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CIRCADIAN RHYTHMS OF MELATONIN AND CORTISOL IN MANIFEST HUNTINGTON'S DISEASE AND IN ACUTE CORTICAL ISCHEMIC STROKE

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Recent studies indicate disruptions to the circadian system in brain injury and neurodegeneration. The results, however, are often not consistent and limited by measurement of only one circadian marker and by infrequent sampling rates. In this study, we examined diurnal rhythmicity in different stages of Huntington (HD) disease and in patients with acute moderate ischemic stroke (AIS) outside the retinohypothalamic pathway by evaluating serum concentrations of melatonin and cortisol at twelve timepoints. All study participants were subjected to the same study protocol of 12-hour light/dark cycle and controlled room conditions. Using cosinor analysis of data and comparing the results with the controls we found melatonin phase delay with lowered amplitude and mesor in stage III HD patients. These changes coexisted with phase advanced rhythm and elevated values of mesor and amplitude for cortisol. Early and mid-stages of HD showed only a phase advance in cortisol secretion. In AIS the circadian rhythm of serum melatonin was sustained without any phase shift and exhibited more flattened profile (lowered mesor and amplitude values), while advanced rhythm with higher mesor for cortisol was present. In conclusion, 1) abnormal pattern of melatonin release in the late stages of HD and in moderate AIS occurs in conjunction with phase-advanced rhythm of cortisol; 2) changes observed in late stages of HD are similar to those that occur with ageing; 3) brain regions other than the presumptive retinopineal neural pathway may play an important role in the pineal production of melatonin in humans; 4) lesion in extrahypothalamic region is related to the strong adrenal stimulation in response to AIS.

Key words: *Huntington disease, ischemic stroke, melatonin, cortisol, circadian rhythm, hypothalamic-pituitary-adrenal axis*

INTRODUCTION

Many physiological functions of human body are related to the circadian rhythms that are primarily regulated by circadian master clock in the hypothalamic suprachiasmatic nucleus (SCN) and also generated at the peripheral level (1). After receiving photic input from the retina, with near-24-hour internal rhythm SCN coordinates performance, endocrine rhythms, behavior, sleep timing and cell cycle progression. Rhythmic direct and indirect outputs from the SCN communicate with surrounding parts of brain and peripheral organs by bidirectional projections with primary negative feedback loops involving the expression of clock genes (2, 3). Although SCN may function as an autonomic clock generating independent circadian oscillations of neuronal firing, the overall effect of its function is more complex since SCN as a neuronal network is highly responsive to and dependent on intercellular signals (4). SCN controls pineal gland and its rhythmic secretion of melatonin, which in turn feeds back on SCN rhythmicity to modulate circadian patterns of activity and other processes (5). It is well known that the consistency of circadian rhythms plays significant role in maintaining body's homeostasis, health and

wellbeing. Recent studies indicate that disruptions to the circadian system are present in neurodegenerative disorders (6, 7, 8). Whether circadian abnormalities represent consequences of the neurodegeneration, or may contribute to the pathogenesis of neurodegenerative process still remain unclear. Thus, understanding the physiological relevance of circadian rhythmicity in neurodegeneration/neuroprotection may bring a new therapeutic perspectives including clock-targeted therapies (9). There are number of biomarkers evaluating the presence of circadian rhythm disturbances including melatonin, core body temperature and cortisol. Melatonin is presently the most accurate marker of circadian rhythmicity and function of SCN in humans. Its rhythm is less or not at all influenced by environmental factors such as sleep-wake state, exercise or mood and provides an information about functional condition of brain pacemaker as well as about neurologic pathway retina-SCN-pineal gland. The known noise factors that affect melatonin phase are light, posture, and ambient temperature (10). Except form being sensitive marker for rhythmicity, melatonin plays role as a neuroprotective factor that improves neuronal survival and function and reduces oxidative stress, inflammation and apoptosis (11, 12). Adrenal secretion of cortisol also exhibits

robust circadian rhythmicity and occurs in response to hypothalamic endocrine efferent outputs. However, the daily pattern of corticosteroid production is influenced not only by SCN activity but also by several interacting systems including adaptive response to acute stress, adrenal intrinsic oscillator and functioning of classical neuroendocrine hypothalamic-pituitary-adrenal axis (HPA) (13). The alterations in both circadian activity of HPA and melatonin secretion have been associated with cognitive impairments and other symptoms related with neurodegeneration in humans, but the results are scarce and sometimes conflicting. Thus, to extend the knowledge about potential disruption of rhythmicity in neurodegeneration and brain injury we decided to characterize diurnal rhythmicity in male patients with acute cortical ischemic stroke outside the retinohypothalamic pathway and with no lesion in the hypothalamic region, and in different stages of Huntington disease by evaluating serum concentrations of melatonin and cortisol at twelve timepoints.

