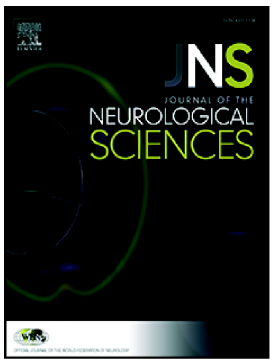

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Multidisciplinary Rehabilitation Reduces Hypothalamic Grey Matter Volume Loss in Individuals with Preclinical Huntington's Disease: A Nine-Month Pilot Study

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Abstract

Background: Hypothalamic pathology is a well-documented feature of Huntington's disease (HD) and is believed 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.

Objective: To evaluate the effects of multidisciplinary rehabilitation on hypothalamic volume, brain-derived neurotrophic factor (BDNF), circadian rhythm and habitual sleep in individuals with preclinical HD.

Methods: Eighteen individuals with HD (ten premanifest and eight prodromal) undertook a nine-month multidisciplinary rehabilitation intervention (intervention group), which included exercise, cognitive and dual task training and social events, and were compared to a community sample of eleven individuals with premanifest HD receiving no intervention (control group). Hypothalamic volume, serum BDNF, salivary cortisol and melatonin concentrations, subjective sleep quality, daytime somnolence, habitual sleep-wake patterns, stress and anxiety and depression symptomatology were evaluated.

Results: Hypothalamus grey matter volume loss was significantly attenuated in the intervention group compared to the control group after controlling for age, gender, Unified Huntington's Disease Rating Scale-Total Motor Score and number of cytosine-adenine-guanine repeats. Serum BDNF levels were maintained in the intervention group, but decreased in the control group following the study period. Both groups exhibited decreases in cortisol and melatonin concentrations. No changes were observed in sleep or mood outcomes.

Conclusions: This exploratory study provides evidence that multidisciplinary rehabilitation can reduce hypothalamic volume loss and maintain peripheral BDNF levels in individuals with preclinical HD but may not impact on circadian rhythm. Larger, randomised controlled trials are required to confirm these findings.

Keywords:

Hypothalamus, brain-derived neurotrophic factor, cortisol, melatonin, circadian rhythm, sleep

Introduction

Hypothalamic pathology, including grey matter volume loss and microglial activation, has been reported as early as a decade prior to clinical manifestation of Huntington's disease (HD) (Kremer et al., 1991; Petersén et al., 2005; Aziz et al., 2008; Politis et al., 2008; Gabery et al., 2010; Soneson et al., 2010). Degeneration within the hypothalamus, particularly the suprachiasmatic nucleus (SCN), which is responsible for controlling the circadian rhythms, is believed to underpin sleep and circadian deficits in individuals with HD (Moore, 1995; Morton et al., 2005; Saper et al., 2005; Aziz et al., 2010; Bartlett et al., 2016).

Disturbances in circadian rhythmicity and sleep are documented in HD mouse models, as well as in humans (Kudo et al., 2011; Morton, 2013; Lazar et al., 2015). 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 delay of the habitual sleep timing have been noted in individuals with premanifest HD (Aziz et al., 2009a; Aziz et al., 2009b; Aziz et al., 2010; van Duijn et al., 2010; Kalliolia et al., 2014). Furthermore, studies in the R6/2 HD mouse model have revealed altered night-day activity ratios, which has also been reported 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). It is not known, however, if these improvements are mediated by changes in the hypothalamus. In patients with HD, our team has previously shown that nine months of multidisciplinary rehabilitation, involving exercise and cognitive training, enhances brain volume in the striatum and prefrontal cortex and improves cognition and motor function (Thompson et al., 2013; Cruickshank et al., 2015). 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 and Berchtold, 2002; Spires et al., 2004; Frazzitta et al., 2014).

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.

Materials and Methods

Study Design

The present investigation was a controlled, non-randomised, non-blinded exploratory study. This study design was adopted due to the rarity of the disease (9.7 per 100,000 in Australia (Rawlins et al., 2016)), the widespread distribution of individuals across Australia and the proof-of-concept nature of the study. The aim was to investigate the effects of nine months of multidisciplinary rehabilitation on hypothalamic volume as the primary outcome and blood-based BDNF, markers of circadian rhythm and habitual sleep-wake outcomes as secondary outcomes in individuals with preclinical HD. Subjective sleep quality, daytime somnolence and stress, anxiety and depression symptomatology were also examined. Study participants were allocated to one of two groups: one receiving nine months of multidisciplinary rehabilitation (intervention group, n = 18) or a group receiving no intervention (control group, n = 11). All outcomes were measured at baseline and following the nine-month study period. The length of the intervention was informed by our previous

work, which showed changes in grey matter volume after nine months of multidisciplinary rehabilitation (Thompson et al., 2013; Cruickshank et al., 2015).

