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The Baseline Cosinus Function: A Periodic Regression Model for Biological Rhythms

T. Ruf

ABSTRACT

A periodic regression model, named the Baseline Cosinus Function (BCF), was designed to fit biological rhythms that show temporal deviations (peaks) above or below an otherwise relatively stable baseline. The BCF model has four parameters only, namely, baseline, peak-height, acrophase, and peak-width. BCF-regressions to daily rhythms in urinary 6-sulphatoxymelatonin (aMT6s), hypothalamic glutamate concentration, and body temperature of hamsters are compared to fits of single (SCF) and complex cosine functions (CCF; using the fundamental and the first harmonic). Goodness of fit statistics show that BCF-regressions to aMT6s-profiles of 36 hamsters resulted in lower residual errors than both SCF and CCF regressions, in particular when rhythms were determined under long photoperiod (n = 18) with relatively short nocturnal peaks (χ^2 = 316.6, 142.7 and 74.5 for SCF, CCF and BCF, respectively). For aMT6s rhythms obtained from hamsters in short photoperiod (n = 18) with prolonged nocturnal peaks, goodness of fit was equivalent in CCF and BCF regressions (χ^2 = 326.3, 107.0 and 101.4, for SCF, CCF, BCF, respectively), while BCF requires one parameter less than CCF. BCF-fits to daily patterns of hypothalamic glutamate and body temperature demonstrate that this model may be applied to various data types and has particular advantages when rhythms are sharply peaked, and when an independent estimate of peak-width, i.e., the total duration of a rise above the baseline, is desired.

Abstracting keywords: Biological rhythms, Cosinor, circadian rhythm analysis, periodic regression, pineal, melatonin, aMT6s, glutamate, body temperature, daily torpor.

INTRODUCTION

Cosinor analysis (Halberg et al., 1972) is probably the most widespread method for analyzing rhythmical biological data, in particular if the number of sampled periods is one, or very small. The initial, and most important step in Cosinor analysis is fitting a sinusoidal function with a given period to a time series of data points, which yields estimates for the goodness of fit, and for the mean, amplitude, and phase angle of the peak of the rhythm studied. These parameters may be subjected to further circular or common statistical analysis (Batschelet, 1981). In the Single Cosinor Analysis (SCA), a single cosine function (SCF) is used for the periodic regression. Since many rhythms are not well described by a simple sinusoidal curve, data are sometimes analyzed by fitting a trigonometric polyno-

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mial that consists of the fundamental period (typically 24 hours for daily rhythms) and the first or further harmonics (12, 8 h, etc.), i.e., Complex Cosinor Analysis (CCA). Although applying these complex cosine functions (CCFs) may result in excellent fits (e.g., Bliss and Blevins, 1959; van Cauter, 1979), the resulting coefficients, in particular for amplitude and phase angle of the non-fundamental harmonics may have no immediate biological meaning. Also, as demonstrated below, for certain types of asymmetric rhythms both SCF and CCF regressions (when restricted to the first harmonic) can fail to result in a satisfactory goodness of fit.

These problems become most pronounced when data series, such as blood concentrations of chemical compounds, or, for example, body temperatures, show significant but brief deviations from an otherwise stable baseline value. For instance, the activity of the pineal gland, causing increased melatonin production in mammals and other vertebrates, is restricted to the night, while during the day, stable minimal amounts of this hormone are secreted (Reiter, 1981). Fitting both single and complex cosine curves to measured values of urinary 6-sulphatoxymelatonin (aMT6s, the main melatonin metabolite; cf., Stieglitz et al., 1995), in particular from animals kept under a long photoperiod with short nocturnal rises of pineal activity, does not always yield adequate model curves and may even result in evidently incorrect estimates for the amplitude and duration of nocturnal elevations in hormone secretion (cf., Lerchl and Partsch, 1994). Similar problems can arise, for instance, if usual periodic functions are regressed to body temperature measurements in mammals or birds that show brief hypothermic episodes, i.e., daily torpor (Lynch et al., 1978; Ruf et al., 1991).