MATERIAL AND METHODS

Study participants

Participant recruitment for the research was carried out during 'dark' months (between October and March) in European Huntington's Disease Network Center in Poznan and in Department of Neurology, Poznan University of Medical Sciences (PUMS), Poland. Eleven patients aged 48.0 ± 3.0 with confirmed Huntington's disease and 8 patients aged 53.0 ± 2.5 with acute ischemic stroke (patients within 2 days of stroke onset) participated in the study. For the control group ($n = 10$) healthy age-matched volunteers were recruited and screened for general medical and psychological health. The control subjects had no history of medical illness. Inclusion criteria for this study were: 1) manifest Huntington disease (HD) confirmed by the presence of trinucleotide repeat in the gene IT-15 and UHDRS Total Motor Score (TMS) ≥ 5 and a total functional capacity (TFC) score < 13 , and 2) acute brain ischemic stroke (AIS) with no lesion in the hypothalamic region. The exclusion criteria were based on one of the following: confirmed depression and other psychiatric disorders, history of a prior neurological condition, such as stroke in patients with HD, blindness, presence of any other factors affecting circadian rhythmicity (e.g. treatment with beta-blockers, jet lag syndrome or being night shift worker). Study participants underwent prestudy screening by standard neurological examination. The motor UHDRS (Total Motor Score, TMS) and functional UHDRS (Total Functional Capacity, TFC), which are standardized rating systems recommended by National Institute for Neurological Disorder and Stroke (NINDS) (14), were used to assess for the severity level of clinical features in manifest HD. Based on the TFC score we classified our HD patients into three categories: early stage (TFC score 11 – 13), mid stage (TFC score 7 – 10) and late/advanced stage (TFC score ≤ 6) of manifest HD.

Computed tomography scanning (noncontrast head CT) was performed on patients with AIS to confirm the diagnosis and to exclude from the study the individuals with ischemic lesions in the hypothalamic region. The lesion area of the CT slices was manually determined. All AIS patients enrolled to the current study suffered from anterior circulation stroke with middle cerebral artery (MCA) occlusion. The other inclusion criteria were: occurrence of first cerebrovascular ischemic stroke, no surgery and neuro-injury up to 3 months prior to ischemic stroke, no current inflammatory disease, absence of liver, kidney and malignant disease. Stroke severity was assessed using Scandinavian Stroke Scale (SSS) that has been widely

used in clinical research to summarize the neurological deficits in stroke patients (15). The SSS evaluates nine items: consciousness, eye movement, arm/hand/leg motor power (each assessed only on the affected side), orientation, speech, facial palsy and gait. Mean SSS score on admission for all patients with AIS was 35.0 ± 3.0 .

The study was conducted with the approval of the Ethical Committee of Poznan University of Medical Sciences. Patients with HD comprised a relatively homogenous study group with almost no comorbidities (only one patient had well controlled diabetes and hypertension) and only two of them with advanced (late) stage of the disease were pharmacologically treated with haloperidol. No other medication management was implemented. HD was recognized according to neuro exam and genetic tests using standard DNA extraction, PCR, and gel-sizing methods (16). Patients with ischemic stroke represented less homogenous group with following pharmacologically controlled comorbidities: hypertension ($n = 6$), dyslipidemia ($n = 6$), diabetes type 2 ($n = 5$), coronary artery disease ($n = 2$). All subjects completed the study without any complaints.

Study design

The experiment was conducted on hospital-in patients (Department of Neurology and Department of Physiology, Poznan University of Medical Sciences, Poland). Sleep pattern and light exposure were controlled for one day before entry into the study. No daytime naps were allowed and patients as well as controls stayed in dim light conditions from 6.00 p.m. to 6.00 a.m. (with complete darkness between 10.00 p.m. and 6.00 a.m.). Given that melatonin secretion could be affected by noising factors that include: light exposure prior to sampling, posture, and ambient temperature, we aimed to minimize them by keeping the same room light and temperature conditions for all study participants, and asking them to stay in the semi-recumbent position for dark period (starting from 2 hours before the time of complete darkness). Participants were scheduled to sleep in dark, sound attenuated, temperature-controlled rooms for approximately 8 hours at their habitual sleep time (between 10.00 p.m. and 6.00 a.m.). In the Department of Neurology, patients were placed in a separate, dimmed rooms, where lighting, temperature (22°C), humidity (60%) and ventilation were controlled. The lighting control (using Beha Digital Lux Tester 93-1065L) aimed to keep the cycle: 12 hours of regular room light (~150 lux) and 12 hours of deem light (less than 10 lux, including complete darkness). By night, light levels were lowered to minimum and samples were collected under the localized yellow light (small torch) directed only on the patients arm. Controls stayed in the air-conditioned chambers in the Department of Physiology under the same experimental conditions. To avoid an unexpected increase in sympathetic stimulation and sleep disruption the intravenous catheter was inserted at least 2 hours before sampling. Blood samples (2 ml each collection) were taken every 2 hours and peripheral venous catheter was immediately flushed through with 5 ml of injectable saline. We preferred blood sampling because the higher melatonin levels present in serum allow greater resolution and sensitivity than sampling by urine or saliva. Serum melatonin and cortisol concentrations were analyzed using enzymatic methods (ELISA) with the test kits provided by IBL Hamburg (Cat No. RE54021 and RE52611, respectively).

Statistics analysis

The parameters for 24-hour period were set using a single cosinor method according to Halberg *et al.* (17) and, in order to summarize results obtained for different individuals from the

same group, rhythm characteristics were further analyzed by the population mean cosinor. This method allows for analyzing 3 constituents characterizing a given frequency: mesor (M, Midline Estimating Statistic of Rhythm that represents average value around which variable oscillates), the amplitude of the oscillation (A, the difference between the peak and the mean value of a wave), and the phase of the maximum in relation to a fixed reference time (ϕ , known as the acrophase or the time at which the peak of a rhythm occurs). Cosinor methodology is based on the cosine function: $f(T) = M + A \cos(\omega T + \phi) + e$, where $f(T)$ is the average hormone concentration at the given time point, ω is an angular frequency, M is mesor, A is an amplitude, ϕ - acrophase and e is the residual from the analysis for the value $f(T)$. Mesor, amplitude and acrophase for both cortisol and melatonin were statistically analyzed to determine significant differences between studied groups using analysis of variance and Kruskal-Wallis tests. The level for statistical significance was $P < 0.05$ throughout the study.

