Neurobiology of Sleep and Circadian Rhythms 6 (2019) 1–8

Contents lists available at ScienceDirect

Neurobiology of Sleep and Circadian Rhythms

journal homepage: www.elsevier.com/locate/nbscr

Research paper

Investigating the relationships between hypothalamic volume and measures of circadian rhythm and habitual sleep in premanifest Huntington's disease

Danielle M. Bartlett[a,*,1], Juan F. Domínguez D[b,1], Alvaro Reyes[c], Pauline Zaenker[a], Kirk W. Feindel[d], Robert U. Newton[e,f], Anthony J. Hannan[g], James A. Slater[h], Peter R. Eastwood[h], Alpar S. Lazar[i], Mel Ziman[a,j], Travis Cruickshank[a,k]

[a] School of Medical and Health Sciences, Edith Cowan University, 270 Joondalup Drive, Joondalup, Western Australia 6027, Australia
[b] School of Psychology, Australian Catholic University, Melbourne, Victoria, Australia
[c] Facultad de Ciencias de la Rehabilitacion, Universidad Andres Bello, Santiago, Chile
[d] Centre for Microscopy, Characterisation and Analysis, University of Western Australia, Crawley, Western Australia, Australia
[e] Exercise Medicine Research Institute, School of Medical and Health Sciences, Edith Cowan University, Joondalup, Western Australia, Australia
[f] University of Queensland Centre for Clinical Research, University of Queensland, Brisbane, Queensland, Australia
[g] The Florey Institute of Neuroscience and Mental Health, University of Melbourne, Parkville, Victoria, Australia
[h] Centre for Sleep Science, School of Human Sciences, Faculty of Science, University of Western Australia, Crawley, Western Australia, Australia
[i] Faculty of Medicine and Health Sciences, University of East Anglia, Norwich, Norfolk, United Kingdom
[j] School of Biomedical Science, University of Western Australia, Crawley, Western Australia, Australia
[k] Peron Institute for Neurological and Translational Science, Perth, Western Australia, Australia

ARTICLE INFO

Keywords:
Huntington's disease
Hypothalamus
Magnetic resonance imaging
Circadian rhythm
Sleep

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.

* Corresponding author.
E-mail address: d.bartlett@ecu.edu.au (D.M. Bartlett).
[1] Danielle M. Bartlett and Juan F. Dominguez D. contributed equally to the manuscript.

https://doi.org/10.1016/j.nbscr.2018.07.001
Received 27 April 2018; Received in revised form 28 June 2018; Accepted 2 July 2018
Available online 03 July 2018

1. 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, the 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.

2. Materials and methods

2.1. 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 $\geq$ 40 and a diagnostic confidence score $<$ 2 on the Unified Huntington's Disease Rating Scale Total Motor Score (UHDRS-TMS) (Reilmann et al., 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.

2.2. 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.

2.3. 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 × 256, 1 mm^3 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 × 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).

2.4. 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 et al., 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 min 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.

2.5. Measures of habitual sleep-wake parameters

2.5.1. 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 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 s epochs as awake or sleep according to the Cole-Kripke algorithm (Cole et al., 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.

2.5.2. 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.

2.5.3. Subjective sleep quality and daytime somnolence

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

2.6. Stress, anxiety and depression symptomatology

The Perceived Stress Scale (Cohen et al., 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 et al., 2010).

2.7. 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 and Benedict, 1918).

2.8. Statistical analysis

2.8.1. Hypothalamic volume

A hypothalamus mask from the WFU Pick Atlas (http://fmri.wfubmc.edu/software/pickatlas), dilated by 3 mm, 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 $\alpha = 0.05$.

2.8.2. Salivary cortisol and melatonin

Missing data points from saliva sampling (removed due to suspected blood contamination) were imputed by calculating the average of the participant's 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 (AUC_G) 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 AUC_G, melatonin AUC_G and Perceived Stress Scale, PSQI and Consensus Sleep Diary scores. Statistical significance was set at $p \leq 0.05$. Statistical analyses were performed using STATA version 9.1.

