Analysis of the circadian rhythm of body temperature

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Currently available methods for the analysis of circadian rhythms provide little help in the evaluation of the circadian rhythm of body temperature because they either assume that the circadian rhythm of body temperature approximates a cosine wave (which for many species is not true) or simply do not address important parameters, such as the amplitude and the shape of the temperature rhythm. The present paper presents methods for the computation and statistical evaluation of the period, amplitude, mean level, and general shape of the circadian rhythm of body temperature. The period is analyzed by the periodogram procedure, whereas the other parameters are analyzed by the histogram method. Both procedures are simple to implement and relatively insensitive to spurious data points. A simplified but fully operational program in BASIC is provided.

The first systematic observations that body temperature oscillates daily, with low values occurring during the period of lower activity and high values during the period of higher activity, were made almost one and a half centuries ago (Davy, 1845; Ogle, 1866). Laboratory studies of the circadian rhythm of body temperature in rodents were started almost 40 years ago (F. Halberg, Zander, Houghlum, & Mühlemann, 1954; Heusner, 1959). As interest in the continuous recording of body temperature grew, several devices for telemetric recording of body temperature in laboratory animals were developed (Cunningham & Peris, 1983; Fryer, Deboo, & Winget, 1966; Georgiev, 1978; Miles, 1962; Rawson, Stolwijk, Graicjem, & Abrams, 1965; Thornhill, Hirst, & Gowdey, 1978). Temperature telemetry devices are now available commercially and are increasingly being adopted by thermal physiologists and chronobiologists (Barnes, 1989; Honma & Hiroshige, 1978; Kittrell & Satinoff, 1988; Kluger, Conn, Franklin, Freter, & Abrams, 1990; Meinrath & D'Amato, 1979; Morley, Conn, Kluger, & Vander, 1990; Ruby, Ibuka, Barnes, & Zucker, 1989; Satinoff, Liran, & Clapman, 1982; Shibata & Nagasaka, 1982; Spencer, Shirer, & Yochim, 1976; Thornhill et al., 1978; Winget, Card, & Hetherington, 1968).

Like any other circadian rhythm, the rhythm of body temperature is characterized mainly by its period, which in the absence of external temporal cues is usually very close to 24 h. Other parameters that are equally important in the temperature rhythm are the mean level of body

temperature (sometimes called the *mesor*), the amplitude of the oscillation, and the shape (or waveform) of the rhythm. Although several procedures for the analysis of circadian rhythms have been described in the literature (e.g., Dörrscheidt & Beck, 1975; Nelson, Tong, Lee, & F. Halberg, 1979; Sokolove & Bushell, 1978), they are not satisfactory because they either assume that the circadian rhythm of body temperature approximates a cosine wave (which may or may not be true) or simply do not address important parameters, such as the amplitude and the shape of the temperature rhythm. Data presented in this paper clearly indicate that the assumption of cosine shape does not hold for all species, and previous research has shown that such parameters as amplitude and mean level have strong physiological significance. For instance, the amplitude of the body temperature rhythm is drastically lower in aged rats relative to young ones (J. Halberg, E. Halberg, Regal, & F. Halberg, 1981; Refinetti, Ma, & Satinoff, 1990). As a contribution to the analysis of circadian rhythmicity, the present paper presents methods for the computation and statistical evaluation of the period, amplitude, mean level, and general shape of the circadian rhythm of body temperature.

METHOD

Computations

For the analysis of the period of the circadian rhythm, the method of the periodogram was used. This method is especially attractive because of its simplicity, intuitive logic, and lack of assumptions about the nature of the data. The logic is as follows: given a set of data collected over time, if one breaks down the set into sections of period P , then the sections will be very similar to each other if the data set has period P , but not if the set has a different period. Thus, differences between sections can be used as an index of how well the chosen period describes the real period.

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Usually, indices for potential periods from 14 to 34 h are calculated, and the highest index is considered to correspond to the true period. For a data set with N values (i.e., X_i for $i=1$ to N), which can be broken down into K sections of period P , Sokolove and Bushell (1978) proposed the index Q_P , which is defined as follows:

$$Q_P = \frac{KN \sum_{h=1}^P (M_h - M)^2}{\sum_{i=1}^N (X_i - M)^2}, \quad (1)$$

where M_h is the mean of P values under each time unit of the period length, and M is the mean of all N values.

