Title
Circadian and ultradian rhythms in normal mice and in a mouse model of Huntington’s disease

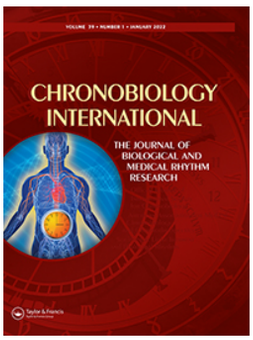
Journal
Chronobiology International, 39(4)

ISSN
0742-0528

Authors
Griffis, Christopher G
Mistry, Janki
Islam, Kendall
et al.

Publication Date
2022-04-03

DOI
10.1080/07420528.2021.2014516



Taylor & Francis
Taylor & Francis Group

Chronobiology International

The Journal of Biological and Medical Rhythm Research

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/icbi20

Circadian and ultradian rhythms in normal mice and in a mouse model of Huntington's disease

Christopher G. Griffis, Janki Mistry, Kendall Islam, Tamara Cutler, Christopher S. Colwell & Alan Garfinkel

To cite this article: Christopher G. Griffis, Janki Mistry, Kendall Islam, Tamara Cutler, Christopher S. Colwell & Alan Garfinkel (2022): Circadian and ultradian rhythms in normal mice and in a mouse model of Huntington's disease, Chronobiology International, DOI: 10.1080/07420528.2021.2014516

To link to this article: https://doi.org/10.1080/07420528.2021.2014516

Published online: 04 Jan 2022.

CHRONOBIOLOGY INTERNATIONAL
https://doi.org/10.1080/07420528.2021.2014516

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

OPEN ACCESS Check for updates

Circadian and ultradian rhythms in normal mice and in a mouse model of Huntington's disease

Christopher G. Griffis[a], Janki Mistry[b,c], Kendall Islam[c,d], Tamara Cutler[e], Christopher S. Colwell[e], and Alan Garfinkel[c,f]

[a]Department of Ophthalmology, David Geffen School of Medicine (DGSOM), University of California, Los Angeles (UCLA), Los Angeles, California, USA; [b]School of Medicine, Medical College of Wisconsin, Milwaukee, Wisconsin, USA; [c]Department of Integrative Biology and Physiology, UCLA, Los Angeles, California, USA; [d]Division of General Internal Medicine, University of California, San Francisco, California, USA; [e]Department of Psychiatry and Biobehavioral Sciences, DGSOM at UCLA, Los Angeles, California, USA; [f]Department of Medicine (Cardiology), DGSOM at UCLA, Los Angeles, California, USA

ABSTRACT

Circadian rhythms in core body temperature (CBT) have been widely studied, but fewer studies have explored higher-frequency (ultradian) rhythms in detail. We analyzed CBT recordings from young and middle-aged wild-type mice as well as from the Q175 model of Huntington's disease (HD), at sufficient temporal resolution to address the question of ultradian rhythms. We used model selection methods to show that the overall circadian pattern was better fit by a square wave than a sine wave. Then, using Fourier analysis of the CBT rhythms, we identified the spectral signature of an 8-hour oscillation that occurs in the night but not the day, an observation that can be confirmed by direct inspection of the rhythms. This diurnal amplitude modulation of the 8-hour rhythm was lost with aging as well as in the HD model. Thus, the impact of aging and disease is seen here in the loss of the ability to separate rhythms into a daytime phase and a nighttime phase. These findings raise the possibility that ultradian rhythms in CBT may be a useful biomarker for the pathology within the central nervous system.

ARTICLE HISTORY
Received 2 August 2021
Revised 23 November 2021
Accepted 30 November 2021

KEYWORDS
Aging; biomarker; core body temperature; circadian; Fourier analysis; Huntington's disease; Q175 mice; ultradian

Introduction

Circadian rhythms play a critical role in organizing cellular and sub-cellular systems, and in ensuring synchronization among physiological processes (Panda et al. 2002; Yamazaki et al. 2000). Of all circadian rhythms, the rhythm in core body temperature (CBT) plays a special role, in that circadian rhythms in CBT "can act as a universal entraining agent for circadian rhythms throughout the body" (Buhr et al. 2010; Edery 2010; Tu et al. 2005).

What is the waveform of these circadian oscillations? There seems to be widespread agreement that the overall waveform of circadian rhythms can be stylized as a sine wave, although occasional studies have argued for square waves, at least in the case of heart rate and blood pressure rhythms (Idema 1996).

Some published records appear to show that the 24-hr (circadian) rhythm is scalloped by additional rhythms at higher, ultradian frequencies (Büttner and Wollnik 1984; Refinetti 1992; Refinetti and Kenagy 2018; Wollnik and Turek 1988). However, despite these findings, most studies have focused on circadian, not ultradian, rhythms in core body temperature. In part, this is because little data exist at sufficient temporal resolution to demonstrate the existence of ultradian rhythms in body temperature. Further, the data that have been published tend to average across multiple individuals (Aschoff et al. 1971), across an entire 24-hr period (Hamilos et al. 1998), or across multiple days (Aschoff et al. 1971; Piccione et al. 2002), impairing the ability to find higher-frequency components. Previous studies have explored ultradian rhythms in other variables, such as locomotor activity (Aschoff 1981), heart rate variability and body temperature in relation to reproductive endocrine rhythms (Grant et al. 2020), and gene expression (van der Veen and Gerkema 2017), among other physiological parameters. One study found persistent ultradian rhythms even post-ablation of the SCN, the body's central pacemaker and circadian rhythm generator, indicating that ultradian rhythms may arise entirely independently from the circadian rhythm (Ruis et al. 1987).

Another major contributor to changes in circadian rhythms is aging. Multiple studies have shown decreased circadian rhythm amplitude of several physiological parameters with age in multiple animals, including

CONTACT Alan Garfinkel agarfinkel@mednet.ucla.edu Department of Medicine, University of California, Los Angeles, CA 90095

Drosophila (Rakshit et al. 2012), mice (Weinert et al. 2002), and humans (Harper et al. 2005). Based on these results in circadian rhythms, we aimed to investigate the effect of aging on ultradian rhythms.

Here we consider circadian rhythms in Core Body Temperature at temporal scales that are sufficiently resolved to address (1) whether the circadian rhythm is, in fact, a sinusoidal process, and (2) whether ultradian components exist. Then, once the character of the circadian waveform has been established, we can explore how aging and disease affect ultradian rhythms.

For example, patients with Huntington's Disease (HD) tend to have disrupted activity patterns including disturbances in sleep and dysfunction of circadian oscillations. Pioneering work done in the R6/2 model of HD found that the mutant mice displayed an increase in daytime activity and a decrease in nighttime activity, which eventually led to the full disruption of the circadian waveform of locomotor activity (Morton et al. 2005). Loh et al. later found that the Q175 model also exhibited age and genotype driven disruption in locomotor activity rhythms compared to wild-type (WT) controls (2013). Importantly, the Q175 models exhibit clear disruption in CBT rhythms as shown by Cutler et al. (2017) and thus can be used as a test case in the present study. Taken together, these results clearly establish the detrimental effect of HD on circadian rhythmicity.