RESULTS

Baseline demographic and clinical characteristics of patients with HD and AIS are summarized in *Table 1*.

Huntington disease (HD)

Based on UHDRS TFC scores illness severity in the HD subjects was distributed as follows: early (stage I; $n = 3$), moderate/mid (stage II; $n = 2$), and late (stage III; $n = 6$) HD. Patients with lower TFC scores were characterized by higher Total Motor Symptom (TMS) scores, and greater impairment on cognitive tasks. There were clear day-night rhythms in circulating serum melatonin and cortisol in all patients with HD. Based on individual endogenous melatonin profile all

participants were classified as high melatonin producers (17). No significant changes in all three chronobiological parameters were observed for patients with early and mid-stage HD when melatonin rhythm was analyzed (*Table 2*). However, in patients with stage III HD we noticed significant melatonin phase delay, lowered amplitude and mesor in comparison with the controls (*Table 2*). *Fig. 1* presents mean serum melatonin concentrations for stage III HD patients (HD advanced) and controls at each time point.

Cortisol concentrations displayed typical pattern with morning peak levels (at about 8.15) and lowest concentrations at around midnight in all the controls. Analysis of early and mid-stage HD patients showed no significant difference in amplitude and mesor (only a trend toward increased mesor was noted) with significantly phase-advanced cortisol rhythm when compared with controls (*Table 2*). In HD patients with stage III (advanced HD) the pattern of cortisol rhythm showed increased mesor and amplitude with phase-advance (*Table 2*).

Fig. 2 shows mean 24-hour cortisol concentration for advanced HD and control groups.

Acute ischemic stroke (AIS)

According to mean SSS score on admission patients with AIS were recognized as being affected by moderate ischemic stroke that applies to less severe condition (not requiring critical care).

The circadian rhythm of serum melatonin was sustained and showed clear periodic patterns but exhibited more flattened profile. *Fig. 3* presents mean serum melatonin concentrations for AIS patients and controls at each time point. Both, melatonin amplitude and mesor represented lower values when compared to the controls (*Table 3*). Using cosinor analysis of AIS patient's data no phase shift was observed. Analyzing cortisol profile in AIS, mean value of mesor was higher and advanced cortisol

Table 1. Baseline demographic and clinical characteristics of patients with HD and AIS.

Variable	HD patients ($n = 11$)			AIS patients ($n = 8$)	Controls ($n = 10$)
Age (y)	48.0 ± 3.0			53.0 ± 2.5	45.0 ± 8.7
Age onset HD/ time from onset AIS (h)	38.2 ± 4.6			30.2 ± 3.7	–
UHDRS TFC (range 0 – 13)	early stage; ($n = 3$) 12.6 ± 1.1	mid stage; ($n = 2$) 8.2 ± 1.2	late stage; ($n = 6$) 4.8 ± 1.1	–	–
UHDRS TMS score (range 0-124)	28.3 ± 13.0	37.8 ± 10.8	48.2 ± 13.4	–	–
Scandinavian Stroke Scale (SSS) (mean SSS score on admission)	–			35.0 ± 3.0	–
Comorbidities (number of patients)	none	none	–	–	none
Hypertension	–			1	6
Diabetes type 2	–			1	5
CAD	–			–	2
Dyslipidemia	–			–	6
others	–			–	None

Data are expressed as mean values ± S.D. HD, Huntington disease; AIS, acute ischemic stroke; UHDRS, Unified Huntington's Disease Rating Scale; TMS, Total Motor Score; TFC, Total Functional Capacity; CAD, coronary artery disease.

Table 2. Circadian rhythm parameters for melatonin and cortisol in control and HD groups.

	Control	Early and mid-stage HD	Late (advanced) stage HD
<i>Melatonin [pg/ml]</i>			
Mesor (pg/ml)	47.8 ± 4.1	43.7 ± 5.2	35.6 ± 6.0*
Amplitude (pg/ml)	42.2 ± 3.7	38.7 ± 4.4	31.4 ± 4.6*
Acrophase (hh:mm)	01:45 ± 00:33	02:07 ± 00:57	03:50 ± 01:12*
<i>Cortisol [ng/ml]</i>			
Mesor (ng/ml)	92.3 ± 8.2	101.2 ± 8.9	149.0 ± 16.2*
Amplitude (ng/ml)	57.2 ± 5.8	62.4 ± 6.7	72.3 ± 8.0*
Acrophase (hh:mm)	08:15 ± 00:38	07:04 ± 00:45*	06:52 ± 00:55*

Values are expressed as mean ± S.D. *P < 0.05.