Q_P is the ratio of two sums of squares with multiplicative constants (K and N) in the numerator. Thus, Q_P is approximately distributed as Fisher's F with $P-1$ and $N-1$ degrees of freedom. However, because N is very large relative to P , the F distribution, in turn, may be ap-

Figure 1. Frequency distribution of 1,000 values of Q_P calculated from sets of 240 random numbers (bars) and chi-square distribution for $P-1$ degrees of freedom (continuous line) for three different periods.

Figure 2. Frequency distribution of 1,000 values of Q_P calculated from sets of random numbers (bars) and chi-square distribution for 23 degrees of freedom (continuous line) for three different sizes of the data set. The data sets had 48 points ($K=2$), 120 points ($K=5$), or 240 points ($K=10$). The period was 24 h in all three cases.

proximated by a chi-square distribution with $P-1$ degrees of freedom. Consequently, the statistical significance of Q_P can be determined by reference to the chi-square distribution (Sokolove & Bushell, 1978). To confirm the chi-square approximation to the distribution of Q_P , its sampling distribution was calculated by computing the statistic for a large number of random samples. Values of Q_P for different values of P were computed for 1,000 samples of 240 random numbers extracted from a uniform distribution in the interval 0 to 1. The sampling distribution of Q_P was then compared to the chi-square distribution (as determined by its standard equation).

Whether or not a given value of Q_P is statistically significant, the significance of differences between means of empirical values of Q_P can be tested using Student's t test or the analysis of variance. Thus, if a group of intact animals have maximal Q_P values at 23, 23, 24, 25, and 25 h, and if a group of brain-lesioned animals have maximal values at 21, 21, 22, 23, and 23 h, the difference

Figure 3. Simulated body-temperature histograms.

between the two means can be considered statistically significant [$t(8) = 3.16$, $p < .02$]. For small samples, or when the assumption of normality is not warranted, non-parametric tests, such as the Mann-Whitney U , are more appropriate (Krauth, 1988; Siegel, 1956). In the example of control and lesioned animals, the two groups can be considered to be different on the basis of the Mann-Whitney U test ($U = 2$, $p < .03$).

For the analysis of mean level, amplitude, and shape, the histogram method was used. As will be evident in the Results section, this method is not only simple and easy to use, but it also helps eliminate noise from the data without making assumptions about the nature of the noise. The procedure consists of preparing a frequency histogram for each value of body temperature (within a preselected range) in the data set. The histogram usually has a bimodal distribution, from which the modal amplitude of the rhythm (the temperature of one mode minus the temperature of the other) and the general shape of the rhythm (Ω , the frequency of the low temperature mode divided by the frequency of the other mode) can be easily calculated. The mean level of the rhythm can be calculated as the mean of all N values or as the mean of the two modes. All three parameters can be used for comparisons between or among groups with t tests and analyses of variance (or their nonparametric equivalents), as mentioned above for the period.

An actual implementation of the computational procedures is shown in Appendix 1. This is a program written in BASIC, which can be run without alterations in most interpreted or compiled versions of BASIC (e.g., GW-BASIC, BASICA, QuickBASIC, etc.). Although users may want to add several features to the program, it is fully operational as is. It assumes that the data are in an ASCII file as real numbers equally spaced in time (with user-selected bin size). The range of acceptable input is set to 34.0°–41.0°C, and values outside this range will be truncated by the program. When the program starts, the file name and the bin size are input and the necessary calculations are performed. The periodogram and histogram analysis are printed in tabular form, which is sufficient for the statistical analyses. Graphic presentation of the histogram and periodogram can be obtained using a graphics program. The period of the rhythm can be identified as the period with the largest Q_P in the periodogram table. Modal amplitude, modal mean, and the shape of the rhythm (Ω) can be calculated from the histogram table. To keep the analysis program short, tests of significance were not included. The significance of the obtained values of Q_P can be determined using the table in Appendix 2. Users may add routines to conduct tests of significance and to calculate the modes of the histogram.

An important consideration about the statistical evaluation of the periodogram is the level of significance to

Figure 4. Body-temperature records over 5 days (left) and the periodograms that describe their circadian periodicity (right). The two top graphs refer to a tau-mutant hamster, whereas the two bottom graphs refer to a normal golden hamster. The dashed line in the periodograms indicates overall significance level of 0.01.

be chosen. Because the significance of many Q_P s in the periodogram is tested simultaneously, the probability of obtaining a significant Q_P is inflated. As indicated by Sokolove and Bushell (1978), the overall level of significance (θ) can be derived from the individual levels of significance (α) as follows:

$$\theta = 1 - (1 - \alpha)^n, \quad (2)$$

where n is the number of periods in the periodogram. Thus, the critical values in Appendix 2 refer to $\alpha = 0.0005$ (for $\theta \approx 0.01$) and $\alpha = 0.0025$ (for $\theta \approx 0.05$). This is a very conservative approach, however, and some researchers may wish to adopt less stringent levels of significance. This is important if a researcher who collects data with 6-min resolution decides to calculate periodograms with 0.1-h resolution rather than with the 1.0-h resolution proposed by Sokolove and Bushell (1978) and discussed in this paper. A periodogram that spans 21 h (e.g., from $P = 14$ to $P = 34$), with 0.1-h resolution, will have 210 values of Q_P , so that α must be set at 0.00005 to obtain $\theta = 0.01$.