Certainly, one possible solution to these obstacles is the calculation of longer partial sums of a Fourier-series that will significantly improve fitted curves (e.g., van Cauter, 1979). However, this approach also results in an increased number of parameters, that may not be of actual biological relevance especially if the rhythm investigated is merely skewed or peaked. Another periodic function, apart from SCF and trigonometric polynomials, suggested by Batschelet (1981) to be used on “flat-topped” or “sharp-peaked” oscillations, can also cause difficulties due to underestimation of the actual amplitude and to the occurrence of secondary peaks, in particular when main peaks are extremely short (Batschelet, 1981). Further, this function contains somewhat complicated parameters for “peakedness” and mesor of the curve.

This paper therefore provides a new periodic function, specifically designed to describe rhythmical, temporary deviations of data series from a generally stable baseline, hence called the Baseline Cosinus Function (BCF). Essentially, this function is based on “cutting off” part of a cosine curve (with a known period) at a certain level. Nonlinear regression of this function to rhythmical data then produces estimates for the base-level, as well as for the height, acrophase and dura-

tion of the peak. Results of this procedure, as applied to measurements of urinary 6-sulphatoxymelatonin excretion, hypothalamic glutamate content, and body temperatures of hamsters, are compared to regressions of SCF and CCF.

METHODS

Data collection

Daily rhythms of urinary 6-sulphatoxymelatonin (aMT6s) excretion in Djungarian hamsters (*Phodopus sungorus*), maintained under either a long photoperiod (LD, 16 h of light per d; n = 18) or a short photoperiod (SD, 8 h of light per d; n = 18), were determined radioimmunologically, and kindly supplied by A. Stieglitz. Details on the experimental conditions and assay procedures are described in Stieglitz et al. (1994, 1995). Due to the small body size of Djungarian hamsters (30 g), the rate of urine production is limited. Therefore, not more than 8 samples per day could be collected. Data on hypothalamic concentrations of glutamate in Syrian hamsters (*Mesocricetus auratus*) were kindly provided by J.D. Glass. Glutamate was measured using the microdialysis technique as described in Glass et al. (1993). Body temperatures were recorded in Djungarian hamsters using temperature transmitters (Minimitter, model XM), as outlined in detail elsewhere (Ruf and Heldmaier, 1987; Ruf et al., 1991).

Data evaluation and statistical analysis

Three periodic functions were fitted to each set of data collected over periods of 24 to 27 h. The first function fitted (SCF) was

$$y[x] = m + A \cdot \cos(x - \phi), \qquad [1]$$

where x represents the time of day (in radians), m the mean of data points, A the amplitude of the rhythm, ϕ the acrophase (the phase of the crest), and y the predicted value of the variable at time x. Here, as in the other functions used, the period of the rhythm analyzed was assumed to be 24 h. Hence, $x = 2\pi \cdot t/24$, when the time t is expressed in hours. The second function used (CCF) was

$$y[x] = m + A_{24} \cdot \cos(x - \phi_{24}) + A_{12} \cdot \cos(2x - \phi_{12}). \qquad [2]$$

Variables in this function are as in eq. 1, except that the 1st harmonic (12 h) is added.Thirdly, periodic regression was performed with the following equation (BCF):

$$y[x] = b + \frac{H}{2 \cdot (1 - c)} \cdot \left(\cos(x - \phi) - c + \left|\cos(x - \phi) - c\right|\right). \qquad [3]$$

In this function, x again represents the time, ϕ indicates the acrophase, b represents a baseline level, and H is the height of sinusoidal peaks above or below this baseline. Like the amplitude and acrophase determined from regressions of the above models, H, which is equivalent to the range of the function, and ϕ, may be used for further plotting and testing of circular distributions.

The rationale for eq. 3 is that, as long as the term $(x - \phi) - c$ is positive, a fraction of a cosine curve (the peak) is added to b, while the model curve remains at the baseline when $(x - \phi) - c$ is negative. The coefficient c determines which fraction of a cosine curve is added to b, and thus determines peak-width. This coefficient has no dimension and varies between –1 and +1. When $c = 0$, the peak-width is equal to half of the total period. Thus, half of the cosine curve remains above the baseline and the peak duration in daily rhythms is 12 h. With $c < 0$, peak duration increases and *vice versa*. Peak duration (*D;* in [h]), i.e., the time interval during which the model curve deviates from the base-level, can be calculated from $D = arc\cos(c) / \pi \cdot 24$. Importantly, if the BCF model is fitted to truly sinusoidal rhythms, c will approach –1 or +1, at which point the BCF generates a simple cosine curve, such as the curve from eq.1.

