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The Association between the Chronotype and Cortisol Levels as well as Gene Expression Levels of *hPER1* in the Evening

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Abstract

Humans usually show 24-hour sleep-wake cycles, which are geared by an inherent, interindividually varying biological clock. The behavioral daytime preferences differ according to the chronotype, which is in turn basically associated with the timing and controlling of the circadian rhythms of biological and psychological parameters. We were interested in the association between chronotype and the levels of cortisol and the clock gene hPER1 in the evening, and therefore examined 29 healthy men. Our data indicate that morning types show lower levels of cortisol and a lower gene expression of hPER1 in the evening.

Keywords

Chronotype; Cortisol; hPER1; Evening Profile; Gene Expression; Biological Clock; Circadian Rhythm; SCN; Sleep

Introduction

Circadian rhythms have in common that they depict an organism's organization over the day. In accordance with the rotation of the earth, organisms show a strongly species-specific and evolutionary very old adaptation to light-and-dark cycles (Gachon F, 2004). In humans, not only biological, but also psychological parameters such as attention, mood, alertness, and learning show changes within 24 hours which are strongly dependent on the time of day and display clear acrophases [(Karni A, 1993), (Kudielka, 2006)]. Vital processes such as cell cycle and apoptosis [(Kudielka, 2004), (Lévi F, 2007)], secretion of hormones (Meyer-Bernstein EL, 1999) or the electrical activity of nerve cells [(Buijs RM, 2003), (Hastings MH, 2008)] follow a clear circadian rhythm. The synchronization of the environment and the organism's internal clock is effectuated via the suprachiasmatic nuclei (SCN), which receive their photic information of brightness/ darkness via the retina and the retino-hypothalamic tract [(Lucas RJ, 1999), (Panda S, 2003)]. This information is in turn imparted to every single cell in the periphery of the body in order to synchronize the organism itself with all of its physiological, biochemical and psychological processes [(Guilding C, 2007), (Schibler U., 2007)]. The cogwheels of these molecular clocks, which are embodied by every cell, are called clock genes, and mainly regulate their own expression via transcriptional/ translational feedback loops (TTFLs) [(Hastings MH, 20008), (Young MW, 2001), (Albrecht U., 2004)]. For the synchronization between the SCN and all of the other cells, different signaling pathways have been proposed, which include hormonal as well as neuronal and autonomous signaling [(Buijs RM, 2003), (Kriegsfeld RJ, 2006), (Kalsbeek A, 2006)]. One of these clock genes in humans is *hPER1*. It has been shown in animal studies that the expression of *Per1* might be strongly influenced and induced by glucocorticoids (Balsalobre A, 2008) via glucocorticoid-responsive elements (GRE) in the DNA (Yamamoto T, 2005). This interconnection implies an important role of glucocorticoids in the regulation of the gene expression of clock genes [(Yamamoto T, 2006), (Dickmeis T, 2007), (Dickmeis T. 2009) , (Abbruzzese EA,)].

The most prominent and well-researched glucocorticoid in humans is the hormone cortisol, which itself shows a very strong circadian rhythm, which peaks in the morning immediately after an individual's awakening and decreases over the day. The synthesis of cortisol is basically induced by the activation of the hypothalamus-pituitary-adrenal (HPA) axis. The corticotropin-releasing hormone (CRH) is released from the paraventricular nucleus (PVN) of the hypothalamus, inducing the synthesis of adrenocorticotropin (ACTH) [(Anders TF., 1982), (Anders TF., 2001)]. The control of the circadian rhythm of cortisol is most probably effectuated by the SCN via a neuroendocrine pathway, since the absence of the SCN results in a rhythmic deficiency of cortisol [6]. More recent research implicates an additional autonomous pathway

between the SCN and the adrenal glands, which might be crucial for the synchronization of the body's peripheral cells and results in the synthesis of cortisol [(Scheer FA,, 1999), (Ishida A,, 2005)].

