

Plasma Melatonin Is Reduced in Huntington's Disease

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ABSTRACT: This study was undertaken to determine whether the production of melatonin, a hormone regulating sleep in relation to the light/dark cycle, is altered in Huntington's disease. We analyzed the circadian rhythm of melatonin in a 24-hour study of cohorts of control, premanifest, and stage II/III Huntington's disease subjects. The mean and acrophase melatonin concentrations were significantly reduced in stage II/III Huntington's disease subjects compared with controls. We also observed a nonsignificant trend toward reduced mean and acrophase melatonin in premanifest Huntington's disease subjects. Onset of melatonin rise was significantly more temporally spread in both premanifest and stage II/III Huntington's disease subjects

compared with controls. A nonsignificant trend also was seen for reduced pulsatile secretion of melatonin. Melatonin concentrations are reduced in Huntington's disease. Altered melatonin patterns may provide an explanation for disrupted sleep and circadian behavior in Huntington's disease, and represent a biomarker for disease state. Melatonin therapy may help the sleep disorders seen in Huntington's disease. © 2014 The Authors. *Movement Disorders* published by Wiley Periodicals, Inc. on behalf of International Parkinson and Movement Disorder Society.

Key Words: Huntington's disease; melatonin; circadian rhythm

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by an expanded CAG repeat in the gene encoding huntingtin.¹ It is char-

acterized by progressive chorea and cognitive and psychiatric disturbance. Other features include weight loss and sleep disturbance.^{2,3} Disturbed sleep is reported in 80% of cases of HD, with reduced sleep efficiency and altered architecture,³ and later increased sleep latency, frequent nocturnal awakenings, and delayed and shortened rapid-eye-movement sleep.⁴⁻⁶ R6/2 HD mice have progressive disruption of day-night circadian behavior, accompanied by dysregulation of suprachiasmatic nucleus (SCN) circadian clock genes.⁷ In another mouse model, reduced rhythms in spontaneous electrical activity in SCN neurons were demonstrated.⁸

Melatonin is secreted by the pineal gland mainly at night, regulating sleep and other circadian processes. The SCN regulates melatonin synthesis via a polysynaptic pathway in response to the light/dark cycle, important in signaling "time of day."⁹ Degeneration of the SCN in HD could influence melatonin secretion, disrupting sleep and circadian rhythms. One study has analyzed melatonin profiles and found a delay in evening rise of melatonin in

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TABLE 1. Demographic, clinical features and melatonin data for Control and HD cohorts expressed as mean (standard deviation)

Stage	Controls	Premanifest HD	Stage II/III HD	<i>p</i> ^a
Number of subjects	15	14	13	
Mean age (range)	52 (29-69)	45 (31-58)	55 (42-70)	
Female:Male	6:9	9:5	5:8	
Mean CAG (range)	—	42 (40-47)	44 (42-47)	
Mean burden score (range)	—	300 (207-434)	435 (273-702)	
UHDRS Total Functional Capacity	—	13 (12-13)	8 (5-12)	
UHDRS Motor Score	—	2 (0-11)	38 (10-65)	
Melatonin concentration (pg/mL):				
Mean over 24 hours	89 (68)	52 (41)	37 (58)	0.007
Nadir	12.9 (10.4)	10.5 (10.8)	7.9 (10.7)	0.47
Acrophase	292 (245)	155 (121)	112 (189)	0.005
Amplitude	140 (121)	72 (57)	52 (91)	0.004
Onset time (hh:mm)	22:34 (01:17)	22:47 (02:47)	22:37 (03:26)	0.96
Offset time (hh:mm)	07:04 (02:01)	07:04 (01:37)	08:00 (04:27)	0.97
Nocturnal duration (h)	8.5 (2.5)	8.3 (3.5)	7.5 (4.8)	0.74

^a*P*-values for differences between means, adjusted for age and sex.

HD, which may not explain the range of HD sleep disorders.¹⁰ As part of a study of neuroendocrine and metabolic factors in HD, we designed a protocol to analyze melatonin circadian rhythms in detail in cohorts of premanifest and moderate HD subjects and controls.

Subjects and Methods

Fourteen premanifest HD gene carriers, 13 stage II/III HD patients, and 15 control subjects were enrolled. Patients were excluded if they had preexistent endocrine disease, central nervous system disorder other than HD, history of alcohol or drug abuse, treatment with corticosteroids, phenothiazine antiemetics or antipsychotic medication (including neuroleptics, selective serotonin reuptake inhibitor drugs), or hypnotic drugs during the preceding 6 months, or night shift working and recent weight change in the preceding 6 months. The study was approved by University College London/University College London Hospital ethics committee.