3. Results

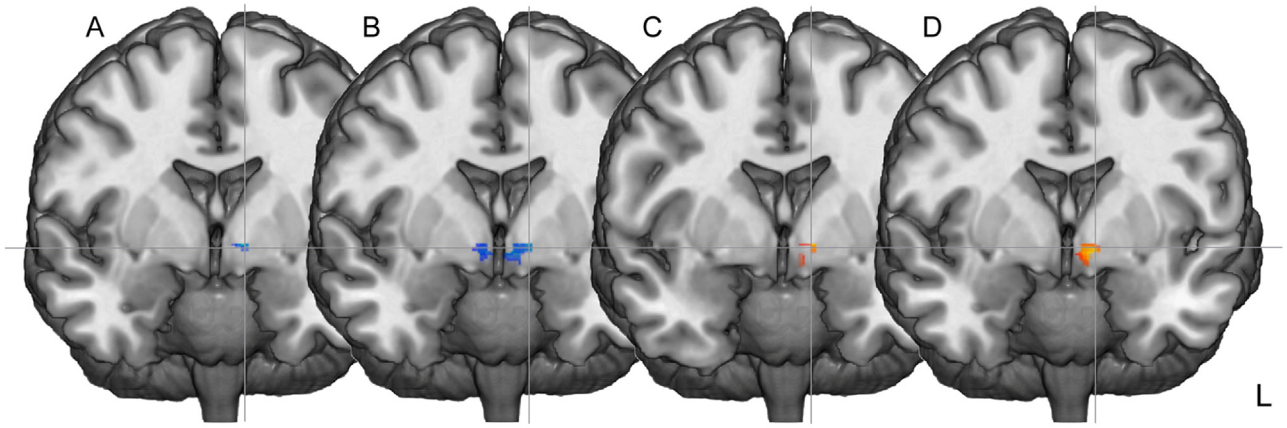
3.1. Participant demographics and clinical characteristics

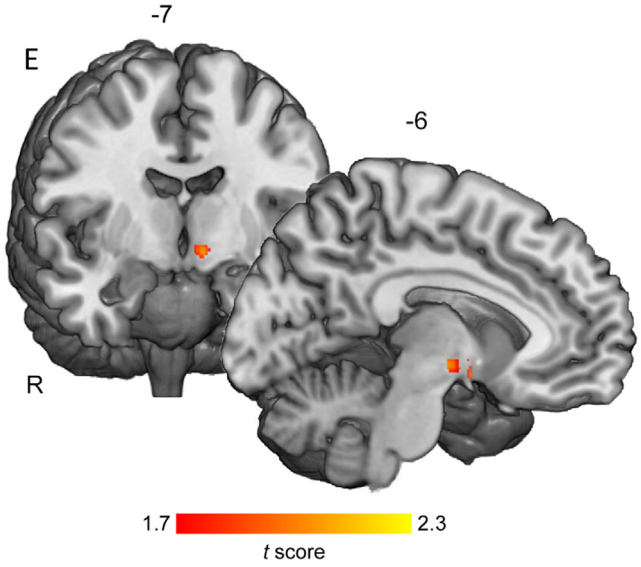
There were no significant differences for age or gender between premanifest HD patients and healthy controls ($p = 0.472$ and 0.283, respectively; Table 1).

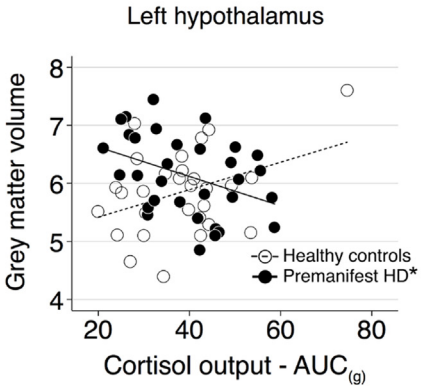
Table 1
Demographic and clinical characteristics of premanifest Huntington's disease and healthy control participants.