Here, we address whether circadian waveforms are sinusoidal (as opposed to square wave) and whether they have identifiable ultradian components, and we examine whether the mice heterozygous and homozygous for the Q175 model of HD showed degraded circadian and ultradian rhythms when compared to the WT mice. We also consider how aging altered the circadian and ultradian rhythms through comparison of young and middle-aged mice.

Materials and methods

Animals

A detailed description of the recording methods has been previously published in Cutler et al. (2017). Mice were obtained from the Jackson Laboratory, (JAX, Bar Harbor, Maine; stock No: 370476)], including WT animals (n = 5), animals heterozygous (n = 7) and homozygous (n = 6) for the Q175 model of HD. The mice were placed on a 12-hr light, 12-hr dark (LD) schedule with food available *ad-lib*. Telemetry studies measuring activity and CBT, and electrocardiograms (ECG) were performed with three different batches of mice aged 4–5 months, and again with the same mice aged 10–11 months. All procedures followed guidelines of the National Institutes of Health and were approved by the UCLA Animal Research Committee.

Telemetry measurements

Telemetry measurements were made as previously described in Cutler et al. (2017). Briefly, mice were surgically instrumented for wireless radio-frequency telemetry (ETA-F20, Data Sciences International, St. Paul, MN), and CBT (degrees Celsius), activity (counts/min), and heart rate (bpm) were sampled continuously for five days and presented as 20 second averages.

Data analysis

Data were analyzed using the SciPy (Version 0.19.1) and NumPy (Version 1.13.3) libraries for the Python (Version 3.6) programming language. The data records had occasional lead failures, ranging in length from 20 seconds to one hour, resulting in values of "NaN" in the data. These gaps were filled in by piecewise linear interpolation, using numpy.interp (Version 1.13). Data were de-meaned, and the power spectrum of the entire waveform was assessed by computing the discrete Fourier transform using the Fast Fourier Transform algorithm from the numpy.fft module (Version 1.13).

We tested the spectral peaks for statistical significance by a resampling-based method (Efron and Tibshirani 1991). The spectral peaks seen in the real data were compared to spectral peaks that were constructed from randomized permutations of the data. In order to choose the time interval to be shuffled, the natural autocorrelation of the data must be considered. The power spectrum is closely related to the autocorrelation of a periodic time series, and random permutation of all individual points, point-by-point, would break this relationship. To preserve the autocorrelation ~ power spectrum relationship, we performed a chunked bootstrap permutation with 20 samples, the length that would be compressed by decimating the data by the first zero-crossing lag of the autocorrelation function.

A peak was considered statistically significant at the $p < .01$ level if it was greater than the 99th percentile of the peaks from the simulated data. As an omnibus test of the significance of the difference between two spectra, we first did Kolmogorov-Smirnov tests to confirm an overall statistically significant difference in each case (see Supplement).

For visualization, we smoothed the raw data by convolution with a Chebyshev kernel, a process equivalent to low pass filtering with a Chebyshev Type II filter at

0.00029 Hz (approximately 1-hr). To account for the differences in the type of records, we selected the following different kernel sizes: 600 points (CBT), 750 points (HR), 700 points (Activity).

Model selection: Square vs. Sine

To compare models, we used resampling to generate distributions of the Akaike Information Criterion (AIC) for fitting the equal-sized parameterized models to a single averaged day for a given condition. Because ultradian rhythms are present in different magnitudes in the day/night cycle, we averaged over all animals of a given genotype and age to a single day and used this as our real fit. The models are parameterized as follows, for the sine wave M_{sin} and the square wave M_{sgn}:

In this model, β_1 is the amplitude, β_2 is the frequency (in Hz) of the main component and β_3 is the phase.

Pre-processed data (i.e., previously cleaned and smoothed) contained 5 days of CBT records from 4 wild-type, young, mice. Each record was split into 5 days (above), and the variance for the light on/off regions was calculated (below). Light was ON for hours between 0 and 12 and OFF thereafter. The day average across all days and mice (in the age and genotype group) was calculated, and the Square and Sine models (described above) were fit using the non-linear least-squares curve fitting method from the lmfit-py package (Neville et al., 2021). The initial 'guess' parameters were derived from the averaged waveform and the Fourier transform of the averaged waveform. β_1 was derived from the domain of the waveform, and β_2 and β_3 were derived from the frequency and phase information of the nearest peak in the FFT corresponding with a 24-hr period. To confirm the best model fit, we generated the sampling distributions on AIC for each model, determined the pairwise differences between the models, and constructed 95% confidence intervals using the percentile method.

"De-squaring"

To study the ultradian rhythms that remain after the removal of the 24-hr square wave component along with its harmonics, we first constructed a square wave fit to the data, using phase and magnitude information from the spectral peak of the data that was nearest to the 24-hr period. We then calculated the complex Fourier transform of this square wave and subtracted its components from the complex Fourier spectrum of the data. We then used the inverse Fourier transform to generate the "de-squared" data. This method removes the square wave and all its harmonics, whereas "low-pass" filtering of a square wave allows the passage of only the fundamental sine wave and spuriously removes its higher harmonics.

Results

After confirming that the WT mice exhibited clear 24-hr cycles in CBT, heart rate and activity (Figure 1), we focused on the rhythm in CBT.

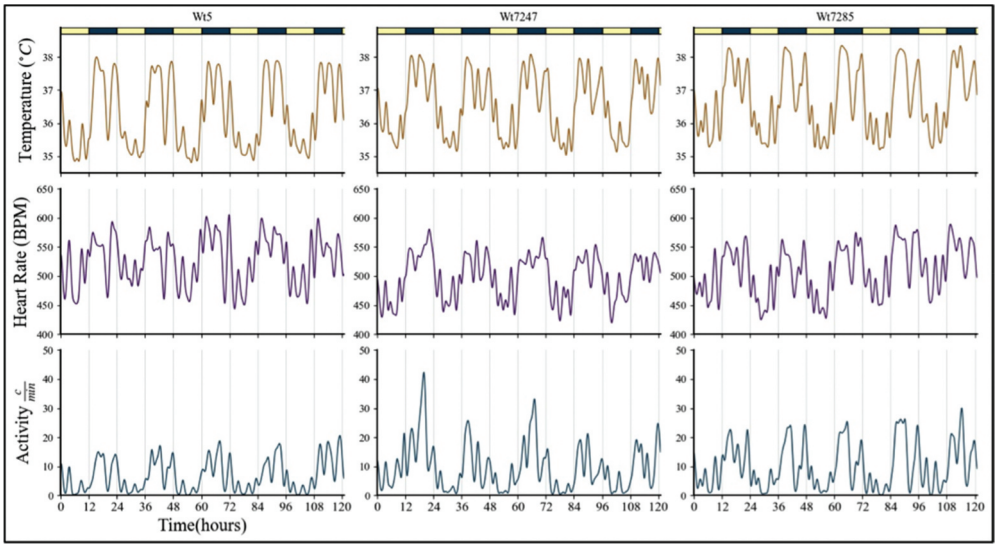


Figure 1. Time series of CBT, heart rate, and activity over five days in three different WT mice, aged 4–5 months. Bars above the core body temperature plot indicate 12-hr periods of light and dark. Note the high-frequency oscillations and non-sinusoidal waveform. Vertical lines show detailed correlations among the high-frequency peaks in core body temperature, heart rate, and activity.