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- 2015000058) and Deakin University (2015-052) human research ethics committees at Perth and Melbourne study sites. All participants provided written informed consent. The study was registered with the Australian New Zealand Clinical Trials Registry (ACTRN12618001717246).

Participants

Twenty-seven premanifest (prior to onset of motor signs) HD and eight prodromal (slight, but no overt, motor signs) 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) a cytosine-adenine-guanine (CAG) repeat length of > 39; 2) a Unified Huntington's Disease Rating Scale (UHDRS) Diagnostic Confidence Level (DCL) of ≤ 2; and 3) a UHDRS Total Motor Score (UHDRS-TMS) of < 5 for premanifest or > 5 for prodromal HD (Reilmann et al., 2014). Exclusion criteria were as follows: 1) concomitant neurological, cardiovascular, musculoskeletal, endocrine or metabolic disorders, 2) shift work, 3) recent or ongoing substance abuse, and 4) the inability to understand written and verbal English.

Multidisciplinary Rehabilitation Intervention

The multidisciplinary rehabilitation intervention was conducted in Perth (with the Melbourne cohort acting as an allocated control group) and was designed by an experienced team of exercise scientists, cognitive training, sleep and circadian rhythm specialists, neuroscientists and a neuropsychiatrist. Details of the intervention can be viewed in detail in supplementary files. Briefly, the intervention was fully supervised by clinical exercise physiologists and cognitive training specialists and consisted of autoregulated periodised aerobic and resistance training, computerised cognitive training, dual-task training, bilingual exercises, healthy lifestyle guidance and social activities. 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 (Jimenez and Paz, 2011; Harries et al., 2015). Supervised computerised cognitive training was informed by a meta-analysis and by previous investigations (Lampit et al., 2014a; 2014b; 2014c; 2015). 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. Dual-task training was performed once weekly for one hour and consisted of combined aerobic and resistance training and cognitive exercises (Yogev-Seligmann et al., 2012; Fritz et al., 2015). Social events were organised every twelve weeks and intervention sessions were conducted in small groups (2-8 participants per session) to encourage social engagement, which has been shown to facilitate adherence to exercise programs in older adults (Thurston and Green, 2004).

Training Adherence and Compliance

Adherence to training sessions and compliance to prescribed exercises were monitored and recorded using a training diary by the exercise physiologists delivering the intervention. The intensity of exercises was monitored using cognitive training software (computerised cognitive training), heart rate monitors (aerobic exercise), the Borg Rating of Perceived Exertion (RPE) scale (Borg, 1982) (both aerobic and resistance exercise) and a visual analogue scale (dual tasking exercises). The volume of aerobic training was monitored using the duration and intensity of exercise. Resistance training volume was calculated using the number of sets and repetitions of exercise performed. An autoregulation periodization model was used to modulate training based on patient motivation and readiness to train in consultation with exercise physiologists (Mann et al., 2010; Zourdos et al., 2016).

MRI Data Acquisition and Pre-processing

T_1-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 consistently 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 consistency (Bartlett et al., 2019). Image pre-processing was conducted according to a longitudinal pipeline (Eshaghi et al., 2014) implemented in SPM12 (http://www.fil.ion.ucl.ac.uk/spm) running on Matlab R2015a (Mathworks, Natick, Massachusetts; see Supplementary Data). A hypothalamus mask from the WFU Pick Atlas (http://fmri.wfubmc.edu/software/pickatlas) was used (dilated by 3mm) 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 template.

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 in the morning at the same time (± 60 minutes) 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 stored at -80ºC until analysis in duplicate using BDNF E_{max} ELISA kits (Promega, Madison, WI) according to the manufacturer's instructions (Ciammola et al., 2007).

Salivary Cortisol and Melatonin Analysis

Saliva samples were collected by participants in their own homes on two consecutive days prior to and following the nine-month study period. 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 (Voultsios et al., 1997; van Duijn et al., 2010). 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 -80ºC until analysis in duplicate using salivary cortisol and melatonin ELISA kits (Salimetrics, USA) according to the manufacturer's instructions.

