD= Consensus Sleep Diary; PSQI= Pittsburgh Sleep Quality Index; HADS= Hospital Anxiety and Depression Scale.
Table S4.6. Results from voxel-based morphometry analysis.

<table>
<thead>
<tr>
<th>Side</th>
<th>k</th>
<th>T-score</th>
<th>p-value</th>
<th>MNI Coordinates (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td><strong>Group Effect</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premanifest HD control vs. premanifest HD intervention</td>
<td>R</td>
<td>284</td>
<td>2.94</td>
<td>0.004</td>
</tr>
<tr>
<td>Premanifest HD control vs. prodromal HD intervention</td>
<td>R</td>
<td>266</td>
<td>2.78</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>87</td>
<td>1.97</td>
<td>0.030</td>
</tr>
<tr>
<td>Premanifest HD control vs. combined premanifest and prodromal HD intervention</td>
<td>R</td>
<td>321</td>
<td>2.90</td>
<td>0.004</td>
</tr>
<tr>
<td><strong>Group x Cortisol Interaction Effect</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premanifest HD control vs. premanifest HD intervention</td>
<td>R</td>
<td>531</td>
<td>3.65</td>
<td>0.001</td>
</tr>
<tr>
<td>Premanifest HD control vs. prodromal HD intervention</td>
<td>R</td>
<td>95</td>
<td>2.59</td>
<td>0.010</td>
</tr>
<tr>
<td>Premanifest HD control vs. combined premanifest and prodromal HD intervention</td>
<td>R</td>
<td>305</td>
<td>2.47</td>
<td>0.012</td>
</tr>
<tr>
<td><strong>Group x Melatonin Interaction Effect</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premanifest HD control vs. prodromal HD intervention</td>
<td>R</td>
<td>233</td>
<td>2.31</td>
<td>0.016</td>
</tr>
<tr>
<td>Premanifest HD control vs. combined premanifest and prodromal HD intervention</td>
<td>R</td>
<td>86</td>
<td>2.36</td>
<td>0.014</td>
</tr>
</tbody>
</table>

Results are significant at \( p < 0.05 \), uncorrected.

\( k \) = No. of voxels; MNI = Montreal Neurological Institute; L = left; R = right.
Chapter 5

The Effects of Multidisciplinary Rehabilitation on Sleep and Sleep-Dependent Memory Consolidation Outcomes in Individuals with Premanifest Huntington’s Disease: An Exploratory Study

Short Communication

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5.1 Abstract

Objective: Disturbances in sleep architecture arise early in the course of Huntington’s disease (HD) and worsen as the disease course lengthens. The aim of this exploratory study was to determine if multidisciplinary rehabilitation could improve sleep architecture and memory consolidation in individuals with premanifest HD.

Methods: Sixteen individuals with premanifest HD completed a nine-month multidisciplinary rehabilitation intervention, consisting of three, two hour sessions per week of cognitive, exercise and dual-task training with social interaction. Outcome measures included polysomnography and sleep-dependent memory consolidation.

Results: A significant increase in the percentage of total sleep time spent in rapid eye movement (REM) sleep, a decrease in the percentage of stage N1 sleep and a decreased time to reach REM sleep were observed following the nine-month intervention. Significant increases in total sleep time and total time in bed were also observed and accompanied by a significantly earlier bed time. No significant differences were observed in memory consolidation after the intervention, as measured by the delayed recall component on the Hopkins Verbal Learning Test-Revised.

Conclusion: This exploratory study provides preliminary evidence that multidisciplinary rehabilitation can improve sleep architecture in individuals with premanifest HD, however these changes were not associated with improvements in memory consolidation. Large randomised controlled trials are however required to confirm and expand on these preliminary findings in individuals with premanifest and manifest HD.
5.2 Introduction

Sleep disturbances are a well-recognised feature of Huntington’s disease (HD) that present several years prior to estimated clinical diagnosis and worsen as the disease course lengthens (Lazar et al., 2015; Morton, 2013). Alterations in sleep architectures are particularly apparent in individuals prior to clinical disease onset and include an increased number of awakenings, a reduction in rapid eye movement (REM) and slow wave sleep, decreased sleep efficiency and insomnia (Arnulf et al., 2008; Lazar et al., 2015; Morton, 2013; Videnovic, Leurgans, Fan, Jaglin, & Shannon, 2009).

Disturbances in sleep have been proposed to exacerbate cognitive impairments, particularly memory deficits, in individuals with HD (Lazar et al., 2015; Walker, 2008). Studies indicate that sleep is integral to memory processing and consolidation (Genzel et al., 2015; Hobson & Pace-Schott, 2002). Investigations in healthy individuals have reported that, although each sleep stage is involved in memory consolidation, REM and slow wave sleep are crucial (Louie & Wilson, 2001; Peigneux et al., 2004; Stickgold, 2005). Treatments aimed at improving or attenuating sleep disturbances may therefore have a positive impact on memory consolidation in individuals with HD.

There are currently no proven strategies for treating sleep disturbances in individuals with HD. Recent evidence indicates that multidisciplinary rehabilitation improves self-reported sleep quality in individuals with Parkinson’s disease (Frazzitta, Maestri, Ferrazzoli, et al., 2015). The effects of multidisciplinary rehabilitation on sleep outcomes in HD individuals is yet to be investigated. However, previous work by our team has shown that multidisciplinary rehabilitation improves verbal learning memory in manifest HD individuals (Cruickshank et al., 2015). While tentative, it is plausible that multidisciplinary rehabilitation enhances verbal learning and memory in individuals with HD by improving sleep quality and architecture.
The purpose of this exploratory study was twofold. Firstly, this study sought to examine, for the first time, whether multidisciplinary rehabilitation improves sleep architecture and sleep-dependent memory consolidation in individuals with premanifest HD. Secondly, this study sought to investigate the role of sleep on memory consolidation in individuals with premanifest HD. It is believed that the implementation of multidisciplinary rehabilitation prior to extensive neuronal loss (during the premanifest stage) would provide maximum benefit for gene positive HD individuals.

5.3 Materials and Methods

5.3.1 Study Design

The aim of this exploratory study was to assess the effects of a nine-month multidisciplinary rehabilitation program on sleep architecture and memory consolidation in individuals with premanifest HD. Outcome measures included polysomnography and memory consolidation measures. Ethical approval for study procedures was granted by the Human Research Ethics Committees at Edith Cowan University (13145), the University of Western Australia and the North Metropolitan Area Mental Health Service (2009_16). All participants provided written informed consent.

5.3.2 Participants

A subset of twenty participants from a concurrently running study with confirmed positive HD gene status, a Unified Huntington’s Disease Rating Scale Total Motor Score (UHDRS-TMS) of <15 and a diagnostic confidence level of <2, no concomitant neurological or cardiovascular disease and no shift work were approached to participate.
5.3.3 Outcome Measures

Polysomnography

Polysomnography was conducted at the Centre for Sleep Science at the University of Western Australia. Polysomnography set-up included electroencephalography (EEG; C4/M1, C3/M2, F4/ M1, F3/M2, O1/M2, O2/M1), electrooculography, submental and bilateral anterior tibial electromyography, electrocardiography, pulse oximetry and respiratory inductive plethysmography in accordance with the American Academy of Sleep Medicine (AASM) recommendations. Signals were acquired and displayed using the Grael system (Compumedics). Sleep stages were scored manually according to the AASM scoring criteria Version 2.1 (Berry et al., 2012).

Memory Consolidation

The Hopkins Verbal Learning Test (HVLT-R) was used to examine immediate and delayed recall. Participants were read a list of words on the evening of the sleep study and were asked to repeat the words in three consecutive trials. Participants were then asked to repeat the same words the following morning for the delayed recall component of the test. The total number of words recalled and retained were recorded for each participant using the delayed recall component and the highest score from the immediate recall component of the test. The HVLT-R has previously been shown to be a sensitive and reliable measure of memory in individuals with premanifest HD (Solomon et al., 2007).

5.3.4 Multidisciplinary Rehabilitation Intervention

Details on the multidisciplinary rehabilitation intervention are described elsewhere (Bartlett et al., 2018 [Chapter 4]). Briefly, participants completed three, two hour supervised multidisciplinary rehabilitation sessions, comprising exercise, computerised cognitive training (NeuroNation, Synaptikon, Germany and Captain’s Log, BrainTrain, USA), dual task training
and social events, per week for nine months in small groups (3-4 participants per session). Training sessions were administered by clinical exercise physiologists.

5.3.5 Statistical Analysis

Normality assumptions were tested using a Shapiro-Wilk test. Within-group comparisons were conducted using paired sample t-tests and Wilcoxon signed-rank tests. A Bonferroni correction was applied to the data to account for multiple comparisons. Hedge’s g was calculated to determine effect sizes for each outcome, where a small, medium and large effect size is considered 0.2, 0.5 and 0.8 respectively. Pearson correlation coefficients were calculated to assess the potential associations between sleep outcomes and sleep-dependent memory consolidation outcomes. Results are reported as mean ± standard deviation (unless stated otherwise) and were considered significant at $p<0.05$. Statistical analyses were performed using SPSS version 24 (IBM Corp., Armonk, NY, USA).

5.4 Results

5.4.1 Baseline Demographic and Clinical Characteristics

Four participants withdrew from the study prior to follow-up testing. Therefore, data from sixteen individuals with premanifest HD were used for analyses. Participant demographics and clinical characteristics are presented in Table 5.1.

5.4.2 Sleep Measures

Following the intervention, significant improvements were observed in bed time, total time spent in bed, REM latency and percentage of the total sleep time spent in stage N1 and REM (Table 5.2). These improvements corresponded to moderate to large effect sizes, suggesting that nine-months of multidisciplinary rehabilitation can reduce the time taken to reach REM sleep, as well as increase the percentage of the total sleep time spent in REM sleep. No other reportable effects were observed in sleep outcomes following the intervention.
Table 5.1. Demographic and clinical characteristics of premanifest HD participants (n=16)

<table>
<thead>
<tr>
<th>Demographic Characteristics</th>
<th>Baseline</th>
<th>Follow-Up</th>
<th>p-value</th>
<th>Effect size (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>40.31 ± 12.29</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>5 (31.3)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Clinical Characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAGn</td>
<td>43.69 ± 3.50</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DBS</td>
<td>302.50 ± 93.53</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CAPs</td>
<td>0.87 ± 0.23</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DCL</td>
<td>0.69 ± 0.87</td>
<td>0.75 ± 0.86</td>
<td>0.739</td>
<td>0.069</td>
</tr>
<tr>
<td>UHDRS-TMS</td>
<td>7.56 ± 9.09</td>
<td>6.31 ± 9.24</td>
<td>0.306</td>
<td>0.136</td>
</tr>
<tr>
<td>BMI</td>
<td>26.13 ± 3.82</td>
<td>26.26 ± 2.98</td>
<td>0.501</td>
<td>0.038</td>
</tr>
<tr>
<td>Smoker, n (%)</td>
<td>4 (25.0)</td>
<td>3 (18.8)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>High alcohol consumption, n (%)†</td>
<td>2 (12.5)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Psychotropic medication, n (%)</td>
<td>2 (12.5)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Categorical variables were analysed using a chi-squared test. Data was tested for normality using a Shapiro-Wilk test. Within group differences were analysed using paired t-tests (for parametric data) and Wilcoxon matched-pairs signed rank tests* (for non-parametric data). Bonferroni correction was applied for multiple comparisons. Data are presented as mean ± standard deviation unless otherwise stated. Values are significant at p ≤ 0.05*.