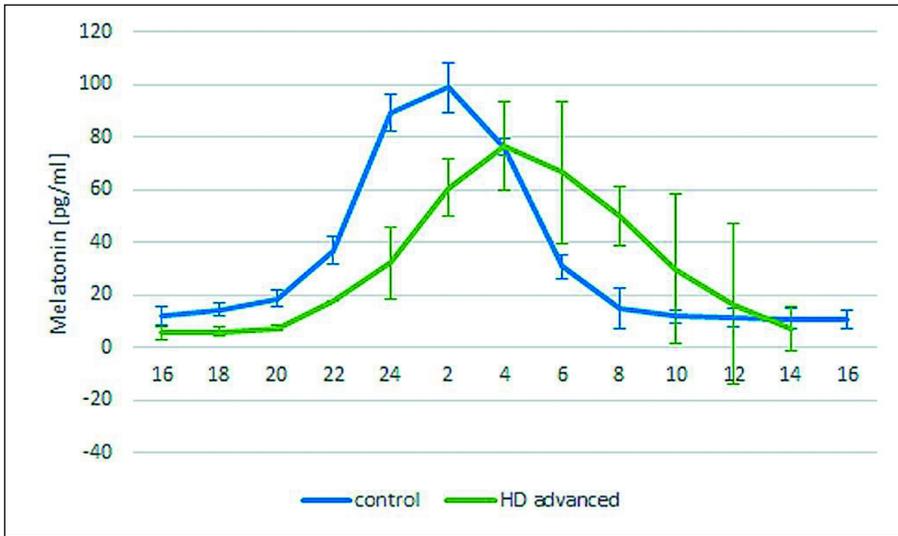


Fig. 1. Twenty-four-hour melatonin concentration of patients with stage III (advanced) HD and controls. Mean (± S.D.).

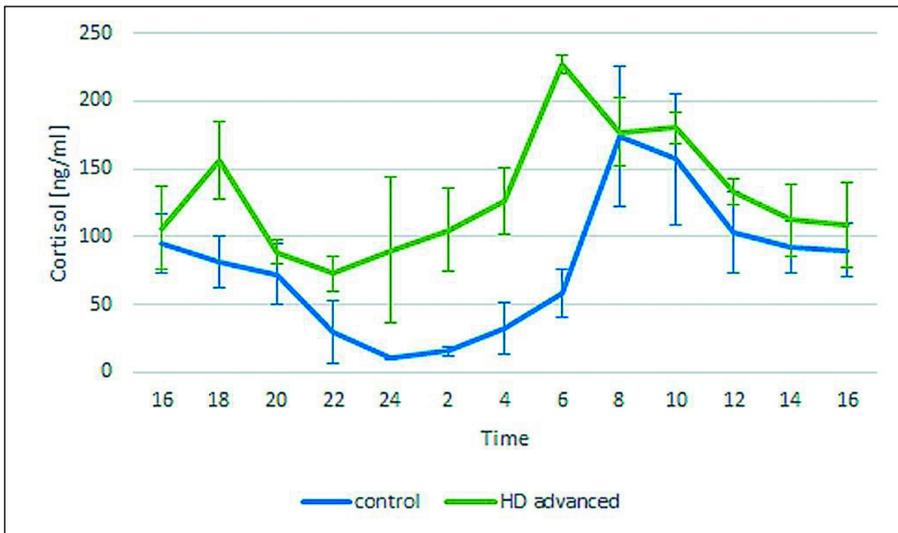


Fig. 2. Twenty-four-hour cortisol concentration of patients with stage III (advanced) HD and controls. Mean (± S.D.).

rhythm was present (Table 3). Figure 4 shows mean serum cortisol circadian profiles for AIS patients and controls. Moreover, patient's cortisol levels were much higher than the

reference range (195.47 ng/ml versus less than 50 ng/ml at 4 p.m. and 341.15 ng/ml versus 150 – 250 ng/ml at 6 a.m., respectively).

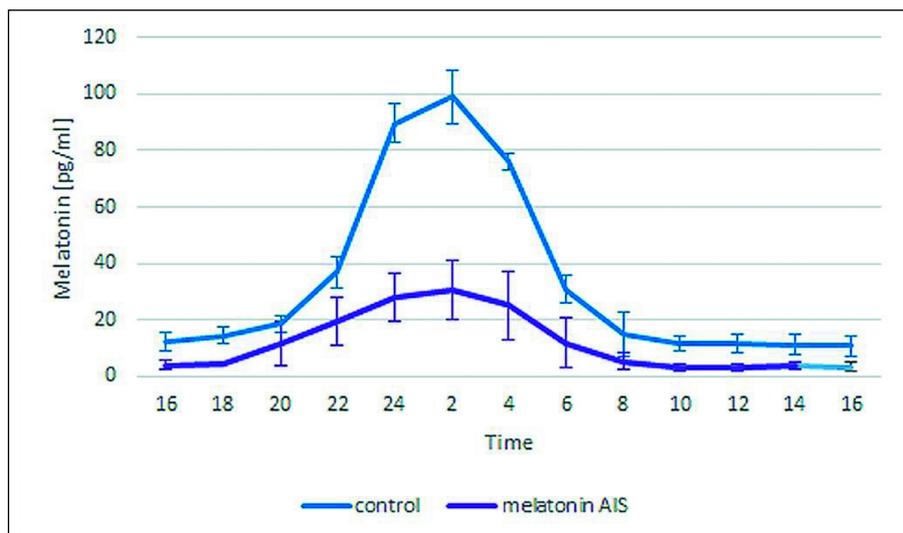


Fig. 3. Twenty-four-hour melatonin concentration of patients with AIS and controls. Mean (\pm S.D.).