Subjective Sleep Assessments

Sleep questionnaires were used to measure habitual sleep/wake timing, sleep quality and daytime somnolence prior to and following the nine-month study period (Aziz et al., 2010; Lazar et al., 2015). 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).

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 and Snaith, 1983). Both scales are deemed valid for use in HD research (De Souza et al., 2010; Downing et al., 2011).

Statistical Analysis

Data from the premanifest and prodromal HD intervention groups were combined. This was based on preliminary analyses that showed no differences in neuroimaging 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 (n = 5 of 504, 0.99%) were interpolated using the average cortisol or melatonin concentration of the participant's previous and subsequent

samples 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 (AUC_G) was calculated using the trapezoid rule for morning cortisol and evening melatonin output (van Duijn et al., 2010; Dijk et al., 2012).

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 analysis of variance (ANOVA), with age, gender, UHDRS-TMS and CAG repeat included as covariates. Two-tailed statistical significance was interpreted at an *a priori* $\alpha = 0.05$.

We then assessed voxel-wise differences in hypothalamic volume change between baseline and nine-month follow-up between intervention and control groups using grey matter Jacobian change images from each respective group. In addition to differences between the control and the intervention group as a whole, we also evaluated differences between the control group and the intervention group split into premanifest and prodromal subgroups (see supplementary data). A general linear model was used to estimate voxel-wise hypothalamic volume change between baseline and the 9-month follow-up (represented in grey matter Jacobian change images) across the three groups. Individual regressors were included for each group. Contrasts comparing controls vs. intervention premanifest, controls vs. intervention prodromal and controls vs. all intervention preclinical participants were evaluated with independent samples t-tests. We also tested for within group volume change over time in areas revealing a group difference. 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). Sex and age were included as covariates of no interest in all analyses. The MarsBaR tool box (Brett et al., 2002) was used to extract parameter estimates from relevant contrasts (the average of all significant voxels in the relevant contrast) to generate plots illustrating the results from the VBM analysis. Given the exploratory nature of this study and our a priori interest in the hypothalamus, all results are reported at $\alpha = 0.05$, uncorrected. Statistical analyses were carried out using STATA (StataCorp., College Station, TX) and the R statistical programming language (R Core Development Team, 2014). Cohen's *d* effect sizes were considered small at 0.2, medium at 0.5 and large at 0.8.

Results

Demographic and Clinical Characteristics

Two participants withdrew from the study prior to baseline testing and four withdrew following baseline testing. Analyses were conducted on data obtained from ten premanifest HD and eight prodromal HD participants in the intervention group and 11 premanifest HD participants in the allocated control group.

Demographic and clinical characteristics for the intervention and control groups are presented in Table 1. Age differed between the intervention group (p = 0.029) and the control group, but no differences were observed in gender, CAG repeat number, disease burden score, CAP score, UHDRS-total functional capacity (UHDRS-TFC), body mass index (BMI), smoking status, alcohol consumption or use of psychotropic medication between the groups at baseline (all p > 0.05). Higher UHDRS-TMS and diagnostic confidence level scores were observed in the intervention group compared to the control group (Table 1 and Supplementary Table 7) due to the inclusion of individuals with prodromal HD in the intervention group. Participants displayed high adherence (attended 78 out of a possible 90 sessions, 87%) and compliance (complied to 77 out of a possible 90 sessions, 85%) to training sessions throughout the intervention.

Table 1. Demographic and clinical characteristics of the HD intervention group and control group at baseline

	Intervention group (n=18)	**Control Group (n=11)**	***p*-value**
Demographic Characteristics			
Age, mean ± SD	40.89 ± 11.73	50.55 ± 9.49	0.029*
Male, n (%)	6 (33)	4 (36)	0.868
Clinical Characteristics			
CAGn, mean ± SD	43.67 ± 3.28	41.91 ± 2.02	0.123
DCL, mean ± SD	0.67 ± 0.84	0.00 ± 0.00	0.000*
UHDRS-TMS, mean ± SD	7.56 ± 8.56	0.09 ± 0.30	0.000*
UHDRS-TFC, mean ± SD	13.00 ± 0.00	13.00 ± 0.00	1.000
Disease burden score, mean ± SD	309.52 ± 91.87	309.36 ± 61.51	0.996
CAPs, mean ± SD	0.89 ± 0.22	0.93 ± 0.13	0.598

BMI, mean ± SD	26.60 ± 3.85	25.29 ± 1.99	0.303
Smoker, n (%)	6 (33)	1 (9)	0.172
High alcohol consumption, n (%)	2 (11)	0 (0)	0.394
Psychotropic medication, n (%)	2 (11)	3 (27)	0.264

*Values are significant at $p \leq 0.05$.
CAGn= cytosine-adenine-guanine repeat number; DCL= diagnostic confidence level; UHDRS-TMS= Unified Huntington's Disease Rating Scale- Total Motor Score; UHDRS-TFC= UHDRS-Total Functional Capacity; CAPs= CAG-age product score; BMI= body mass index.

