Chapter 13

SYNOPSIS, CONCLUSIONS & FUTURE PERSPECTIVES
With them the seed of Wisdom did I sow,
And with mine own hand wrought to make it grow;
And this was all the Harvest that I reap’d,
“'I came like Water, and like Wind I go.”

Omar Khayyam (1048-1131 CE)

(Translation from Persian by Edward FitzGerald in 1859)
SYNOPSIS & CONCLUSIONS

The nuclear symptoms and signs of Huntington’s disease (HD) consist of motor, cognitive and behavioural disturbances. Other less well-known, but prevalent and debilitating features of HD include unintended weight loss, sleep and circadian rhythm disturbances, as well as autonomic nervous system dysfunction. However, the pathogenesis of these less well-known features of HD is poorly understood and currently no effective treatment options are available. It is thus of paramount importance to elucidate the pathological basis of these symptoms and signs in order to design and apply more effective therapeutic interventions. Recently, substantial dysfunction of the hypothalamus was reported in both human studies and various knock-in and transgenic animal models of HD. The hypothalamus consists of groups of interconnected neuronal nuclei located at the base of the brain that regulate a broad array of physiologic, homeostatic and behavioural activities. Therefore, in this thesis we attempt to substantiate the premise that hypothalamic dysfunction per se, as well as secondary (neuro)endocrine and metabolic alterations could contribute to the pathogenesis of several non-motor symptoms and signs of HD (Chapter 1).

Part I: Secondary signs of HD

The first part of the thesis is largely devoted to the exploration of the nature and extent of weight loss, sleep and circadian rhythm disturbances, and autonomic complaints in HD patients. In addition, an effort is made to identify the clinical predictors associated with these secondary signs.

A significant decrease in body weight was found over the course of three years in a large group of early stage HD patients (Chapter 2). However, no single motor, cognitive or behavioural score was independently associated with weight loss, suggesting that loss of body weight in HD is not secondary to hyperactivity or other symptoms, but likely results from a metabolic defect. As both HD patients and transgenic mice showed a higher rate of weight loss with greater CAG repeat number, this metabolic defect possibly stems directly from polyglutamine length dependent interference of the mutant protein with cellular homeostasis in central (e.g. hypothalamus) or peripheral (e.g. muscle, fat) tissues that are involved in body weight regulation. Moreover, these findings indicate that patients with a higher number of CAG repeats are at an increased risk of unintended weight loss.

The effects of the interaction between mutant and normal HTT on clinical phenotype are presented in Chapter 3. We found that normal and mutant CAG repeat sizes interact to influence age of onset, and the severity or progression of motor, cognitive and functional symptoms in HD patients, but not body weight. As the effect of the interaction on basal ganglia volume could already be detected in premanifest subjects, these data suggest that the interplay between normal and mutant huntingtin (fragments) directly influences neuronal atrophy or loss and is thus an integral feature of HD pathogenesis. The underlying mechanism may involve interaction of the polyglutamine domains of normal and mutant huntingtin (fragments) and needs further elucidation. These findings may have predictive value and are essential for the design and interpretation of future therapeutic trials.
Data from a systematic evaluation of subjective sleep quality and daytime somnolence in a large cohort of HD patients and premanifest mutation carriers are reported in Chapter 4. The findings indicate that while nighttime sleep impairment is more prevalent in HD patients, daytime sleepiness appears unlikely to be a major issue in HD. We also show that depression is the most important clinical predictor of sleep impairment in HD patients. Moreover, our findings suggest a delayed sleep phase syndrome-like circadian rhythm disorder in HD patients which appears to be associated with lower cognitive performance.

Findings from a questionnaire study indicated that HD patients experience various symptoms suggestive of autonomic nervous system dysfunction (Chapter 5). The complaints particularly concerned the gastrointestinal, urinary, cardiovascular and male sexual domains, and some of them were also present in premanifest mutation carriers. These findings indicate that, contrary to common belief, autonomic symptoms are highly prevalent in HD patients and may even precede the onset of motor signs. Moreover, we found that a greater degree of autonomic dysfunction in HD patients was associated with more functional disability as well as more depressive symptoms, suggesting that adequate management of autonomic symptoms in HD patients could be evaluated as a potential strategy to improve their quality of life.