Along with the general circadian rhythm, humans show large interindividual differences in the organization of their behavior within the course of a 24h-day, which can be summarized in the concept of chronotypes (Roenneberg T, 2003). According to the individual daytime preference, chronotypes can be distinguished into morning or evening types. The preference is easily stated in the structure of the sleep-wake cycle and might be explained by the interindividually varying adjustment of the internal biological clock to the external daytime and nighttime (Merrow M, 2005). Accordingly, morning types show earlier bedtimes and waking-up times compared to evening types [(Roenneberg T, 2003),(Rosenthal L, 2001), (Korczak AL, 2008)]. The chronotype, in turn, seems to be closely associated with the controlling and timing of the pulsatile and rhythmic secretion of hormones such as cortisol [(Bailey SL, 1991), (Bailey SL, 2001), (Roenneberg T, 2007)]. These differences in the adjustment might be partially anchored in polymorphisms of different clock genes [(Carpen JD, 2006), (llebrandt KV, 2008)] and several other factors such as the intensity of light irradiation and entrainment (Duffy JF, 2001).

Clearly, the rhythmic functioning and interplay of physiological, biochemical and psychological parameters seems to be crucial for mental and physical health. By contrast, arrhythmic processes such as the growth of cancer cells are associated with disease, since the fine-tuning of vital processes is disrupted. A long-term study was able to show that nurses who had carried out shift work for more than 30 years had a significantly increased risk of breast cancer (Schernhammer ES, 2001)

The concept of chronotherapy is also gaining in importance in the field of medicine, through the assumption that the metabolism of medication is likewise subject to the circadian clock and should therefore be tightly timed. More than thirty anticancer medications were tested in animals, with results showing that toxicity as well as effectiveness of medication varied considerably depending on the time of administration (Lévi F., 2001). An increasing amount of data supports the effectiveness of chronotherapy (Lévi F, 2001). Therapeutic implications such as the consideration of the chronotype would be a further step towards personalized and more effective medicine.

Evening types in particular often experience the problem of a deficient fit between their inherent biological clock and the social clock (e.g. work schedules etc.). The discrepancy between working and free days might therefore result in a sleep deficit during the week, which is compensated at weekends. It is not yet clear what the health-related consequences of such "social jetlags" might be (Wittmann M,, 2009).

We were therefore interested in the research of the association between the observable chronotype and the levels of cortisol and *hPER1*, respectively, in the evening.

Material and Methods

Ethics

This study was conducted according to the declaration of Helsinki (adopted by the 55th General Assembly of the World Medical Association, Tokyo, 2004). The study protocol was approved by the ethics committee of the Canton of Zurich (Department of Internal Medicine of the University Hospital of Zurich) and all participants provided written informed consent regarding participation in the study.

Subjects, Recruitment Criteria

Subjects were recruited at the University of Zurich. Inclusion criteria were male sex, good mental and physical health, and age between 20 and 30. Exclusion criteria were jetlag, intake of medication, sleep disorders, intake of psychotropic substances, psychological or physical illness, hospitalization, smoking and shift work within four months prior to the data acquisition and psychiatric history in general. Forty-nine men who responded to the flyers or placards were screened using standardized interviews and questionnaires (SCID-II; ADS; SCL-90; general health problems) for their physical and mental health. From this initial sample size, 18 subjects were excluded after the screening due to health problems, intake of medication or if the values of the psychometric data indicated a possible mental problem. The data regarding mRNA of hPER1 for two participants could not be analyzed at all, which led to the decision to completely exclude these subjects from the calculations. The final sample size was n=29. This sample is part of a larger study including more parameters. Further results are published elsewhere (Abbruzzese EA).

Study Protocol

In order to best control for their circadian rhythm, the study participants were asked to adhere to a strict sleeping and eating schedule for one week before participating in the investigation. Eating and sleeping times were documented in a diary. Furthermore, they were requested not to exercise and to avoid daily hassles during the study day.