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 2 and Figure 1A). The 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. This result was replicated when the intervention group was divided into premanifest and prodromal sub-groups (see Supplementary Table 8). Importantly, the volume loss over the nine-month period in the right hypothalamus in the control group was statistically significant (see Table 2), whereas there was no statistically significant volume loss in the intervention group.

Table 2. Voxel-based morphometry analysis of the hypothalamus and its relationship with cortisol and melatonin output.

	Side	k	Peak Voxel				
			T-score	*p*-value	MNI Coordinates (mm)		
					x	y	z
Hypothalamus Volume							
Volume loss in control group	R	311	3.39	0.001*	8	3	-13
Greater volume loss in control group (n=12) vs intervention group (n=17)	R	321	2.90	0.004*	5	2	-7
Cortisol and Melatonin							
Group x Cortisol Interaction Effect							
Control group (n=10) vs. intervention group	R	305	2.47	0.012*	6	-4	-10

(n=17)						
Group x Melatonin Interaction Effect						
Control group (n=10) vs. intervention group (n=17)	R	86	2.36	0.014*	8	-2 -14

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

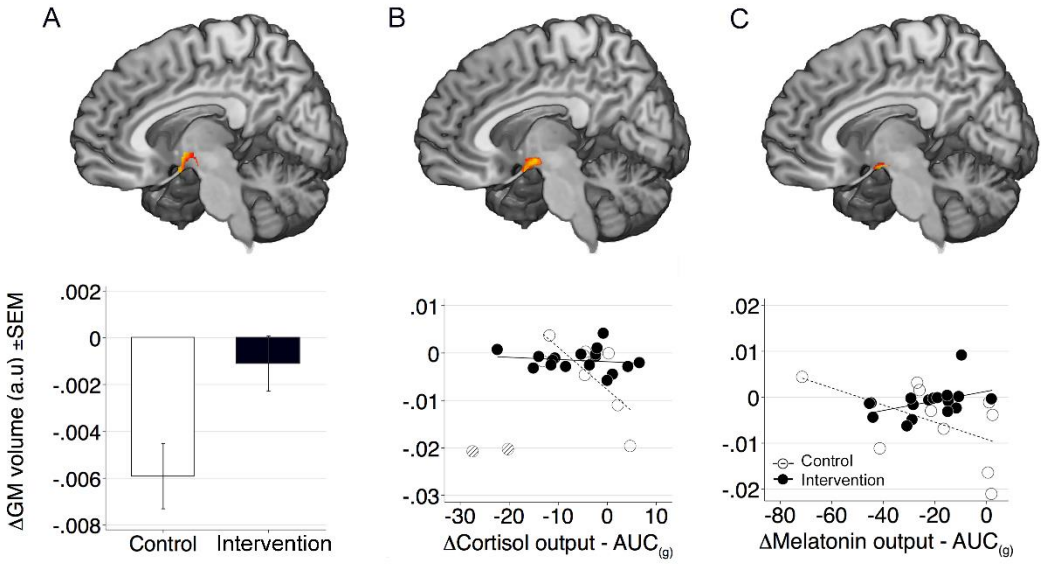

Figure 1. Effects of nine months of multidisciplinary therapy on hypothalamic grey matter (GM) volume as revealed by VBM analysis. (A) Group effect on GM volume and bar graphs illustrating the effect. Values in the bar graphs correspond to the mean volume change in each group (after averaging across 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 = ~8 (MNI) and are scaled to the peak T-score for the relevant analysis (see Table 2).

Serum BDNF Analysis

A within × between group interaction ($p = 0.030$; Table 3 and Supplementary Table 9) was observed for serum BDNF. This was denoted by a 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 preclinical HD.