Core body temperature: Square vs. Sine

While circadian rhythms are often thought of and stylized as sinusoidal, on visual inspection, the overall circadian rhythm of CBT in these mice seems to be shaped more like a square wave than a smoothly changing sine wave. One factor likely partly responsible for this is the experimental set-up, in which the LD cycle was maintained as a square wave with 12-hrs lights-on and 12-hrs lights-off. It is known that the light-dark cycle can entrain the circadian rhythm (Duffy and Wright 2005), providing a mechanism for this forcing. The effect of the square-wave forcing by the LD cycle can be seen clearly in the CBT rhythm: in every case, the turning on of the lights was immediately followed by a sharp decline in CBT (Figure 1).

To give a rigorous answer to the question of whether a square pulse or a sine wave best describes the CBT rhythms, we used a Model Selection approach, employing the Akaike Information Criterion (AIC), to choose between a sine function and a square wave (Figure 2a). Based on the AIC, the best model fit is the square wave (Figure 2b). The ΔAIC, the difference in AIC between the square and sine waves was −1402 (−1840,-377)

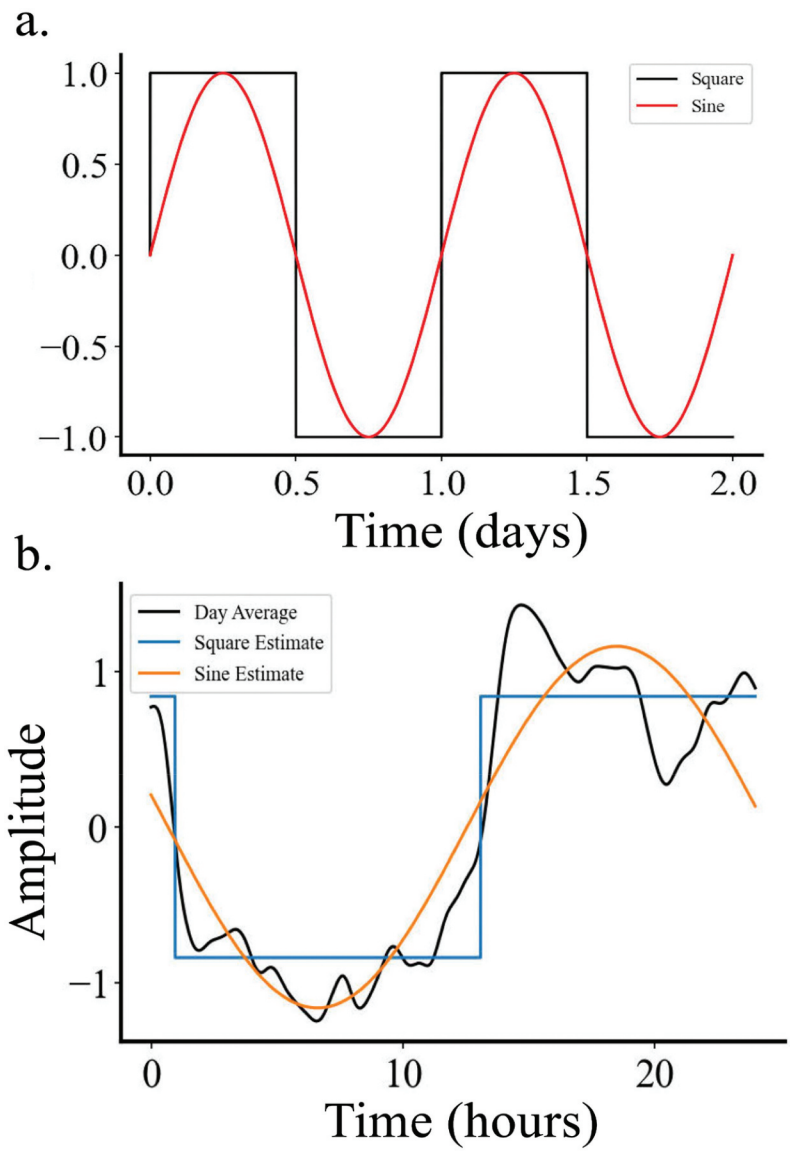


Figure 2. (a) Model selection candidates. (b) Example of best fit model selection results superimposed on the average core body temperature waveform.

[mean, (95% CI)] (see Methods and Supplemental Figure S3), indicating that the square wave is a statistically significantly better model.

Ultradian oscillations

In addition to the 24-hr rhythm, there appear to be oscillations at several ultradian frequencies, producing highly scalloped waveforms. In the WT mice, these ultradian peaks and troughs, for CBT, heart rate, and activity line up exactly synchronously with each other (Figure 1).

Another important feature of the circadian rhythm in CBT, visually apparent, is the obvious difference between the rhythms seen in the daytime as opposed to those seen at night: the nighttime was marked by large-amplitude ultradian oscillations, giving the impression of large scallopings of the night-time waveform. These ultradian oscillations were much less pronounced in the daytime (Figure 1).

Fourier analysis

To quantify the periodic contributions to the waveform, we carried out Fourier analysis of the CBT records. Fourier analysis revealed several highly statistically significant peaks, as confirmed by resampling-based significance testing (see Methods) (Figure 3). The largest and leftmost peak represents the 24-hr circadian rhythm. In addition, in each wild-type mouse, there are statistically highly significant

