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Circadian and extracircadian exploration during daytime hours of circulating corticosterone and other endocrine chronomes

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Abstract

During 7 consecutive days, blood and several tissues were collected during daytime working hours only, three times per day at 4-h intervals from inbred Wistar rats, which had been previously standardized for 1 month in two rooms on a regimen of 12 h of light (L) alternating with 12 h of darkness (LD12:12). In one room, lights were on from 09:00 to 21:00 and in the other room, lights were on from 21:00 to 09:00 (DL12:12; reversed lighting regimen). This setup provides a convenient design to study circadian and extracircadian variations over long (e.g. 7-day) spans. Prior checking of certain circadian rhythms in animals reared in the room on reversed lighting (DL) as compared with animals in the usual (LD) regimen provided evidence that the 180° phase-shift had occurred. These measurements were limited to the circadian (and not extended to infradian) variation. As marker rhythm, the core temperature of a subsample of rats was measured every 4 h around the clock (by night as well as by day) before the start of the 7-day sampling. An antiphase of the circadian rhythm in core temperature was thus demonstrated between rats in the LD vs. DL rooms. A sex difference in core temperature was also found in each room. A reversed rhythm in animals kept in DL and an antiphase between rats kept in DL vs. LD was again shown for the circulating corticosterone rhythm documented in subsamples of 8 animals of each sex sampled around the clock during the first ~1.5 day of the 7-day sampling. The findings were in keeping with the proposition that sampling rats at three timepoints 4 h apart during daytime from two rooms on opposite lighting regimens allows the assessment of circadian changes, the day-time samples from animals kept on the reversed lighting regimen accounting for the samples that would have to be obtained by night from animals kept in the room with the usual lighting regimen. During the 7-day-long follow-up, circadian and extracircadian spectral components were mapped for serum corticosterone, taking into account the large day-to-day variability. A third check on the synchronization of the animals to their respective lighting regimen was a comparison (and a good agreement) between studies carried out earlier on the same variables and the circadian results obtained on core temperature and serum

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corticosterone in this study as a whole. The present study happened to start on the day of the second extremum of a moderate double magnetic storm. The study of any associations of corticosterone with the storm is beyond our scope herein, as are the results on circulating prolactin, characterized by a greater variability and a larger sex difference than corticosterone. Sex differences and extracircadian aspects of prolactin and endothelin determined in the same samples are reported elsewhere, as are results on melatonin. Prior studies on melatonin were confirmed insofar as a circadian profile is concerned by sampling on two antiphasic lighting regimens, as also reported elsewhere. Accordingly, a circadian map for the rat will eventually be extended by the result of this study and aligned with other maps with the qualification of the unassessed contribution in this study of a magnetic storm.

Keywords

Circadian and extracircadian exploration; Circulating corticosterone; Endocrine chronomes

1. Introduction

The chronome (time structure) of corticosterone in the rat is of basic interest as a gauge of an adrenocortical cycle. It also constitutes a model for the clinical assessment of cyclic steroidal functions. It has long been known that steroids can be characterized by an ever-broadening spectrum of rhythms with many components other than circadian, as yet used neither in basic research nor in the clinic. At best there is the (under) use of morning-vs. evening-cortisol, regarded as an acceptable clinical shortcut in accounting for circadian cortisol variability in clinical practice. Herein, the slightly broader sources of variability along time scales longer than 24 h were explored to start focus on other components of variation that have been found in the ever-broadening spectrum of human, but not as yet murine components of variation. Sampling for extracircadians here implemented also allows for gauging any variation in characteristics of circadian rhythm parameters determined by cosinor as well as of infradians by getting data covering seven 24-h cycles by work only during regular hours.

The lighting regimen is a known powerful synchronizer of circadians, as shown by inferential statistical testing for several variables [1], including the rhythm in circulating blood eosinophil count by 1953 [2], and for circadians in many other functions, still in the 1950s [3–5], including the changes in circulating corticosterone by 1958, Fig. 1. But the adjustment of the circadian acrophase to a new lighting regimen is not immediate, as shown for the rhythm in liver-glycogen, Fig. 2, and for that in circulating corticosterone as well [5]. The circadian rhythm in mitoses may not be fully reversed after much more than a week on the new schedule [3]. In our hands, core temperature and serum corticosterone are considered as consistently demonstrable marker rhythms, serving as a guide and a check of the system state for other variables that are to be collected as part of a survey. This assumption is best tested in each new study. Phase-shifts and/or other chronome alterations can be brought about by usually uncontrolled factors such as magnetic storms [6,7]. The topic of the mechanisms of phase-shifts and of the underlying presumably hypothalamic mechanisms was reviewed in 1959 [8] and the influencing role of the suprachiasmatic nuclei on the amplitude (A) and acrophase (ϕ) is summarized elsewhere with a broader view of chronomes [9–11].