CAGn= cytosine-adenine-guanine repeat number, DCL= diagnostic confidence level, UHDRS-TMS= Unified Huntington’s Disease Rating Scale Total Motor Score, DBS= disease burden score, CAPs= CAG age product score, BMI= body mass index.

†High alcohol consumption is described as consumption of more than 14 alcoholic beverages per week.

5.4.3 Memory Consolidation (Hopkins Verbal Learning Test)

A large effect size was observed in the total number of words recalled using the HVLT-R following multidisciplinary rehabilitation. No significant differences were observed however, in delayed recall or word retention components of the HVLT-R (Table 5.2). Upon assessment of correlation values between sleep and memory outcomes, significant positive correlations of moderate strength were observed between the change in sleep efficiency and changes in delayed recall (d = 0.50, p = 0.049) and retention of words (d = 0.50, p = 0.050), indicating that an increase in sleep efficiency is moderately associated with a greater propensity to recall words from memory. However, the lack of significant change in sleep efficiency and delayed recall or retention of words following the intervention period somewhat limits the conclusions
that can be drawn from these findings. No other significant correlations were identified between sleep and memory outcomes.

Table 5.2. Sleep and memory consolidation outcomes following a nine-month multidisciplinary rehabilitation intervention (n=16).

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Follow-Up</th>
<th>p-value</th>
<th>Effect size (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Polysomnography</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bed time, hh:mm</td>
<td>22:38 ± 00:18</td>
<td>22:01 ± 00:37</td>
<td>0.001*</td>
<td>1.315</td>
</tr>
<tr>
<td>Time of awakening, hh:mm</td>
<td>06:07 ± 00:41</td>
<td>06:13 ± 00:26</td>
<td>0.356</td>
<td>0.175</td>
</tr>
<tr>
<td>Total Sleep Time, min</td>
<td>350.88 ± 63.46</td>
<td>390.31 ± 65.76</td>
<td>0.010†</td>
<td>0.610</td>
</tr>
<tr>
<td>Total Time in Bed, min</td>
<td>448.81 ± 44.91</td>
<td>492.56 ± 40.67</td>
<td>0.001*</td>
<td>1.021</td>
</tr>
<tr>
<td>Sleep Efficiency, %</td>
<td>77.90 ± 10.34</td>
<td>79.16 ± 11.48</td>
<td>0.501</td>
<td>0.115</td>
</tr>
<tr>
<td>Number of Awakenings</td>
<td>22.13 ± 8.75</td>
<td>25.13 ± 8.25</td>
<td>0.129</td>
<td>0.353</td>
</tr>
<tr>
<td>WASO, min</td>
<td>74.81 ± 39.18</td>
<td>68.22 ± 40.30</td>
<td>0.406</td>
<td>0.166</td>
</tr>
<tr>
<td>Sleep Latency, min</td>
<td>21.72 ± 18.04</td>
<td>26.81 ± 22.95</td>
<td>0.776</td>
<td>0.247</td>
</tr>
<tr>
<td>REM Latency, min</td>
<td>199.66 ± 77.68</td>
<td>117.53 ± 44.57</td>
<td>0.004†</td>
<td>1.297</td>
</tr>
<tr>
<td>Stage N1 (%)</td>
<td>17.35 ± 9.52</td>
<td>12.75 ± 7.10</td>
<td>0.043*</td>
<td>0.548</td>
</tr>
<tr>
<td>Stage N2 (%)</td>
<td>46.74 ± 7.32</td>
<td>44.36 ± 7.94</td>
<td>0.287</td>
<td>0.312</td>
</tr>
<tr>
<td>Stage N3 (%)</td>
<td>18.76 ± 7.28</td>
<td>17.52 ± 4.93</td>
<td>0.387</td>
<td>0.199</td>
</tr>
<tr>
<td>REM (%)</td>
<td>17.16 ± 6.91</td>
<td>25.38 ± 8.35</td>
<td>0.002†</td>
<td>1.073</td>
</tr>
<tr>
<td>Number of arousals</td>
<td>108.13 ± 43.21</td>
<td>128.94 ± 48.61</td>
<td>0.074</td>
<td>0.452</td>
</tr>
<tr>
<td>Arousal Index</td>
<td>14.51 ± 5.61</td>
<td>15.76 ± 6.05</td>
<td>0.304</td>
<td>0.214</td>
</tr>
<tr>
<td>Apnoea/Hypopnoea Index</td>
<td>6.70 ± 6.08</td>
<td>6.30 ± 5.77</td>
<td>0.408</td>
<td>0.067</td>
</tr>
<tr>
<td>PLM Index, per hour</td>
<td>2.71 ± 4.67</td>
<td>4.11 ± 3.92</td>
<td>0.182</td>
<td>0.325</td>
</tr>
<tr>
<td><strong>Sleep-Dependent Memory Consolidation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HVLT-R Total Recall</td>
<td>24.63 ± 4.88</td>
<td>27.69 ± 6.04</td>
<td>0.018*</td>
<td>0.557</td>
</tr>
<tr>
<td>HVLT-R Delayed Recall</td>
<td>7.25 ± 2.52</td>
<td>7.31 ± 4.27</td>
<td>0.617</td>
<td>0.017</td>
</tr>
<tr>
<td>HVLT-R Retention (%)</td>
<td>71.40 ± 19.46</td>
<td>65.48 ± 35.03</td>
<td>0.366</td>
<td>0.209</td>
</tr>
</tbody>
</table>

Data was tested for normality using a Shapiro-Wilk test. Within group differences were analysed using paired t-tests (for parametric data) and Wilcoxon matched-pairs signed rank tests (for non-parametric data). Bonferroni correction was applied for multiple comparisons. Data are presented as mean ± standard deviation unless otherwise stated. Values are significant at p ≤ 0.05*. WASO= wake after sleep onset, REM= rapid eye movement, N1= stage 1 of non-REM sleep, N2= stage 2 of non-REM sleep, N3= stage 3 of non-REM sleep, TST= total sleep time, PLM= periodic limb movement, HVLT-R= Hopkins Verbal Learning Test- Revised.
5.5 Discussion

Sleep disturbances are an early feature of HD that worsen as the disease course lengthens (Lazar et al., 2015; Morton, 2013). Disturbances in sleep are known to impair cognitive function (Lowe, Safati, & Hall, 2017; Walker, 2008) and have even been purported to hasten neurodegenerative processes (Musiek & Holtzman, 2016). Despite the disabling consequences of sleep disturbances, particularly on the cognition of patients, no treatment options have been developed to alleviate sleep disturbances in individuals with HD. In this exploratory study, we evaluated the effects of multidisciplinary rehabilitation on sleep quality and architecture and memory consolidation in individuals with premanifest HD.

Previous studies show that individuals with HD have an increase in REM latency and a decrease in REM sleep (Arnulf et al., 2008). In the current study, increases in the percentage of REM sleep and decreases in REM latency were observed following the intervention. To the authors’ knowledge, this is the first study to find significant improvements in REM sleep in individuals with premanifest HD after a non-pharmaceutical intervention. Our findings suggest that multidisciplinary rehabilitation may favourably modulate sleep architecture, which is of clinical interest, especially considering the known role of sleep in the maintenance of brain structures and function (Malhotra & Desai, 2010); however, controlled studies are required to determine the extent to which multidisciplinary rehabilitation may impact on sleep architecture.

REM sleep has been proposed to have functions in the reorganisation of memories (Genzel et al., 2015). Therefore, it is possible that an improvement in REM sleep could have beneficial implications for memory consolidation. Cognitive decline, including impairments in learning and memory, have been reported in individuals with HD (Stout et al., 2012). Previous work by our team has shown that multidisciplinary rehabilitation improves verbal learning and memory in individuals with manifest HD. Together with the improvements in REM sleep following such an intervention in premanifest HD, it was anticipated that memory consolidation outcomes...
would improve following the nine-month intervention. However, contrary to expectation, the results presented here demonstrate that, although improvements in REM sleep and verbal learning and memory were observed, these two outcomes were not associated. Furthermore, no improvements in the delayed recall or retention components of the memory consolidation tasks were noted. This suggests that the improvements in REM sleep may not be a mediating factor in the improvement of verbal learning and memory following multidisciplinary rehabilitation. However, it is important to note that the present study used the delayed recall and retention components of the HVLT-R to assess memory consolidation after sleep, whereas previous studies assessing memory consolidation have typically utilised a paired word association test (Genzel, Dresler, Wehrle, Grözinger, & Steiger, 2009; Mander, Rao, Lu, Saletin, Lindquist, et al., 2013). While both tests have the capacity to measure memory consolidation, it is possible that the paired word association test is more sensitive to changes than the HVLT-R. Nevertheless, larger randomised controlled studies are required to confirm these preliminary findings and further assess the clinical sensitivity of these tests for assessing memory consolidation in individuals with HD.

While the results of the study appear promising, a number of limitations exist within this exploratory study. The small sample size and lack of control group limits the generalisability of these results to the wider HD population. Additionally, participants spent only one night in the sleep laboratory, which has been suggested to not be sufficient for acclimatisation, which is essential for good sleep (Toussaint et al., 1995). While significant changes in sleep timing occurred following the intervention, it is important to note that changes in sleep timing may have occurred as a result of laboratory practices (i.e. termination of sleep studies by laboratory staff) and should therefore be interpreted with caution. Finally, alcohol consumption and medication use have been reported to impact on sleep architecture, including REM sleep and REM latency (Garcia & Salloum, 2015). While alcohol consumption and medication use were
not measured at follow-up in this study, significance was maintained for REM sleep percentage and REM latency after removal of individuals that reported high alcohol consumption and medication use at baseline, indicating that the use of medications and alcohol did not have a large effect on REM latency and REM sleep percentage. Nevertheless, future studies should include medication use and alcohol consumption as a confounding factor in studies assessing sleep.

Despite these methodological shortcomings, this study offers preliminary evidence on the positive effects of multidisciplinary rehabilitation on sleep architecture and provides impetus for further studies assessing the effects of multidisciplinary rehabilitation on sleep architecture and quality and associated effects on clinical outcomes in individuals with HD.
Chapter 6

6.1 General Discussion

Huntington’s disease (HD) is a rare, autosomal dominant neurodegenerative disease caused by an expansion in the number of cytosine-adenine-guanine (CAG) repeats in exon one of the huntingtin gene (The Huntington's Disease Collaborative Research Group, 1993). While the classic triad of features includes motor, cognitive and mood disturbances, circadian rhythm and sleep disturbances are becoming increasingly recognised as features of HD that require treatment (Aziz et al., 2010; Lazar et al., 2015; Morton, 2013; Morton et al., 2005). Recent evidence suggests that sleep disturbances emerge together with cognitive deficits during the premanifest stage of HD and worsen as the disease course lengthens (Lazar et al., 2015; Morton et al., 2005), suggesting that both features are related. This supposition is supported by preclinical evidence, where disturbances in circadian rhythmicity and sleep/wake activity have been shown to cause cognitive, metabolic, psychological and cardiovascular consequences (Briançon-Marjollet et al., 2015; Morton et al., 2005), and have been purported to occur as a result of hypothalamic pathology. Despite this supposition, only one study to date has examined the neurobiological underpinnings of sleep disturbances in HD (Baker et al., 2016), using the sleep component of a depression questionnaire. Understanding the mechanisms that mediate circadian rhythm and sleep disruption will enable identification of potential avenues for therapeutic targets. Furthermore, identifying such mechanisms during the premanifest stage of HD will allow for the implementation of therapies prior to extensive neuronal damage, potentially leading to greater benefits.