Participants were admitted to a private clinical room, intravenous cannula inserted, and clinical assessment and HD rating scales (Unified Huntington's Disease Rating Scale [UHDRS] and total functional capacity [TFC]¹¹) performed. The subject could walk freely, watch TV, but not fall asleep or snack outside scheduled times. At 10:00 PM, they retired to bed and lights were turned off until 6:00 AM. Hourly blood samples were taken over 24 hours, using a long line to minimize sleep disruption. Melatonin samples were placed on ice before centrifugation at 4°C at 175 g for 10 min. Plasma was immediately placed on dry ice and stored at -80°C, before analysis.

Plasma Analyses

Plasma melatonin was measured in duplicate by radioimmunoassay (Labor Diagnostika Nord GmbH

& Co. KG, Nordhorn, Germany). Detection limit of the assay was 3 pg/mL, with 3 to 1000 pg/mL range, interassay variability of 9.6 to 16.2%, and 78.3% recovery.

Statistical Analysis

Results are expressed as mean ± standard deviation (SD) unless otherwise specified. Intergroup differences were assessed by linear regression on disease group (in three categories), age, and sex. Logarithms were taken where this improved normality of residuals. Regression was conducted with and without outlying values, and *P*-values were little changed by their exclusion. Semi-partial correlation coefficients were calculated between melatonin levels and disease burden/functional capacity, adjusted for age and sex. All tests were two-tailed. Analyses were performed using SPSS for Windows (16.0, SPSS, Inc., Chicago, IL).

Circadian variation of plasma melatonin was quantified by best-fit curve obtained using locally weighted linear regression with Gaussian kernel with a regression window of 4 h.¹² The following definitions were used: Nadir and acrophase concentrations, minimum and maximum of best-fitting curve; amplitude concentration, half the difference between acrophase and nadir values; onset of melatonin rise, time of first plasma level exceeding mean +3 SDs of baseline values over the 11:00 AM to 3:00 PM period, not followed by lower concentrations before acrophase; melatonin offset, time of last value occurring after acrophase exceeding -3 SDs of baseline levels¹²; nocturnal duration, length of time between melatonin onset and offset.

Fourier Transformation (FT) analysis was used to measure spectral power of melatonin oscillations, enabling analysis of the strength/power of hormonal

oscillations at different frequencies.¹³ Signal–noise ratio was improved by using a 3-point moving average, and any trend in the data was removed by a difference + mean regression. Data were analyzed by using the EASY-TSA program¹⁴ (Oxford University,

UK: Nathan.hill@phc.ox.ac.uk). Comparisons between waveforms of time series analysis were performed by probability of standard error of mean (SEM) at any discrete time point, overlapping the SEM of the comparative group.

Results

Table 1 shows demographic and clinical data for the cohorts. Analysis of mean plasma melatonin concentrations for the three groups over 24 hours indicates reduced nocturnal concentrations, acrophase, and amplitude concentrations in premanifest and affected subjects (Table 1, Fig. 1A, 1B). *P* values in Table 1 reflect a joint test of differences between all three groups (after adjustment for age and sex).

Adjusted for age and sex, mean 24-hour melatonin concentrations in Stage II/III HD are 82% lower (95% confidence interval, 49–94% lower, *P* = 0.002) than controls, and 68% lower (90% lower to 2% higher, *P* = 0.053) than pre-manifest disease (results are obtained in percentage terms, because logs were taken before analysis). Acrophase concentrations in Stage II/III HD are 86% lower (56–95% lower, *P* = 0.001) than controls, and 71% lower (2–92% lower, *P* = 0.047) than pre-manifest disease. Amplitudes in Stage II/III HD are 87% lower (59–96% lower, *P* = 0.001) than controls, and 75% lower (9–93% lower, *P* = 0.04) than pre-manifest disease. Mean, acrophase, and amplitude concentrations for pre-manifest HD were lower compared with controls, but did not reach significance.