To ease visualization of rhythms, all graphs show double-plots of both data and fitted curves. Regression procedures and statistics were, however, restricted to single data-sets. Nonlinear regressions were carried out using the Levenberg-Marquardt method to minimize the sum of squared residuals. The overall significance of the model fitted was tested by ANOVA. For data sets without replicates, coefficients of determination (r^2 ; in %) are given as a measure for goodness of fit. In data sets with replicates, i.e., aMT6s rhythms from hamsters adapted to LD and SD for at least 6 wks (n = 18 each), the χ^2 -test on the sum of weighted squared residuals was used to assess the goodness of fit (Press et al., 1992; Bardsley et al., 1995). The sequences of residuals from fits of all models, in the order of original measurements, were also subjected to the Runs test conditional upon the number of positive and negative residuals (Draper and Smith, 1981; Bardsley et al., 1995). Since the number of runs in residuals from regressions generally tends to be larger than expected from the theoretical distribution (Bardsley et al., 1995), one-tailed Run statistics were calculated to test for a clustered distribution only. Further, models were compared by calculating F-statistics on the residuals as described by Bardsley et al. (1995).

RESULTS

Examples for fits of the different periodic functions applied to daily rhythms of aMT6s in hamsters are shown in Fig. 1. It is typical for the duration of nocturnal peaks in this melatonin-metabolite to gradually increase after transfer of the an-