Salivary Cortisol and Melatonin Analysis

No within × between group interactions existed across the cortisol and melatonin measures (p = 0.355 and p = 0.809, respectively; Table 3 and Supplementary Table 9). However, both the intervention and control groups exhibited a decrease in cortisol and melatonin AUC_G following the study period and the magnitude of change was not different between the two groups, suggesting that the multidisciplinary rehabilitation program did not have an effect on morning cortisol or evening melatonin release.

Table 3. Mean serum BDNF and salivary cortisol and melatonin concentrations, subjective sleep outcomes and affective symptomatology outcomes in the preclinical HD intervention and control groups

Outcome	Intervention Group (n=18)		Control Group (n=11)		Within × Between Groups Interaction		Within Group		Between Groups	
	Baseline	Follow-Up	Baseline	Follow-Up	*p*-Value	Partial η^2	*p*-Value	Partial η^2	*p*-Value	Partial η^2
Serum BDNF (pg/mL)	26920.8 ± 1613.4	27227.4 ± 1073.7	30126.5 ± 2041.1	23358.9 ± 1358.3	0.030*	0.188	0.933	0.000	0.846	0.002
Cortisol (nmol/L)										
Awakening (hh:mm)	6:06 ± 00:14	6:02 ± 00:13	6:05 ± 00:18	7:00 ± 00:17	0.020*	0.213	0.774	0.004	0.157	0.085
Time of sample relative to awakening:					0.328	0.042	0.398	0.031	0.951	0.000
+15 mins	11.6 ± 1.0	9.1 ± 0.8	11.4 ± 1.3	8.7 ± 1.0						
+30 mins	14.6 ± 1.1	11.6 ± 0.8	13.4 ± 1.4	9.4 ± 1.0						
+45 mins	14.0 ± 0.8	12.3 ± 0.7	14.1 ± 1.1	11.0 ± 0.9						
+60 mins	12.3 ± 0.9	11.2 ± 0.8	12.6 ± 1.2	9.4 ± 1.0						
AUC_G	40.5 ± 2.4	34.0 ± 1.8	39.5 ± 3.1	29.4 ± 2.2	0.355	0.037	0.714	0.006	0.347	0.039
Melatonin (pg/mL)										
Bedtime (hh:mm)	22:00 ± 00:12	21:58 ± 00:12	22:12 ± 00:15	21:54 ± 00:15	0.281	0.053	0.013*	0.247	0.825	0.002
Time of sample relative to bedtime:					0.719	0.006	0.326	0.042	0.346	0.039
-2 hrs	13.9 ± 2.3	8.9 ± 2.8	11.9 ± 2.9	7.2 ± 3.6						
-1 hr	15.6 ± 2.8	8.6 ± 1.9	13.1 ± 3.5	6.6 ± 2.4						
+0 hrs	21.2 ± 3.0	12.8 ± 2.5	15.7 ± 3.8	8.5 ± 3.1						
+1 hr	23.1 ± 3.6	13.5 ± 2.8	16.4 ± 4.6	9.4 ± 3.5						
AUC_G	53.5 ± 7.6	32.6 ± 6.6	42.0 ± 9.6	23.5 ± 8.4	0.809	0.003	0.437	0.027	0.353	0.038
CSD										
Total time in bed (min)	423.0 ± 12.9	424.1 ± 20.1	433.5 ± 16.3	496.8 ± 25.2	0.025*	0.228	0.874	0.001	0.109	0.124
Total sleep time (min)	378.6 ± 14.4	379.0 ± 15.2	389.4 ± 18.0	453.2 ± 19.1	0.008*	0.303	0.837	0.002	0.067	0.158
Sleep onset latency (min)	24.3 ± 6.0	22.6 ± 5.2	8.0 ± 7.5	10.8 ± 6.5	0.697	0.008	0.290	0.056	0.066	0.159
Wake after sleep onset (min)	18.2 ± 6.4	11.8 ± 4.8	7.0 ± 8.1	5.3 ± 6.0	0.667	0.009	0.644	0.011	0.262	0.063
Number of awakenings	1.6 ± 0.4	1.4 ± 0.4	0.5 ± 0.5	1.3 ± 0.5	0.218	0.075	0.276	0.059	0.252	0.065
Sleep efficiency (%)	89.8 ± 3	89.9 ± 2.7	90.2 ± 3.8	92.2 ± 3.4	0.610	0.013	0.823	0.003	0.759	0.005
Restorative quality of sleep	3.5 ± 0.3	3.5 ± 0.2	3.7 ± 0.3	4.0 ± 0.3	0.420	0.035	0.706	0.008	0.468	0.028
PSQI Global Score	5.8 ± 0.8	5.4 ± 0.8	5.2 ± 1.0	4.9 ± 1.0	0.925	0.000	0.633	0.011	0.674	0.008
Epworth Sleepiness Scale	5.7 ± 1.0	4.6 ± 0.8	4.8 ± 1.2	5.3 ± 1.0	0.099	0.119	0.795	0.003	0.924	0.000