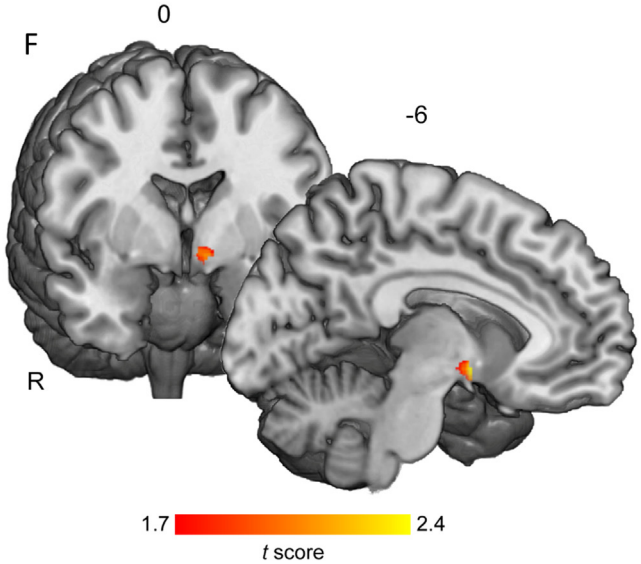
	Premanifest HD (n = 32)	Healthy Controls (n = 29)	*p*-value
Demographic Characteristics			
Age, mean ± SD	44.5 ± 11.4	44.3 ± 10.8	0.472
Male, n (%)	11 (34.4)	8 (27.6)	0.283
Clinical Characteristics			
CAGn, mean ± SD	42.8 ± 2.8	N/A	N/A
Estimated age of onset, median (IQR)	47.4 (44.9–55.7)	N/A	N/A
Diagnostic Confidence Level, mean ± SD	0.38 ± 0.70	N/A	N/A
UHDRS-TMS, mean ± SD	4.35 ± 7.24	N/A	N/A
Disease burden score, mean ± SD	305.0 ± 76.8	N/A	N/A
CAPs, mean ± SD	0.89 ± 0.18	N/A	N/A
BMI, mean ± SD	26.47 ± 4.5	26.75 ± 5.9	0.417
Smoker, n (%)	6 (18.8)	0 (0.0)	0.007*
High alcohol consumption, n (%)**	5 (15.6)	6 (20.7)	0.303
Psychotropic medication, n (%)	3 (9.4)	5 (17.2)	0.181
Monthly METs, mean ± SD	3278.0 ± 6272.5	7481.2 ± 9584.5	0.022*
METs (previous day), mean ± SD	168 ± 359.2	85.1 ± 211.8	0.141

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.
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.
* Results are significant at $p < 0.05$
** High alcohol consumption equates to in excess of 14 alcoholic drinks per week









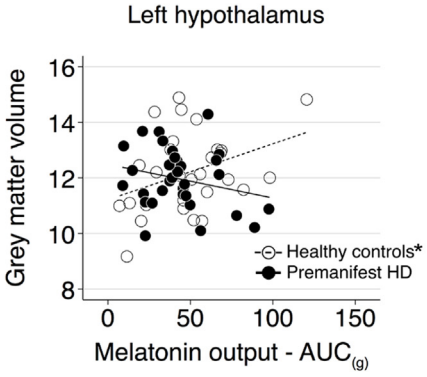


Fig. 1. Results from voxel-based morphometry analyses showing overlap between maps of (A) the hypothalamic volume group difference, (B) association between hypothalamus loss and CAPs in premanifest HD, (C) group by cortisol output interaction and (D) group by melatonin output interaction. (E) shows the 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.

Table 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.