The case of about-weekly (circaseptan) and half-weekly (circasemiseptan) changes documented for a host of variables [12–21] is much more complex. Thus far, only adjustments after a transmeridian round-trip of circaseptan end-points of human blood pressure and heart rate have been studied, and by contrast, in the first two cases investigated, a lasting phase jump was recorded for these variables [19,20], Fig. 3. In several follow-up round-trip flights by the same man (FH) in his 80s and by another man in his 70s (GSK) during another solar cycle, a circaseptan blood pressure variation was not consistently demonstrable before or after the

flights. It is already documented that heart rate circaseptans are amplified in the presence of corresponding components in the physical environment and are dampened in the absence of corresponding frequencies in solar activity [21].

For the case of circulating corticosterone in the mouse, the discovery of the circadian rhythm in adrenal corticosteroids coincided with the demonstration of its amenability to phase-shifting, Fig. 1 [1]. The corticosterone rhythm has been confirmed repeatedly in rodents and other species, as a model for hormonal changes in human glucocorticoids, that were also quite consistent in the hands of various investigators on different continents, as summarized by 1968 in Fig. 4, as are acrophases in electrolyte metabolism, another aspect of adrenocortical function, Fig. 5. Moreover, the human mapping of circadian adrenocortical functions, as seen in Figs. 4 and 5, is not limited to a single 24-h cycle, whereas in rodents, studies cover usually only a single cycle. A notable exception quantified a circannual rhythm by cosinor, and in that context, assessed the speed of phase-shift of the serum corticosterone rhythm after lighting reversal [5]. The phase-shift of the circadian rhythm in circulating corticosterone in the C mouse was achieved fully within 7 days, as documented time-microscopically as well as by the naked eye [5].

In any event, by sampling for a week on a fixed antiphasic lighting regimen, clearly this study aims at assessing the variability in period length as well as in other characteristics, and thus the uncertainty of the circadian period, phase, amplitude and waveform by six replications of an original profile, while thus also examining any infradian variation in the same rats by sampling in a way deemed as approximating that around the clock if not that along a 7-day scale. To facilitate the practicability of the study, antiphasic lighting regimens were used during standardization and sampling as done earlier [22] (cf. [23]), using a model new to our laboratory in Pecs. Any infradian, e.g. about-weekly (circaseptan) and/or half-weekly (circasemiseptan) changes were thus also to be investigated to the extent that they were detectable on the antiphasic regimens. Not anticipated was the fact that inadvertently the first day of sampling coincided with the second extremum of a moderate magnetic double storm, as gauged by an equatorial Dst index of -81 and was just preceded by a planetary magnetic index Kp of 6.3, Fig. 6.

2. Materials and methods

2.1. Animals

According to our design, animals were kept during standardization for 1 month in two rooms on opposite regimens: one room on a “usual” regimen of 12 h of light (L) from 09:00 to 21:00 alternating with 12 h of darkness (D) from 21:00 to 09:00 (L_{09:00–21:00}D_{21:00–09:00}); the other room on a “reversed” (DL) regimen, L_{21:00–09:00}D_{09:00–21:00}. This was done to facilitate the sampling of blood and tissues during regular working hours for 7 consecutive days, in order to thereby cover six circadian timepoints at 4-h intervals without staying up by night. The three circadian stages that would have required work by night were presumably provided by the rats on the reversed LD12:12 schedule. The results were expressed in hours after light onset (HALO), representing six circadian stages in relation to light onset. The core temperature of a randomly picked subsample of animals was first measured around the clock to check on the synchronization of the animals to their respective lighting regimens. Thirteen male and 16 female rats served to determine the extent of synchronization to each of two lighting regimens (LD: n = 16 and DL [reversed]: n = 13). Core temperature was measured around the clock every 4 h for 24 h (seven samples, the first timepoint being repeated 24 h later).