Perceived Stress Scale	18.3 ± 1.8	15.3 ± 1.9	16.6 ± 2.2	17.0 ± 2.4	0.305	0.048	0.492	0.022	0.815	0.003
HADS total score	8.9 ± 1.3	6.3 ± 1.0	6.1 ± 1.6	6.4 ± 1.3	0.079	0.128	0.300	0.047	0.448	0.025
Anxiety	5.8 ± 0.8	5.0 ± 0.8	4.3 ± 1.0	5.1 ± 1.0	0.155	0.086	0.655	0.009	0.587	0.013
Depression	3.1 ± 0.6	1.3 ± 0.3	1.8 ± 0.8	1.3 ± 0.4	0.147	0.089	0.179	0.077	0.360	0.037

Data are presented as mean ± standard deviation. *Values are significant at $p < 0.05$. Partial η^2, 0.01, 0.06 and 0.14 are defined as small, medium and large effects, respectively. CSD= Consensus Sleep Diary; PSQI= Pittsburgh Sleep Quality Index; HADS= Hospital Anxiety and Depression Scale. The sleep efficiency component of the CSD was calculated as follows: total sleep time/total time in bed x 100. Values for the restorative quality of sleep component of the CSD range from 1-5, with lower values indicating worse sleep quality. Possible ranges for questionnaires are as follows: PSQI (0-21), Epworth Sleepiness Scale (0-24), Perceived Stress Scale (0-40), HADS-anxiety (0-21), HADS-depression (0-21).

Subjective Sleep Assessments

Within × between group interactions were observed for the time of awakening (p = 0.020), total time in bed (p = 0.025) and total sleep time (p = 0.008; Table 3 and Supplementary Table 9). Sleep outcomes did not change over the nine-month study period in the intervention group, however the total time in bed and total sleep time increased in the control group (Table 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.

No other differences were observed between groups in habitual sleep/wake timing (CSD), subjective sleep quality (PSQI scores) or daytime somnolence (ESS scores) (Table 3 and Supplementary Table 9). These results suggest that multidisciplinary rehabilitation did not alter subjective sleep quality or daytime somnolence; however, it may have the potential to mediate sleep/wake timing.

Affective Symptoms

No between × within group interactions were observed for affective symptomatology (Table 3 and Supplementary Table 9). Anxiety and depression scores on the HADS were subthreshold (< 8) for both groups, indicating no clinically meaningful anxiety symptomatology (Zigmond and Snaith, 1983).

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

An interaction effect was observed between group (intervention or control) and change in cortisol output (Table 2 and Supplementary Table 8). 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. However, two potential influential cases were also identified, both from the control group. Regression diagnostics (Cook's distance and leverage) confirmed these cases as influential. Re-running the voxel-wise analysis excluding these cases showed a reversal in the association between right hypothalamic grey matter volume loss and change in morning cortisol output in the control group: the association was now negative, in comparison to a flat slope for the intervention group (Figure 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 now 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 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 1C, Table 2 and Supplementary Table 8). 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).

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 attenuated grey matter volume loss within the hypothalamus in individuals with preclinical HD.

Studies by our team and others show that individuals with premanifest and prodromal HD have reduced grey matter volume in the hypothalamus as early as ten years prior to predicted clinical disease onset (Soneson et al., 2010; Bartlett et al., 2019). Results from the present study suggest that multidisciplinary rehabilitation reduces hypothalamic grey matter volume loss, indicating that multidisciplinary rehabilitation may act to preserve the hypothalamus. Interestingly, this reduced volume loss occurred on the right side of the hypothalamus, suggesting that the intervention may have exhibited an asymmetric effect on the hypothalamus. However, studies with larger sample sizes are required to confirm this preliminary finding. To the authors' knowledge, this is the first study to document a 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 increase grey matter volume in the caudate and dorsolateral prefrontal cortex in symptomatic HD individuals (Cruickshank et al., 2015). Together, these findings provide compelling evidence that adds to the growing number of preclinical and clinical studies 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 (Spires et al., 2004; Lazic et al., 2006; Nithianantharajah and Hannan, 2006).