No significant differences were seen in melatonin secretion onset, offset, or duration of nocturnal secretion (Fig. 1C, Table 1). However, whereas in most control subjects, melatonin onset occurred between 9:00 PM and midnight, in HD gene carriers, the melatonin onset was more temporally spread (*P* = 0.006 and *P* = 0.001 for pre-manifest HD and moderate HD, respectively, each compared with controls, by variance ratio test). Fourier transformation (FT) analysis (Fig. 1D) showed a dominant pulsatility at 132 min for all groups (control, 7.5, SEM, 3.0%; pre-manifest, 3.8, SEM, 1.6%; Moderate, 2.1, SEM, 1.0%) with minor repetition of periodicity at 660 minutes. The strength of spectral power at 132 minutes was lower in the stage II/III and pre-manifest

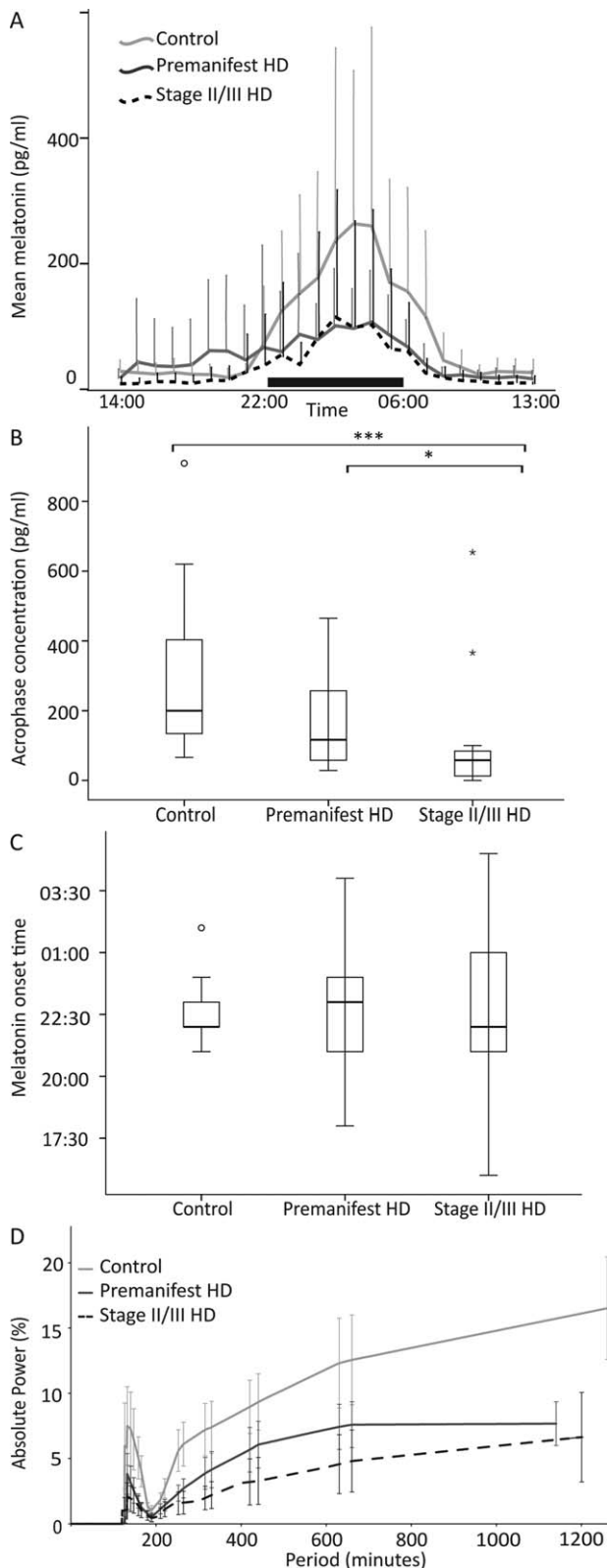


FIG. 1. Analysis of melatonin in control, pre-manifest, and stage II/III HD cohorts. **(A)** Mean melatonin concentrations over 24-h sampling period in the three groups. The black bar on the abscissa indicates the dark period (10:00 PM–6:00 AM). **(B)** Box plot showing distributions of acrophase concentrations in healthy controls, pre-manifest, and Stage II/III subjects. The bars with bold stars indicate statistical significance ($*P \leq 0.05$, $***P \leq 0.001$). **(C)** Box plot showing distribution of melatonin onset time in the three groups. **(D)** FT analysis of melatonin, plotting strength/power (%) against frequency (minutes) of melatonin oscillations for the three groups. Circle and stars in **B** and **C** represent outlying values.

groups in comparison with controls. Pairwise comparison showed significance between the stage II/III and control groups ($P < 0.03$), but the overall between-group comparison did not reach statistical significance.