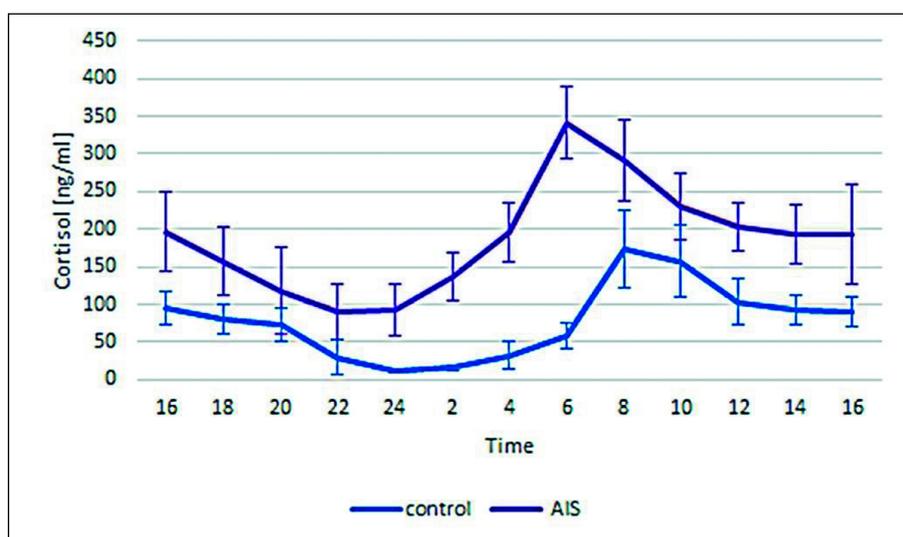


Fig. 4. Twenty-four-hour cortisol concentration of patients with AIS and controls. Mean (\pm S.D.).

DISCUSSION

Data regarding cortisol and melatonin rhythmicity in Huntington disease and ischemic stroke are often conflicting. The lack of consistent findings is probably attributed to the use of different experimental and analytical methods, seasons of blood/urine collection, small sample size and non-cohesive study groups. In this study, an important methodological issue was to investigate both circadian markers in HD and AIS patients, using blood from the same and frequent collections and analyzing the results with cosinor method. Moreover, participants for the tested groups were of similar age and males only. Thus, we have attempted to validate rhythms' synchronization eliminating factors that might possibly contribute to the final results, such as heterogeneity of study group, time/season of scheduled experiment and differences in experimental procedure when investigating two circadian markers.

Here we report that abnormal pattern of melatonin release in the late stages of HD and in moderate AIS occurs in conjunction with phase-advanced rhythm of cortisol.

Table 3. Circadian rhythm parameters for melatonin and cortisol in control and AIS groups.

	Control	AIS
<i>Melatonin [pg/ml]</i>		
Mesor (pg/ml)	47.8 \pm 4.1	18.2 \pm 8.5*
Amplitude (pg/ml)	42.2 \pm 3.7	16.7 \pm 6.3*
Acrophase (hh:mm)	01:45 \pm 00:33	01:58 \pm 00:55
<i>Cortisol [ng/ml]</i>		
Mesor (ng/ml)	92.3 \pm 8.2	214.4 \pm 21.12*
Amplitude (ng/ml)	57.2 \pm 5.8	64.1 \pm 6.2
Acrophase (hh:mm)	08:15 \pm 00:38	06:53 \pm 00:50*

Values are expressed as mean \pm S.D. *P < 0.05.

All patients represented a clear day-night rhythms in circulating melatonin and no changes, compared with controls, were observed in early and mid-stage of HD. Moreover, our patients and controls were high melatonin secretors, which may possibly indicate the presence of genetically determined high sensitivity beta receptors of the pineal gland to noradrenergic stimulation or high activity of enzymes involved in melatonin production (18). We conclude that phase and amplitude of melatonin secretion in high melatonin producers with neurodegeneration is preserved for relatively long time and remains unchanged within the first two stages of HD. Azis *et al.* reported no change in amplitude and mesor together with the delay in timing of melatonin evening rise in nine patients with early stage of HD (19). Based on our results, we suggest that phase change becomes apparent afterwards, when the disease progresses into stage III. The persistence of a good melatonin amplitude in early and mid-stage HD indicates stability of the circadian system function of these patients. Although some scientists suggest that HD can be recognized as a disorder affecting the whole brain (20), the macro- and microscopic examinations of postmortem human brains demonstrate characteristic for HD neuronal changes (astrogliosis and neuronal loss) specifically in the neostriatum (putamen and caudate nucleus CN, with more evident changes in CN), globus pallidus and, to a very low degree, in cerebral cortex (21). Recent studies have revealed that in early stages of neurodegeneration (grades 1 and 2) non-striatal structures of the brain are apparently normal or may show only mild atrophy (22). On the other hand, imaging findings have highlighted that HD pathology also constitutes of hypothalamic changes in the grey matter regions early, in the prodromal stages (23, 24). Given that disrupted circadian rhythmicity in HD arise mainly from hypothalamic SCN pathology (25), we speculate that sustained normal expression of melatonin rhythm in early and mid-stage HD may be related to low degree of SCN damage and to still well responding pineal adrenergic receptors as they can increase in number in response to denervation in an up-regulation mechanism (26). Moreover, studies on animal models have brought an evidence that in the animals with partial SCN ablation only a very small portion of SCN is sufficient to coordinate melatonin rhythm (27). The greater degree of SCN neurodegenerative lesion (late stage HD) may affect melatonin rhythmic secretion by reduced stimulatory signals from SCN during scotophase (28, 29) and/or functioning of SCN melatonin receptors (MT1 and MT2) responding to feedback inhibitory melatonin signal (30).