The biological mechanisms mediating the attenuation of hypothalamic volume loss after multidisciplinary therapy are unknown. However, it is possible that preservation of BDNF levels observed in the intervention group may have contributed to the attenuation of hypothalamic volume loss. BDNF plays a crucial role in the development, protection and plasticity of neurons (Bemelmans et al., 1999; Rossi et al., 2006; Bekinschtein et al., 2011) and exhibits a reduced level of expression in the hypothalamus of individuals with HD at post-mortem (Baldo et al., 2018). Therefore, the maintenance of serum BDNF levels as a result of regular exercise and cognitive training could contribute to the support and preservation of the hypothalamus (Bartlett et al., 2016). However, the role that BDNF plays in neuroplasticity is yet to be robustly confirmed. Furthermore, BDNF has been shown to cross the blood-brain barrier in animals (Pan et al., 1998a; Pan et al., 1998b; Pan and Kastin, 1999), but the extent to which this occurs in humans has not yet been established. As such, our supposition that the preservation of BDNF levels following multidisciplinary rehabilitation may lead to maintenance of hypothalamic volume must be taken with caution.

Attenuation of grey matter volume loss in the hypothalamus following multidisciplinary rehabilitation was positively associated with changes melatonin release. Interestingly, this finding occurred in the basal hypothalamus, a region that contains the suprachiasmatic nucleus. Similarly, bilateral attenuation of hypothalamic grey matter volume loss was also positively associated with the changes in cortisol release following the intervention. However, contrary to our expectations, the reduced loss of hypothalamic grey matter volume 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 observe reduced daytime somnolence in the group receiving the multidisciplinary rehabilitation intervention, which was accompanied by a reduction in anxiety and depression symptomatology, however the 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 an attenuation of hypothalamic volume loss to positively coincide with habitual sleep outcomes. Evidence indicates that a network of brain structures, including the thalamus and parts of the brain stem, are responsible for regulating the sleep/wake cycle (Saper et al., 2005; Fuller et al., 2006; Luppi et al., 2013).

While multidisciplinary rehabilitation was beneficial for the hypothalamus, it may not have widespread positive effects on this broader network of structures necessary for the regulation of circadian rhythm and sleep. Therefore, future studies should assess the effects of multidisciplinary rehabilitation on these other structures regulating the sleep/wake cycle and use objective measures to assess the effects of multidisciplinary rehabilitation on sleep architecture rather than the subjective measures used here.

A number of limitations need to be considered when interpreting our findings. Due to the rarity of the disease, the specific stage of the disease investigated and the widespread distribution of the individuals across Australia, a non-randomised cohort study design was adopted. The nature of the group allocation prevented blinding of assessors, which may have introduced bias. However, the objective nature of the primary and secondary outcome measures and the use of a statistician unaware of group allocation to conduct statistical analyses would have reduced the incidence of bias in the study. While this limits the generalisations that can be made to the wider HD community, 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. Furthermore, to the authors' knowledge, this is the first study to investigate the effects of multidisciplinary rehabilitation in preclinical HD. It is also the first study to use an autoregulated, periodized aerobic and resistance training intervention in this population and shows feasibility in implementing such approaches. It is important to note that brain imaging occurred across two study sites (Perth and Melbourne), which could potentially lead to between-site variation in MR images. However, a between-site consistency analysis was conducted, whereby three individuals underwent MRI at both locations to ensure that the MRI scans were obtained consistently across sites (Bartlett et al., 2019). In addition, we tested for within group differences in the region of the hypothalamus that showed group differences. We found that while there was statistically significant volume loss in the control group, there was no significant change in the intervention group. Consequently, even if there is a site effect potentially confounding the group comparison, we can nevertheless assert from the within group analyses that the control group exhibited volume loss in an area of the right hypothalamus, while the intervention group did not. This supports our intervention having a beneficial effect on hypothalamus volume. Finally, melatonin was only assessed at four time points at hourly intervals within the three hours surrounding the participants' usual bedtime.

Future studies should measure the dim light melatonin onset and its relationship with hypothalamic volume in individuals with preclinical HD.

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

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Conflict of Interest Statement

The Authors declare that there is no conflict of interest.

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