2.2. Experimental procedure

Body weight and body core temperature were recorded a day before the experiment. On the first day of experiment (April 5, 2004), samples were obtained every 4 h at six different timepoints of the day (at 09:00, 13:00, 17:00, 21:00, 01:00, and 05:00) from one male and one female rat at each timepoint from both LD and DL rooms, respectively. During the dark span (LD: 21.00–09.00, DL: 09.00–21.00), blood was drawn under a dim red light. From the second day on, samples were collected at 4-h intervals during the working hours, from 09:00 to 17:00 on a daily basis for 6 additional days from both groups of rats kept on LD and DL, respectively. After a 1-day interruption, additional samples were collected at two of the three clock hours (four HALO times). Under a short ether anesthesia, the blood was withdrawn by cardiac puncture (2 ml per animal) and promptly stored on ice. The blood was centrifuged (4000 rpm, 4 °C, 20 min) and plasma was separated and stored at –20 °C. After bleeding, the animals were killed by cervical dislocation, the pituitary and the brain, and during the last few days the duodenum, were removed and placed on dry ice. Blood and tissues were kept frozen at –20 °C until required for analysis.

2.3. Measurement of corticosterone

Corticosterone concentration from serum was determined by radioimmunoassay (RIA). A mixture of 5 µl of serum and 100 µl assay buffer (ASB, 0.5 M PBS containing 1 g/l gelatine and 1 g/l sodium azide, pH 7.4) was extracted with 1 ml diethyl-ether. The dried extract was reconstituted with 1.25 ml ASB from which two parallel determinations were made. Each tube contained 500 µl extract (content of 2 µl sample), 12,000 cpm tritiated corticosterone (NEN, NET-399 1,2,6,7-3H Corticosterone, 90–120 Ci/mmol) and 15 nl per tube CS-RCS-57 antibody (own product, 1:47,000 final dilution) in a total volume of 700 µl. For standard, Calbiochem corticosterone was used in a nine-step series ranging from 15 to 4000 fmol. After an overnight incubation at 4 °C, the bound and free steroids were separated with dextran coated charcoal. The radioactivity was measured in a two-phase liquid scintillation system. The sensitivity of the assay is 30 fmol per tube. The antibody showed 19%, 7.1%, 5.5%, 5.1% and 3.0% cross-reactions with progesterone, desoxycorticosterone, testosterone, 20 α -dihydroprogesterone and androstenedione, respectively. With other natural steroids, the antibody showed <1% cross-reactions. The inter- and intra-assay coefficients of variation were 9.2% and 6.4%, respectively.

2.4. Statistics

The data were analyzed by linear-nonlinear rhythmometry [24–26] and by parameter tests [27]. More specifically, the data for each variable were analyzed by single cosinor, involving the least squares fit of cosine curves with fixed periods in the range of 7 days to 12 h, linearly in frequency. Estimates were thus obtained for the **midline estimating statistic of rhythm** (MESOR, M, a chronome [time structure]-adjusted mean), the double amplitude (2A, a measure of the extent of predictable change within a cycle), and the acrophase, a measure of the timing of overall high values recurring in each cycle. Original data were log₁₀-transformed when indicated. Results were summarized globally and separately for males and females on each lighting regimen by single (corticosterone) or population-mean (temperature) cosinor. Parameter tests compared the circadian characteristics of male and female rats, as well as rats of each sex kept on opposite lighting regimens sampled around the clock on the first day of study. In the latter case, in order to test for an anticipated antiphase, 180° (12 h) were added to the acrophase of animals kept in DL before testing the equality of acrophases of rats kept in LD or DL.

The data were also analyzed separately for rats of each sex by multiple-component single cosinor. The presence of a sex difference prompted us to express the data as a percentage of

the series mean value, computed separately for males and females in each room, so that the relative data could be pooled for a global analysis.

3. Results

3.1. Validation of exclusive daytime murine sampling on antiphasic lighting regimens by circadian rhythmic core temperature

To determine whether the circadian acrophase of core temperature of rats kept on a reversed lighting regimen (DL) differed from that of rats kept in LD, and to determine whether any difference in the circadian variation of core temperature between male and female rats could be found, core temperature was recorded from rats of both genders kept on LD and DL schedules.