Analyzing advanced HD we have found that the patterns of circadian changes for both, melatonin and cortisol, are similar to the changes that occur with ageing, when the production of melatonin declines and is shifted to later hours while the production of cortisol increases and its peak occurs earlier in the night (31). Several studies suggest that different neuronal dysfunctions linked with increased basic level of cortisol may result from disinhibition of HPA-axis due to declined levels of melatonin indicating its role as an antiadrenocortical or antistress factor (32-35). In our study, impaired melatonin secretion (phase delay, decreased amplitude and mesor) in advanced HD coexisted with high mean value of cortisol, but because of small sample size no correlations between changes in chronobiologic parameters of cortisol and melatonin were calculated. Nevertheless, because melatonin feedbacks SCN and acts through network of interconnected signaling pathways we believe that reciprocal relation between melatonin and corticoids may play role as an additional mechanism in the neuropathological changes leading to the dysfunction of HPA-axis in late stages of HD.

Pineal production of melatonin is controlled by a number of brain regions that include the SCN, paraventricular nucleus of the hypothalamus (PVN), and intermediolateral nucleus of the spinal cord (IML) (36). Interestingly, we have found that ischemic stroke outside these structures impacts circadian timekeeping within 24 hours of onset in terms of decreased melatonin mesor and amplitude. Because our patients demonstrated anterior circulation stroke and showed no detectable in MRI injury in the PVN and SCN regions, direct neuronal defects in stimulatory pathway of melatonin secretion can be ruled out as the cause. We suggest that brain regions other than the presumptive retinopineal neural pathway may play an important role in the pineal production of melatonin in humans.

Additionally, in contrast to the experiments describing the disruption in timing of melatonin onset and offset in experimental animals (37), we did not observe any significant melatonin phase shift. Thus, in agreement with the human study by Rizenthaler *et al.* (38), we consider another explanatory possibility - depressed melatonin levels may reflect its rapid utilization as a free radical scavenger reducing oxidative damage within first 30 hours of cerebral ischemia. During acute phase of ischemic stroke formation of reactive oxygen and nitrogen species by endothelial cells and infiltrating leucocytes is particularly significant during reperfusion phase, the phase often called 'cerebral reperfusion injury' (39). Melatonin and its metabolites actively stimulate variety of neuroprotective pathways reducing oxidative damage, positively modulating anti-apoptotic, anti-inflammatory and other mechanisms (40, 41). Therefore, decreased serum melatonin due to its accelerated turnover seems to be possible, in addition to the fact that in humans melatonin metabolism is rapid and half-life is short, ranging between 10 and 60 min (42). To confirm, however, this hypothesis more studies on biology of melatonin and its metabolites are required.

Another possible explanation for the relationship between lowered melatonin and incidence of AIS may refer to the pineal calcification. It has been shown that fibrous tissue and calcium deposits largely replace the pineal gland of some older people (43). As a consequence, reduced melatonin levels, as calculated by secretional capacity of the pineal gland, may occur (44). A recent retrospective study has indicated that ischemic stroke incidence is associated with pineal calcification (45). These data have, however, been questioned a year after by Del Brutto and co-workers in a population-based cross-sectional study (46). Clearly, further research is needed to evaluate the above assumptions.

A number of authors report HPA-axis disruption following an acute stroke and several mechanisms explaining elevated cortisol have been hypothesized, including central activation of HPA-axis, increased adrenal responsiveness to ACTH, or proinflammatory cytokines release (47-49). In this study, acute moderate stroke was related to phase-advanced rhythm and elevated level of cortisol together with lowered melatonin concentration. It has been proposed that melatonin directly (50) or by sleep-related processes might inhibit nocturnal HPA secretory activity (51). On this background, it seems attractive to assume that in AIS patients melatonin loss accelerates and potentiates adrenal secretory activity leading to hypercortisolism which, in turn, might increase the likelihood of developing serious stroke-related consequences. Moreover, regarding the site of injury, our findings support the kinetic study by Fassbender *et al.* demonstrating that patients without lesions of the hippocampal region represent a strong adrenal stimulation in response to ischemic stroke (52).

Limitations

Several limitations of this study need to be acknowledged. The size of the screened population was small, thus any interpretations should be made with caution and further replicate experiments are required. Small sample size did not allow for calculations of some meaningful associations with adequate statistical power (e.g. correlations between tested variables and clinical features of our patients). Additionally, the study period was short and did not include long-term observations concerning patients with stroke. Furthermore, it would be interesting to compare the results obtained from our AIS cohort with changes in more severe stroke cases. Lastly, to support our speculations more variables should be studied, for example blood/urine level of melatonin metabolites such as N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK) which is regarded to be an efficient scavenger of reactive oxygen species (53). Despite these limitations, we believe that our work may bring an additional scientific background to the neuropathology studies and serve as an inspiration for the next experiments evaluating potential mechanistic insights to investigate circadian disruptions in detail.

In summary, disruptions of circadian rhythm in HD patients, as evidenced by cortisol and melatonin blood levels, are more manifest in late stages of this disease and patterns of circadian change are similar to those observed with ageing. Acute ischemic stroke in the extra-hypothalamic brain structures is characterized by lowered blood melatonin concentration accompanied by hypercortisolemia and phase advance shift of the cortisol rhythm.