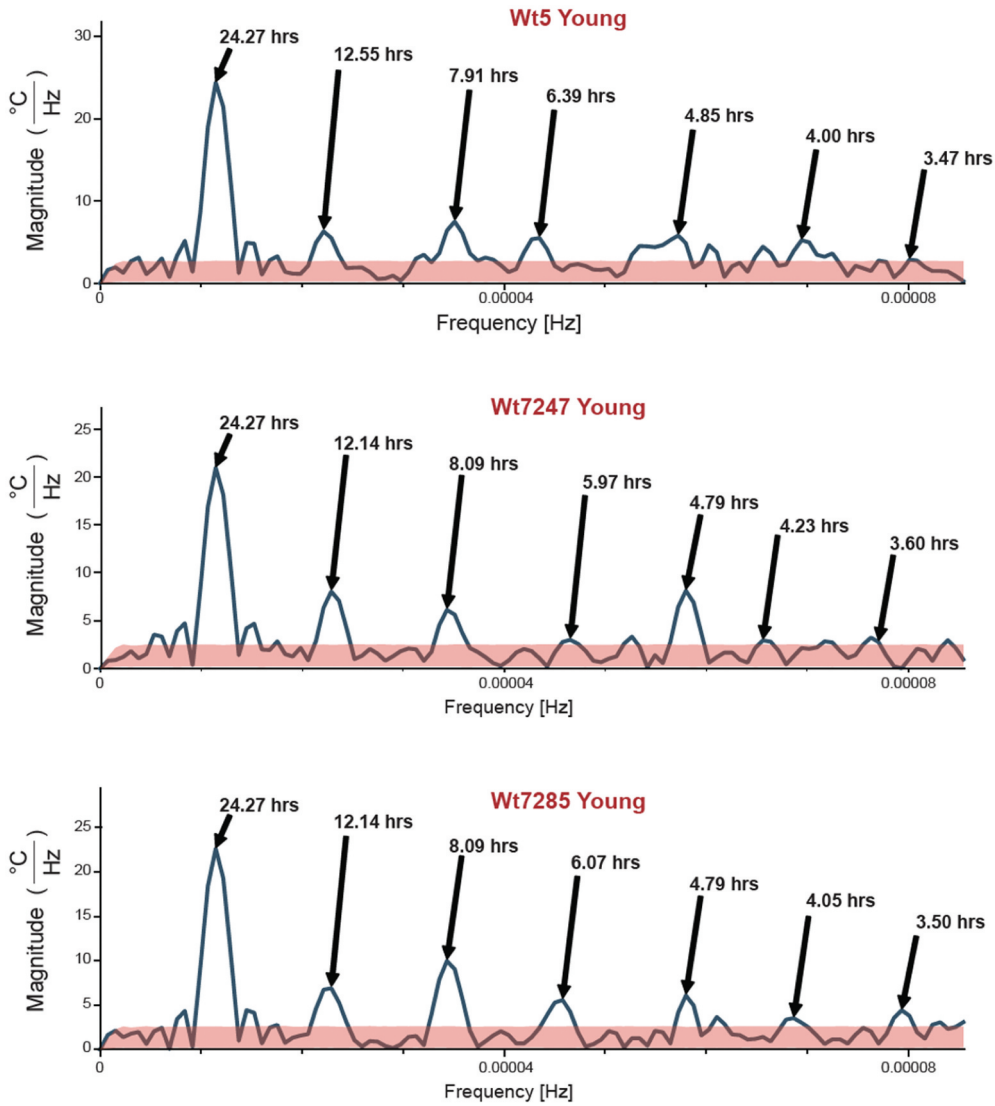


Figure 3. Fourier spectra of core body temperature in 3 WT mice, aged 4–5 months. Pink shaded area indicates the cutoffs for the 0.005 (bottom edge) and 99.995 (top edge) percentiles of 10,000 repetitions of the Fourier transform on permutations of the data (see Methods). Peaks rising above the pink area are therefore statistically significant at the 0.01 level.

peaks ($p < .01$) at 12-hrs, 8-hrs, 6–6.4-hrs, and 4.8-hrs. The Fourier analysis therefore confirms the existence of multiple ultradian peaks.

WT: the effects of aging

In the CBT rhythm, the middle-aged WT mice exhibited a robust rhythm, as seen in both waveform and Fourier analyses. There were, however, significant differences in the expression of the higher-frequency rhythms. In particular, there was a marked loss of the 12-hr peak and a diminution of the 6–6.4-hr peak in these middle-aged mice (Figure 4a). By contrast, the peak corresponding to the 8-hr rhythm was relatively spared. Thus, the primary impact of the aging in these animals appears to be a loss of some of the higher frequency, ultradian, rhythms rather than a simple loss of the circadian rhythm itself.

Young healthy versus young HD model

Both heterozygous and homozygous HD young mice showed a loss of the 12-hr peak (similar to the aged wild-type). The 12-hr peak was diminished in young heterozygous mice (Figure 4b, top) and diminished or absent altogether in the young homozygous mice (Figure 4b, bottom).

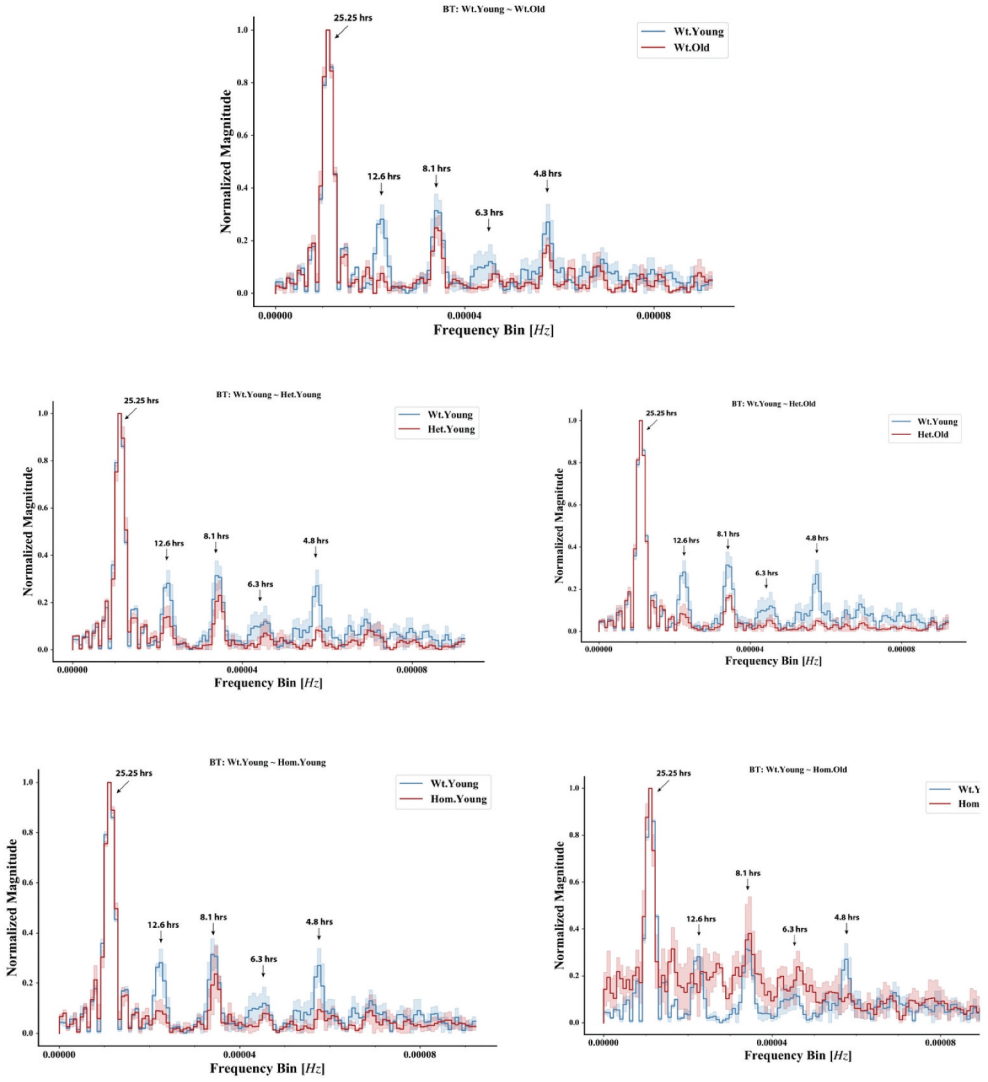


Figure 4. (a) Fourier spectra of core body temperature in 3 WT mice (solid line indicates the average of the 3 mice, while shaded areas indicate 95% confidence intervals). The blue line represents mice aged 4–5 months, while the red line represents the same mice aged 10–11 months. (b) Fourier spectra of core body temperature in 3 WT, 4 Het, and 4 Hom mice. Blue lines represent young WT; red lines represent young Het (top) and young Hom (bottom). In both groups of diseased mice, the 12-hr and 6-hr peaks were lost: a sign indicating the presence of amplitude modulation of the 8-hr ultradian rhythm by the 24-hr fundamental. (c) Fourier spectra of core body temperature in WT, Het, and Hom mice. Blue lines represent young WT; red lines represent aged Het (top), and aged Hom (bottom). The aged Hom mice had such irregular body temperature rhythms that the Fourier spectra of these records all had high noise floors, making interpretation of these results difficult.