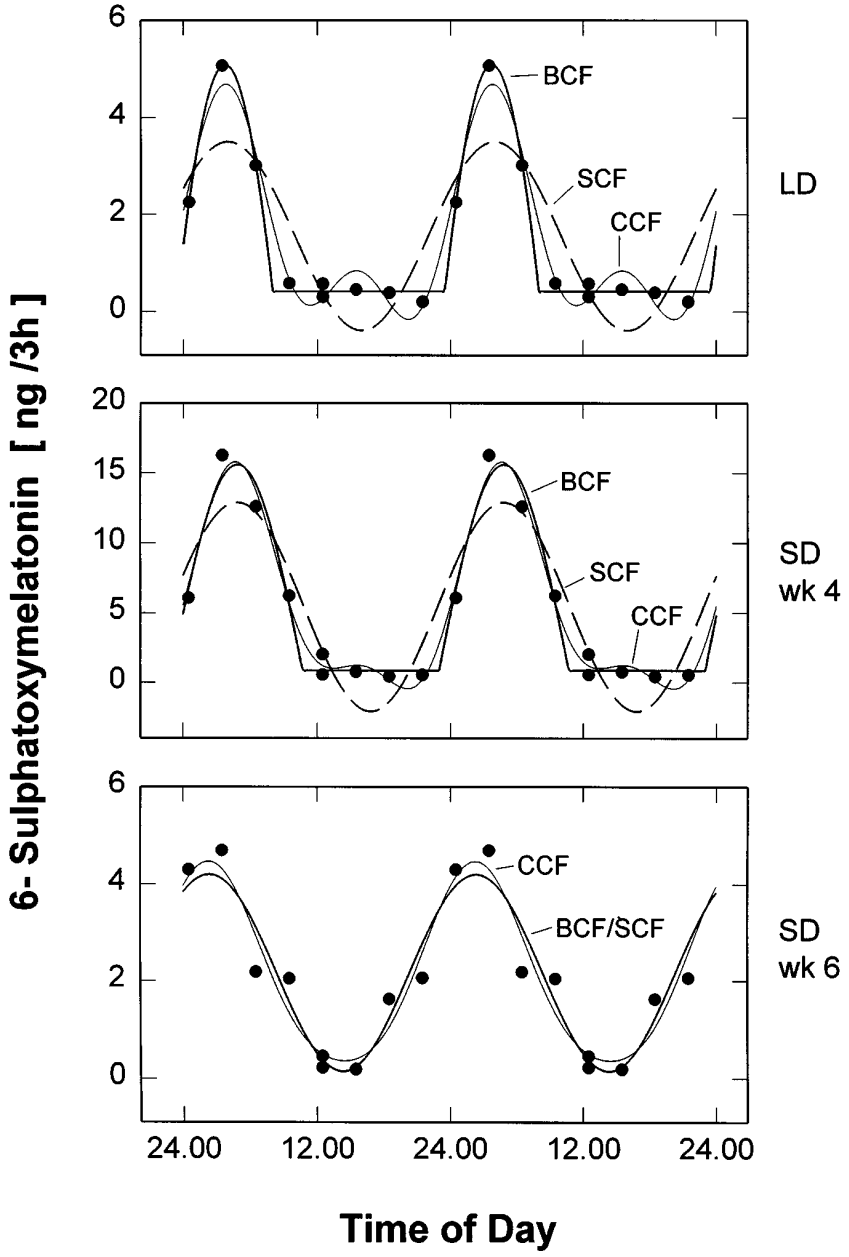

Fig 1. Double plots of individual profiles of urinary 6-sulphatoxymelatonin excretion in hamsters, and fitted model curves for SCF, CCF, and BCF. Data were determined for hamsters adapted to LD (top) and after 4 wks (middle) and 6 wks (bottom) in SD. While BCF yields best fits to rhythms consisting of sharp peaks above stable baselines (LD and SD wk 4), all three functions result in almost identical model curves when data are sinusoidal (SD wk 6).

imals from LD to SD. Differences in the goodness of fit between SCF, CCF, and BCF regressions were most pronounced when nocturnal aMT6s peaks were short, as under LD (Fig.1, top panel). For this type of daily pattern, SCF regressions clearly showed a poor goodness of fit and particularly tended to underestimate peak-amplitudes. CCF model curves on data from LD hamsters often showed secondary peaks (Fig. 1) that were not justified by the data profiles. Even for measurements from hamsters under SD, BCF regression frequently resulted in best χ^2s, particularly when compared to SCF fits (Fig. 1, middle; Table 1). Only in a relatively small number of individuals that showed an apparently sinusoidal pattern of aMT6s excretion, all three functions generated almost identical curves (Fig. 1, bottom). In these cases, BCF converged into a plain cosine function, with $c = -1$.

For a more detailed comparison, SCF, CCF and BCF were fitted to a total of 36 daily rhythms of urinary aMT6s excretion in hamsters maintained under either LD (n = 18) and SD (n = 18). In none of the residual sequences from either model was the number of runs significantly smaller than expected. Thus, Runs tests gave no indication for any of the models tested to be severely inappropriate for these types of rhythms. As summarized in Table 1, the goodness of fit, however, clearly differed between models. In regressions on both LD and SD data, χ^2 was smallest in BCF fits. Since the probability Q of large χ^2s occurring by chance is based on the number of parameters in each model (3 in SCF, 5 in CCF and 4 in BCF), differences in this quantitative measure for goodness of fit were even more pronounced.

A direct comparison of models using the F-statistic indicates that for both LD and SD aMT6s profiles, fitting CCF instead of SCF significantly improved the goodness of fit (LD: $F_{(CCF/SCF)} = 22.5$, $p < 0.0001$; SD: $F_{(CCF/SCF)} = 37.9$,

Table 1. Goodness-of-fit statistics for nonlinear regressions of SCF, CCF and BCF models to daily rhythms of aMT6s excretion in hamsters adapted to long (LD; n = 18) and short (SD; n = 18) photoperiod for 6 to 8 wks. Statistics are χ^2, the sum of squared residuals normalized to unit variance, and the probability Q that a value of χ^2 as large as determined should occur by chance.

	LD		SD	
	χ^2	Q	χ^2	Q
SCF	316.6	$2.5 \cdot 10^{-7}$	326.3	$2.9 \cdot 10^{-10}$
CCF	142.7	$1.4 \cdot 10^{-6}$	107.0	0.005
BCF	74.5	0.881	101.4	0.193

$p < 0.0001$). As expected, a significant further reduction of residual errors after the application of BCF was found in regressions on LD-profiles, with relatively short nocturnal peaks of aMT6s excretion. ($F_{(BCF/CCF)} = 17.4$, $p < 0.001$). BCF regressions to SD rhythms did not significantly improve fits as compared to CCF ($F_{(BCF/CCF)} = 1.03$, n.s.).