The hypothalamus contains the SCN, which is responsible for generating and regulating the circadian rhythm. The direct and indirect connections between the SCN and other key hypothalamic nuclei control the circadian timing of cortisol and melatonin release and the sleep-wake cycle. The impact of degenerative processes on these nuclei could therefore
facilitate disintegration of the circadian release of hormones and sleep-wake timing. However, the implications of hypothalamic degeneration have not yet been extensively studied.

Several studies have reported disintegration of circadian rhythm and sleep parameters in individuals with premanifest HD compared to healthy controls (Aziz et al., 2010; Aziz, Pijl, Frölich, Schroder-van der Elst, et al., 2009; Lazar et al., 2015; Morton, 2013; Morton et al., 2005) and these differences in circadian rhythm and sleep have been purported to be mediated by hypothalamic pathology as reviewed in Chapter 2 (Bartlett et al., 2016). Despite this supposition, no studies have attempted to assess the relationship between hypothalamic volume and biological markers of circadian rhythm and sleep. One study to date has assessed the relationship between hypothalamic volume and subjective sleep, in individuals with premanifest and manifest HD (Baker et al., 2016). This study did not report a relationship between hypothalamic volume and subjective sleep outcomes, however this study used questions on sleep quality from a depression inventory, highlighting the need for further studies aimed at assessing the relationship between hypothalamic volume and circadian rhythm and habitual sleep.

The study conducted as part of this thesis (Chapter 3) suggests that even during the premanifest stages of HD individuals present with reduced hypothalamic grey matter volume compared to age- and gender-matched healthy controls. This finding aligns with previous reports of hypothalamic pathology in individuals with premanifest HD, including reduced grey matter volume and increased microglial activation in the hypothalamus (Politis et al., 2008; Soneson et al., 2010). In our study, the nature of this reduced hypothalamic volume was leftward biased. Interestingly, leftward biased atrophy has also been reported in the striatum in individuals with HD (Thieben et al., 2002). The exact reason for this is unknown, however studies postulate that this could merely be due to cumulative lifetime activity (Jenkins et al., 1998; Mühlau et al.,
It is possible that leftward biased degeneration of the hypothalamus is the first sign of hypothalamic degeneration in individuals with premanifest HD.

The pathological mechanisms responsible for reduced hypothalamic volume in individuals with HD are not yet entirely understood. Evidence from post-mortem investigations and studies in mouse models provides some insight into the pathological mechanisms mediating hypothalamic changes, as well as the mechanisms by which these changes could impact on circadian rhythm and habitual sleep outcomes. Neuronal inclusions of mutant huntingtin in suprachiasmatic nucleus (SCN) tissue at post-mortem (Aziz et al., 2008), may directly mediate changes in the functioning of the SCN. These neuronal inclusions of mutant huntingtin within the SCN could result in the reduction in the number of vasoactive intestinal polypeptide and arginine vasopressin expressing neurons, which are crucial in the regulation of SCN activity (Aton et al., 2005; Hofman & Swaab, 1994), as well as post-transcriptional changes in these neuropeptides, which have been reported in HD (van Wamelen et al., 2013). This, together with the loss of orexin-releasing neurons in the lateral hypothalamus that has been reported in individuals with HD at post-mortem and in mouse models of HD (Aziz et al., 2008; Petersén et al., 2005), could result in impaired functioning of the hypothalamic nuclei and lead to the disruption of the circadian rhythm and sleep-wake cycle, however this supposition needs to be investigated in greater detail.

Despite observing reduced hypothalamic volume in individuals with premanifest HD, no differences in circadian regulated hormones were noted (Chapter 3). Morning cortisol and evening melatonin levels remained similar to those in healthy controls. The reduced hypothalamic grey matter volume in individuals with premanifest HD, taken together with the lack of differences in cortisol and melatonin output, suggest that neural compensatory networks may exist to maintain neuroendocrine output. Alternatively, the lack of differences in cortisol and melatonin output between individuals with premanifest HD and healthy controls could be
explained by a sex-dependent loss of circadian rhythmicity in HD. Evidence from mouse models of HD, which recapitulate the circadian rhythm disturbances observed in individuals with HD (Kudo et al., 2011; Morton et al., 2005), shows that female HD mice exhibit a delayed or less severe circadian phenotype compared to male HD mice (Kuljis et al., 2016). As the cohort presented in Chapter 3 consisted primarily of females (64.4%), it is possible that circadian deficits were not yet apparent and may manifest later in the disease course. Further studies are required to examine this tentative speculation.

Although individuals with premanifest HD exhibited habitually reduced sleep efficiency and increased awakenings compared to healthy controls, no consistent relationships were observed between habitual sleep outcomes and hypothalamic volume. This was contrary to expectations; it was anticipated that hypothalamic volume would be associated with sleep outcomes, particularly as the hypothalamus contains key nuclei that regulate the sleep-wake cycle (Saper et al., 2005). However, it is important to acknowledge that other brain structures, such as the thalamus and the locus coeruleus, are also involved in regulating the sleep-wake cycle and perhaps provide a compensatory role for the hypothalamus when pathologically affected.

Despite intense scientific efforts, the treatment of HD remains symptomatic, with currently approved medications indicated for the treatment of motor or psychiatric features of the disease, often with only partial relief (Dominguez & Munoz-Sanjuan, 2014; Mason & Barker, 2016; Pidgeon & Rickards, 2013). While a number of studies have identified potential therapeutic interventions to treat circadian rhythm and sleep disturbances in other clinical populations, none have been trialled to date in individuals with HD (Paus et al., 2007; Rutten et al., 2012; H.-B. Wang et al., 2017). However, evidence from HD mouse models suggests that treatment of circadian rhythm and sleep disturbances prior to clinical onset of HD could have beneficial outcomes on other disease features, particularly motor deficits, and could even delay the progression of the disease (H.-B. Wang et al., 2018; H.-B. Wang et al., 2017).
studies suggest that early intervention is crucial to effectively treat circadian rhythm and sleep disturbances in HD.

Preclinical studies in HD mouse models have shown that behavioural therapy, particularly bright light therapy and voluntary wheel running, delays the progression of circadian dysfunction and restores synchronisation of the light-dark cycle in the R6/2 mouse model (Cuesta et al., 2014). Temporally scheduled feeding and blue light therapy have also been shown to favourably modulate the circadian rhythm and improve motor features in the Q175 and BACHD mouse models of HD (H.-B. Wang et al., 2018; H.-B. Wang et al., 2017). Together, this data suggests that the circadian rhythm can be entrained in HD by means of targeting various zeitgebers, including physical activity, indicating that environmental interventions could improve circadian rhythm outcomes in HD. Furthermore, multidisciplinary rehabilitation, an environmental intervention comprising physical and cognitive training, has been shown to improve sleep outcomes in individuals with Parkinson’s and Alzheimer’s disease (Nascimento et al., 2014). These findings provide impetus for the investigation of the therapeutic effects of multidisciplinary rehabilitation as a strategy for treating circadian rhythm and sleep disturbances in individuals with HD, particularly in those with premanifest HD.

Previous studies conducted by our team have demonstrated that multidisciplinary rehabilitation can improve brain volume and clinical outcomes in individuals with manifest HD. In particular, increases in the striatum and dorsolateral prefrontal cortex were observed following a nine-month multidisciplinary rehabilitation intervention, which coincided with improvements in verbal learning and memory, strength, fat-free mass, mood and quality of life outcomes (Cruickshank et al., 2015; Thompson et al., 2013). The data presented in Chapter 4 of this thesis adds weight to these studies and confirms that multidisciplinary therapy has positive effects on brain volume. In our more recent study, we provide evidence of significant hypothalamic degeneration over a nine month period in individuals with premanifest HD. Furthermore, we
show that following nine-months of multidisciplinary rehabilitation, a reduced loss of hypothalamic grey matter volume was observed in individuals with premanifest HD (intervention group) when compared to a cohort of individuals with premanifest HD receiving standard care (control group). Taken together, this suggests that multidisciplinary rehabilitation has the potential to maintain, and even improve, grey matter volume in the hypothalamus of individuals with premanifest HD. Furthermore, preliminary studies show increases in volume in other brain regions in premanifest HD (Cruickshank et al., submitted 2018) and in manifest HD (Cruickshank et al., 2015) following multidisciplinary rehabilitation.

Understanding the mechanisms mediating the maintenance or increases in grey matter volume after multidisciplinary rehabilitation will enable further optimisation of these programs in the future treatment for individuals with HD. The mechanisms underpinning maintenance or increases in brain volume following multidisciplinary rehabilitation are yet to be determined. Studies conducted in mice have demonstrated that brain-derived neurotrophic factor (BDNF) is essential for neurogenesis following interventions comprising exercise (Bekinschtein et al., 2011; Rossi et al., 2006). Our results demonstrate that nine months of multidisciplinary rehabilitation attenuates the loss of serum BDNF concentrations and this coincides with a reduction in the loss of grey matter volume in the hypothalamus (Chapter 4). BDNF is known to cross the blood-brain barrier (Pan, Banks, Fasold, et al., 1998; Pan, Banks, & Kastin, 1998; Pan & Kastin, 1999) in animal models and our observed increases in BDNF in the intervention group may indicate that BDNF mediated the neuroprotective effects in the present study, however these findings require confirmation.

In addition to improving brain outcomes, environmental interventions, particularly those encompassing exercise training, have been shown to regulate the circadian rhythm in animal models of HD (Cuesta et al., 2014) and improve subjective sleep quality in individuals with Alzheimer’s disease and Parkinson’s disease (Nascimento et al., 2014). Based on initial studies
using subjective sleep assessments (Chapter 4), no changes in sleep outcomes were identified in the intervention group compared to the control group. However, when objective sleep measures were used to assess sleep outcomes following the intervention, significant differences were observed in REM latency, the percentage of total sleep time spent in REM and in stage N1 sleep (Chapter 5). It is possible that individuals have no perceived deterioration in habitual sleep despite pathological alterations in sleep architecture, a phenomenon which has previously been reported in individuals with HD (Goodman et., 2011). Therefore, objective assessment of sleep using polysomnography should be conducted in future studies to provide further insight into these features in premanifest HD.

Compared to healthy controls, individuals with HD exhibit a decrease in the percentage of total sleep time spent in REM and an increase in REM latency (Arnulf et al., 2008). In the study presented in Chapter 5, increases in the percentage of REM sleep and decreases in REM latency were observed following the intervention period, suggesting that multidisciplinary rehabilitation can improve sleep outcomes in individuals with premanifest HD. Furthermore, given that REM sleep has been proposed to be a key mediator of the neuroplasticity associated with reorganisation of memories (Genzel et al., 2015), it is possible that an increase in REM could have beneficial implications for memory consolidation. However, the results presented here demonstrate that, although increases in REM sleep were observed, no improvements in the delayed recall or retention of words were noted, indicating that the improvements in REM did not mediate improvements in memory consolidation in this group. It should be noted that the sample size in this study was relatively small. In addition, the measure used to quantify memory consolidation in this study may not be sufficiently sensitive to detect small changes. Larger studies are therefore required, with more robust measures, to comprehensively assess the effects of increased REM sleep on memory consolidation.
Although speculative, it is possible that the effects of multidisciplinary rehabilitation on grey matter volume could be mediated by improved sleep regulation. Recent studies suggest that prolonged sleep disturbances could exacerbate neurodegenerative processes (Musiek, 2015; Musiek & Holtzman, 2016). The mechanisms by which sleep disturbances could drive neurodegenerative processes are yet to be robustly examined, however it has been suggested that oxidative damage as a result of circadian dysregulation could contribute to neurodegeneration (Musiek, 2015). Furthermore, while studies are yet to be conducted in humans regarding the effects of treating sleep disturbances on clinical outcomes, evidence from animal models suggest that treating sleep disturbances can also have a favourable impact on clinical features of the disease (Kudo et al., 2011). It is therefore plausible that the improvements in clinical outcomes observed following multidisciplinary rehabilitation in individuals with HD could be mediated by improvements in sleep. Further studies are required to assess this hypothesis in individuals with HD.