Middle-aged HD mice

In the aged heterozygous mice, while there is a clear 24-hr cycle that is present both visually and on Fourier analysis, there is also a loss of the peaks at 12- and 6–6.4-hrs (Figure 4c, top). The loss of the 12- and 6–6.4-hr rhythms is also seen in the aged wild-type mice (Figure 4a).

In the aged homozygous mice, there is a complete loss of the 12- and 6–6.4-hr components, and in about 50% of the cases, a degradation of the overall 24-hr waveform (Figure 4c, bottom).

Fourier analysis of the rhythms

The Fourier spectrum of the CBT rhythm had highly significant peaks at 24-, 12-, 8-, 6- and 4.8-hrs. These periods are the fundamental 24-hr period and its 2nd, 3rd, 4th, and 5th harmonics.

There will be harmonics in the spectrum of *any* square wave. In our wild-type mice, as we saw, the 24-hr rhythm in CBT resembled a square wave; we will stylize it as a square wave with a 12-hr 'on' (= 1) and a 12-hr 'off' (= 0) pattern (Figure 5a). The Fourier analysis of this pure square wave consists of peaks at 24-hr, 8-hr, 4.8-hr, and higher frequencies (Figure 5b), the higher harmonics of the square wave that are required to create the square shape.

But note that these are all, and only, *odd* harmonics: The fundamental period (1st harmonic) is 24-hrs, and the 8- and 4.8-hr rhythms are the 3rd and 5th harmonics, respectively. *Therefore, the existence of the 24-hr square wave cannot account for the peaks at 12- and 6-hrs, which are even harmonics.*

The most important example of a process that will produce a spectrum like Figure 2 is the phenomenon of *amplitude modulation*. Suppose, for example, that a sine wave with an 8-hr period is amplitude modulated by a 24-

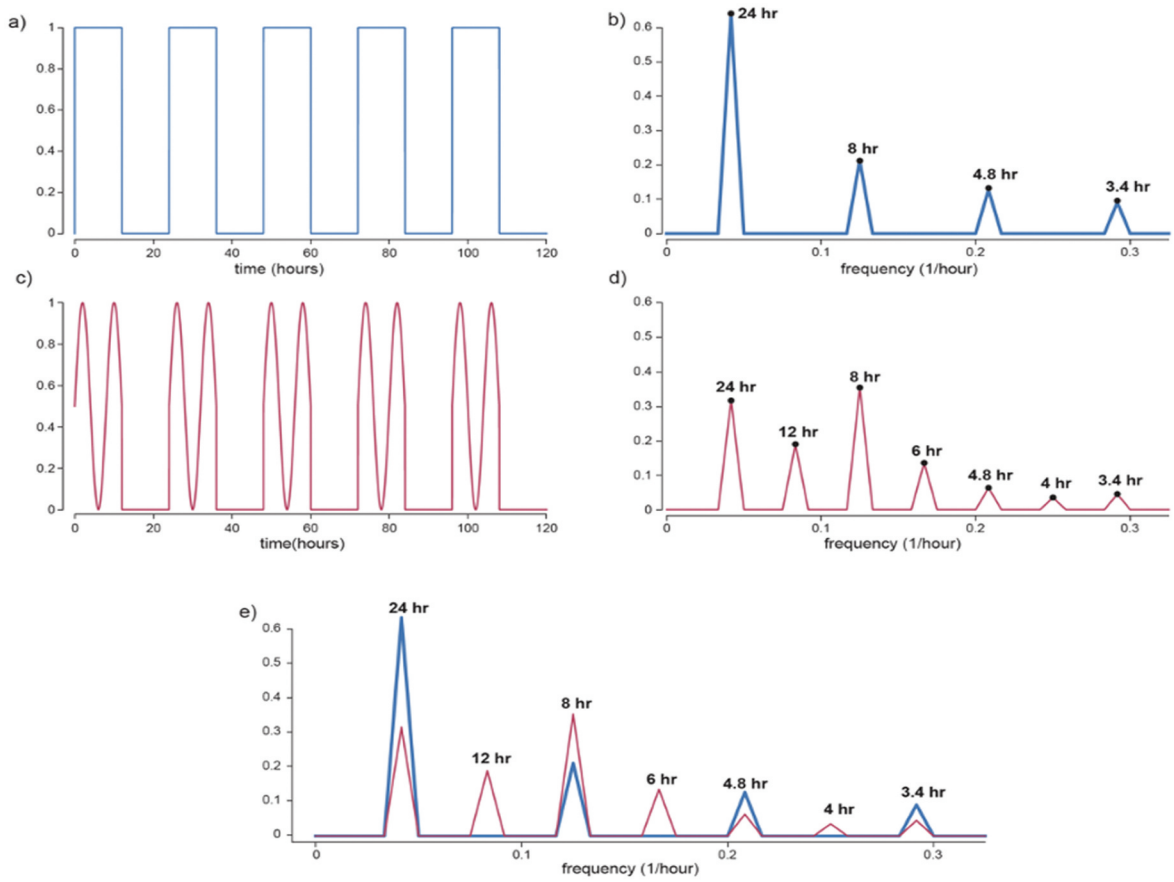


Figure 5. Time series (a) and Fourier spectrum (b) of a 24-hr square wave that is 12 hours 'on' and 12 hours 'off.' Time series (c) and Fourier spectrum (d) of an 8-hr sinusoidal rhythm that is amplitude modulated by the 24-hr square-wave. (e) Superimposed spectra of 12-hr square wave and modulated wave. Note that the 8-hr peak is higher in the modulated wave than in the square wave.

hr square wave that is 12-hrs on and 12-hrs off. The result is a wave that has the 8-hr rhythm expressed for half the day (Figure 5c).

The Fourier spectrum of the resulting wave (Figure 5d) shows a large peak at the 8-hr frequency, plus two 'sidebands' at 12- and 6-hrs. These sidebands are explained by Fourier analysis: if a wave A is amplitude-modulated by a slower wave B, the Fourier spectrum has a peak at the frequency of A, plus two additional sidebands at (frequency of A) *minus* (frequency of B) and (frequency of A) *plus* (frequency of B).

In this case the high frequency is 1/8-hr, and the low frequency is 1/24-hr, so the predicted side bands would be at 1/8 – 1/24 = 1/12 and 1/8 + 1/24 = 1/6, that is, at periods of 12- and 6-hrs, exactly what was observed in the real CBT records.

In other words, *the simplest model for the observed spectrum is that of a 24-hr on/off square wave modulating an 8-hr process that is more sinusoidal in shape, resulting in an 8-hr rhythm that is on during the night and off during the day.*

"de-squared" waveforms

As a further demonstration of the presence of 24-hr modulation of the ultradian rhythms, we constructed a "de-squared" waveform from the wild-type CBT records. Beginning with the CBT record, a 24-hr square wave was constructed from each trace, and we calculated the phase and magnitude of its Fourier transform. We then subtracted these components from the original spectrum and used the inverse Fourier transform to produce the "de-squared" waveform, that is, the original waveform minus the (square) 24-hr component (Figure 6). Note that the de-squared waveform removes the high- and low-amplitude effects, but still shows a clear separation of nighttime and daytime rhythms in their waveforms. In the nighttime, lower-frequency rhythms (i.e., the 8-hr component) dominate, whereas in the daytime, higher frequencies predominate, even after the amplitude variation due to the 24-hr rhythm is removed.