The study participants were instructed to arrive at our laboratory at 19:30 h at the latest. They were led to a quiet room, where they spent the rest of the evening reading (no stimulating reading was approved). The first sample was taken at 20:00 h. The following four measurements were collected at 30-min intervals at 20:30 h, 21:00 h, 21:30 h, and 22:00 h. The study was conducted in the evening, based on the assumption that the cortisol and hPER1 levels would be lowest during that time and would therefore best depict differences in chronotypes.

Sampling Methods for Psychometric as well as Biological Parameters and Analysis of mRNA and Cortisol

For the assessment of their chronotype, participants had to fill in the Munich chronotype questionnaire [27], which consists of 12 questions concerning preferences of sleeping habits. One of the advantages of the MCTQ compared to other chronotype questionnaires is the calculation of the midsleep of working as well as free days, which considers the correction of a possible social jetlag in subjects. In order to retain statistical power, the original seven categories of chronotypes were summarized into three main categories: morning type, normal type and evening type.

mRNA levels of hPER1 were assessed in oral mucosa [40] in ratio to the housekeeping gene GAPDH as previously described e.g. by Cajochen and colleagues for human samples [41]. The biochemical analysis took place in our biochemical laboratory at the Institute of Psychology of the University of Zurich. Samples were taken with a pipette tip (ep Dualfilter T.I.P.S. 50-1000, Eppendorf, Hamburg, Germany) and immediately pipetted into a mixture of 0.7 µl of β-Mercaptoethanol and 100 µl Lysis buffer (Absolutely RNA Nanoprep Kit, Stratagene). Samples were subsequently frozen at -80°C. RNA extraction was performed using the Absolutely RNA Nanoprep Kit (Stratagene), followed by reverse transcription with Superscript III First Strand Synthesis Super Mix for qRT-PCR (Invitrogen). The quantitative real-time PCR was conducted using TaqMan Universal PCR Mastermix (Applied Biosystem) on an ABI 7700 Sequence Detection System (Applied Biosystems). The mRNA was measured in duplicates. The following primer and probes were used: Hs00242988 for hPER1 and 4352934E for the endogenous control hGAPDH [41] (both Applied Biosystems). The amount of target, normalized to the endogenous reference (GAPDH) and relative to a calibrator, was calculated by $2^{-\Delta\Delta CT}$.

Cortisol samples were collected using Salivettes (Sarstedt, Sevelen, Switzerland) and subsequently frozen at -20°C. Saliva samples were assayed in the Laboratory of Biopsychology of the Technical University of Dresden, Germany (Luminescence Immunoassay, IBL).

Statistics

Statistics were calculated using SPSS 19.0 for Mac. The chronotype was assumed as independent variable, while the repeated measurements of cortisol and hPER1 were considered as dependent variable. Psychometric and cortisol data were complete. Single time points in the data of mRNA of hPER1 were missing due to poor analyzability. This led to the decision to use linear mixed models for the statistical calculation of the repeated measures, since this procedure also includes incomplete cases in the analysis compared to the general linear model for repeated measures, which excludes cases with single missing data points. Furthermore, general linear models assume independence of repeated measurements, while linear mixed models seem to be far more appropriate for dependent and therefore nested data.

Post-hoc tests between the groups were calculated with t-tests for independent samples corrected according to Bonferroni statistics.

Normal distributions of the single measurement time points of cortisol and hPER1 were tested with the Kolmogorov-Smirnov test and could be assumed (all $p>0.05$).

Results

According to their score on the MCTQ, participants were classified into three groups, resulting in 11 subjects of the morning type (37.9%), 12 subjects of the normal type (41.4%) and six subjects of the evening type (20.7%). For cortisol (all measured in nmol/ l), the mean of the five measurement time points over the evening varied from 1.42 to 4.10 for morning types (mean 2.51, standard error of mean 0.264), from 1.49 to 4.46 for normal types (mean 2.54, standard error of

mean 0.253), and from 1.74 to 6.60 for evening types (mean 3.71, standard error of mean 0.357). For hPER1 (all measured in ratio to GAPDH) the measurement time points over the evening varied from 2.81 to 6.13 for early types (mean 2.46, standard error of mean 1.270), from 4.86 to 6.81 for normal types (mean 6.69, standard error of the mean 1.101) and from 3.98 to 8.19 for evening types (mean 6.47, standard error of the mean 1.623).