There are several limitations to the research presented in this thesis. Firstly, the studies presented in Chapters 4 and 5 were not randomised. Together with the small sample size, this limits the generalisability of the studies to the wider HD community. Secondly, the studies were conducted in individuals with premanifest HD, limiting the translation of the intervention to other, more severe disease stages. Additional studies in manifest populations are required for translation to this disease stage. Finally, a washout period was not included, preventing the assessment of the residual effects of multidisciplinary rehabilitation. Future studies should include a washout period to determine the length of duration of the effects of multidisciplinary rehabilitation in HD.
6.2 Future Directions

The work presented in this thesis is the first to assess the neurobiological underpinnings of circadian rhythm and habitual sleep disturbances in premanifest HD. It is also the first to evaluate the effects of multidisciplinary rehabilitation on circadian rhythm and sleep in individuals with premanifest HD. Findings from this thesis show that hypothalamic pathology and sleep disturbances are key features of HD and are remediable to multidisciplinary rehabilitation. Following multidisciplinary rehabilitation, individuals with premanifest HD had significant improvements in sleep architecture and attenuated grey matter volume loss in the hypothalamus. This adds to the growing body of evidence supporting the use of multidisciplinary rehabilitation as a strategy to improve neurobiological and clinical outcomes in HD (Cruickshank et al., 2015; Piira et al., 2013; Thompson et al., 2013; Veenhuizen et al., 2011; Zinzi et al., 2007). Further studies are nevertheless required to build upon this preliminary data.

Chapter 3 of this thesis details the association between hypothalamic volume and circadian markers in individuals with premanifest HD. The hypothalamus contains the circadian pacemaker, the SCN, which is responsible for mediating the circadian rhythm. However, there are multiple structures that are involved in the release of cortisol and melatonin (Bartlett et al., 2016; Saper et al., 2005). Future studies should therefore consider imaging other structures involved in the release of cortisol and melatonin. Furthermore, cortisol and melatonin are subject to variation due to various environmental factors. The inclusion of additional indicators of circadian rhythm, such as thermoregulation, heart rate and blood pressure, would provide a more holistic view of circadian rhythm in premanifest HD (Bellosta Diago et al., 2017; Cagnacci, Elliott, & Yen, 1992). In addition, while the studies presented in this thesis assessed melatonin levels around the participants’ usual bedtime, future studies should assess the dim
light melatonin onset as an additional measure of circadian rhythm changes in individuals with HD (Pandi-Perumal et al., 2007).

Circadian rhythm and sleep disturbances are common features amongst neurodegenerative disease and have recently been purported to drive neurodegenerative processes (Musiek, 2015; Musiek & Holtzman, 2016; Pillai & Leverenz, 2017). Despite this knowledge, the associations between circadian rhythm and sleep disturbances and brain volume in individuals with HD are yet to be robustly investigated. Future studies should longitudinally assess the relationship between circadian rhythm and sleep disturbances and brain volume in individuals with HD.

While improvements were observed in sleep architecture following the nine-month intervention, future studies should assess the inclusion of bright light therapy into the multidisciplinary rehabilitation model, with the aim to improve circadian rhythm. Light therapy has been shown to improve the rest-activity profile in HD mouse models (H.-B. Wang et al., 2017), sleep, mood and motor symptoms in individuals with Parkinson’s disease (Paus et al., 2007; Rutten et al., 2012) and sleep-wake cycles in individuals with Alzheimer’s disease (Ancoli-Israel, Gehrman, et al., 2003; Burns, Allen, Tomenson, Duignan, & Byrne, 2009; Lyketsos, Lindell Veiel, Baker, & Steele, 1999; McCurry et al., 2011). Bright light therapy has the potential to improve circadian rhythmicity in individuals with HD and should therefore be assessed for inclusion into the multidisciplinary rehabilitation model.

Circadian rhythm and sleep disturbances arise early in HD and worsen as the disease course lengthens. Chapter 5 of this thesis demonstrates that multidisciplinary rehabilitation can improve sleep architecture in individuals with premanifest HD. Multidisciplinary rehabilitation has previously been shown to be feasible and beneficial in individuals with manifest HD. It is plausible that multidisciplinary rehabilitation could improve sleep outcomes in individuals with manifest HD. Therefore, future studies should assess the effects of multidisciplinary
rehabilitation on sleep outcomes in individuals with manifest HD. Evidence from HD mouse models suggests that behavioural interventions, such as restricted feeding and light therapy, improve circadian rhythm and sleep outcomes, as well as motor outcomes (H.-B. Wang et al., 2018; H.-B. Wang et al., 2017). However, it is not known if the improvements in circadian rhythm and sleep mediate the improvements in motor outcomes. Future studies should assess the relationship between improved sleep as a result of multidisciplinary rehabilitation and motor outcomes in individuals with manifest HD.

The results presented in this thesis also provide impetus for the evaluation of multidisciplinary rehabilitation in other neurodegenerative diseases. For example, a recent study by Breen et al (2016) reported hypothalamic volume loss in individuals with Parkinson’s disease. Furthermore, individuals with Parkinson’s disease exhibit reduced REM density (Schroeder et al., 2016). Given that multidisciplinary rehabilitation reduces the loss of hypothalamic volume and increases REM in individuals with HD, it is plausible that similar effects could be seen in individuals with Parkinson’s disease.
6.3 Conclusion

In conclusion, individuals with premanifest HD exhibit reduced hypothalamic volume compared to healthy controls. Contrary to expectation, hypothalamic volume was not strongly associated with the output of circadian-regulated hormones, cortisol and melatonin in the population studied here. However, it is possible that the circadian rhythm is maintained by compensatory mechanisms, until these mechanisms are overcome, after which circadian dysregulation ensues.

The research presented in this thesis provides preliminary evidence to suggest that multidisciplinary rehabilitation can reduce the loss of hypothalamic grey matter volume in individuals with premanifest HD. The mechanisms underpinning this reduced loss of volume are yet to be determined, however the reduced loss of hypothalamic volume coincided with maintenance of BDNF levels. It is plausible that hypothalamic volume is somewhat preserved by maintenance of BDNF levels in premanifest HD. Furthermore, significant improvements in sleep architecture, but not in subjective sleep outcomes or circadian release of hormones, were observed in individuals with premanifest HD following nine months of multidisciplinary rehabilitation. It is possible that these improvements in sleep architecture also contribute to the maintenance of grey matter volume observed following multidisciplinary rehabilitation in HD, but not improvements in memory consolidation. However, these findings need to be confirmed in a larger cohort of individuals with premanifest HD. Future studies should assess the effects of multidisciplinary rehabilitation on sleep and circadian rhythm outcomes in a larger, randomised controlled trial in individuals with premanifest HD.
References


Miyazaki, T., Hashimoto, S., Masubuchi, S., Honma, S., & Honma, K.-I. (2001). Phase-advance shifts of human circadian pacemaker are accelerated by daytime physical


Nishino, H., Koizumi, K., & Brooks, C. M. (1976). The role of suprachiasmatic nuclei of the hypothalamus in the production of circadian rhythm. *Brain Res, 112*(1), 45-59. doi:[http://dx.doi.org/10.1016/0006-8993(76)90333-4](http://dx.doi.org/10.1016/0006-8993(76)90333-4)


van der Marck, M. A., Munneke, M., Mulleners, W., Hoogerwaard, E. M., Borm, G. F., Overeem, S., . . . the IMPACT study group. (2013). Integrated multidisciplinary care in
Parkinson's disease: a non-randomised, controlled trial (IMPACT). The Lancet Neurology, 12(10), 947-956. doi:http://dx.doi.org/10.1016/S1474-4422(13)70196-0


HEROs (Huntington’s disease Exercise Rehabilitation Optimisation study):

The Effects of Multidisciplinary Rehabilitation Therapy on Clinical Measures of Disease Progression and Quality of Life for Patients with Huntington’s Disease.

PARTICIPANT INFORMATION SHEET

Prof Mel Ziman, Dr Travis Cruickshank, Mrs. Maggie Speirs, Prof Nellie Georgiou-Karistianis, Prof Brian Power, Prof Anthony Hannan, Prof Andrew Churchyard, Dr Wei Peng Teo, Ms Linda Hoult, Ms Danielle Bartlett, Ms Niamh Mundell, Mr Andrew Govus, Mr Timothy Pulverenti and Ms Catarina Kordsachia, Prof Peter Eastwood, Dr Juan Dominguez, Dr Kirk Feindel, Ms Sophia Quick, Dr Alpar Lazar, Dr Amit Lampit

Please take time to read the following information carefully and discuss it with your friends, family and clinician if you wish. Ask us any question if some part of the information is not clear to you or if you would like more information. Please do this before you sign this consent form.

Who is funding this study and where will it be conducted?

This study has been supported by a grant from Lotterywest, Huntington’s WA (Inc.) and Edith Cowan University. This project will be conducted at Edith Cowan University, Monash University and Deakin University as well as at clinical exercise centres and in participant’s homes.

Contact persons: Should you have any questions about the study please contact:

Dr Travis Cruickshank, Ph: 6304 3416 Email: t.cruickshank@ecu.edu.au;
Mrs Maggie Speirs, Ph: 9346 7599 Email: maggie@huntingtonswa.org.au;
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Prof Nellie Georgiou-Karistianis: Phone 9905 1575 Email: nellie.georgiou-karistianis@monash.edu

All study participants will be provided with a copy of the Participant Information Sheet and Participant Consent Form for their personal records. You may decide to provide or not provide your consent for the prospective study. Your decision will not lead to any penalty or affect your regular medical care or any benefit to which you are entitled.

What is the purpose of this study?

The purpose of this study is to examine the clinical utility of multidisciplinary therapy compared to standard care in HD gene positive individuals. This is a pilot study. The study will involve allocation to a multidisciplinary therapy (Perth) or standard care group (Melbourne). The multidisciplinary therapy program encompasses cognitive, psychological, physical and social exercises aimed at slowing disease progression, enhancing neural stimulation, functional
independence and quality of life. Participants allocated to the multidisciplinary therapy group will be required to commit to a four hour program of multidisciplinary therapy every week for 12 months. The therapy will be delivered as a three times weekly supervised therapy at a clinical exercise centre. The researchers aim to advance multidisciplinary therapy for individuals with Huntington’s disease in collaboration with teams locally, nationally and internationally.

**Why was I selected to take part in this study?**

This study is suitable for individuals diagnosed as gene positive (either presymptomatic or symptomatic) for Huntington’s disease.

**Do I have to participate?**

There is no obligation and or requirement to participate in this study. Declining to participate in this research project will not influence your regular medical treatment in any way.