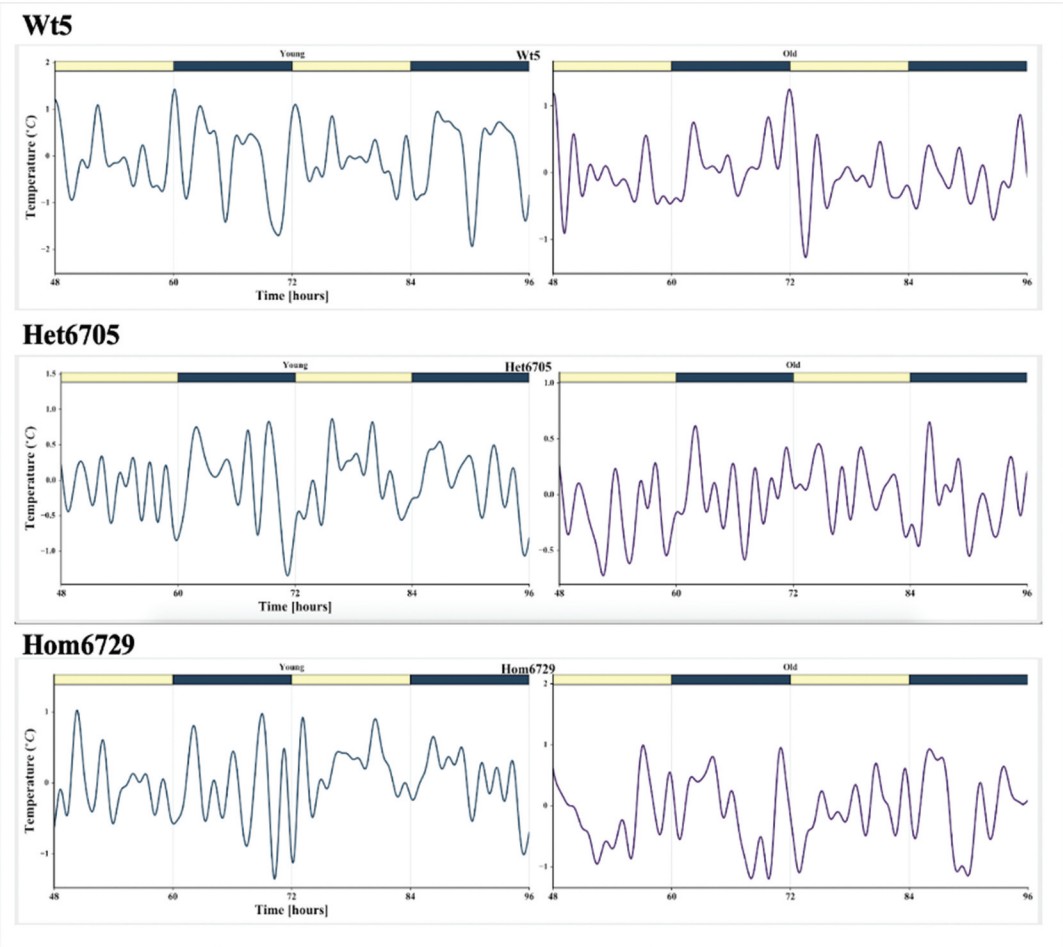


Figure 6. De-squared core body temperature time series for one mouse for each genotype at 4–5 months (left) and 10–11 months (right).

When we computed the power spectral density from the resulting signal, there were highly significant peaks at the 12-, 8- and 6-hr periods, indicating that the modulation effects on the 8-hr rhythm persisted even after the 24-hr wave itself (and its harmonics) were taken out of the record.

The 8-hr peak: 'Harmonic' or independent process?

There are two distinct ways in which the Fourier analysis of a 24-hr rhythm can have a significant peak at 8-hrs: (1) the 8-hr peak is a 3rd harmonic of a 24-hr non-sinusoidal waveform, necessary to shape the 24-hr waveform, or (2) over and above the 24-hr rhythm, there is a second, causally independent process that is occurring with an 8-hr period.

To illustrate, we created a 24-hr square wave with 12-hrs on and 12-hrs off, and allowed it to modulate an 8-hr sine wave (Figure 5a,c). Then we took the Fourier spectrum of the modulated signal and compared it to the spectrum of the square wave. (Figure 5b,d). Note that the 8-hr component of the modulated wave is significantly larger than the 8-hr peak from the square wave, indicating that the 8-hr peak in the modulated wave is due to something over and above the square wave's 8-hr harmonic (Figure 5e).

We then applied this analysis to the CBT data (see Methods). In each case, the 8-hr peak as well as the side bands corresponding to 12-hrs and 6-hrs, which both remained after subtraction of the 3rd harmonic of the 24-hr square wave, were still statistically significant, indicating that there is an independent 8-hr rhythm in the CBT signal (data not shown).

Discussion

We found that daily rhythms in CBT in WT mice are square in shape and, in addition, are sculpted by several higher-frequency rhythms. The square shape may well be due to the square wave in the LD condition, acting as a forcing on the CBT rhythm, although at least one study, using careful methodology, argued that the circadian rhythm in humans in heart rate and Mean Arterial Pressure is better modeled as a square wave than as a sinusoid (Idema et al. 1992). Further research is necessary to resolve whether the square wave would persist under LL or ramped-LD forcing.

Fourier analysis found several significant spectral peaks; interestingly, these occurred at both odd and even harmonics of the 24-hr fundamental. However, the Fourier analysis of a 24-hr square wave produces only odd harmonics, the 1st ('fundamental,' 24-hr), 3rd (8-hr), 5th (4.8-hr), etc. (Figure 5b). This follows from the Principle of Asymmetric Harmonic Distortion (Smith 2013), which says that if harmonics appear uniformly in all phases of the overall cycle, then the Fourier spectrum will have only odd harmonics. The presence of even harmonics in our spectrum, at 12-hrs (2nd) and 6-hrs (4th), therefore indicates that some harmonics are present in one phase (e.g., nighttime) but not the other (daytime). Thus, the principle explains in the Fourier spectrum *exactly what we observed in the time series record: large oscillations in the nighttime but not in the day*. The loss of these even harmonics with age and disease implies that in these conditions, the mice lose their ability to segregate rhythms into daytime and nighttime components.

The general phenomenon of an ultradian rhythm that is modulated into a "nighttime phase" in which it is expressed and a "daytime phase" in which it is quiescent, (or vice versa), may be common in circadian physiology. Consider, for example, the expression of estradiol in a healthy 27-year-old female (Licinio et al. 1998). Here, there is a clear high-amplitude 1-hr rhythm in the daytime which is absent at night (Supplemental Figure S2). Previous work has also informally supported the notion of ultradian rhythm modulation by the circadian rhythm: for example, in an investigation of ultradian rhythmicity of several physiological parameters in Q175 mice, including heart rate, CBT, and locomotor activity, Smarr and colleagues concluded that "ultradian rhythm amplitude is modulated by time of day" (2019).