The circadian rhythm in core temperature was tested using six or seven timepoints (without or with the repeated first timepoint). On an individual basis, a circadian rhythm could be demonstrated with statistical significance ($P < 0.05$) in seven out of 29 rats. On a group basis, the circadian rhythm was invariably demonstrated ($P < 0.05$). Parameter tests found that female rats had a higher MESOR of core temperature than male rats, whether the analyses were based on six timepoints (LD: 37.2° vs. 36.8° , $F = 3.189$, $P = 0.096$; DL: 37.5° vs. 37.0° , $F = 5.547$, $P = 0.038$) or on seven timepoints (LD: 37.2° vs. 36.7° , $F = 4.170$, $P = 0.060$; DL: 37.4° vs. 37.0° , $F = 6.408$, $P = 0.028$). A test of the equality of acrophases adjusted to the respective lighting regimens (HALO time) was statistically significant for males ($F = 5.812$, $P = 0.035$) but not for females ($F = 0.289$, $P = 0.600$) when considering only six timepoints. When all seven timepoints were considered, the test was not significant for both males and females, suggesting that the animals were fully synchronized to their respective lighting regimens. A small acrophase difference detected with six but not with seven timepoints only in the case of male rats may be interpreted to indicate that females, with a statistically significantly higher core temperature (and more active adrenals, as documented by corticosterone determinations; see also 28), adjust faster to the daily routine and/or are less disturbed by external noise than males.

3.2. Circadian and infradian changes in circulating corticosterone

The feasibility of using staggered lighting regimens was further examined for the case of corticosterone assessed in the same animals by around the clock sampling from both rooms during the first ~1.5 days. A circadian rhythm is demonstrated with borderline statistical significance ($P < 0.10$) in both series of male rats (from LD and DL), but only in one series from the female rats. The use of one animal per timepoint per regimen allowed rhythm demonstration at the 10% probability level in three of four series and suggests that with larger numbers a full validation of the approach may become possible. Indeed, expression of the data as % of mean, to reduce the effect of sex differences by transformation and pooling, fully validates the reversal on this sub-sample of rats since the only exception in Fig. 7a is the series on LD in females. The satisfactory agreement of the data from the two rooms is best seen after the data are plotted as a function of HALO-time in Fig. 7b. Furthermore, the overall results based on all data collected during the study support the assumption of a complete phase-shift, Fig. 8a.

Analysis of the around the clock data collected over the first ~1.5 days already shows that corticosterone is higher in female than in male rats. The difference is observed in both rooms kept on opposite lighting regimens (LD12:12:670 $\pm$ 118 vs. 279 $\pm$ 64 nmol/l, $t = 2.920$, $P = 0.011$; DL12:12:868 $\pm$ 200 vs. 380 $\pm$ 97 nmol/l, $t = 2.200$, $P = 0.045$). A sex difference is also observed when considering all animals in the study (LD12:12:530 $\pm$ 64 vs. 235 $\pm$ 27, $t = 4.226$, $P < 0.001$; DL12:12:826 $\pm$ 87 vs. 474 $\pm$ 51, $t = 3.541$, $P < 0.001$). A circadian rhythm is

demonstrated with statistical significance in both male and female rats, Fig. 8b, as well as overall ($P < 0.001$), Fig. 8a. Parameter tests confirm the MESOR difference between males and females ($F = 34.65$, $P < 0.001$). Analyses of pooled data are performed on relative data, expressed as a percentage of the mean values calculated separately for male and female rats in each room, before and after their $\log_{10}$ -transformation.

Least squares spectra in the frequency range of one cycle per week to two cycles per day indicate the presence of a prominent circadian rhythm accounting for 40% of the overall variability as well as of a statistically significant 12-h component ($P = 0.036$) qualifying the circadian waveform of corticosterone. Further modeling of the data yields a composite model consisting of cosine curves with periods of 7 days ($P = 0.061$), 24 h ($P < 0.001$) and 12 h ($P = 0.004$), accounting for 49% of the variance ($P < 0.001$), Fig. 9a. Similar analyses on the $\log_{10}$ -transformed data account for 51% of the variance ($P < 0.001$) when considering cosine curves with periods of 7 days ($P = 0.038$), 3.5 days ($P = 0.039$) and 1-day ($P < 0.001$), Fig. 9b.