In accordance with the well-established circadian rhythm of cortisol, a decrease over time could be clearly observed. This was not the case for hPER1, which strongly fluctuated between the measurement time points. The linear mixed model for repeated measures showed that the factor time significantly influenced the course of cortisol over the five measurement time points ($F=13.818$; $p<0.000$). Furthermore, there was a significant influence of the three chronotypes on the measured levels of cortisol ($F=4.327$; $p=0.015$).

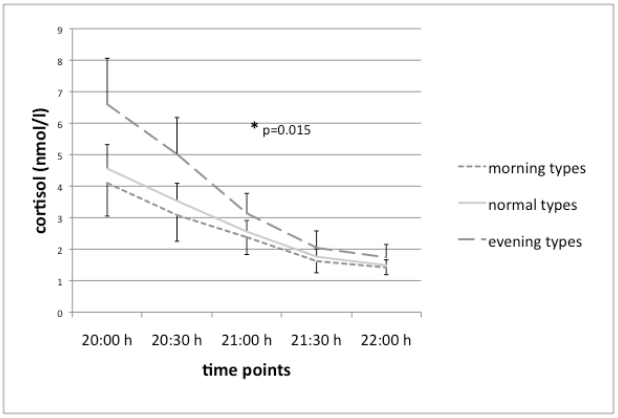

FIG. 1 THE COURSE OF CORTISOL IN THE EVENING BETWEEN 20:00 h AND 22:00 h

The course of cortisol in the evening differs significantly according to the chronotypes: Evening types show the highest levels compared to normal and morning types. All chronotypes show a parallel decreasing pattern. Measurement time points are given in mean and standard deviation.

The course of cortisol showed parallel decreasing patterns for all three chronotypes, indicating the lowest levels of cortisol in the morning types, while the evening types showed the highest levels of cortisol. Normal types were in-between. Post-hoc tests showed significant differences in the pairwise comparison of morning and evening types (difference of mean -1.194, $p=0.024$) as well as the comparison of normal and evening types (difference of mean -1.168; $p=0.026$). The interaction effect of time and chronotype showed no significant difference.

The factor time had no significant influence on the course of the expression levels of hPER1, whereas the factor chronotype showed a significant influence on the expression levels of hPER1 ($F=4.327$; $p=0.045$). The morning types showed the lowest levels at all measurement time points, followed by the normal types.

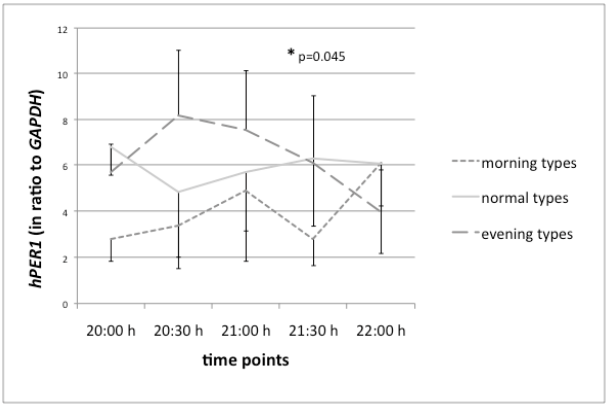

FIG. 2 THE COURSE OF hPER1 IN THE EVENING BETWEEN 20:00 h AND 22:00 h

The course of all chronotypes shows strong fluctuations in the expression of hPER1 over the evening. Nevertheless, a significant difference can be discerned between the chronotypes, indicating a lower expression in morning types compared to normal types. Measurement time point are given in mean and standard deviation.