The segregation into "daytime rhythms" and "nighttime rhythms" can be seen as an example of a "gating" phenomenon, in which a phenomenon is permitted in one phase of a cycle but not another. Gating has been observed at a variety of time scales: Sato and Numata reported a circadian gating of the sensitivity of emergence times to entrainment by moonlight (2014). At a much longer time scale, Olive and Garwood reported a circa-*annual* gating of the final stages of gametogenesis (1983).

In our mice, it is precisely the circadian gating function which is lost in the aged and diseased mice. With disease and age, there was a clear loss of the ultradian components of CBT, specifically the 12-hr and 6-hr components of the 24-hr rhythm. Interestingly, the 8-hr rhythm was generally preserved with age, at least in part, but lost its modulation into day and night modes.

The findings that are reported most frequently in the literature on aging and circadian rhythms are a decrease in the amplitude or an outright loss of the circadian rhythm. We did not see an overall loss of circadian rhythmicity in our middle aged (10–11 mo.) wild-type animals. We also did not see a diminution or loss of the overall circadian rhythm in young (3 mo.) animals with the HD mutation, whether homozygous or heterozygous. In older, diseased mice, however, there was

a highly significant loss of the day/night modulation and in some cases overall degradation of the circadian rhythm. Consequently, the principal effects of aging in our mice consisted in the loss of the higher-frequency peaks and their modulation by the 24-hr cycle. Moreover, age and the HD phenotype acted synergistically, resulting in a marked loss of both circadian and ultradian rhythmicity in the aged heterozygous and homozygous animals.

The frequency which was most greatly diminished or lost with age and disease was the 12-hr rhythm, the second harmonic of the 24-hr rhythm. It is the largest component of asymmetric harmonic distortion, and its loss is the sign in the Fourier spectrum that the modulation segregating "daytime oscillations" from "nighttime oscillations" is diminished in these aging mice. Overall, our results align with the understanding that HD mice display an accelerated aged phenotype (Kudo et al. 2011).

Our findings also indicate that the 8-hr rhythm was preserved with age and mutation, but its gating, or modulation, by the 24-hr rhythm (i.e., the 12-hr and 6-hr rhythms) was lost with aging and disease. This caused a loss of segregation between daytime and nighttime rhythms in CBT.

The question remains: what is the 8-hr rhythm? Previous work has found evidence of several physiological parameters which may be regulated by or contribute to an 8-hr ultradian process. Hughes et al. discovered an independent 8-hr rhythm of zinc finger protein 560 gene expression in murine hepatocytes *in vivo* (2009). van der Veen and Gerkema (2017) confirmed the finding of 8-hr rhythms in murine hepatocyte gene expression *in vitro*, using data from Hughes et al. (2009). Their intention was to "[unmask] optional ultradian rhythms and [prohibit] the occurrence of harmonics of a fundamental circadian signal." However, one of the authors' criteria, which they deemed essential for the existence of an independent ultradian process, was the continuous expression of the ultradian process throughout the dataset. This precludes the possibility of day-night segregation in ultradian rhythms, which we have found in our data and appears to be visibly present in the data from van der Veen and Gerkema (2017).

In contrast to removing only the 24-hr component, our methodology eliminates the 24-hr square wave *as well as its harmonics* (which are necessarily odd). After the square wave was removed, a significant peak in the Fourier spectra corresponding to an 8-hr frequency remained, which supports our conclusion that an intrinsically driven 8-hr rhythm exists. Additionally, two sidebands of the 8-hr peak, corresponding to 12- and 6-hrs, remained after the square wave removal, indicating that the independent 8-hr ultradian rhythm is modulated by the circadian rhythm. The presence of a robust, coexistent 8-hr ultradian rhythm in addition to the circadian rhythm demonstrates clear evidence that CBT oscillates on an ultradian scale in addition to its circadian regulation. The idea that CBT regulation incorporates asymmetric harmonic distortion, or, to speak physiologically, the segregation of rhythms into one phase but not the other, presents a new framework for our understanding of CBT rhythmicity. The loss of circadian modulation of the 8-hr rhythm with aging and disease also may indicate potential pathophysiological mechanisms associated with age and Huntington's disease.

The control of CBT is mediated by a hierarchically organized set of hypothalamic structures with the preoptic area and the median preoptic nucleus at the top of it (Saper and Lowell 2014). Circadian rhythms in CBT are independent of locomotor activity but dependent upon an intact SCN (Ruby et al. 2002; Scheer et al. 2005; Stephan and Nunez 1977). Pre-symptomatic HD patients were reported to have an elevated daytime CBT (Schultz et al. 2021), but rhythms were not measured. Therefore, future studies will need to determine whether these ultradian rhythms that are so prominent in the mouse models are also present in the patient population. New technologies allow CBT to be continuously measured through wireless capsules, and perhaps even wearable devices, which should facilitate measurements in patient populations.

Acknowledgements

We are grateful to Aram Derbedrossian, Se-Young (Myki) Lee, Natalia Vargas, Sarah Popelka, and Lauryn Robinson for technical assistance in assembling and processing the data. The data were originally collected in the UCLA cardiovascular core headed by Dr. K. Roos.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

The author(s) reported there is no funding associated with the work featured in this article.

References

Aschoff J. 1981. A survey of biological rhythms. In: Aschoff J, editor. Biological rhythms. New York (NY): Plenoff Press, pp. 3–10.

Aschoff J, Fatranská M, Giedke H, Doerr P, Stamm D, Wisser H. 1971. Human circadian rhythms in continuous darkness: Entrainment by social cues. Science. 171:213–215. doi:10.1126/science.171.3967.213

Buhr ED, Yoo SH, Takahashi JS. 2010. Temperature as a universal resetting cue for mammalian circadian oscillators. Science. 330:379–385. doi:10.1126/science.1195262

Büttner D, Wollnik F. 1984. Strain-differentiated circadian and ultradian rhythms in locomotor activity of the laboratory rat. Behav Genet. 14:137–152. doi:10.1007/BF01076410

Cutler TS, Park S, Loh DH, Jordan MC, Yokota T, Roos KP, Ghiani Ca, Colwell CS. 2017. Neurocardiovascular deficits in the Q175 mouse model of Huntington's disease. Physiol Rep. 5. doi:10.14814/phy2.13289.

Duffy JF, Wright KP Jr. 2005. Entrainment of the human circadian system by light. J Biol Rhythms. 20:326–338. doi:10.1177/0748730405277983

Edery I. 2010. Circadian rhythms. Temperatures to communicate by. Science. 330:329–330. doi:10.1126/science.1197747

Efron B, Tibshirani R. 1991. Statistical data analysis in the computer age. Science. 253:390–395. doi:10.1126/science.253.5018.390

Grant AD, Newman M, Kriegsfeld LJ. 2020. Ultradian rhythms in heart rate variability and distal body temperature anticipate onset of the luteinizing hormone surge. Sci Rep. 10:1–12. doi:10.1038/s41598-020-76236-6

Hamilos DL, Nutter D, Gershtenson J, Redmond DP, Clementi JD, Schmaling KB, Make BJ, Jones JF. 1998. Core body temperature is normal in chronic fatigue syndrome. Biol Psychiatry. 43:293–302. doi:10.1016/S0006-3223(97)83214-3

Harper DG, Volicer L, Stopa EG, McKee AC, Nitta M, Satlin A. 2005. Disturbance of endogenous circadian rhythm in aging and Alzheimer disease. Am J Geriatric Psychiatry. 13:359–368. doi:10.1097/00019442-200505000-00004

Hughes ME, DiTacchio L, Hayes KR, Vollmers C, Pulivarthy S, Baggs JE, Panda S, Hogenesch JB. 2009. Harmonics of circadian gene transcription in mammals. PLoS Genet. 5:e1000442. doi:10.1371/journal.pgen.1000442

Idema RN. 1996. Ambulatory blood pressure monitoring: modeling of circadian blood pressure variation by square wave fitting. Rotterdam, Netherlands: Erasmus University Rotterdam.