We did not find evidence of associations between the melatonin variables investigated in Table 1 and UHDRS motor score, TFC, CAG repeat, or repeat burden score¹⁵ when corrected for age, sex, and multiple comparisons.

Discussion

This is the first study to demonstrate that plasma melatonin concentrations are reduced in subjects with HD, suggesting that melatonin secretion is disrupted. Our data also show that melatonin acrophase concentrations and amplitude of secretion are reduced. Melatonin secretion declined with disease progression, with lower concentrations and a trend for reduced pulsatility of secretion in the stage II/III patients compared with controls. The study also found lower melatonin concentrations in premanifest HD, although this did not reach significance but showed a temporal spread of onset of melatonin nocturnal rise in both premanifest and stage II/III HD subjects. We corrected for age and sex, because the pre-manifest cohort was not as closely matched to the pre-manifest group as the stage II/III. Sampling hourly throughout the night could represent a source of error, but individual and best-fit plots for controls show a typical melatonin increase overnight, suggesting no disruption of nocturnal secretion.

A previous study of a single time point measurement of melatonin (in the morning after an overnight fast) in advanced HD patients did not detect a difference compared with controls.¹⁶ Our data show that single measures cannot reflect the dynamic range of melatonin over 24 hours. Additionally, in Figure 1A, early morning melatonin levels are similar in all three groups, which may explain this negative finding. Indeed, between 8:00 AM and 1:00 PM, the melatonin concentrations in controls, pre-manifest HD, and stage II/III HD subjects are similar, suggesting that measuring melatonin during this period is unlikely to show a difference.

Aziz et al.¹⁰ studied a small group ($n = 9$) of early stage HD subjects and controls and found no difference in melatonin concentration, but correlated diurnal melatonin levels with motor score and TFC,¹⁰ suggesting an effect of HD on melatonin concentrations not detectable in their small cohorts. They also identified an 80-minute delay in evening rise of melatonin. Although we also observed a delay in evening melatonin rise in some HD gene carriers, we also noted a similar number of HD subjects with an advanced melatonin rise; therefore, we conclude that

the rise of melatonin is temporally altered. We showed a decrease in melatonin concentrations from control to pre-manifest and moderate HD, but no correlation with clinical indicators of disease severity (UHDRS motor score, TFC) and CAG repeat burden, possibly reflecting that melatonin is a specific marker for SCN/pineal function. The larger patient groups and inclusion of subjects with more advanced HD (stage II/III) may explain why lower melatonin concentrations were found in our study.

The SCN keeps local circadian clocks synchronized with both each other and the solar cycle.¹⁷ Disrupted and reduced melatonin secretion may indicate dysfunction or degeneration of SCN neurons, with abnormal circadian rhythm and integration of light/dark cycle. A study of knock-in transgenic mice with a 175 CAG repeat identified both gene dosage and age-dependent circadian disruption, although histological analysis of the SCN did not identify cell loss.¹⁸ However, a study in human HD post-mortem tissue found evidence of post-transcriptional changes in SCN neuropeptides, suggesting a functional abnormality.¹⁹ This may play a role in the melatonin changes we found and sleep abnormalities in HD. Restoring nocturnal melatonin levels may prove therapeutic, and a trial of melatonin for HD sleep disturbance is warranted. Melatonin may also be neuroprotective and can delay disease onset and mortality in R 6/2 mice.²⁰

The precise cause of the sleep abnormalities in HD is not known and may involve complex interplay between the hypothalamus, basal ganglia, cortex, and brain stem.¹⁷ Recent studies in the R 6/2 mouse identified significant circadian behavior, sleep, and electroencephalogram disturbance in the transgenic mice, detectable at even a presymptomatic stage.^{21,22} However, neuroendocrine factors were not analyzed in these studies.

Sleep disorders are seen in other neurodegenerative disorders, including Parkinson's disease (PD), and since submission of this paper two reports of reduced melatonin levels in PD subjects have been published, also suggesting a link with sleep disorders.^{23,24} Thus, disruption of melatonin synthesis and release may be seen more widely in neurodegeneration, although involvement of the SCN in PD pathology is not known.

Our data demonstrate for the first time a significant abnormality in melatonin concentrations in HD patients, offering therapeutic strategies and potential as state biomarker to track disease progression in HD. A biomarker for disease trait is not needed for HD, because it can be clearly differentiated from other neurodegenerative disorders with the CAG repeat genetic test. ■

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