Underlying the greatest fluctuation, the evening types showed the highest expression levels at time points two and three, but intermediate levels at time points one and four, and finally, the lowest levels at time point five. Corresponding to this observation, pairwise post-hoc tests revealed only a nearly significant difference between morning and normal types (difference of mean -4.235, $p=0.058$).

Discussion

The daily recurring sleep-wake pattern is taken as a matter of course. Independently of a health-protective and efficient circadian rhythm, humans show different preferences in the organization of their day, which is mirrored in their chronotype. As stated, this preference seems to be partly anchored in the human's genome and additionally regulated by the environment and lifestyle. Our data showed significant differences in the levels of cortisol as well as in the levels of gene expression of hPER1 in the evening according to the self-reported chronotype. In general, lower levels in both cortisol and hPER1 were observed in morning types compared to normal types.

Cortisol levels were highest in evening types compared to morning types over the evening. This effect was not clearly seen in hPER1, which might be due to the small number of participants and the considerable variance between the measurement time points of evening types. It might also be hypothesized that evening types undergo a higher variability, since they have to chronically adapt to (too) early social clocks during the week (e.g. reflected in early working hours, etc.), ignoring their internal clock. It would be interesting to see whether under free-running conditions, their expression would vary less. These deliberations would be in line with data reporting an association between evening types and a more irregular lifestyle (Monk TH, 2004). According to Guilding and Piggins (Guilding C, 2007), the peak of mRNA of Per1 in the SCN can be found four to six hours after the subjective onset of the day (light exposure). Based on this assumption, it could be supposed that the levels of hPER1 decrease earlier in morning types compared to normal or evening types. This would clearly support our findings. The difference between chronotypes in the gene expression of elements of the internal clock might explain parts of the interindividual time shift of biological and psychological processes.

A similar effect can be seen in cortisol, since the earlier the chronotype, the lower the cortisol levels in the evening. These findings confirm previous results postulating higher cortisol levels in evening types at 20:00 h (Kudielka BM, 2007). It is interesting to observe that the measurement time points assimilate within the course of the evening. This might be interpreted as an entrainment effect, which particularly affects evening types and induces a faster decrease of cortisol in the evening due to the onset of darkness outside, triggering the readjustment of the light-dark cycle (Herzog ED, 2001).

In general, it can be suggested that depending on the individual chronotype, there are differences in the gene expression of hPER1 and cortisol in the evening. This emphasizes the importance of considering this trait not only in everyday life by adapting time schedules to different needs in order keep an ideal fit between one's organism and the environment, but also in future research into personalized medicine, in which individual differences should be better accounted for in the design of individualized treatments.

Shortcomings of our study were certainly the poor analyzability of hPER1 and the relatively small number of participants. Further studies should include more subjects in order to increase the statistical power and to enable a differentiation into subgroups of morningness and eveningness types. Additionally, it would be interesting to compare subjects over a longer timeframe in order to better record the 24-hour rhythm. Furthermore in a larger sample the question could be addressed of whether the variation of hPER1 is a correlate of the decrease of cortisol, since the gene expression of hPER1 might be influenced via glucocorticoid-receptive elements in the DNA (Yamamoto T, 2005).

It cannot yet be discerned how far-reaching the damage due to our society's rhythmical mismatch is. In the long term, further research is needed to provide an understanding of the aftermath of an induced and chronic arrhythmia in the circadian circle. Hypothesized effects on molecular and epigenetic levels might be the key to understanding several psychological as well as physical health problems.

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REFERENCES

Abbruzzese EA, Birchler T, Ehlert U. (submitted). Effects of psychosocial stress on the gene expression of the clock genes hPER1 and hPER2 in humans.

Albrecht U. The mammalian circadian clock: a network of gene expression. Front Biosci 2004; 9: 48-55.

Allebrandt KV, Roenneberg T. The search for circadian clock components in humans: new perspectives for association studies. Braz J Med BIol Res 2008; 41: 716-721.

Anders TF. Biological rhythms in development. Psychosom Med 1982; 44: 61-72.