DISCUSSION

The present results indicate that, for certain types of rhythm profiles, BCF regressions can have significant overall advantages over both SCF and CCF models. As compared to SCF, fitting BCF can substantially reduce the residual error and usually results in more realistic estimates of peak amplitude, in particular when the rhythm is characterized by relatively short peaks, as in aMT6s profiles of hamsters under LD. However, it was a desired feature of the BCF to "automatically" generate a simple cosine curve (once *c* becomes -1) if the rhythm showed a completely sinusoidal pattern. Thus, whenever there is variability in the shape of rhythms studied, as among the SD aMT6s rhythms analyzed above, no *a priori* assumptions about the appropriate model have to be made, and the BCF model can be used safely. This may compensate for the fact that those cases resulting in simple cosine curves are clearly over-determined by the number of parameters used in the BCF model.

As compared to the CCF model, BCF has two clear advantages. Firstly, all coefficients have an immediate and easy-to-interpret biological meaning. Secondly, BCF requires one parameter less, which avoids overfitting of data and leads to larger values of Q (Table 1), indicating an improved goodness of fit even if the residual error is similar to CCF fits. The problems caused by large numbers of parameters were also the reason why the CCF regressions investigated here were restricted to the fundamental plus the first harmonic.

Another benefit from applying the BCF model is the inherent, independent estimation of peak duration which often is an important parameter of interest. For example, Lerchl and Partsch (1994) used CCF regressions to calculate the duration of nocturnal rises of melatonin secretion in humans. Peak duration was defined by the authors as the time period during which fitted CCF curves were greater than mean daytime-levels plus 3 standard deviations. When these data (taken from Fig. 2 in Lerchl and Partsch, 1994) are subjected to BCF regression, resulting curves show an almost identical goodness of fit (CCF: $r^2 = 96.4$ %; $F = 278.8$; $p < 0.0001$; BCF: $r^2 = 96.9$ %; $F = 460.0$; $p < 0.0001$). However, the BCF additionally provides an objective estimate for peak duration, of 12.08 h in this particular case. Hence, the use of BCF can avoid possible bias due to arbitrary selections of appropriate factors for threshold determination.