	Premanifest HD	Healthy Controls	*p*-value
Cortisol nmol/L (mean ± SD)			
Time of awakening (hh:mm)	6:06 ± 1:01	6:21 ± 0:45	0.141
Time of sample relative to awakening:			
+15 min	11.08 ± 4.40	11.28 ± 3.32	0.421
+30 min	13.84 ± 4.59	13.42 ± 4.29	0.357
+45 min	13.69 ± 3.56	13.30 ± 4.41	0.352
+60 min	12.26 ± 3.47	11.62 ± 3.72	0.244
AUC_G	38.17 ± 11.30	39.20 ± 10.61	0.357
Melatonin (pg/mL)			
Reported usual bedtime (hh:mm)	22:05 ± 0:50	22:10 ± 0:31	0.322
Time of sample relative to bedtime:			
-2 h	10.87 ± 7.68	10.02 ± 6.19	0.319
-1 h	12.16 ± 7.07	13.71 ± 7.84	0.210
+0 h	17.10 ± 9.30	19.44 ± 11.10	0.187
+1 h	19.42 ± 12.02	22.07 ± 11.11	0.188
AUC_G	49.20 ± 25.88	44.41 ± 21.77	0.219
Actigraphy measures:			
Total time in bed (min)	464.28 ± 46.73	458.58 ± 43.10	0.680
Total sleep time (min)	403.17 ± 45.20	415.12 ± 40.25	0.365
Sleep onset latency (min)	7.10 ± 6.80	4.39 ± 3.77	0.106
Wake after sleep onset (min)	54.02 ± 25.97	39.07 ± 16.87	0.028*
Number of awakenings	18.47 ± 6.72	14.55 ± 5.33	0.039*
Average duration of awakenings (min)	2.87 ± 0.64	2.75 ± 0.87	0.625
Sleep efficiency (%)	86.98 ± 5.42	90.68 ± 3.75	0.012*
PSQI global score	5.76 ± 3.02	4.97 ± 2.44	0.134
Epworth Sleepiness Scale Score	4.69 ± 3.61	6.76 ± 3.37	0.025*
Consensus Sleep Diary:			
Total time in bed (min)	430.42 ± 61.33	434.84 ± 68.48	0.372
Total sleep time (min)	385.82 ± 53.90	387.92 ± 74.77	0.453
Sleep onset latency (min)	16.65 ± 19.90	14.35 ± 13.92	0.303
Wake after sleep onset (min)	19.04 ± 25.37	16.93 ± 22.89	0.412
Sleep efficiency	90.52 ± 5.17	89.32 ± 9.96	0.487
Number of awakenings	1.25 ± 1.47	1.75 ± 1.03	0.057
Average duration of awakenings (min)	10.18 ± 23.46	8.97 ± 11.11	0.059
Restorative quality of sleep	3.75 ± 0.81	3.80 ± 0.92	0.411
Perceived Stress Scale	17.12 ± 6.72	19.86 ± 6.85	0.062
HADS:			
Total score	7.54 ± 5.30	8.38 ± 5.69	0.276
Anxiety	5.06 ± 3.48	6.00 ± 3.97	0.164
Depression	2.49 ± 2.52	2.38 ± 2.14	0.427

Data are reported as mean and standard deviation, unless stated otherwise. Group differences were analysed using t-tests. AUC_G 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.
HD = Huntington's disease; AUC_G = area under the curve with respect to ground; PSQI = Pittsburgh Sleep Quality Index; HADS = Hospital Anxiety and Depression Scale.
* Results are significant at $p < 0.05$.

3.2. 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 Fig. 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 and Fig. 1B).

3.3. 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 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 (AUC_G; Table 2).

3.4. Habitual sleep outcomes

3.4.1. 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 2).

3.4.2. Consensus sleep diary

No significant differences were observed in self-report sleep outcomes using the Consensus Sleep Diary (Table 2).

3.4.3. 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).

3.5. 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 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.

3.6. Physical activity

The premanifest HD group reported significantly less monthly physical activity (METs) than healthy controls ($p = 0.022$; Table 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.