Bailey SL, Heitkemper MM. Circadian rhythmicity of cortisol and body temperature: morningness-eveningness effects. Chronobiol Int 2001; 18: 249-261.

Bailey SL, Heitkemper MM. Morningness–eveningness and early-morning salivary cortisol levels. Biol Psychol 1991; 32: 181-192.

Balsalobre A, Brown SA, Marcacci L, Tronche F, Kellendonk C, Reichardt HM, Schütz G, Schibler U. Resetting of Circadian Time in Peripheral Tissues by Glucocorticoid

Signaling. Science 2000; 289: 2344-2347.

Bjarnason, G.A., Jordan, R.C.K., Wood, P.A., Li, Q., Lincoln, D.W., Sothern, R.B., Hrushesky, W.J.M., Ben-David, Y. Circadian Expression of Clock Genes in Human Oral Mucosa and Skin. Am. J. Clin. Pathol 2001; 158: 1793-1801.

Buijs RM, van Eden CG, Goncharuk VD, Kalsbeek A. The biological clock tunes the organs of the body: timing by hormones and the autonomic nervous system. J Endocrinol 2003; 177: 17-26.

Cajochen C, Jud C, Münch M, Kobialka S, Wirz-Justice A, Albrecht U. Evening exposure to blue light stimulates the expression of the clock gene PER2 in humans. Eur J Neurosci 2006; 23: 1082-1086.

Carpen JD, von Schantz M, Smits M, Skene DJ, Archer SN. A silent polymorphism in the PER1 gene associates with extreme diurnal preference in humans. J Hum Genet 2006; 51: 1122-1125.

Dallmann R, Touma C, Palme R, Albrecht U, Steinlechner S. Impaired daily glucocorticoid rhythm in Per1Brd mice. J Comp Physiol A 2006; 192: 769-775.

Dickmeis T, Lahiri K, Nica G, Vallone D, Santoriello C, Neumann CJ, Hammerschmidt M, Foulkes NS. Glucocorticoids play a key role in circadian cell cycle rhythms. PLoS Biol 2007; 5: e78.

Dickmeis T. Glucocorticoids and the circadian clock. J Endocrinol 2009; 200: 3-22.

Duffy JF, Rimmer DW, Czeisler CA. Association of intrinsic circadian period with morningness-eveningness, usual wake time, and circadian phase. Behav Neurosci 2001; 115: 895-899.

Ehlert U, Gaab J, Heinrichs M. Psychoneuroendocrinological contributions to the etiology of depression, posttraumatic stress disorder, and stress-related bodily disorders: the role of the hypothalamus-pituitary-adrenal axis. Biol Psychiatry 2001; 57: 141-152.

Gachon F, Nagoshi E, Brown SA, Ripperger J, Schibler U. The mammalian circadian timing system: from gene expression to physiology. Chromosoma 2004; 113: 103-112.

Granda TG, Liu XH, Smaaland R, Cermakian N, Filipski E, Sassone-Corsi P, Lévi F. Circadian regulation of cell cycle and apoptosis proteins in mouse bone marrow and tumor. FASEB J 2004; 19: 304-306.

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.

Hastings MH, Maywood ES, Reddy AB. Two decades of circadian time. J Neuroendocrinol 2008; 20: 812-819.

Herzog ED, Tosini G. The mammalian circadian clock shop. Semin Cell Dev Biol 2001; 12: 295-303.

Ishida A, Mutoh T, Ueyama T, Bando H, Masubuchi S, Nakahara D, Tsujimoto G, Okamura H. Light activates the adrenal gland: timing of gene expression and glucocorticoid release. Cell Metab 2005; 2: 297-307.

Kalsbeek A, Palm IF, La Fleur SE, Scheer FA, Perreau-Lenz S, Ruiter M, Kreier F, Cailotto C, Buijs RM. SCN outputs and the hypothalamic balance of life. J Biol Rhythms 2006; 21: 458-469.

Karni A, Sagi D. The time course of learning a visual skill. Nature 1993; 365: 250-252.