Separate analyses of male and female rats indicate the presence of a prominent circadian rhythm ($P < 0.001$) with the contribution to the waveform by the second harmonic with a period of 12 h (Males: $P = 0.048$; Females: $P = 0.058$) and a circaseptan component of borderline statistical significance for males ($P = 0.057$) or a questionable circasemiseptan component for females ($P = 0.180$). The model accounts for 65% of the variance for males ($P < 0.001$) and for 38% of the variance for females ($P = 0.002$).

Nonlinear analyses of the pooled $\log_{10}$ -transformed (relative) data confirm the presence of both the circadian rhythm, with an estimated period of 24.03 h (95% CI: 23.30–24.75 h), and an infradian component with an estimated period of 103.8 h (4.3 days) (95% CI: 79.5–128.1 h). These results are similar for male and female rats: for males, the circadian and infradian periods are estimated as 24.00 (23.20–24.79) and 108.2 (61.2–155.1) hours; for females, the respective periods are estimated as 24.03 (22.57–25.49) and 101.9 (71.5–132.3) hours. An about 4.3-day component was validated by nonlinear least squares for circulating corticosterone.

4. Discussion

This study validates the model and experimental design based on staggered lighting regimens. It shows the feasibility of assessing circadian rhythms in the laboratory based on sampling restricted to the usual working hours by housing animals in rooms on staggered lighting regimens. Such a protocol is of particular interest when sampling is prolonged over many consecutive days, e.g. to determine the uncertainty of the circadian period, as done herein. The around the clock measurements of marker rhythms such as the circadian core temperature and corticosterone rhythms serve a useful purpose to ascertain that animals are properly synchronized prior to the start of a large experiment involving multiple sampling of blood and various tissues, aimed at assessing not only the circadian but also any about-weekly and half-weekly components of variation. Being able to work during regular hours while still covering all circadian stages in relation to light onset is particularly important when experiments are not limited to the study of circadian rhythms but focus also on extracircadian, notably infradian changes requiring sampling to continue for seven consecutive days, as in this study or for even longer spans.

Findings of this study include a sex difference, female rats having higher circulating corticosterone concentrations than male rats, in keeping with a finding made nearly half a century ago on the mouse [28] with the count of circulating eosinophil cells, known to be depressed by steroids with a certain chemical structure [29] then used as a gauge of adrenocortical function. Any infradian component of about 4.3 days found in a data pool from

both male and female rats together with a prominent circadian rhythm has to be qualified by the moderate double magnetic storm that coincided with the first days of this study.

Melatonin was also determined in this study, among other variables, as reported elsewhere [30–37]. A major lesson, in addition to controlling the lighting regimen and avoiding all disturbance of the animals prior to study, including prior venisections [38], is to be flexible in the future in planning a study depending on whether one does or does not wish to study a magnetic storm such as that which occurred before and again at the start of this study, hence qualifying the results herein.