3.7. Associations between hypothalamic volume and circadian markers

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 (Fig. 1E and Table 3). This group difference in the association between hypothalamic volume and morning cortisol output

Table 3
Association analyses between hypothalamic volume and CAPs, cortisol and melatonin.

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

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.

was statistically significant, as shown by interaction analysis (Fig. 1C, Fig. 1E and Table 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). 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 (Fig. 1F and Table 3). This group difference in the correlation between hypothalamic volume and evening melatonin output was statistically significant (Fig. 1D, Fig. 1F and Table 3). The group by melatonin output interaction encompassed the region extending from the superior tuberal hypothalamus to the anterior-inferior hypothalamus.

3.8. 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.

4. 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 outcomes 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 et al., 2005; Hofman and 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 et al., 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 et al., 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 et al., 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 (Kalliolia et al., 2014; Stothard 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.

Acknowledgements

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

Sources of funding

This study was supported by Lotterywest (MZ and TC; #G0002718). PRE was supported by a NHMRC Senior Research Fellowship (#513704). AJH was supported by a NHMRC Principal Research Fellowship (#1117148). ASL is supported by grant from the Wellcome trust (207799/Z/17/Z).

Conflicts of interest

None.

Author contributions

D.M.B, M.Z and T.M.C conceptualised and ran the study. J.F.D.D analysed brain imaging data. A.R assisted with statistical analyses. P.Z assisted with sample collection and interpretation of data. K.W.F assisted with collection, analysis and interpretation of brain imaging data. R.N and J.A.S assisted with actigraphy data collection and analysis. A.J.H, P.R.E and A.S.L contributed to the design of the study and interpretation of data. D.M.B, J.F.D.D, M.Z and T.M.C wrote the manuscript. All authors contributed to the writing and revision of the manuscript.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.nbscr.2018.07.001.

References

Ainsworth, B.E., Haskell, W.L., Herrmann, S.D., Meckes, N., Bassett Jr, D.R., Tudor-Locke, C., Greer, J.L., Vezina, J., Whitt-Glover, M.C., Leon, A.S., 2011. 2011 Compendium of Physical Activities: a second update of codes and MET values. Med. Sci. Sport. Exerc. 43, 1575–1581.

Ancoli-Israel, S., Cole, R., Alessi, C., Chambers, M., Moorcroft, W., Pollak, C.P., 2003. The role of actigraphy in the study of sleep and circadian rhythms. Sleep 26, 342–392.

Aton, S.J., Colwell, C.S., Harmar, A.J., Waschek, J., Herzog, E.D., 2005. Vasoactive intestinal polypeptide mediates circadian rhythmicity and synchrony in mammalian clock neurons. Nat. Neurosci. 8, 476–483.

Aziz, N.A., Anguelova, G.V., Marinus, J., Lammers, G.-J., Roos, R.A.C., 2010. Sleep and circadian rhythm alterations correlate with depression and cognitive impairment in Huntington's disease. Park. Relat. Disord. 16, 345–350.

Aziz, N.A., Fronczek, R., Maat-Schieman, M., Unmehopa, U., Roelandse, F., Overeem, S., Van Duinen, S., Lammers, G.J., Swaab, D.F., Roos, R.A.C., 2008. Hypocretin and melanin-concentrating hormone in patients with Huntington disease. Brain Pathol. 18, 474–483.

Baker, C.R., Dominguez D, J.F., Stout, J.C., Gabery, S., Churchyard, A., Chua, P., Egan, G.F., Petersen, A., Georgiou-Karistianis, N., Poudel, G.R., 2016. Subjective sleep problems in Huntington's disease: a pilot investigation of the relationship to brain structure, neurocognitive, and neuropsychiatric function. J. Neurol. Sci. 364, 148–153.

Bartlett, D.M., Cruickshank, T.M., Hannan, A.J., Eastwood, P.R., Lazar, A.S., Ziman, M.R., 2016. Neuroendocrine and neurotrophic signaling in Huntington's disease: implications for pathogenic mechanisms and treatment strategies. Neurosci. Biobehav. Rev. 71, 444–454.