Korczak AL, Martynhak BJ, Pedrazzoli M, Brito AF, Louzada FM. Influence of chronotype and social zeitgebers on sleep/wake patterns. Braz J Med Biol Res 2008; 41: 914-919.

Kriegsfeld RJ, Silver R. The regulation of neuroendocrine function: Timing is everything. Horm Behav 2006; 49: 557-574.

Kudielka BM, Bellinggrath S, Hellhammer DH. Further support for higher salivary cortisol levels in "morning" compared to "evening" persons. J Psychosom Res 2007; 62: 595-596.

Kudielka, BM, Federenko IS, Hellhammer DH, Wust S. Morningness and eveningness: the free cortisol rise after awakening in "early birds" and "night owls". Biol Psychol 2006; 72: 141-146.

Lévi F, Schibler U. Circadian rhythms: mechanisms and therapeutic implications. Annu Rev Pharmacol Toxicol 2007; 47: 593-628.

Lévi F. Circadian chronotherapy for human cancers. Lancet Oncology 2001; 2: 307-315.

Lucas RJ, Freedman MS, Munoz M, Garcia-Fernandez J, Foster RG. Non-rod, non-cone ocular photoreceptors regulate the mammalian pineal. Science 1999; 284: 505-507.

Merrow M, Spoelstra K, Roenneberg T. The circadian cycle: daily rhythms from behaviour to genes. EMBO Rep 2005; 6: 930-935.

Meyer-Bernstein EL, Jetton AE, Matsumoto SI, Markuns JF, Lehman MN, Bittman EL. Effects of suprachiasmatic transplants on circadian rhythms of neuroendocrine function in golden hamsters. Endocrinology 1999; 140: 207-218.

Monk TH, Buysse DJ, Potts JM, DeGrazia JM, Kupfer DJ. Morningness-eveningness and lifestyle regularity. Chronobiol Int 2004; 21: 435-443.

Panda S, Provencio I, Tu DC, Pires SS, Rollag MD, Castrucci AM, Pletcher MT, Sato TK, Wiltshire T, Andrahazy M, Kay SA, Van Gelder RN, Hogenesch JB. Melanopsin is required for non-image-forming photic responses in blind mice. Science 2003; 301: 525-257.

Roenneberg T, Kuehnle T, Juda M, Kantermann T, Allebrandt K, Gordijn M, Merrow M. Epidemiology of the human circadian clock. Sleep Med Rev 2007; 11: 429-438.

Roenneberg T, Wirz-Justice A, Merrow M. Life between clocks: daily temporal patterns of human chronotypes. J Biol Rhythms 2003; 18: 80-90.

Rosenthal L, Day R, Gerhardstein R, Meixner R, Roth T, Guildo R, Fortier J. Sleepiness/ alertness among healthy evening and morning type individuals. Sleep Med 2001; 2: 243-248.

Scheer FA, Buijs RM. Light affects morning salivary cortisol in humans. J Clin Endocrinol Metab 1999; 84: 3395-3398.

Schernhammer ES, Laden F, Speizer FE, Willett WC, Hunter DJ, Kawachi I, Colditz GA. Rotating night shifts and risk of breast cancer in women participating in the nurses' health study. J Natl Cancer Inst 2001; 93: 1563-1568.

Schibler U. The daily timing of gene expression and physiology in mammals. Dialogues Clin Neurosci 2007; 9: 257-272.

Wittmann M, Dinich J, Merrow M, Roenneberg T. Social Jetlag: Misalignment of Biological and Social Time. Chronobiol Int 2006; 23: 497-509.

Yamamoto T, Nakahata Y, Tanaka M, Yoshida M, Soma H, Shinohara K, Yasuda A, Mamine T, Takumi T. Acute physical stress elevates mouse period1 mRNA expression in mouse peripheral tissues via a glucocorticoid-responsive element. J Biol Chem 2005; 280: 42036-42043.

Young MW, Kay SA. Time zones: a comparative genetics of circadian clocks. Nat Rev Genet 2001; 2: 702-715.