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REFERENCES

- Weaver DR. The suprachiasmatic nucleus: a 25-year retrospective. *J Biol Rhythms* 1998; 13: 100-112.
- Guilding C, Piggins HD. Challenging the omnipotence of the suprachiasmatic timekeeper: are circadian oscillators present throughout the mammalian brain? *Eur J Neurosci* 2007; 25: 3195-3216.
- Takahashi JS, Hong HK, Ko CH, McDearmon EL. The genetics of mammalian circadian order and disorder: implications for physiology and disease. *Nat Rev Genet* 2008; 9: 764-775.
- Welsh DK, Takahashi JS, Kay SA. Suprachiasmatic nucleus: cell autonomy and network properties. *Annu Rev Physiol* 2010; 72: 551-577.
- Gillette MU, McArthur AJ. Circadian actions of melatonin at the suprachiasmatic nucleus. *Behav Brain Res* 1996; 73: 135-139.
- Hatfield CF, Herbert J, Van Someren EJ, Hodges JR, Hastings MH. Disrupted daily activity/rest cycles in relation to daily cortisol rhythms of home-dwelling patients with early Alzheimer's dementia. *Brain* 2004; 127: 1061-1074.
- Morton AJ. Disintegration of the sleep-wake cycle and circadian timing in Huntington's disease. *J Neurosci* 2005; 25: 157-163.
- Videnovic A, Lazar AS, Barker RA, Overeem S. 'The clocks that time us' - circadian rhythms in neurodegenerative disorders. *Nat Rev Neurol* 2014; 10: 683-693.
- Bubenik GA, Konturek SJ. Melatonin and aging: prospects for human treatment. *J Physiol Pharmacol* 2011; 62: 13-19.
- Benloucif S, Burgess HJ, Klerman EB, et al. Measuring melatonin in humans. *J Clin Sleep Med* 2008; 4: 66-69.
- Escribano BM, Colin-Gonzalez AL, Santamaria A, Tunez I. The role of melatonin in multiple sclerosis, Huntington's disease and cerebral ischemia. *CNS Neurol Disord Drug Targets* 2014; 13: 1096-1119.
- Adamczyk-Sowa M, Sowa P, Adamczyk J, et al. Effect of melatonin supplementation on plasma lipid hydroperoxides, homocysteine concentration and chronic fatigue syndrome in multiple sclerosis patients treated with interferons-beta and mitoxantrone. *J Physiol Pharmacol* 2016; 67: 235-242.
- Chung S, Son GH, Kim K. Circadian rhythm of adrenal glucocorticoid: its regulation and clinical implications. *Biochim Biophys Acta* 2011; 1812: 581-591.
- Huntington Study Group. Unified Huntington's Disease Rating Scale: reliability and consistency. *Mov Disord* 1996; 11: 136-142.
- Askim T, Bernhardt J, Churilov L, Indredavik B. The Scandinavian Stroke Scale is equally as good as The National Institutes of Health Stroke Scale in identifying 3-month outcome. *J Rehabil Med* 2016; 48: 909-912.
- Ranen NG, Stine OC, Abbott MH, et al. Anticipation and instability of IT-15 (CAG)_n repeats in parent-offspring pairs with Huntington disease. *Am J Hum Genet* 1995; 57: 593-602.
- Halberg F, Tong YI, Johnson EA. Circadian system phase as aspect of temporal morphology: procedure and illustrative examples. In: The Cellular Aspects of Biorhythms, von Mayerbach H (ed). Berlin, Springer Verlag, 1967, pp. 20-48.
- Wetterberg L, Bergiannaki JD, Paparrigopoulos T, et al. Normative melatonin excretion: a multinational study. *Psychoneuroendocrinology* 1999; 24: 209-226.
- Aziz NA, Pijl H, Frolich M, et al. Delayed onset of the diurnal melatonin rise in patients with Huntington's disease. *J Neurol* 2009; 256: 1961-1965.
- Carroll JB, Bates GP, Steffan J, Saft C, Tabrizi SJ. Treating the whole body in Huntington's disease. *Lancet Neurol* 2015; 14: 1135-1142.
- Vonsattel JP, Myers RH, Stevens TJ, Ferrante RJ, Bird ED, Richardson EP. Neuropathological classification of Huntington's disease. *J Neuropathol Exp Neurol* 1985; 44: 559-577.
- Waldvogel HJ, Kim EH, Tippett LJ, Vonsattel JP, Faull RL. The Neuropathology of Huntington's Disease. *Curr Top Behav Neurosci* 2015; 22: 33-80. doi: 10.1007/7854_2014_354.
- Politis M, Pavese N, Tai YF, Tabrizi SJ, Barker RA, Piccini P. Hypothalamic involvement in Huntington's disease: an in vivo PET study. *Brain* 2008; 131: 2860-2869.
- Soneson C, Fontes M, Zhou Y, et al. Early changes in the hypothalamic region in prodromal Huntington disease revealed by MRI analysis. *Neurobiol Dis* 2010; 40: 531-543.
- van Wamelen DJ, Aziz NA, Anink JJ, et al. Suprachiasmatic nucleus neuropeptide expression in patients with Huntington's disease. *Sleep* 2013; 36: 117-125.
- Weiss B, Greenberg LH, Cantor E. Denervation supersensitivity and beta-adrenergic receptors as a function of age. *Adv Biochem Psychopharmacol* 1980; 21: 461-472.
- Reppert SM, Perlow MJ, Ungerleider LG, et al. Effects of damage to the suprachiasmatic area of the anterior hypothalamus on the daily melatonin and cortisol rhythms in the rhesus monkey. *J Neurosci* 1981; 1: 1414-1425.
- Kallioli E, Silajdzic E, Nambron R, et al. Plasma melatonin is reduced in Huntington's disease. *Mov Disord* 2014; 29: 1511-1515.