**What will happen if I decide to participate in this research project?**

Prior to commencement of the study you will be required to sign a consent form.

As a participant you will be asked to participate in two assessment periods, one at baseline and one after 12 months. We will organise a suitable time and place for your assessments. At these periods you will be assessed using neurological (Unified Huntington’s Disease Rating Scale Total Motor Score (UHDRS-TMS)), physical, cognitive, psychological, physiological and quality of life assessments. The neurological assessment (UHDRS) will be performed to ensure that participants fulfill the inclusions criteria. The results of this neurological examination will not be disclosed to participants and will only be used for research purposes. You will be asked to undergo magnetic resonance imaging (MRI) to assess brain volume and Dual Energy X-Ray Absorptiometry (DEXA) scans to assess body composition. You will also be asked to provide saliva, hair and a small 36 ml blood sample for acute and chronic assessment of biological measures.

Physical methods of assessment will include fine and gross motor movements, your balance and walking and your strength. Maximum physical capacity will be examined using reliable and clinically valid measurements.

Cognitive methods of assessment will involve a variety of tests that measure your problem solving capability and your memory and emotional recognition. Psychological assessments will be used to assess your anxiety and depression levels. Physiological methods of assessment will measure your body composition, your sleep, your body temperature control, exercise capacity, and your brain volume by MRI.

The results of these assessments will not be disclosed to participants and will only be used for research purposes.

After baseline assessment, individuals who decide to participate will undertake a multidisciplinary therapy intervention (Perth) or maintain their standard care (Melbourne). The multidisciplinary therapy intervention will require you to participate in both physical and mental exercises every week for 12 months. Supervised multidisciplinary therapy (physical
and mental) will require you to attend a specific clinical gym for 60-90 minutes three times a week.

Participant attendance at gym sessions will be monitored by clinical exercise specialists.

After 12 months you will be required to once again undertake a neurological examination (UHDRS), as well as physical, cognitive, psychological and physiological assessments as indicate above.

There may be a video recording of one of your clinical assessments and you will be informed of this so you can provide your consent for this to take place.

**What are the costs to me?**

Travel costs and time taken to perform the assessments and multidisciplinary therapy intervention are the only costs involved in the prospective study.

**What are the potential benefits associated with participating in this study?**

The proposed multidisciplinary therapy may exert positive therapeutic changes in physical, cognitive, social, psychological and/or physiological functioning. If successful, the research program may be routinely implemented for individuals with Huntington’s disease worldwide.

**What are the potential disadvantages and or risks of taking part in this study?**

Please read carefully the following sections for MRI, DEXA (dual energy x-ray absorptiometry) scan, maximum physical capacity and donation of a blood sample for more detailed information. There are time commitments and travel commitments associated with the study.

**What information do I need to know about having an MRI scan?**

As a participant you will be required to have an MRI scan. MRI stands for magnetic resonance imaging. An MRI scanner is a machine that can take clear pictures of the inside of the body. The machine uses an electric field and a strong magnet to produce electromagnetic radiation and radio waves which are not harmful. The pictures taken by the machine are called MRI scans. Prior to the scanning two healthy volunteers will undergo scans to ensure the safety and reliability of the MRI procedures. The machine uses an electric field and a strong magnet to produce electromagnetic radiation and radio waves which are not harmful. The pictures taken by the machine are called MRI scans. The scan will take 20 for one scan or up to 60 minutes for three different kinds of scans and you will be informed at the time we make your appointment whether you are required for one or three scans. There is no special preparation and you can eat and drink as normal before the scan and you can take your normal medications. We will ask you to lie on a table and this will be moved backwards into the MRI scanner. The scanner will record information about your brain. It is very important that you keep very still during the scanning. When you lie on the table, we will make sure you are as comfortable as possible. The scanner makes loud tapping noises and we can give you some earphones to reduce the noise.
Some people (approximately 3-5%) experience symptoms of claustrophobia in the scanner. If you experience discomfort at any time during the scan, you will be able to alert staff by pressing on a call button provided to you.

Because a large magnet is used we will ask you if you have any metal implanted in your body, such as a pacemaker or metal pins and to remove any metal items such as watches, jewellery, hairpins, dentures etc.

Very occasionally (in approximately 2% of cases), the images of normal participants may show anatomical abnormalities. It may be necessary to do further tests to establish whether an abnormality is truly present. Some findings may have no negative implications for your health, and are called incidental. However, in about 1% of scans, the imaging abnormality may represent a risk to your health, and is called an adverse finding. In many cases, there are effective treatments available for adverse findings, but sometimes there are adverse findings for which no effective treatment is currently available.

Before your scan, you will be asked to indicate whether you wish to be informed about (i) all incidental findings; or (ii) only those adverse findings that would usually lead directly to treatment. You will also be asked whether you would like any incidental and/or adverse findings to be discussed with you by your usual General Practitioner (GP), another doctor of your choice, or by the MBI radiologist. You are entitled to be informed of the results of the scan but we understand that you may not wish to receive the report yourself.

You may consent to copies of the report being released to the research team, and/or to a doctor of your choice. Further investigation and treatment of adverse findings is unable to be funded by Monash Biomedical Imaging or by the researchers conducting the study. Knowledge of an incidental finding may also have implications for your private health insurance.

We will use these brain scans to compare your brain volume before and after the intervention which will increase our understanding of how the intervention affects your mental and physical symptoms of Huntington’s disease.

**What information do I need to know about performing tests of my maximum physical capacity?**

In order for us to test your maximal physical capacity, you will be asked to complete an incremental exercise test on a cycle ergometer until self-perceived exhaustion. The test will begin at an easy workload of 60-80 watts and thereon increase after every minute by 20 watts until self-perceived exhaustion. During the test you will be required to wear a mouthpiece that is attached to a gas analyser. This analyser measures gas exchange and how much oxygen you consume. Your heart rate will also be measured using a wireless strap around your chest. In addition, changes in haemoglobin concentration will be measured non-invasively in the front part of your brain using near infrared resonance spectroscopy. Finally, blood lactate will be measured before and after the test using a Lactate Pro 2.

You will also be asked to perform 3 maximal isometric foot extensions against a rigid foot platform on an isokinetic dynamometer. During the maximal isometric foot extensions your muscle activity will be assessed using non-invasive electrodes placed on the front and back of your lower limbs.
These maximal physical assessments will enable us to measure:

- Gas exchange using a mouthpiece connected to a gas analyser
- Blood lactate levels before and after exercise using the Lactate Pro 2
- Haemoglobin concentration changes in the front part of your head
- Muscle activity using non-invasive electrodes placed on your lower limbs

**What are the potential risks? Will you experience any discomfort or inconvenience?**

- Pain and discomfort from shaving, abrading and cleaning with alcohol before applying the electrodes.
- During the incremental cycling performance test there is a rare chance (1 in 100000) of cardiovascular complications. However, if the ECG test, or the test by your GP, or medical questionnaires shows any potential risks against maximal exercise, you will be excluded from this testing procedure.
- Maximal muscle contractions during the strength measurement may lead to muscle soreness directly after or in the days that follow the test.

**What information do I need to know about having a DEXA scan?**

As a participant in this project you will be required to have a DEXA scan which takes approximately 15 minutes to determine your body composition, such as your bone density and muscle mass.

The DEXA scan will expose you to a very small amount of radiation. As part of everyday living, everyone is exposed to naturally occurring background radiation and receives a dose of about 2 millisieverts (mSv) each year. The effective dose from this research project is about 0.001 mSv/year. At this dose level, no harmful effects of radiation have been demonstrated, as any effect is too small to measure. Therefore, the risk from radiation as a result of this project is believed to be minimal.

However, it is important for the researchers to know if you have received any other doses of ionising radiation during your medical care as cumulative doses may be harmful.

**What information do I need to know about giving a blood sample?**

Some possible disadvantages and risks associated with blood testing include bruising, bleeding, fainting, dizziness, haematoma, and infection. We need to ask you to provide a sample of blood for this research project as we would like to measure genetic sequences that may be associated with disease progression or your ability to respond to multidisciplinary therapy. We would also like to measure the levels of biological markers in your blood sample such as hormones and antioxidants.

Your blood and DNA sample will not have your name on it. It will be stored in a locked freezer in a secure research facility, and will only be accessed by staff connected to this study.

Your genetic and biochemical material and de-identified information will not be released for other uses without your prior consent, unless required by law.
With your consent your de-identified genetic and biochemical sample may be used for future research projects looking at multidisciplinary therapy and/or Huntington’s disease. Your samples will be kept for a maximum of seven years after completion of this project. If however you decline to allow us to use your DNA and biochemical samples for future research we will destroy them seven years after the completion of this research.

A small portion of your blood sample (sufficient for a genetic test) may be transferred to Monash University for genetic testing and we will keep a portion here in Perth to assess other gene sequences. Although we cannot be directly responsible for the samples once they have left our facility, Monash University has confirmed with us that your samples will not be sold or shared with anyone else.

In addition, your sample will not be utilised for any genetic test other than stated above or for any test that determines the health risk of your family and will not be disclosed to insurance or employment companies.

What information do I need to know about having polysomnography sleep assessment?

Stress and sleep disturbances have been reported in premanifest HD individuals and these factors have been shown to impact on brain function and even reduce volume of certain brain structures. As these factors can be manipulated through an individual’s environment, it is likely that multidisciplinary therapy will regulate stress and sleep patterns in these individuals.

In order to assess changes in stress outcomes and sleep quality in premanifest HD individuals as a result of the multidisciplinary therapy intervention, we will require the participants to provide morning and evening saliva samples via passively drooling into a tube and a 36mL blood sample in the morning as well as providing 20 strands of hair. These measures will provide a biological indication of changes in stress and sleep hormonal patterns.

The participants will also be required to complete short questionnaires regarding self-perceived stress levels, as well as questionnaires detailing their sleep patterns and perception of daytime sleepiness in order to assess clinically relevant outcomes of the intervention on stress and sleep patterns.

Finally, to further analyse the effects of the intervention on sleep patterns, participants will be asked to undertake polysomnography, the gold standard measure of sleep architecture and quality. This will be accompanied by the use of actigraphy wrist monitors to observe sleep patterns of participants in their own homes to avoid any confounding effects of sleeping in a new environment.

Polysomnography studies will require the participant to stay overnight in a sleep clinic facility. The UWA Centre for Sleep Science is a specialised research facility with 5 separate bedrooms. Each participant will have their own bedroom. The rooms are all air-conditioned and have temperature control. Participants are encouraged to bring their own pillow and anything they would prefer to sleep with (apart from a bed partner). It is advised that participants wear pyjamas of loose, light fabric—something that is comfortable to sleep in. Participants will arrive at 7pm and will go about their normal evening routine before going to bed around their usual bedtime. In order to measure breathing patterns, heart rate, oxygen levels and brain activity during sleep, wires and electrodes will need to be attached to the body and then connected to a computer. These are to measure and record electric impulses generated by your body; the
electrodes themselves do not create any electricity. These electrodes are required for: electroencephalography (EEG, which measures brain activity), electromyography (EMG, which measures skeletal muscle movement), electrocardiography (ECG, which measures heart rhythm) and electrooculography (EOG, which measures eye movements).