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References

1. Halberg F, Barnum CP, Silber RH, Bittner JJ. 24-h rhythms at several levels of integration in mice on different lighting regimens. *Proc Soc exp Biol (NY)* 1958;97:897–900.
2. Halberg F, Visscher MB, Bittner JJ. Eosinophil rhythm in mice: range of occurrence; effects of illumination, feeding and adrenalectomy. *Am J Physiol* 1953;174:109–22. [PubMed: 13065505]
3. Halberg F, Bittner JJ, Smith D. Belichtungswechsel und 24-Stundenperiodik von Mitosen im Hantepithel der Maus. *Z Vitamin-, Hormon- u Fermentforsch* 1957;9:69–73.
4. Halberg F, Albrecht PG, Barnum CP Jr. Phase-shifting of liver-glycogen rhythm in intact mice. *Am J Physiol* 1960;199:400–2. [PubMed: 13710666]
5. Haus E, Halberg F. Circannual rhythm in level and timing of serum corticosterone in standardized inbred mature C-mice. *Environ Res* 1970;3:81–106. [PubMed: 5436927]
6. Chibisov, S.; Halberg, F.; Cornélissen, G.; Breus, TK. Amplitude changes of biorhythms as a sign of desynchronization and magnetic storm. Abstract, 2nd International Symposium, Problems of Rhythms in Natural Sciences; Moscow. March 1–3, 2004; Moscow: Russian People's Friendship University; 2004. p. 493-6.
7. Chibisov SM, Cornélissen G, Halberg F. Magnetic storm effect on the circulation of rabbits. *Biomed Pharmacother* 2004;58(Suppl 1):S 15–9.
8. Halberg, F.; Halberg, E.; Barnum, CP.; Bittner, JJ. Physiologic 24-h periodicity in human beings and mice, the lighting regimen and daily routine. In: Withrow, RB., editor. *Photoperiodism and related phenomena in plants and animals*. Washington, DC: AAAS; 1959. p. 803-78. Ed. Publ. No. 55
9. Cornélissen, G.; Halberg, F. Introduction to chronobiology; Medtronic Chronobiology Seminar #7. Apr. 1994 p. 52 (Library of Congress Catalog Card #94-060580; URL <http://www.msi.umn.edu/~halberg/>)
10. Halberg, F.; Cornélissen, G.; Katinas, G.; Syutkina, EV.; Sothorn, RB.; Zaslavskaya, R., et al. Transdisciplinary unifying implications of circadian findings in the 1950s; *J Circadian Rhythms*. 2003. p. 61 www.JCircadianRhythms.com/content/pdf/1740-3391/1/2.pdf
11. Halberg F, Cornélissen G, Wang ZR, Wan C, Ulmer W, Katinas G, et al. Chronomics: circadian and circaseptan timing of radiotherapy, drugs, calories, perhaps nutraceuticals and beyond. *J Exp Ther Oncol* 2003;3:223–60. [PubMed: 14641812]
12. Dérier L. Rhythm and proliferation with special reference to the 6-day rhythm of blood leukocyte count. *Neoplasma* 1960;7:117–34. [PubMed: 13816020]
13. Halberg F, Engeli M, Hamburger C, Hillman D. Spectral resolution of low-frequency, small-amplitude rhythms in excreted 17-ketosteroid; probable androgen induced circaseptan desynchronization. *Acta Endocrinol (Kbh)* 1965;50(Suppl 103):5–54.
14. Touitou, Y.; Hans, E., editors. *Biological rhythms in clinical and laboratory medicine*. Berlin: Springer-Verlag; 1992. p. 730
15. Hildebrandt, G.; Bandt-Reges, I. *Chronobiologie in der Naturheilkunde: Grundlagen der Circaseptanperiodik*. Heidelberg: Haug; 1992. p. 102