Breen, D.P., Nombela, C., Vuono, R., Jones, P.S., Fisher, K., Burn, D.J., Brooks, D.J., Reddy, A.B., Rowe, J.B., Barker, R.A., 2016. Hypothalamic volume loss is associated with reduced melatonin output in Parkinson's disease. Mov. Disord.: Off. J. Mov. Disord. Soc. 31, 1062–1066.

Briançon-Marjollet, A., Weiszenstein, M., Henri, M., Thomas, A., Godin-Ribuot, D., Polak, J., 2015. The impact of sleep disorders on glucose metabolism: endocrine and molecular mechanisms. Diabetol. Metab. Syndr. 7, 25.

Buysse, D.J., Reynolds 3rd, C.F., Monk, T.H., Berman, S.R., Kupfer, D.J., 1989. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res. 28, 193–213.

Carney, C.E., Buysse, D.J., Ancoli-Israel, S., Edinger, J.D., Krystal, A.D., Lichstein, K.L., Morin, C.M., 2012. The Consensus Sleep Diary: standardizing prospective sleep self-monitoring. Sleep 35, 287–302.

Cohen, S., Kamarck, T., Mermelstein, R., 1983. A global measure of perceived stress. J. Health Social. Behav. 24, 385–396.

Cole, R.J., Kripke, D.F., Gruen, W., Mullaney, D.J., Gillin, J.C., 1992. Automatic sleep/wake identification from wrist activity. Sleep 15, 461–469.