**Part II: Hypothalamic pathology in HD**

In this section (Chapter 6) we report a significant reduction by about 30% in the total number of hypocretin-1 immunoreactive neurons in the lateral hypothalamus of HD patients. This decrease appears to be relatively specific as the total number of melanin-concentrating hormone (MCH) immunoreactive neurons was not significantly altered. Hypocretin-1 levels in the prefrontal cortex were reduced to the same extent, but ventricular cerebrospinal fluid levels were unchanged. It remains to be shown whether this moderate decrease in hypocretin signalling could contribute to clinical symptoms. As MCH cell number was not clearly affected in HD patients, alterations in MCH neurotransmission are unlikely to have clinical effects in HD. Interestingly, neuronal intranuclear and cytoplasmic inclusions were not uniformly present in various hypothalamic and adjacent structures in HD patients. This finding may indicate that various hypothalamic nuclei are differentially affected by inclusion formation despite their close anatomical juxtaposition in the hypothalamus.

**Part III: Endocrine studies in HD**

A detailed description of cortisol secretory dynamics in patients with HD is presented in Chapter 7. We found that the total 24 h cortisol production rates were significantly elevated in HD patients. The increase in cortisol production was primarily confined to the morning and early afternoon period. In addition, circadian rhythmicity analysis revealed a significantly higher amplitude of the diurnal cortisol concentration profile in HD patients. These findings point towards a disturbed central glucocorticoid feedback regulation in HD patients and indicate that hypothalamic-pituitary-adrenal axis dysfunction is an early feature of the disease.

We found no significant differences in growth hormone and ghrelin secretion characteristics between HD patients and controls (Chapter 8). However, in HD patients, both growth hormone secretion and its irregularity as well as the degree of postprandial ghrelin suppression significantly increased with worsening motor and functional impairment. Moreover, postprandial ghrelin suppression also increased with decreasing body
weight and higher CAG repeat number. These findings suggest subtle changes in the regulation of growth hormone and ghrelin secretion dynamics in early stage HD patients that may become more pronounced in the later stages of the disease.

Investigation of the thyrotropic and lactotropic axes function (Chapter 9) revealed a mild hyperactivity of the hypothalamic-pituitary-thyroid axis, as well as a more irregular pattern of prolactin secretion in HD patients compared with matched controls. These findings are consistent with disrupted hypothalamic-pituitary dopamine signaling in HD. Interestingly, higher free T$_4$ levels were associated with larger mutant CAG repeat sizes. In addition, there was an inverse trend for the relation between total T$_4$ levels and body mass index (BMI) in HD patients. As thyroid hormones are known to increase energy expenditure, elevated thyroid hormone levels in early stage HD patients that seem to increase with mutant CAG repeat size, may contribute to the lower BMI in HD mutation carriers, and possibly account for the association between mutant $HTT$ CAG repeat size and weight loss in HD.

The plasma levels and diurnal rhythmicity of the adipokines leptin, adiponectin and resistin did not significantly differ between HD patients and controls (Chapter 10). However, when corrected for fat mass, both mean plasma leptin concentration and secretion rate significantly increased with the size of the CAG repeat mutation in HD patients. As leptin is an anorexigenic hormone that stimulates energy expenditure, enhanced leptin production rate in HD patients with higher CAG repeat lengths in the mutant allele could lead to decreased appetite and hypermetabolism, thereby contributing to the higher rate of weight loss in these subjects. Interestingly, unlike in controls, neither BMI nor body fat mass was significantly related to leptin production in HD patients. These findings suggest that the HD mutation interferes with adipose tissue function, which may contribute to weight loss in HD patients.

The timing of the evening rise in melatonin levels was significantly delayed by more than one and a half hours in early stage HD patients compared with control subjects (Chapter 11). Moreover, despite similar mean diurnal melatonin levels between HD patients and controls, we found strong negative associations between mean diurnal melatonin levels and both motor and functional disability in these patients. These findings suggest a delayed sleep phase syndrome-like circadian rhythm disorder in early stage HD patients and suggest that melatonin levels may progressively decline with advancing disease.

**Part IV: Metabolic studies in HD**

Compared with controls, we found a significantly higher basal resting energy expenditure in HD patients which was primarily due to an increased fat oxidation rate (Chapter 12). Moreover, unlike in controls, hyperinsulinemia induced a further increase in energy expenditure in HD patients which was now primarily due to an elevated rate of glucose oxidation. Although we did not find any evidence for insulin resistance in HD patients, higher CAG repeat size was associated with lower insulin sensitivity. These findings suggest sympathetic hyperactivity, possibly due to dysfunction of the hypothalamic ventromedial nucleus, as well as peripheral polyglutamine length dependent interference of mutant huntingtin with insulin signaling that may become clinically relevant in carriers of mutations with large CAG repeat sizes.
FUTURE PERSPECTIVES