A sleep technician will ensure the following are attached:

- Six electrodes and wires attached to the scalp using medical paste (easily removed in the morning upon having a shower)
- One stick-on electrode behind each ear, one next to each eye, three under the chin and two on the upper chest
- A band around the chest and another around the abdomen to measure breathing
- Tape on one finger to measure oxygen saturation
- A soft wire and tubing under the nose and an airflow sensor to measure breathing
- Two electrodes on each leg to record movement
- A microphone to record sound
- Two activity monitors- one around the wrist and one soft belt around the hips to record movement
- A video camera to record movements whilst asleep (turned on once you are settled and it is dark. This is an infra-red camera and does not record details)
- All of these leads will be connected to one box that can be easily unhooked and taken with you, should you want to get up during the night

This set up process takes approximately 45 minutes. A sleep technician will be in the office next door to your bedroom all night and you will be able to communicate with this technician through an intercom system next to your bed. Although most people think it will be difficult to fall asleep in a different place whilst being hooked up to machines, generally, almost everyone falls asleep relatively quickly. The following morning you will be given the results of your sleep patterns and sleep quality.

These techniques are required to assess participants for sleep abnormalities. All these measures will be repeated both before and after the intervention and will provide a holistic insight into any changes in stress and sleep patterns of these individuals following the intervention.

**What happens when the research study stops?**

Your regular medical treatment will continue as normal. We will advise you of the general study outcomes by newsletter, websites and public seminar. We also intend to publish our results in medical research journals and present them at research conferences locally, nationally and internationally. We may also provide general information to participating individuals if they wish to receive it. Please note that your name or any other identifying information will not be included in any of the publications or presentations.

**What will happen to my data, blood sample and DNA samples?**
Your biological samples will be stored in locked freezers in secure laboratories for a maximum of seven years after the end of the project. These samples will be permanently destroyed by chemical and heat treatments.

Your data will be stored on password protected computers for a maximum of seven years then the data will be permanently destroyed.

**Will my taking part in this research project be kept confidential?**

If it is relevant you will be asked to provide consent for researchers to access your clinical information for use in this study. The information that we wish to access, is your CAG (cytosine, adenine guanine – DNA) repeat number, UHDRS (United Huntington’s Disease Rating Scale) score, your disease duration and your age at diagnosis.

The clinical information along with your study data will be available to the researchers only and will be securely stored in ECU locked filing cabinets for analysis. Electronic and recorded data obtained will be transferred onto ECU computers and stored on password protected hard drives. All data will be entered into a solitary excel database where it will be encrypted and only available to individuals working on this project. All data obtained from you will be de-identified for use in publications.

Researchers / clinicians will be monitoring changes in physical and mental health as a result of the disease or multidisciplinary therapy. Changes which indicate that the participant’s safety is at risk will be reported to their treating physician or the Neurosciences Unit so that appropriate treatment can be provided.

**What if I would like extra information or independent advice about participation in research?**

The contact details for the project staff, Huntington’s WA staff, Edith Cowan University (ECU), Monash University and North Metropolitan Health Service Mental Health (NMHS MH) review bodies are included at the end of this information sheet.

**What if new information regarding multidisciplinary therapy becomes available throughout the study?**

Sometimes during the course of a research project, new information becomes available about the treatment that is being studied. If this happens, we will tell you about it and discuss with you whether you want to continue in the research project. If you decide to withdraw your regular health care will continue.

**What if I decide to no longer participate?**

You may withdraw from the study at any time without prejudice and do not need to provide a reason. There will be no changes to your routine medical treatment.

**What will happen to the results upon conclusion of the study?**

The results of the study will be written in medical and scientific literature and presented at research seminars and forums. If you so desire, you may be given a report of your personal results.
Who has reviewed the study?

Approval to conduct this research has been provided by the Human Research Ethics Committees of Edith Cowan University (ECU), Monash University, Deakin University and the North Metropolitan Mental Health Service Research Ethics and Governance Office (NHMS MH REGO) in accordance with their ethics review and approval procedures. Any person considering participation in this research project, or agreeing to participate, may raise any questions or issues with the researchers at any time.

If you have any questions or require further information about the research, please contact:

Mrs Maggie Speirs: 9346 7599 Email maggie@huntingtonswa.org.au
Dr Travis Cruickshank: Phone 6304 3416 Email t.cruickshank@ecu.edu.au
Prof Mel Ziman: Phone 6304 3640 Email m.ziman@ecu.edu.au
Prof Nellie Georgiou-Karistianis: Phone 9905 1575 Email: nellie.georgiou-karistianis@monash.edu

In addition, any person not satisfied with the response of researchers may raise ethics issues or concerns, and may make any complaints about this research project by contacting either the Human Research Ethics Office at ECU on (08) 6304 2170 or research.ethics@ecu.edu.au.
HEROS (HUNTINGTON’S DISEASE EXERCISE REHABILITATION OPTIMISATION STUDY):

PARTICIPANT CONSENT FORM

1. I (the participant) have read the information above and any questions I have asked have been answered to my satisfaction. I understand that my participation is voluntary and that I may withdraw at any time without penalty or affect to my regular medical care.

2. I understand that the information I provide will be kept in the strictest confidence by the researchers, unless obliged to release this information by law.

3. I understand that the study involves the following procedures:
   a. I will be asked to attend gym sessions three times per week
   b. I may be asked to give a blood, saliva and hair sample for biochemical analysis.
   c. I will be asked to perform a variety of non-invasive neurological (UHDRS), physical, cognitive and psychological tests and to answer questionnaires and I understand that some of the tests may be video-recorded for analysis purposes.
   d. I agree to my carer or partner participating in this research project by completing questionnaires about my health status, and give permission for them to do so
   e. I understand that I may be asked to undertake an MRI brain scan and / or a DEXA scan.
   f. I understand I may be required to undertake an overnight stay at a sleep clinic and wear an actigraphy bracelet to measure sleep quality.
   g. I understand that I will be required to undertake a maximal aerobic and strength test. I understand that my skin will be prepared to record muscle activity throughout the strength test. I understand that my heart rate, gas exchange, blood lactate and haemoglobin concentration changes will be measured throughout the aerobic test.
   h. I understand that the maximal strength measurements and cycling exercises may lead to muscle soreness if I am unaccustomed to the mode and intensity of the exercise performed.

4. I agree the research data gathered for this study may be published provided my name and any other identifying information is not used

5. I agree to allow this project to access my CAG (cytosine, adenine guanine – DNA) repeat number, and where relevant my UHDRS (United Huntington’s Disease Rating Scale) score, disease duration and my age at diagnosis which is held in my case notes at the Neurosciences Unit.

6. I agree that data gathered as part of this study can be used in future research projects that have approval from the appropriate Institutional Ethics Committees, as long as my name or any other identifying information is not made available to these projects.

7. I confirm that I do not suffer from any neurological condition other than HD, or any physical or mental condition that would affect my ability to participate in this study and I provide permission for the researchers to contact my treating physician or the Neurosciences Unit to arrange appropriate treatment if they identify any physical or mental health concerns before or during the study.
8. I wish to be informed about
   a. all incidental findings; or
   b. only those adverse findings that would usually lead directly to treatment.

9. Would you like any incidental and/or adverse findings to be discussed with
   a. your usual General Practitioner,
   b. another doctor of your choice,
   c. or by the radiologist.

10. Are you currently participating in any other research project?

Name of Participant (please print) ____________________________________________

Signed ___________________________ Date ____________________

Phone ________________

Investigator (Name, please print) ____________________________________________

Signed ___________________________ Date _________________________________

Approval to conduct this research has been provided by the Human Research Ethics Committees of Edith Cowan University (ECU) and the North Metropolitan Mental Health Service Research Ethics and Governance Office (NHMS MH REGO) in accordance with their ethics review and approval procedures. Any person considering participation in this research project, or agreeing to participate, may raise any questions or issues with the researchers at any time.

Any questions concerning the project entitled “HEROs: The effects of Multidisciplinary Therapy on clinical measures of disease progression and quality of life for patients with Huntington’s disease” can be directed to:

Prof Mel Ziman; Phone: 6304 3640, Email: m.ziman@ecu.edu.au;
Linda Hoult; Phone: 6304 3401, Email: l.hoult@ecu.edu.au
Dr Travis Cruickshank; Phone: 6304 3416, Email: t.cruickshank@ecu.edu.au
Huntington’s WA: Maggie Speirs; Phone: 9346 7599, Email: maggie@huntingtonswa.org.au

In addition, any person not satisfied with the response of researchers may raise ethics issues or concerns, and may make any complaints about this research project by contacting either the Human Research Ethics Office at ECU on (08) 6304 2170 or research.ethics@ecu.edu.au or The NMHS MH REGO Executive Officer on (08) 9347 6502 or NMAHSMHREGO@health.wa.gov.au.
HEROs (Huntington’s disease Exercise Rehabilitation Optimisation study):

The effects of multidisciplinary rehabilitation therapy on clinical measures of disease progression and quality of life for patients with Huntington’s disease.

PARTICIPANT INFORMATION SHEET

Prof Mel Ziman, Dr Travis Cruickshank, Ms Linda Hoult, Ms Danielle Bartlett, Mr Andrew Govus, Mr Timothy Pulverenti, Mr Tim Ball

Please take time to read the following information carefully and discuss it with your friends, family and clinician if you wish. Ask us any question if some part of the information is not clear to you or if you would like more information. Please do this before you sign this consent form.

Who is funding this study and where will it be conducted?

This study has been supported by a grant from Lotterywest, Huntington’s WA (Inc.) and Edith Cowan University. This project will be conducted at Edith Cowan University and at participant homes.

Contact persons:

Prof Mel Ziman, Ph: 6304 3640, Email: m.ziman@ecu.edu.au
Dr Travis Cruickshank, Ph: 6304 3416, Email: t.cruickshank@ecu.edu.au
Ms Linda Hoult, Ph: 6304 3401, Email: l.hoult@ecu.edu.au
Ms Danielle Bartlett, Ph: 6304 3568, Email: d.bartlett@ecu.edu.au

All study participants will be provided with a copy of the Participant Information Sheet and Participant Consent Form for their personal records. You may decide to provide or not provide your consent for the prospective study. Your decision will not lead to any penalty or affect your regular medical care or any benefit to which you are entitled.

What is the purpose of the study?

The purpose of this study is to assess healthy individuals using physical, cognitive and biological measures. The data collected from these measures will be used to validate measures in people with Huntington’s Disease (HD).

Why was I selected for this study?

This study is suitable for healthy individuals. Participation will assist in the validation of novel motor, cognitive and biological measures for use in HD studies.

Do I have to participate?
There is no obligation and or requirement to participate in this study. Declining to participate in this research project **will not** influence your health or your regular medical treatment in any way.

**What will happen if I decide to participate in this research study?**

Prior to the commencement of the study you will be required to sign a consent form.

As a participant you will be asked to perform assessments that assess your physical function, exercise capacity, cognition and sleep quality. You will also be asked to provide a small hair sample for biochemical analyses. All results are for the research project only and not for clinical assessment.

Physical measures include fine and gross motor assessments. Balance, walking and strength measures will also be used. Exercise capacity will be measured using a measure of self-perceived maximal exertion in a specialised exercise physiology lab. Cognitive methods of assessments will involve a variety of tests that measure your problem solving capacity and ability to recognise and perceive visual and auditory emotions. Your quality of sleep will also be examined using actigraph wrist monitors and validated sleep questionnaires.

All assessments will be administered by trained specialists. The results of these assessments will not be disclosed to individuals not involved with the research study and will only be used for research purposes.

**What are the costs to me?**

Travel costs and time taken to perform the assessments are all that is involved.