Idema RN, Gelsema ES, Wenting GJ, Man In't Veld AJ. 1992. Modeling blood pressure profiles. Hypertension. 19:496–498. doi:10.1161/01.HYP.19.5.496

Kudo T, Schroeder A, Loh DH, Kuljis D, Jordan MC, Roos KP, Colwell CS. 2011. Dysfunctions in circadian behavior and physiology in mouse models of Huntington's disease. Exp Neurol. 228:80–90. doi:10.1016/j.expneurol.2010.12.011

Licinio J, Negrão AB, Mantzoros C, Kaklamani V, Wong ML, Bongiorno PB, Mulla A, Cearnal L, Veldhuis JD, Flier JS, et al. 1998. Synchronicity of frequently sampled, 24-h concentrations of circulating leptin, luteinizing hormone, and estradiol in healthy women. Proc Natl Acad Sci U S A. 95:2541–2546. doi:10.1073/pnas.95.5.2541

Loh DH, Kudo T, Truong D, Wu Y, Colwell CS. 2013. The Q175 mouse model of Huntington's disease shows gene dosage- and age-related decline in circadian rhythms of activity and sleep. PLoS One. 8:e69993. doi:10.1371/journal.pone.0069993

Morton AJ, Wood NI, Hastings MH, Hurelbrink C, Barker RA, Maywood ES. 2005. Disintegration of the sleep-wake cycle and circadian timing in Huntington's disease. J Neurosci. 25:157–163. doi:10.1523/JNEUROSCI.3842-04.2005

Neville, M; Stensitzki, T, Otten, R, et al. 2021. Non-Linear Least-Squares Minimization and Curve-Fitting for Python. San Francisco (CA): Github; [accessed 2021 June 18]. https://lmfit.github.io/lmfit-py/

Olive PJW, Garwood PR. 1983. The importance of long term endogenous rhythms in the maintenance of reproductive cycles of marine invertebrates: A reappraisal. Int J Invertebr Reprod. 6(5–6):339–347. doi:10.1080/01651269.1983.10510059

Panda S, Antoch MP, Miller BH, Su AI, Schook AB, Straume M, Schultz PG, Kay SA, Takahashi JS, Hogenesch JB. 2002. Coordinated transcription of key pathways in the mouse by the circadian clock. Cell. 109:307–320. doi:10.1016/S0092-8674(02)00722-5

Piccione G, Caola G, Refinetti R. 2002. The circadian rhythm of body temperature of the horse. null. 33:113–119. doi:10.1076/brhm.33.1.113.1322.

Rakshit K, Krishnan N, Guzik EM, Pyza E, Giebultowicz JM. 2012. Effects of aging on the molecular circadian oscillations in Drosophila. Chronobiol Int. 29:5–14. doi:10.3109/07420528.2011.635237

Refinetti R. 1992. Analysis of the circadian rhythm of body temperature. Behav Res Methods Instrum Comput. 24:28–36. doi:10.3758/BF03203466

Refinetti R, Kenagy GJ. 2018. Circadian rhythms of body temperature and locomotor activity in the antelope ground squirrel, Ammospermophilus leucurus. J Therm Biol. 72:67–72. doi:10.1016/j.jtherbio.2018.01.001

Ruby NF, Dark J, Burns DE, Heller HC, Zucker I. 2002. The suprachiasmatic nucleus is essential for circadian body temperature rhythms in hibernating ground squirrels. J Neurosci. 22:357–364. doi:10.1523/JNEUROSCI.22-01-00357.2002

Ruis JF, Rietveld WJ, Buys PJ. 1987. Effects of suprachiasmatic nuclei lesions on circadian and ultradian rhythms in body temperature in ocular enucleated rats. J Interdiscipl Cycle Res. 18:259–273. doi:10.1080/09291018709359952

Saper CB, Lowell BB. 2014. The hypothalamus. Curr Biol. 24:R1111–1116. doi:10.1016/j.cub.2014.10.023

Satoh A, Numata H. 2014. Circatidal rhythms and their entrainment to the tidal cycle in insects. In: Annual, lunar, and tidal clocks. In Numata, H, and Helm, B. eds. Tokyo: Springer. p. 25–39.

Scheer FA, Pirovano C, Van Someren EJ, Buijs RM. 2005. Environmental light and suprachiasmatic nucleus interact in the regulation of body temperature. Neuroscience. 132:465–477. doi:10.1016/j.neuroscience.2004.12.012

Schultz JL, Harshman LA, Kamholz JA, Nopoulos PC. 2021. Autonomic dysregulation as an early pathologic feature of Huntington disease. Auton Neurosci. 231:102775. doi:10.1016/j.autneu.2021.102775

Smarr B, Cutler T, Loh DH, Kudo T, Kuljis D, Kriegsfeld L, Ghiani CA, Colwell CS. 2019. Circadian dysfunction in the Q175 model of Huntington's disease: Network analysis. J Neurosci Res. 97:1606–1623. doi:10.1002/jnr.24505

Smith S. 2013. Fourier transform properties. In: Digital signal processing: A practical guide for engineers and scientists. Boston, MA: Elsevier, pp. 185–208.

Stephan FK, Nunez AA. 1977. Elimination of circadian rhythms in drinking, activity, sleep, and temperature by isolation of the suprachiasmatic nuclei. Behav Biol. 20:1–61. doi:10.1016/S0091-6773(77)90397-2

Tu BP, Kudlicki A, Rowicka M, McKnight SL. 2005. Logic of the yeast metabolic cycle: Temporal compartmentalization of cellular processes. Science. 310:1152–1158. doi:10.1126/science.1120499

van der Veen DR, Gerkema MP. 2017. Unmasking ultradian rhythms in gene expression. FASEB J. 31:743–750. doi:10.1096/fj.201600872R

Weinert H, Weinert D, Waterhouse J. 2002. The circadian activity and body temperature rhythms of mice during their last days of life. Biol Rhythm Res. 33:199–212. doi:10.1076/brhm.33.2.199.1311

Wollnik F, Turek FW. 1988. Estrous correlated modulations of circadian and ultradian wheel-running activity rhythms in LEW/Ztm rats. Physiol Behav. 43:389–396. doi:10.1016/0031-9384(88)90204-1

Yamazaki S, Numano R, Abe M, Hida A, Takahashi R, Ueda M, Block GD, Sakaki Y, Menaker M, Tei H. 2000. Resetting central and peripheral circadian oscillators in transgenic rats. Science. 288:682–685. doi:10.1126/science.288.5466.682