16. Halberg F. The week in phylogeny and ontogeny: opportunities for oncology. *In Vivo* 1995;9:269–78. [PubMed: 8555425]
17. Hildebrandt, G.; Moser, M.; Lehofer, M. Chronobiologie und Chronomedizin: Biologische Rhythmen/Medizinische Konsequenzen. Stuttgart: Hippokrates; 1998. p. 141
18. Mikulecky, M.; Duris, I. Chronocosmobiology and chronocosmomedicine in Slovakia: the legacy of Academician Ladislav Derer. *Proc. 3rd International Symposium of Chronobiology and Chronomedicine*; Kunming, China. October 7–12, 1998; p. 56-57.
19. Halberg F, Hillman D, Halberg E. Circaseptan (about 7-day) cardiovascular adjustment after transmeridian round-trip (west-east-west vs. east-west-east) flights. *Chronobiologia* 1988;15:247–9.
20. Hillman D, Halberg E, Halberg F. More on about-weekly (circaseptan) cardiovascular variation after transmeridian (7-h shift of schedule) east-west-east flights. *Chronobiologia* 1988;15:249–50.
21. Cornélissen G, Halberg F, Wendt HW, Bingham C, Sothorn RB, Hans E, et al. Resonance of about-weekly human heart rate rhythm with solar activity change. *Biologia (Bratislava)* 1996;51:749–56.
22. Halberg E, Halberg F. Chronobiologic study design in everyday life, clinic and laboratory. *Chronobiologia* 1980;7:95–120. [PubMed: 7438897]
23. Sanchez de la Pefia S, Halberg F, Halberg E, Ungar F, Cornélissen G, Sanchez E, et al. Pineal modulation of ACTH 1–17 effect upon murine corticosterone production. *Brain Res Bull* 1983;11:117–25. [PubMed: 6313140]
24. Halberg F. Chronobiology. *Annu Rev Physiol* 1969;31:675–725. [PubMed: 4885778]
25. Halberg F, Carandente F, Cornélissen G, Katinas GS. Glossary of chronobiology. *Chronobiologia* 1977;4(Suppl 1):189.
26. Cornélissen, G.; Halberg, F. Chronomedicine. In: Armitage, P.; Colton, T., editors. *Encyclopedia of biostatistics*. 1. Chichester, UK: John Wiley & Sons Ltd.; 1998. p. 642-9.
27. Bingham C, Arbogast B, Cornélissen Guillaume G, Lee JK, Halberg F. Inferential statistical methods for estimating and comparing cosinor parameters. *Chronobiologia* 1982;9:397–439. [PubMed: 7168995]
28. Halberg F, Hamerston O, Bittner JJ. Sex difference in eosinophil counts in tail blood of mature B₁ mice. *Science* 1957;125:73. [PubMed: 13390968]
29. Halberg F. Some correlations between chemical structure and maximal eosinopenia in adrenalectomized and hypophysectomized mice. *J Pharmacol Exp Ther* 1952;106:135–49. [PubMed: 12981636]
30. Jozsa, R.; Kaszaki, J.; Cornélissen, G.; Olah, A.; Nagy, G.; Csernus, V., et al. Extracircadian variation of endothelin-1 in murine plasma and pituitary and human blood. In: Cornélissen, G.; Kenner, R.; Fiser, B.; Siegelova, J., editors. *Proceedings, Symposium: Chronobiology in Medicine. Dedicated to the 85th Anniversary of Professor Franz Halberg*. Brno: Masaryk University; 2004. p. 102-3.
31. Zeman, M.; Cornélissen, G.; Balazova, K.; Jozsa, R.; Olah, A.; Nagy, G., et al. Circadian rhythm of melatonin in rat duodenum. In: Cornélissen, G.; Kenner, R.; Fiser, B.; Siegelova, J., editors. *Proceedings, Symposium: Chronobiology in Medicine. Dedicated to the 85th Anniversary of Professor Franz Halberg*. Brno: Masaryk University; 2004. p. 95-7.
32. Stebelova K, Zeman M, Cornélissen G, Bubenik G, Jozsa R, Hardeland R, et al. Chronomics reveal and quantify circadian rhythmic melatonin in duodenum of rats. *Biomed Pharmacother* 2005;59(Suppl 1):S209–S212. [PubMed: 16275496]
33. Zeman M, Jozsa R, Cornélissen G, Stebelova K, Bubenik G, Olah A, et al. Chronomics: circadian lead of murine extrapineal vs. pineal melatonin rhythms with an infradian hypothalamic exploration. *Biomed Pharmacother* 2005;59(Suppl 1):S213–S219. [PubMed: 16275497]
34. Nagy, G.; Jozsa, R.; Cornélissen, G.; Olah, A.; Zeman, M.; Kaszaki, J., et al. Circadian and circasemiseptan changes in circulating prolactin in rats. In: Cornélissen, G.; Kenner, R.; Fiser, B.; Siegelova, J., editors. *Proceedings, Symposium: Chronobiology in Medicine. Dedicated to the 85th Anniversary of Professor Franz Halberg*. Brno: Masaryk University; 2004. p. 98-9.
35. Olah, A.; Jozsa, R.; Cornélissen, G.; Zeman, M.; Nagy, G.; Kaszaki, J., et al. Validation of exclusive daytime murine sampling on antiphasic lighting regimens by circadian rhythmic core temperature behavior. In: Cornélissen, G.; Kenner, R.; Fiser, B.; Siegelova, J., editors. *Proceedings, Symposium: Chronobiology in Medicine. Dedicated to the 85th Anniversary of Professor Franz Halberg*. Brno: Masaryk University; 2004. p. 100-1.

36. Csernus, V.; Jozsa, R.; Cornélissen, G.; Zeman, M.; Olah, A.; Nagy, G., et al. Circadian and circaseptan changes in circulating corticosterone of rats. In: Cornélissen, G.; Kenner, R.; Fiser, B.; Siegelova, J., editors. Proceedings, Symposium: Chronobiology in Medicine. Dedicated to the 85th Anniversary of Professor Franz Halberg. Brno: Masaryk University; 2004. p. 108-9.
37. Poeggeler G, Cornélissen G, Huether G, Hardeland R, Jozsa R, Zeman M, et al. Chronomics affirm extending scope of lead in phase of duodenal vs. pineal melatonin rhythms. *Biomed Pharmacother* 2005;59(Supp 1):S220–S224. [PubMed: 16275498]
38. Halberg F. Changes in eosinophil count of mice with venisections repeated at intervals of several days. *Proc Soc Exp Biol (NY)* 1953;82:160–2.