**What are the potential benefits associated with participating in this study?**

The results of this study could be of interest to you, your family, acting clinicians and the HD community at large. The findings of this study could lead to more specialised diagnostic and prognostic assessment tools leading to more effective treatments for individuals suffering with HD.

**What are the potential disadvantages and or risks of taking part in this study?**

Please read carefully the following sections for maximum physical capacity for more detailed information. There are time commitments and travel commitments associated with the study. Importantly, your hair and exercise data will be deidentified (not have your name on it), thereby maintaining your privacy. This information will not be released for other uses without your prior consent, unless required by law.

**What information do I need to know about performing tests of my maximum physical capacity?**

In order for us to test your maximal physical capacity, you will be asked to complete an incremental exercise test on a cycle ergometer until self-perceived exhaustion. The test will begin at an easy
workload of 60-80 watts and thereon increase after every minute by 20 watts until self-perceived exhaustion. During the test you will be required to wear a mouthpiece that is attached to a gas analyser. This analyser measures gas exchange and how much oxygen you consume. Your heart rate will also be measured using a wireless strap around your chest. In addition, changes in haemoglobin concentration will be measured non-invasively in the front part of your brain using near infrared resonance spectroscopy. Finally, blood lactate will be measured before and after the test using a Lactate Pro 2.

You will also be asked to perform 3 maximal isometric foot extensions against a rigid foot platform on an isokinetic dynamometer. During the maximal isometric foot extensions your muscle activity will be assessed using non-invasive electrodes placed on the front and back of your lower limbs.

These maximal physical assessments will enable us to measure:

- Gas exchange using a mouthpiece connected to a gas analyser
- Blood lactate levels before and after exercise using the Lactate Pro 2
- Haemoglobin concentration changes in the front part of your head
- Muscle activity using non-invasive electrodes placed on your lower limbs

**What are the potential risks? Will you experience any discomfort or inconvenience?**

- Pain and discomfort from shaving, abrading and cleaning with alcohol before applying the electrodes.
- During the incremental cycling performance test there is a rare chance (1 in 100000) of cardiovascular complications. However, if the ECG test, or the test by your GP, or medical questionnaires shows any potential risks against maximal exercise, you will be excluded from this testing procedure.
- Maximal muscle contractions during the strength measurement may lead to muscle soreness directly after or in the days that follow the test.

**What happens when the research study stops?**

We will advise you of the general study outcomes by newsletter, websites and public seminar. We also intend to publish our results in medical research journals and present them at research conferences locally, nationally and internationally. We may also provide general information to participating individuals if they wish to receive it. Please note that your name or any other identifying information will not be included in any of the publications or presentations.

**What will happen to my data and biochemical samples?**

Your biological samples will be stored in locked freezers in secure laboratories for a maximum of seven years after the end of the project. These samples will be permanently destroyed by chemical and heat treatments.

Your data will be stored on password protected computers for a maximum of seven years then the data will be permanently destroyed.

**Will taking part in this research project be kept confidential?**
Your information along with your study data will be available to the researchers only and will be securely stored in ECU locked filing cabinets for analysis. Electronic and recorded data obtained will be transferred onto ECU computers and stored on password protected hard drives. All data will be entered into a solitary excel database where it will be encrypted and only available to individuals working on this project. All data obtained from you will be de-identified for use in publications.

What if I would like extra information or independent advice about participation in research?

The contact details for the project staff and Edith Cowan University are included at the end of this information sheet.

What if I decide to no longer participate?

You may withdraw from the study at any time without prejudice and do not need to provide a reason.

What will happen to the results upon conclusion of the study?

The results of the study will be written in medical and scientific literature and presented at research seminars and forums. If you so desire, you may be given a report of your personal results.

Who has reviewed the study?

Approval to conduct this research has been provided by the Edith Cowan University (ECU) Human Research Ethics Committee. Any person considering participation in this research project, or agreeing to participate, may raise any questions or issues with the researchers at any time.

If you have any questions or require further information about the research, please contact:

Dr Travis Cruickshank; Phone: 6304 3416, Email: t.cruickshank@ecu.edu.au
Prof Mel Ziman; Phone: 6304 3640, Email: m.ziman@ecu.edu.au
Ms Linda Hoult; Phone: 6304 3401, Email: l.hoult@ecu.edu.au
Ms Danielle Bartlett; Phone: 6304 3568, Email: d.bartlett@ecu.edu.au

In addition, any person not satisfied with the response of researchers may raise ethics issues or concerns, and may make any complaints about this research project by contacting either the Human Research Ethics Office at ECU on (08) 6304 2170 or research.ethics@ecu.edu.au.
HEROS (HUNTINGTON’S DISEASE EXERCISE REHABILITATION OPTIMISATION STUDY):

HEALTHY CONTROL PARTICIPANT CONSENT FORM

11. I (the participant) have read the information above and any questions I have asked have been answered to my satisfaction. I understand that my participation is voluntary and that I may withdraw at any time without penalty.

12. I understand that the information I provide will be kept in the strictest confidence by the researchers, unless obliged to release this information by law.

13. I understand that the study involves the following procedures:
   
i. I will be required to give hair samples for biochemical analysis.

j. I will be asked to perform a variety of non-invasive physical and cognitive tests and to answer questionnaires

k. I will be asked to wear an actigraph monitor and complete questionnaires to monitor sleep quality.

a. I will be required to undertake a maximal aerobic and strength test. I understand that my skin will be prepared to record muscle activity throughout the strength test. I understand that my heart rate, gas exchange, blood lactate and haemoglobin concentration changes will be measured throughout the aerobic test.

b. I understand that the maximal strength measurements and cycling exercises may lead to muscle soreness if I am unaccustomed to the mode and intensity of the exercise performed.

14. I agree the research data gathered for this study may be published provided my name and any other identifying information is not used.

15. I agree that data gathered as part of this study can be used in future research projects that have approval from the appropriate Institutional Ethics Committees, as long as my name or any other identifying information is not made available to these projects.

16. I confirm that I do not suffer from any neurological condition or any physical or mental condition that would affect my ability to participate in this study.

17. I wish to be informed about
   
a. all incidental findings; or
   
b. only those adverse findings that would usually lead directly to treatment.
18. Would you like any incidental and/or adverse findings to be discussed with
   a. your usual General Practitioner,
   b. another doctor of your choice,
   c. or by the radiologist.

19. Are you currently participating in any other research project?

   Name of Participant (please print) _______________________________________________
   Signed _________________________________ Date __________________
   Phone ______
   Investigator (Name, please print) ______________________________________________
   Signed __________________________________Date ___________________

Approval to conduct this research has been provided by the of Edith Cowan University (ECU) Human Research Ethics Committee. Any person considering participation in this research project, or agreeing to participate, may raise any questions or issues with the researchers at any time.

Any questions concerning the project entitled “HEROs: The effects of Multidisciplinary Therapy on clinical measures of disease progression and quality of life for patients with Huntington’s disease” can be directed to:

Prof Mel Ziman, Phone: 6304 3640, Email: m.ziman@ecu.edu.au ;
Dr Travis Cruickshank, Phone: 63043416, Email: t.cruickshank@ecu.edu.au ;
Ms Linda Hoult, Phone: 6304 3401, Email: l.hoult@ecu.edu.au
Ms Danielle Bartlett, Phone: 6304 3568, Email: d.bartlett@ecu.edu.au

In addition, any person not satisfied with the response of researchers may raise ethics issues or concerns, and may make any complaints about this research project by contacting either the Human Research Ethics Office at ECU on (08) 6304 2170 or research.ethics@ecu.edu.au.
Appendix 3: Salivary Sampling Instructions for Participants

Information for Participants- Biological Sampling and Questionnaires

Thank you for agreeing to provide us with biological samples for the HEROs 2.0 research project. In this sample collection package, you will find:

- 2 x saliva sampling packs, each containing 4 red-capped tubes, 4 blue-capped tubes, 8 straws and 8 labels for tubes
- 1 x set of detailed instructions describing how to collect your saliva samples (below)
- 1 x set of picture instructions for providing saliva samples
- 1 x biological specimen storage bag to store your samples in before placing them in the freezer
- 2 x saliva collection questionnaires to note down the time you went to bed, the time you woke up and the time you provided your samples
- 1 x set of questionnaires to fill out at various stages of sampling (containing the Perceived Stress Scale, the Epworth Sleepiness Scale, the Pittsburgh Sleep Quality Index and the Consensus Sleep Diary)

Travis and Danielle will make an appointment to come to your home to carry out cognitive testing. This will occur in the morning on two days that are suitable for you. During this time, Danielle will also take 4 tubes of blood and pick up your saliva samples that you would have already collected.

The night before our visit, we ask that you begin taking your night-time saliva samples. **IT IS ESSENTIAL THAT NIGHT TIME SALIVA SAMPLES ARE TAKEN IN DIM-LIGHT CONDITIONS. PLEASE WEAR YOUR SUNGLASSES DURING NIGHT TIME SALIVA SAMPLE COLLECTION BEGINNING FROM THE FIRST SAMPLE AND FOR THE REMAINDER OF THE THREE HOUR SAMPLE COLLECTION PERIOD AND REFRAIN FROM HAVING ANY LIGHTS ON DURING THIS TIME. LIGHT WILL RESET THE RELEASE OF THE SLEEP HORMONE MELATONIN AND WILL NOT PROVIDE AN ACCURATE INDICATION OF MELATONIN LEVELS. PLEASE DO NOT FALL ASLEEP DURING THIS TIME AS THIS WILL AFFECT HORMONE LEVELS.** You may watch TV during this sampling period, provided you wear your sunglasses and reduce the brightness of your TV using the settings menu.

The following morning, you will provide your morning saliva samples. You should also fill out the Consensus Sleep Diary, Perceived Stress Scale, Pittsburgh Sleep Quality Index and Epworth Sleepiness Scale. **After** you have provided your morning saliva samples, it is recommended that you have something to eat and **drink** prior to your blood being taken. Each of these processes will be repeated again on a second day. This ensures that the samples we take are reliable. On this second day, we will also require a hair sample (20 strands).

The **exact times** of saliva collection and **colour of the tube caps** are critical for analysis of the saliva samples. The **blue**-capped tubes will be for **morning** saliva and the **red**-capped tubes for saliva **before bed-time**. It is important that we collect saliva in these tubes so that they can be analysed appropriately. So, **blue**= **morning** and **red**= **night-time**.
We ask that you collect your saliva in the tubes by drooling into them using the straws provided, then **label the tubes with the time and date the samples were collected and your full name** and then **store them in your freezer** in the biological sample bag provided until we come to collect them.