29. Klein DC, Moore RY. Pineal N-acetyltransferase and hydroxyindole-O-methyltransferase: control by the retinohypothalamic tract and the suprachiasmatic nucleus. *Brain Res* 1979; 174: 245-262.
30. Korf HW, von Gall C. Circadian physiology. In: Textbook of Neuroscience in the 21st Century: Basic and Clinical, D.W. Pfaff (ed). New York, Springer 2012, pp. 1813-1845.
31. Zisapel N, Tarrasch R, Laudon M. The relationship between melatonin and cortisol rhythms: clinical implications of melatonin therapy. *Drug Dev Res* 2005; 65: 119-125.
32. Mori W, Aoyama H, Mori N. Melatonin protects rats from injurious effects of a glucocorticoid, dexamethasone. *Jpn J Exp Med* 1984; 54: 255-261.
33. Torres-Farfan C, Richter HG, Rojas-Garcia P, et al. mt1 Melatonin receptor in the primate adrenal gland: inhibition of adrenocorticotropin-stimulated cortisol production by melatonin. *J Clin Endocrinol Metab* 2003; 88: 450-458.
34. Campino C, Valenzuela FJ, Torres-Farfan C, et al. Melatonin exerts direct inhibitory actions on ACTH responses in the human adrenal gland. *Horm Metab Res* 2011; 43: 337-342.
35. Torres-Farfan C, Richter HG, Germain AM, et al. Maternal melatonin selectively inhibits cortisol production in the primate fetal adrenal gland. *J Physiol* 2004; 554: 841-856.
36. Borjigin J, Li X, Snyder SH. The pineal gland and melatonin: molecular and pharmacologic regulation. *Annu Rev Pharmacol Toxicol* 1999; 39: 53-65.
37. Meng H, Liu T, Borjigin J, Wang MM. Ischemic stroke destabilizes circadian rhythms. *J Circadian Rhythms* 2008; 6: 9. doi:10.1186/1740-3391-6-9
38. Ritzenthaler T, Nighoghossian N, Berthiller J, et al. Nocturnal urine melatonin and 6-sulphatoxymelatonin excretion at the acute stage of ischaemic stroke. *J Pineal Res* 2009; 46: 349-352.
39. Pan J, Konstas AA, Bateman B, Ortolano GA, Pile-Spellman J. Reperfusion injury following cerebral ischemia: pathophysiology, MR imaging, and potential therapies. *Neuroradiology* 2007; 49: 93-102.
40. Andrabi SS, Parvez S, Tabassum H. Melatonin and ischemic stroke: mechanistic roles and action. *Adv Pharmacol Sci* 2015; 2015: 384750. doi:10.1155/2015/384750
41. Adamczyk-Sowa M, Pierzchala K, Sowa P, Polaniak R, Kukla M, Hartel M. Influence of melatonin supplementation on serum antioxidative properties and impact of the quality of life in multiple sclerosis patients. *J Physiol Pharmacol* 2014; 65: 543-550.
42. Fourtillan JB, Brisson AM, Gobin P, Ingrand I, Decourt JP, Girault J. Bioavailability of melatonin in humans after day-time administration of D(7) melatonin. *Biopharm Drug Dispos* 2000; 21: 15-22.
43. Ongkana N, Zhao XZ, Tohno S, et al. High accumulation of calcium and phosphorus in the pineal bodies with aging. *Biol Trace Elem Res* 2007; 119: 120-127.
44. Nolte I, Lutkhoff AT, Stuck BA, et al. Pineal volume and circadian melatonin profile in healthy volunteers: an interdisciplinary approach. *J Magn Reson Imaging* 2009; 30: 499-505.
45. Kitkhuandee A, Sawanyawisuth K, Johns NP, Kanpittaya J, Johns J. Pineal calcification is associated with symptomatic cerebral infarction. *J Stroke Cerebrovasc Dis* 2014; 23: 249-253.
46. Del Brutto OH, Mera RM, Lama J, Zambrano M. Stroke and pineal gland calcification: lack of association. Results from a population-based study (The Atahualpa Project). *Clin Neurol Neurosurg* 2015; 130: 91-94.
47. Olsson T, Marklund N, Gustafson Y, Nasman B. Abnormalities at different levels of the hypothalamic-pituitary-adrenocortical axis early after stroke. *Stroke* 1992; 23: 1573-1576.
48. Johansson A, Ahren B, Nasman B, Carlstrom K, Olsson T. Cortisol axis abnormalities early after stroke - relationships to cytokines and leptin. *J Intern Med* 2000; 247: 179-187.
49. Mitchell A. Clinical implications of poststroke hypothalamic-pituitary adrenal axis dysfunction: a critical literature review. *J Stroke Cerebrovascular Dis* 1997; 6: 377-388.
50. Rao NV, Raza B, Prasad JK, et al. Melatonin decreases glucocorticoid blood concentration in the rat and palm squirrel, acting directly on the adrenal gland. *Biomed Res* 2001; 22: 115-117.
51. Perras B, Ozcan S, Fehm HL, Born J. Melatonin does not inhibit hypothalamic-pituitary-adrenal activity in waking young men. *J Neuroendocrinol* 2005; 17: 811-816.
52. Fassbender K, Schmidt R, Mossner R, Daffertshofer M, Hennerici M. Pattern of activation of the hypothalamic-pituitary-adrenal axis in acute stroke. Relation to acute confusional state, extent of brain damage, and clinical outcome. *Stroke* 1994; 25: 1105-1108.
53. Jaworek J, Szklarczyk J, Bonior J, et al. Melatonin metabolite, N(1)-acetyl-N(1)-formyl-5-methoxykynuramine (AFMK), attenuates acute pancreatitis in the rat: in vivo and in vitro studies. *J Physiol Pharmacol* 2016; 67: 411-421.

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