TIMING OF CIRCADIAN VARIATION OF MURINE CORTICOSTERONE DEPENDS ON LIGHTING REGIMEN*

* LD12:12 = 12 hours of light alternating with 12 hours of darkness;
2A = double amplitude; ϕ = acrophase (timing of overall high values)
 $H_0: \phi_R = \phi_U + 12h$; $P < 0.05$.
F. Halberg et al., Proc. Soc. Exp. Biol. & Med. 97:897-900, 1958.

Fig. 1.

Inversion of the temporal placement along the 24-h scale of the circadian rhythm in circulating corticosterone of mice by reversal of regimen of light alternating with darkness every 12 h [1]. © Halberg.

Fig. 2.
Different stages in the uneven (first slow, then faster) adjustment of the circadian rhythm in liver-glycogen of mice after the reversal of the lighting regimen [4]. © Halberg.

Lasting phase-shift of a circaseptan rhythm (top) following a round-trip flight from Minneapolis, Minnesota, to Nice, France (1), and return (2), with rapid resynchronization of the circadian rhythm after the return flight (bottom)

Fig. 3. Phase jump of circaseptan acrophase following a round-trip from Minnesota to Nice, France, and back. © Halberg.

**AGREEMENT OF CIRCADIAN ACROPHASES (Φ)
OF HUMAN HYDROXYCORTICOSTEROID EXCRETION
IN SERIES FROM DIFFERENT GEOGRAPHIC LOCATIONS
UP TO 1968**

* Φ (dots) with 95% confidence intervals (horizontal bars) estimated by least squares fit of 24-hour cosine curve; next to Φ s are N of subjects, (N of days), [sampling interval (hours)].
NOTE: Φ s in excellent agreement with subsequent cortisol Φ s in blood and saliva over ensuing decades. Source: F. Halberg et al., *Experientia* 1969; 25: 107 - 112.

Fig. 4.
Relative agreement within and among geographic areas in studies over several days of a gauge of the adrenocortical cycle. © Halberg.

**AGREEMENT OF CIRCADIAN ACROPHASES (Φ)
OF HUMAN POTASSIUM EXCRETION
IN SERIES FROM DIFFERENT GEOGRAPHIC LOCATIONS
UP TO 1968**

* Φ (dots) with 95% confidence intervals (horizontal bars) estimated by least squares fit of 24-hour cosine curve; next to Φ s are N of subjects, (N of days), [interval (hours)] covered by sample. Source: F. Halberg et al., *Experientia* 2969; 25: 107-112.

Fig. 5.

Relative agreement within geographic locations and among different sites of analyses based on series of different lengths, covering up to 52 days. © Halberg.

**EQUATORIAL (Dst, top) and PLANETARY (Kp, bottom)
GEOMAGNETIC DISTURBANCE INDICES
BEFORE, DURING and AFTER
AN ENDOCRINE STUDY IN PECS HUNGARY***

*** Framed areas cover the time spans of 4-hourly sampling on antiphase lighting regimens from 00:00 April 5 to 16:00 April 13 with gap between 00:00 April 12 to 00:00 April 13**

Fig. 6.

Geomagnetic activity during sampling in this study and during the preceding and following weeks assessed by an equatorial (top) and a planetary (bottom) index. © Halberg

Fig. 7.

a. Antiphasic rhythms validating the synchronization of animals kept in two rooms on opposite lighting regimens for long-term sampling on this variable, *b.* Replot of corticosterone data from rats in each room as a function of hours after light onset (HALO). © Halberg.

Fig. 8.

Summary of 7 days' data assayed in original units stacked over idealized 24-h day. *a.* All animals. *b.* Separately for male and female rats. © Halberg.

Fig. 9.

a. Circadian-infradian profile of the circulating corticosterone in Wistar rats of both sexes pooled after conversion to relative data expressed as % of mean separately for each gender.

b. Results after $\log_{10}$ -transformation of the relative data. The data await scrutiny in terms of any effects of a double magnetic storm, found to have involved the first days of this study, as documented in Fig. 6. © Halberg.