Before providing your saliva sample, it is essential that you:

- **Avoid** alcohol 12 hours prior to, and during, saliva sample collection
- **Do not** brush your teeth before, and during, saliva sample collection
- Avoid smoking within the hour leading up to, and during, sample collection
- **Do not** eat or drink (except water) **60 minutes prior** to providing a saliva sample and **during** sample collection
- If you need water, please ensure that it is consumed **no less than 10 minutes before** collecting each of your saliva samples to avoid diluting the sample
- **Avoid strenuous exercise in the day leading up to and during sample collection**

**Day one of saliva sample collection:**

**The evening before our visit**

Prior to going to bed, please drool into the **red-capped** tubes at the times indicated below (again, please ensure you are wearing your **sunglasses** from the start of your sample collection and during the remainder of the three hour sample collection period and that you **refrain from turning on any lights** during this time):

- 2 hours before your normal bed time
- 1 hour before your normal bed time
- At your normal bed time
- 1 hour after your normal bed time (we have to ask that you stay up an hour past your usual bed time for this sample)
- Please set an alarm for each of these time points to ensure the samples are taken at the correct time
- **PLEASE REMAIN AWAKE DURING THIS TIME.** You may watch TV, however please ensure you wear your sunglasses and reduce the brightness of your TV using the settings menu
- Ensure all tubes are **labelled** with your full name, the time and the date and please fill out the **Saliva Sample Collection Questionnaire**
- Please ensure all tubes are placed in the freezer

**On the morning of our visit**

After you wake up, please drool into the **blue-capped** tubes at the following times:

- 15 minutes after awakening
- 30 minutes after awakening
- 45 minutes after awakening
- 60 minutes after awakening
Please set an alarm for each of these time points to ensure the samples are taken at the correct time

- Ensure all tubes are labelled with your full name, the time and the date and please fill out the **Saliva Sample Collection Questionnaire**
- Please ensure all tubes are placed in the freezer

Whilst you are waiting between saliva sample collections in the morning, please fill out the following questionnaires:

1. The Perceived Stress Scale
2. The Pittsburgh Sleep Quality Index
3. The Epworth Sleepiness Scale
4. The Consensus Sleep Diary

Travis and Danielle will schedule, in advance, a time to come to your house that same morning. At this visit, Danielle will also take **36ml (4 tubes) of blood** and **20 strands of hair** and pick up the saliva tubes. These samples will provide invaluable information regarding the benefits of the intervention.

**Day 2 of saliva sample collection:**

This will be a repeat of the first day of saliva sampling. There will be no need to fill out the Perceived Stress Scale, Epworth Sleepiness Survey or Pittsburgh Sleep Quality Index on the second day. You will, however, need to fill out the Consensus Sleep Diary and the Saliva Collection Questionnaire on the second day.

The evening before our visit

Prior to going to bed, please drool into the **red-capped** tubes at the times indicated below:

- 2 hours before your normal bed time
- 1 hour before your normal bed time
- At your normal bed time
- 1 hour after your normal bed time (we have to ask that you stay up an hour past your usual bed time for this sample)
- Please set an alarm for each of these time points to ensure the samples are taken at the correct time
- Ensure all tubes are labelled with your full name, the time and the date and please fill out the **Saliva Sample Collection Questionnaire**
- Please ensure all tubes are placed in the freezer

Again, please ensure you are wearing your sunglasses from the start of your sample collection and during the remainder of the three-hour sample collection period and that you **refrain from turning on any lights or falling asleep** during this time. You may watch TV, however please be sure to wear your sunglasses and reduce the brightness of your TV using the settings menu.

**On the morning of our visit**

After you wake up, please drool into the **blue-capped** tubes at the following times:

- 15 minutes after awakening
- 30 minutes after awakening
- 45 minutes after awakening
- 60 minutes after awakening
- Please set an alarm for each of these time points to ensure the samples are taken at the correct time
- Ensure all tubes are labelled with your full name, the time and the date and placed in the freezer, and please fill out the Saliva Sample Collection Questionnaire

Whilst you are waiting between saliva sample collections in the morning, please fill out the Consensus Sleep Diary questionnaire. Travis and Danielle will schedule, in advance, a time to come to your house that same morning. At this visit, Danielle will also take 36ml (4 tubes) of blood.

**What happens if you forget/miss a sample?**

We will send you a reminder text message/phone call the evening before our visit to remind you to begin your sampling two hours before your bedtime. **If you miss a sample, please DO NOT provide a “make up” sample. Timing is essential in the analysis of these samples.** If you do miss a sample, we will send out more saliva collection tubes and we will ask you to repeat the sampling on another day.

If you require further information, please contact Danielle Bartlett by email at d.bartlett@ecu.edu.au or by phone on 6304 3568 or 0448 524 696.
MORNING SALIVA COLLECTION

- No eating or drinking (60 mins before and during)
- No smoking (60 mins before)
- No alcohol (12 hrs before)
- No exercise (the day before and during)

Wake up at the usual time

1. Provide sample 15 mins after waking (into blue-capped tube)
2. Wait 15 mins
3. Provide sample 30 mins after waking (into blue-capped tube)
4. Wait 15 mins
5. Provide sample 45 mins after waking (into blue-capped tube)
6. Wait 15 mins
7. Provide sample 60 mins after waking (into blue-capped tube)

Instructions:
1. Drool into a blue-capped tube through a straw
2. Ensure tube is filled with saliva to just below the top
3. **Label the tube with your full name, the date and the time of collection**
4. Store tube in the bag provided in your freezer
5. Please fill out the saliva collection questionnaire

On **day 1** please fill out the:
1. Perceived Stress Scale
2. Epworth Sleepiness Scale
3. Pittsburgh Sleep Quality Index
4. Consensus Sleep Diary

On **day 2** please fill out the:
1. Consensus Sleep Diary

Place samples in your freezer
**EVENING SALIVA COLLECTION**

Instructions:
1. Drool into a red-capped tube through a straw
2. Ensure tube is filled with saliva to just below the top
3. **Label the tube with your full name, the date and the time of collection**
4. Store tube in the bag provided in your freezer
5. Please fill out the saliva collection questionnaire

**IMPORTANT NOTE:** IT IS ESSENTIAL THAT THESE SAMPLES ARE TAKEN IN DIM LIGHT CONDITIONS. PLEASE WEAR YOUR SUNGLASSES STARTING FROM THE FIRST SAMPLE COLLECTION AND FOR THE REMAINDER OF THE THREE HOUR COLLECTION PERIOD AND REFRAIN FROM TURNING ON ANY LIGHTS DURING THIS TIME
Appendix 4: Saliva Sampling Questionnaire

Saliva Sample Collection Questionnaire - Day 1

This questionnaire is designed to provide us with as much information as possible about the saliva samples you are providing us so that we can analyse them in the appropriate manner. Please be completely honest when completing this survey.

Please fill out directly after providing your night-time saliva samples:

1. What is your usual bedtime (UBT)? _______________________
2. What time did you provide your first saliva sample (2hrs before UBT)? ______ pm
3. What time did you provide your second saliva sample (1hr before UBT)? ______ pm
4. What time did you provide your third saliva sample (at UBT)? ______ pm
5. What time did you provide your fourth saliva sample (1 hr after UBT)? ______ pm
6. Did you wear your sunglasses during the three hour period of night time saliva collection? Yes/No
7. Did you avoid turning on any lights during the three hour period of night time saliva collection? Yes/No
8. How long before the first sample did you eat food? _________ hours
9. Did you avoid smoking in the hour leading up to the first sample collection, as well as during the three hour sample collection period? (if non-smoker, please indicate) ________ hours
10. Did you have a drink of any sort within the 10 minutes prior to each sample collected? Yes/No

Please fill out directly after providing your morning saliva samples:

1. What time did you wake up this morning? ______ am
2. What time did you provide your first saliva sample (15 mins after waking)? _____ am
3. What time did you provide your second saliva sample (30 mins after waking)? ___am
4. What time did you provide your third saliva sample (45 mins after waking)? ____ am
5. What time did you provide your fourth saliva sample (60 mins after waking)? ___ am
6. Did you have a drink of any sort within the 10 minutes prior to each sample collected? __________________________
7. Did you avoid brushing your teeth during morning saliva sample collection? Yes/No
8. Did you avoid smoking during morning sample collection? (if non-smoker, leave blank) ________________

9. Have you participated in regular physical activity over the past month? Yes/No
   If yes,
   a. How many days per week?
      __________________________________________________________
   b. What exercise did you mostly do?
      __________________________________________________________
   c. How long did you participate in physical activity (at any one time)?
      ___ hrs ___ mins
   d. At what intensity?

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<td>e.g. Leisurely walking (minimum exertion)</td>
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10. Have you participated in physical activity this week? Yes/No
    If yes,
    a. How many days this week?
       __________________________________________________________
    b. What exercise did you mostly do?
       __________________________________________________________
    c. How long did you participate in physical activity (at any one time)?
       ___ hrs ___ mins
    d. At what intensity?

    | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
    |---|---|---|---|---|---|---|---|---|----|
    | e.g. Leisurely walking (minimum exertion) | e.g. Cycling at a regular pace (medium exertion) | e.g. Fast cycling/aerobics (maximum exertion) |

11. Did you participate in any physical activity yesterday? Yes/No
    If yes,
    a. What exercise did you mostly do?
       __________________________________________________________
    b. How long did you participate in physical activity (at any one time)?
___ hrs ___ mins

c. At what intensity?

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Thank you for taking the time to complete this questionnaire. If you have any queries, please contact Danielle Bartlett by email at d.bartlett@ecu.edu.au or by phone on 6304 3568 or 0448 524 696.
Saliva Sample Collection Questionnaire- Day 2

This questionnaire is designed to provide us with as much information as possible about the saliva samples you are providing us so that we can analyse them in the appropriate manner. Please be completely honest when completing this survey.

Please fill out directly after providing your night-time saliva samples:

11. What is your usual bedtime (UBT)? ___________________
12. What time did you provide your first saliva sample (2hrs before UBT)? _______ pm
13. What time did you provide your second saliva sample (1hr before UBT)? _______ pm
14. What time did you provide your third saliva sample (at UBT)? _______ pm
15. What time did you provide your fourth saliva sample (1 hr after UBT)? _______ pm
16. Did you wear your sunglasses during the three hour period of night time saliva collection? Yes/No
17. Did you avoid turning on any lights during the three hour period of night time saliva collection? Yes/No
18. How long before the first sample did you eat food? ________ hours
19. Did you avoid smoking in the hour leading up to the first sample collection, as well as during the three hour sample collection period? (if non-smoker, please indicate) ________ hours
20. Did you have a drink of any sort within the 10 minutes prior to each sample collected? Yes/No

Please fill out directly after providing your morning saliva samples:

12. What time did you wake up this morning? _______ am
13. What time did you provide your first saliva sample (15 mins after waking)? _______ am
14. What time did you provide your second saliva sample (30 mins after waking)? ___am
15. What time did you provide your third saliva sample (45 mins after waking)? ___ am
16. What time did you provide your fourth saliva sample (60 mins after waking)? ___ am
17. Did you have a drink of any sort within the 10 minutes prior to each sample collected? __________________________
18. Did you avoid brushing your teeth during morning saliva sample collection? Yes/No
19. Did you avoid smoking during morning sample collection? (if non-smoker, leave blank) __________________________
20. Have you participated in regular physical activity over the past month? Yes/No
If yes,
a. How many days per week?
____________________________________________________________________________________

b. What exercise did you mostly do?
____________________________________________________________________________________

c. How long did you participate in physical activity (at any one time)?
___ hrs ___mins

d. At what intensity?

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21. Have you participated in physical activity this week? Yes/No
If yes,
a. How many days this week?
____________________________________________________________________________________

b. What exercise did you mostly do?
____________________________________________________________________________________

c. How long did you participate in physical activity (at any one time)?
___ hrs ___mins

d. At what intensity?

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22. Did you participate in any physical activity yesterday? Yes/No
If yes,
a. What exercise did you mostly do?
____________________________________________________________________________________

b. How long did you participate in physical activity (at any one time)?
___ hrs ___mins

c. At what intensity?
Thank you for taking the time to complete this questionnaire. If you have any queries, please contact Danielle Bartlett by email at d.bartlett@ecu.edu.au or by phone on 6304 3568 or 0448 524 696.