The studies described in this thesis provide fertile ground for further basic as well as clinical research into several facets of HD. First, however, alike every other claim of scientific advance, our assertions should be subjected to scrutiny and attempts should be undertaken to replicate the findings in other, preferably larger, cohorts of HD patients. Moreover, it would be interesting to assess patients in various stages of the disease, although medication use as well as functional and mental disabilities in more advanced stage patients posit major feasibility challenges. In this regard, it is particularly encouraging to note that several of our results, such as the inverse association between body weight and mutant CAG repeat size and increased cortisol levels, have already been replicated in independent investigations implicating relatively large numbers of HD patients, while confirmational studies are underway for several other of our findings, including the effect of normal and mutant HTT interaction on disease severity that will be assessed in the TRACK-HD cohort. With this conditional stipulation in mind, in the remaining part of this section we will put forth a few lines of thought that may serve to direct future research endeavours in this field. The mechanism underlying the negative association between body weight and mutant CAG repeat length needs further elucidation, as this may facilitate the generation of therapeutics aiming to restore cellular energy homeostasis in HD. In particular, it remains to be shown to what extent the relation between body weight and mutant CAG repeat size is due to polyglutamine length dependent pathology of brain structures involved in energy homeostasis (e.g. the hypothalamus), or due to pathology of peripheral tissues such as muscle, fat and pancreatic tissue.

Evaluation of the association between CAG repeat size and the functional integrity of the hypothalamus, for instance as assessed by various imaging techniques, combined with hormone challenge tests and muscle/fat biopsies may greatly advance our understanding of this issue. Likewise, the pathways mediating the effect of the interaction between normal and mutant HTT CAG repeat sizes on disease severity await further clarification. Several models could account for this phenomenon, including competitive polyglutamine length dependent interaction of normal and mutant huntingtin with numerous protein binding partners, mitochondrial energy production or transcriptional mechanisms. Regardless of the responsible mechanisms, however, an important corollary of this study is that future clinical trials aiming to assess the efficacy of a particular therapy in HD patients should consider adjusting the outcome for the CAG repeat sizes in both HTT alleles. Findings from our inventory of sleep disturbances, as well as cortisol and melatonin rhythms in HD indicate a delayed sleep-phase syndrome-like circadian rhythm disturbance that requires further scrutiny by means of other measures of circadian rhythm such as 24 h body temperature recordings and actigraphy. This is important as HD patients may benefit from similar strategies applied for the re-entrainment of the circadian rhythms in subjects suffering from a delayed-sleep phase syndrome. We also found that HD patients experience a large number of symptoms that, at least partly, could be manifestations of autonomic nervous system dysfunction. Application of more extensive, standardized questionnaires in combination with objective measures of autonomic nervous system function may help to elucidate to what degree putative symptoms of autonomic origin actually stem from autonomic failure in HD, rather than being expressions of pathognomonic deilities such as motor impairment. Neuropathological assessment of the hypothalamus revealed that neuronal intranuclear and cytoplasmic inclusions are not uniformly present in various hypothalamic and adjacent structures in HD. Characterization of the processes mediating this heterogeneity may illuminate why certain neuronal populations are more susceptible to HD pathology than others. In this respect, it would be interesting to assess whether Rhes (Ras homologue enriched in striatum), a protein that is thought to mediate the relative selectivity of striatal pathology in HD, is present in the hypothalamus, whether its distribution differs between various hypothalamic nuclei, and whether its expression is altered in HD hypothalami. The findings from our endocrine and metabolic
studies in early stage HD patients are consistent with hypothalamic pathology, and especially implicate the suprachiasmatic nucleus in the disease process. Employing immunocytochemical and in situ hybridization techniques, systematic neuropathological evaluation of the suprachiasmatic and other hypothalamic nuclei in HD are currently underway in close collaboration with the Netherlands Institute for Neurosciences. In addition, regarding the progressive nature of HD, longitudinal studies of endocrine (particularly cortisol and growth hormone secretion) and metabolic (especially energy expenditure and response to insulin challenge) parameters in conjunction with clinical assessments may provide more insight into potential dynamic alterations which might prove useful as biomarkers of disease progression. Last but not least, it should be noted that certain of our findings, such as the association between the secretion of the adipocyte specific hormone leptin and mutant CAG repeat size, are better explained by peripheral, rather than central, pathology in HD. Hence, dictated by the expression of mutant huntingtin throughout the body, a holistic approach with the evaluation of both central as well as peripheral tissues appears a prudent strategy for the identification of the pathological basis of secondary signs in HD.10

REFERENCES