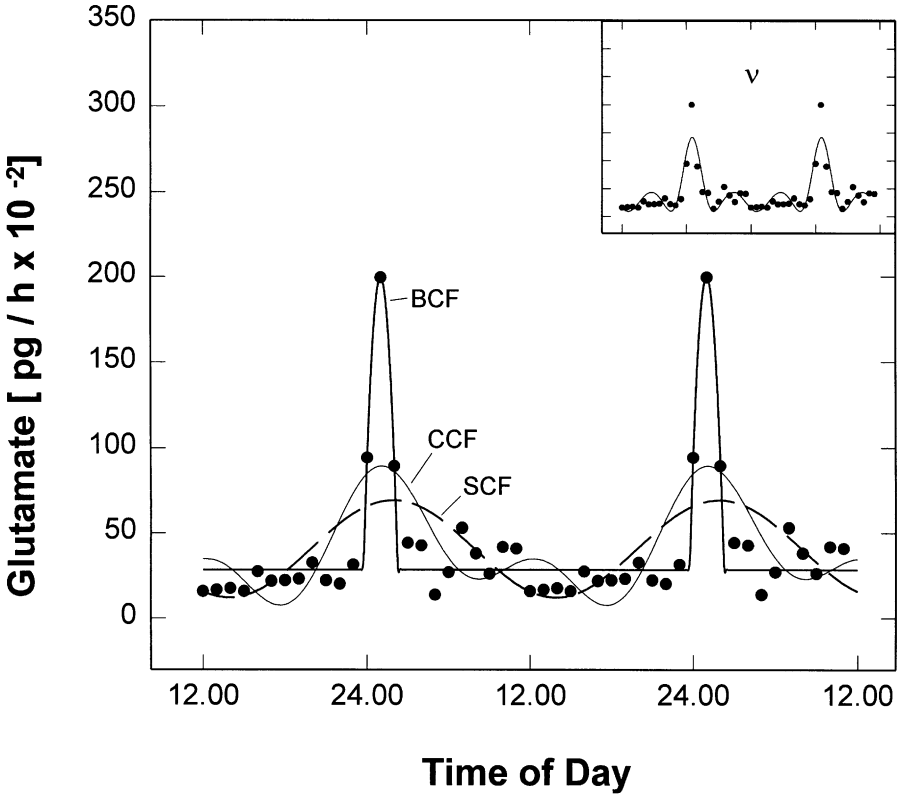

Fig. 2. Periodic regressions to a rhythm in hypothalamic glutamate concentration in Syrian hamsters (means from 7 hamsters; data from J.D. Glass) The main graph shows fits of SCF (r^2 = 28.4 %; F = 4.0; p = 0.035), as well as CCF (r^2 = 41.7 %; F = 3.2; p=0.037) and BCF (r^2 = 93.5 %; F = 91.4; p < 0.0001) . The inset graph shows the result of fitting a function suggested by Batschelet (1981). Goodness-of-fit statistics for this function were: r^2 = 75.67 %; F = 19.7; p < 0.0001). In both SCF and CCF fits the number of runs among sequences of residuals was significantly smaller than to be expected (p < 0.02 and p < 0.04 respectively), indicating that these models are inappropriate to describe the rhythm.

The most powerful field of BCF applications are certainly rhythms with significant, but extremely short peaks, that depart from a flat base-level. An example for this kind of rhythm is the time course of hypothalamic glutamate content in Syrian hamsters (Glass et al., 1993) as illustrated in Fig. 2. As nocturnal rises in glutamate in this species last less than 2 h, only the BCF model provides good estimates of peak-height and duration. Fig. 2 also includes predictions from another, less common function (labeled ν), that has been suggested by Batschelet (1981; eq. 8.3.6) to be used on “sharply-peaked” or “flat-topped” oscillations. This function has the form

$$y[x] = M + A \cdot \cos\left(x - \phi + v \cdot \sin\left(x - \phi\right)\right). \quad [4]$$

with parameters as in eq. 1, except for v which determines the 'peakedness' (Batschelet, 1981) of the curve and for M which is equal to the mean only if v=0. This equation was found to result in excellent fits to many of the aMT6s rhythms analyzed above. However, in particular for extremely "peaked" data, such as the pattern shown in Fig. 2, it not only underestimates the actual amplitude of peaks (as do SCF and CCF fits), but also tends to create substantial secondary peaks and troughs that are unwarranted by the data (cf., Batschelet, 1981). Moreover, while having the same number of coefficients, parameters in this equation are not as easily interpreted as in the BCF model suggested here. Finally, eq. 4, like SCF and CCF models, does not provide an objective estimate for the duration of rhythmic departures from the baseline.