De Souza, J., Jones, L.A., Rickards, H., 2010. Validation of self-report depression rating scales in Huntington's disease. Mov. Disord. 25, 91–96.
Dickmeis, T., 2009. Glucocorticoids and the circadian clock. J. Endocrinol. 200, 3–22.
Dijk, D.-J., Duffy, J.F., Silva, E.J., Shanahan, T.L., Boivin, D.B., Czeisler, C.A., 2012. Amplitude reduction and phase shifts of melatonin, cortisol and other circadian rhythms after a gradual advance of sleep and light exposure in humans. PLOS One 7, e30037.
Downing, N., Smith, M.M., Beglinger, L.J., Mills, J., Duff, K., Rowe, K.C., Epping, E., Paulsen, J.S., the PREDICT-HD Investigators of the Huntington Study Group, 2011. Perceived stress in prodromal Huntington disease. Psychol. Health 27, 196–209.
Fisher, S.P., Black, S.W., Schwartz, M.D., Wilk, A.J., Chen, T.-M., Lincoln, W.U., Liu, H.W., Kilduff, T.S., Morairty, S.R., 2013. Longitudinal analysis of the electroencephalogram and sleep phenotype in the R6/2 mouse model of Huntington's disease. Brain 136, 2159–2172.
Fisher, S.P., Schwartz, M.D., Wurts-Black, S., Thomas, A.M., Chen, T.-M., Miller, M.A., Palmerston, J.B., Kilduff, T.S., Morairty, S.R., 2016. Quantitative electroencephalographic analysis provides an early-stage indicator of disease onset and progression in the zQ175 knock-in mouse model of Huntington's disease. Sleep 39, 379–391.
Gregory, S., Long, J.D., Klöppel, S., Razi, A., Scheller, E., Minkova, L., Papoutsi, M., Mills, J.A., Durr, A., Leavitt, B.R., 2017. Operationalizing compensation over time in neurodegenerative disease. Brain 140, 1158.
Harris, J.A., Benedict, F.G., 1918. A Biometric Study of Human Basal Metabolism. Proc. Natl. Acad. Sci. USA 4, 370–373.
Hofman, M.A., Swaab, D.F., 1994. Alterations in circadian rhythmicity of the vasopressin-producing neurons of the human suprachiasmatic nucleus (SCN) with aging. Brain Res. 651, 134–142.
Hubers, A.A., van der Mast, R.C., Pereira, A.M., Roos, R.A., Veen, L.J., Cobbaert, C.M., van Duijn, E., Giltay, E.J., 2015. Hypothalamic-pituitary-adrenal axis functioning in Huntington's disease and its association with depressive symptoms and suicidality. J. Neuroendocr. 27, 234–244.
Jack, C.R., Bernstein, M.A., Fox, N.C., Thompson, P., Alexander, G., Harvey, D., Borowski, B., Britson, P.J., Whitwell, J.L., Ward, C., Dale, A.M., Felmlee, J.P., Gunter, J.L., Hill, D.L.G., Killiany, R., Schuff, N., Fox-Bosetti, S., Lin, C., Studholme, C., DeCarli, C.S., Gunnar, K., Ward, H.A., Metzger, G.J., Scott, K.T., Mallozzi, R., Blezek, D., Levy, J., Debbins, J.P., Fleisher, A.S., Albert, M., Green, R., Bartzokis, G., Glover, G., Mugler, J., Weiner, M.W., 2008. The Alzheimer's disease neuroimaging initiative (ADNI): mri methods. J. Magn. Reson. Imaging 27, 685–691.
Johns, M.W., 1991. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep 14, 540–545.
Kalliolia, E., Silajdzic, E., Nambron, R., Hill, N.R., Doshi, A., Frost, C., Watt, H., Hindmarsh, P., Bjorkqvist, M., Warner, T.T., 2014. Plasma melatonin is reduced in Huntington's disease. Mov. Disord. 29, 1511–1515.
Kantor, S., Szabo, L., Varga, J., Cuesta, M., Morton, A.J., 2013. Progressive sleep and electroencephalogram changes in mice carrying the Huntington's disease mutation. Brain 136, 2147–2158.
Kudo, T., Schroeder, A., Loh, D.H., Kuljis, D., Jordan, M.C., Roos, K.P., Colwell, C.S., 2011. Dysfunctions in circadian behavior and physiology in mouse models of Huntington's disease. Exp. Neurol. 228, 80–90.
Kuljis, D.A., Gad, L., Loh, D.H., MacDowell Kaswan, Z., Hitchcock, O.N., Ghiani, C.A., Colwell, C.S., 2016. Sex differences in circadian dysfunction in the BACHD Mouse Model of Huntington's disease. PLOS One 11, e0147583.
Lazar, A.S., Panin, F., Goodman, A.O., Lazic, S.E., Lazar, Z.I., Mason, S.L., Rogers, L., Murgatroyd, P.R., Watson, L.P., Singh, P., Borowsky, B., Shneerson, J.M., Barker, R.A., 2015. Sleep deficits but no metabolic deficits in premanifest Huntington's disease. Ann. Neurol. 78, 630–648.