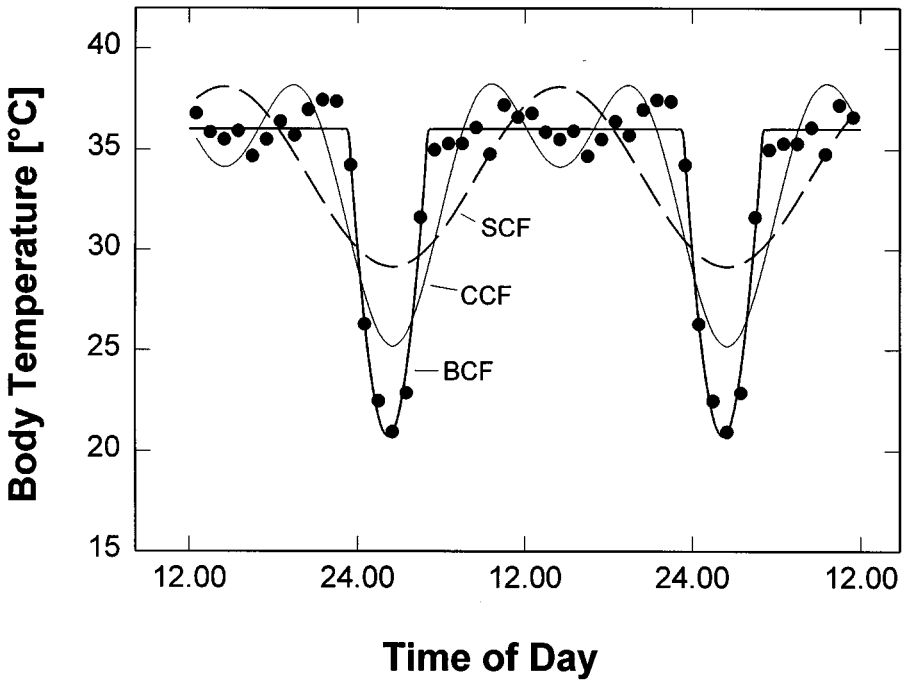

Fig. 3. SCF, CCF, and BCF regressions of the time course of telemetrically recorded body temperatures in a Djungarian hamster (double plot). The animals exhibits a bout of daily torpor, during which body temperature is lowered to about 21 °C. Goodness-of-fit statistics were: r^2 = 41.8 %, 74.7 % and 97.2 %, and F = 16.2, 31.7 and 512.6 for SCF, CCF and BCF, respectively. All regressions were highly significant ($p < 0.0001$), but the number of runs in residual sequences indicated a clustered distibution of positive and negative residuals resulting from both SCF ($p < 0.02$) and CCF ($p < 0.01$) regressions.

The preceding examples only concerned applications of the BCF model to measurements of chemical substances showing temporal rises above baselines. Of course, this function may be useful for other physiological measures, with negative amplitudes as well. For instance, Fig. 3 shows SCF, CCF and BCF fits to hourly means of body temperatures in a Djungarian hamster displaying voluntary hypothermia (daily torpor). Similar to the example shown in Fig. 2, a reasonable estimate of the actual magnitude of temporal changes in body temperature can be found using the BCF regression only. Also, this type of data illustrates another case in which arbitrary thresholds for the calculation of "peak duration", specifically the length of torpor bouts that were previously defined as the time spent below 30°C or 32°C (Lynch et al., 1978; Ruf et al., 1991), could be replaced by an independent statistic, namely, the coefficient c in eq. 3.

Evidently, the BCF as described in eq. 3 appears relatively complicated compared to equations 1, 2, or 4. However, it should be noticed that the underlying concept of "temporal windows" during which deviations from, and returns to an otherwise constantly regulated (or simply minimal) baseline occur, may not be uncommon for many biological rhythms. Hence, in certain cases this model could represent actual regulatory processes, such as daily rhythms in pineal activity or body temperature control, more realistically than the other functions discussed here. Also, the easy-to-understand meaning of parameters used in BCF may compensate for the somewhat complicated equation. For practical purposes, BCF fits pose no problem at all, since powerful nonlinear regression functions are available in many commercially available programs, such as SPSS© and MATHCAD©[1] or "shareware" programs like NONLIN. When the BCF model is applied, care should be taken to constrain the parameter c to numbers between –1 and + 1, because false minimal sums of squared residuals may occur if c is changed to values outside this range. Initial guesses for c may be obtained by estimating peak duration (D; in hours) from data plots and calculating $c = \cos(\pi \cdot D / 24)$. Good initial estimates of c, as well as of ϕ, will significantly improve the regression procedure and avoid false convergence.

It should be emphasized, that least-square fits of the BCF alone of course do not constitute a sufficient statistical analysis of time series. Although BCF fits usually result in not-converging regression attempts or in flat lines (with $H = 0$) when data fluctuate randomly, independent tests for randomness of time series may be used beforehand (see, for example, Zar, 1984). Also, the acrophase and peak-height obtained from individual BCF-fits may be combined to calculate vectors that can be subjected to tests for randomness of directions and further circular statistics as summarized by Batschelet (1981). If rhythm periods are un-

[1] A MATHCAD© 5.0 document that calculates and plots BCF regressions and goodness-of-fit statistics is provided in the appendix.

known, a periodogram method may be applied as in Single and Complex Cosinor procedures (Anderson, 1971). Since the BCF model is inappropriate for the detection of multiple ultradian fluctuations, more complicated procedures should be considered if data profiles contain substantial pulsatile or episodic components (e.g., van Cauter, 1979).