Loh, D.H., Kudo, T., Truong, D., Wu, Y., Colwell, C.S., 2013. The Q175 mouse model of Huntington's disease shows gene dosage-and age-related decline in circadian rhythms of activity and sleep. PLoS One 8, e69993.
Makris, N., Swaab, D.F., van der Kouwe, A., Abbs, B., Boriel, D., Handa, R.J., Tobet, S., Goldstein, J.M., 2013. Volumetric parcellation methodology of the human hypothalamus in neuroimaging: normative data and sex differences. NeuroImage 69, 1–10.
Minkova, L., Gregory, S., Scahill, R.I., Abdulkadir, A., Kaller, C.P., Peter, J., Long, J.D., Stout, J.C., Reilmann, R., Roos, R.A.C., Durr, A., Leavitt, B.R., Tabrizi, S.J., Klöppel, S., the TRACK-HD Investigators, 2018. Cross-sectional and longitudinal voxel-based grey matter asymmetries in Huntington's disease. NeuroImage: Clin. 17, 312–324.
Morton, A.J., 2013. Circadian and sleep disorder in Huntington's disease. Exp. Neurol. 243, 34–44.
Morton, A.J., Wood, N.I., Hastings, M.H., Hurelbrink, C., Barker, R.A., Maywood, E.S., 2005. Disintegration of the sleep-wake cycle and circadian timing in Huntington's disease. J. Neurosci. 25, 157–163.
Mühlau, M., Gaser, C., Wohlschläger, A.M., Weindl, A., Städtler, M., Valet, M., Zimmer, C., Kassubek, J., Peinemann, A., 2007. Striatal gray matter loss in Huntington's disease is leftward biased. Mov. Disord. 22, 1169–1173.
Petersén, Å., Gil, J., Maat-Schieman, M.L.C., Björkqvist, M., Tanila, H., Araújo, I.M., Smith, R., Popovic, N., Wierup, N., Norlén, P., 2005. Orexin loss in Huntington's disease. Human. Mol. Genet. 14, 39–47.
Piano, C., Mazzucchi, E., Bentivoglio, A.R., Losurdo, A., Calandra Buonaura, G., Imperatori, C., Cortelli, P., Della Marca, G., 2017. Wake and sleep EEG in patients with Huntington disease: an eLORETA study and review of the literature. Clin. EEG Neurosci. 48, 60–71.
Politis, M., Pavese, N., Tai, Y.F., Tabrizi, S.J., Barker, R.A., Piccini, P., 2008. Hypothalamic involvement in Huntington's disease: an in vivo PET study. Brain 131, 2860–2869.
Reilmann, R., Leavitt, B.R., Ross, C.A., 2014. Diagnostic criteria for Huntington's disease based on natural history. Mov. Disord.: Off. J. Mov. Disord. Soc. 29, 1335–1341.
Saper, C.B., Scammell, T.E., Lu, J., 2005. Hypothalamic regulation of sleep and circadian rhythms. Nature 437, 1257–1263.
Scheller, E., Minkova, L., Leitner, M., Kloppel, S., 2014. Attempted and successful compensation in preclinical and early manifest neurodegeneration - a review of task FMRI studies. Front. psychiatry 5, 132.
Soneson, C., Fontes, M., Zhou, Y., Denisov, V., Paulsen, J.S., Kirik, D., Petersen, A., 2010. Early changes in the hypothalamic region in prodromal Huntington disease revealed by MRI analysis. Neurobiol. Dis. 40, 531–543.
Steiger, A., 2002. Sleep and the hypothalamo–pituitary–adrenocortical system. Sleep. Med. Rev. 6, 125–138.
Stothard, E.R., McHill, A.W., Depner, C.M., Birks, B.R., Moehlman, T.M., Ritchie, H.K., Guzzetti, J.R., Chinoy, E.D., LeBourgeois, M.K., Axelsson, J., Wright, K.P., 2017. Circadian Entrainment to the Natural Light-Dark Cycle across Seasons and the Weekend. Curr. Biol. 27, 508–513.
van Duijn, E., Selis, M.A., Giltay, E.J., Zitman, F.G., Roos, R.A., van Pelt, H., van der Mast, R.C., 2010. Hypothalamic-pituitary-adrenal axis functioning in Huntington's disease mutation carriers compared with mutation-negative first-degree controls. Brain Res. Bull. 83, 232–237.
van Wamelen, D.J., Aziz, N.A., Anink, J.J., van Steenhoven, R., Angeloni, D., Fraschini, F., Jockers, R., Roos, R.A.C., Swaab, D.F., 2013. Suprachiasmatic nucleus neuropeptide expression in patients with Huntington's disease. Sleep 36, 117–125.
Voultsios, A., Kennaway, D.J., Dawson, D., 1997. Salivary melatonin as a circadian phase marker: validation and comparison to plasma melatonin. J. Biol. Rhythm. 12, 457–466.
Weckesser, L.J., Plessow, F., Pilhatsch, M., Muehlhan, M., Kirschbaum, C., Miller, R., 2014. Do venepuncture procedures induce cortisol responses? A review, study, and synthesis for stress research. Psychoneuroendocrinology 46, 88–99.