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APPENDIX

The following Mathcad 5.0 document fits the BCF function and displays the fitted curve, results for parameters and goodness-of-fit statistics. Example data are mean numbers of mice (*Apodemus flavicollis*) trapped at two hour intervals. Data are read from an AXCII-file containing two colums, time of day (t) and number of mice (y)

ORIGIN ≡, Start fields at 1, not 0.

data: = READPRN (datafile)

n : = 12, i : =…n, t : = data$^{<1>}$ y : = data

x_i : = $\frac{t_i}{24}\cdot 2\pi$ Calculate x in radians.

t =

	1
1	12
2	14
3	16
4	18
5	20
6	22
7	24
8	2
9	4
10	6
11	8
12	10

y =

	1
1	4
2	3
3	1
4	5
5	4
6	13
7	21
8	28
9	23
10	9
11	3
12	2

Define the BCF function:

$$F_{BCF}(x, \phi, b, H, c) : b + \frac{H}{2\cdot(1-c)} \cdot (\cos(x-\phi) - c + \left|\cos(x-\phi) - c\right|).$$

Sum of squares to be minimized:

$$SSE(\phi, b, H, c) : = \sum_{i=1}^{n} (y_i - F_{BCF}(x_i, \phi, b, H, c))^2, \text{ where}$$

ϕ : = 1, b: = 5, H : = 20, c : = 0.3. Initial guesses for parameters

Given

SSE (ϕ, b, H, c)=0, $c \le 1$, $\phi \ge 0$ $\phi \le (2\cdot\pi)$ Constraints.

1=1 2=2 3=3 Dummy equations required by Mathcad.

$$\begin{bmatrix} \phi \\ b \\ H \\ c \end{bmatrix} : = \text{Minerr}(\phi, b, H, c).$$ Find minimum sum of errors.

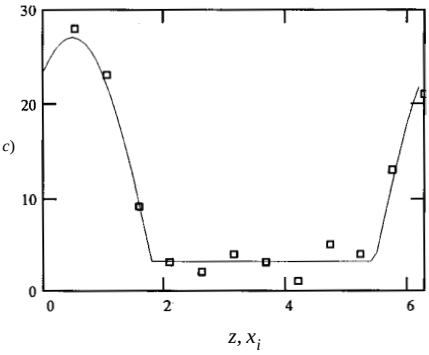
$\phi_h := \frac{\phi}{2\cdot\pi}\cdot 24$ $D := \frac{\arccos(c)}{\pi}\cdot 24$ Calculate peak phase [*h*] and peak duration [*h*].

Parameters:

$z \equiv 0,0.1\ldots 2\cdot\pi.$

$\phi = 0.491$	Acrophase
$\phi_h = 1.875$	Acrophase in h
$b = 3.144$	Baseline
$H = 23.903$	Peak height
$c = 0.264$	Cut-off constant
$D = 9.962$	Peak duarion in h

$\frac{F_{BCF}(z,\phi,b,H,c)}{y_i}$ □



Goodness of fit statistics:

Total_ SS := var(*y*)·*n* Resd_SS := SSE(ϕ,*b*,H,*c*,) Regr_SS := Total_SS – Resd_SS

$Regr\_MS := \frac{Regr\_SS}{3}$ $Resd\_MS := \frac{Resd\_SS}{n-4}$

$r\_square := \frac{Regr\_SS}{Totaal\_SS}\cdot 100$ $F := \frac{Resd\_MS}{Resd\_MS}$ $d1 := 3$ $d2 := n - 1$ Degrees of freedom

$r := \sqrt{}\ r\_square$

$x := \frac{d2}{d2+d1\cdot F}$ $a := \frac{d2}{2}$ $b := \frac{d1}{2}$ $p := \frac{\Gamma(a+b)}{\Gamma(a)+\Gamma(b)}\cdot\int_0^x u^{a-1}\cdot(1-u)^{b-1}\,du$

Results:

$r^2 = 97.94$	Percentage of variance explained.
$F = 126.801$	F statistic.
$p = 7.142\cdot 10^{-9}$	Probability $[F > F_{crit}]$.