Example

Body temperature data were obtained from 1 female golden hamster (*Mesocricetus auratus*), 2 female tau-mutant hamsters (see Ralph & Menaker, 1988), 1 male tree shrew (*Tupaia belangeri*), and 1 male human volunteer.

The nonhuman subjects were kept in individual plastic cages (23 × 45 × 20 cm) under conditions of constant il-

lumination at either 23°C (hamsters) or 30°C (tree shrew). Small temperature-sensitive radio transmitters were implanted intra-abdominally under Nembutal anesthesia several days before the beginning of data collection. The signal from the transmitter was captured by an RA-1010 receiver and connected to a Dataquest Data Acquisition System (Mini-Mitter Co., Sunriver, OR), which sampled body temperature every 6 min. Data were collected for at least 10 consecutive days (240 readings per day) for each animal. Intra-abdominal temperature is significantly but regularly lower than colonic temperature by 0.5°–1.0°C.

Sublingual temperature of the human subject was measured at irregular intervals over a period of 8 days (eight readings per day) with an electronic clinical thermometer. No attempt was made to obtain a free-running circadian rhythm. For measurements during the period of sleep, the subject was awakened briefly (never more than once in any given night) and then allowed to resume sleep.

RESULTS AND DISCUSSION

Test of Periodogram

Figure 1 shows the sampling distribution of Q_P for different values of P . As noted earlier, the distribution of this statistic is approximated by a chi-square distribution with $P-1$ degrees of freedom. The adherence (fit) of the Q_P distribution to the chi-square distribution was tested with a chi-square goodness-of-fit test. Adherence was poor for $P = 10$ [$\chi^2(49) = 75$, $p < .02$], but was

Figure 5. Hourly means of body temperature calculated from multiple hourly measurements over 5 days (left) and histogram of the frequency of each body temperature (at 0.1°C resolution) over all data points (right) for 3 different species.

much better for values of P closer to the circadian range [for $P = 20$, $\chi^2(49) = 44$, $p > .6$; for $P = 30$, $\chi^2(49) = 41$, $p > .8$].

Because N (the total number of data points) is constant, increases in P (the length of the period) produce decreases in K (the number of data sections of size P). For this reason, and because an experimenter sometimes may have data for only 2 or 3 days, it is important to investigate the effects of changes in K when P is kept constant by varying N . Examples for three values of K are given in Figure 2. As suggested by Sokolove and Bushell (1978), a minimum of 10 days of data seems to be necessary for Q_P to maintain its chi-square distribution. The quality of the adherence of the Q_P distribution to the chi-square distribution was tested with a chi-square goodness-of-fit test. Adherence was good for $K = 10$ [$\chi^2(49) = 36$, $p > .9$],

but poor for smaller values [for $K = 2$, $\chi^2(49) = 240$, $p < .001$; for $K = 5$, $\chi^2(49) = 91$, $p < .001$].

Test of Histogram

Figure 3 provides four sets of simulated histograms based on artificial data. Graph A has $\Omega = 2.8$ (i.e., $0.11 \div 0.04 = 2.75$). This Ω larger than 1 indicates an animal (e.g., the hamster) that is inactive (or, to be more precise, has a lower level of body temperature) during most of the day. Graph B has $\Omega = 0.4$, which indicates an animal (e.g., the human) that is active during most of the day. Graph C has $\Omega = 1.0$, indicating a symmetrical distribution between (subjective) day and night. Graph D has $\Omega = 2.8$, which is similar to Graph A, because Ω is based only on the two modes. Values of Ω can be compared by usual statistical tests. For instance, a group of

8 animals with mean Ω ($\pm SE$) of 3.1 (± 0.5) is significantly different from a group of 8 animals with mean Ω of 1.2 (± 0.4) [$t(14) \approx 3.80$, $p < .01$]. The other two parameters of the circadian rhythm can also be easily calculated from the histogram. For instance, the two modes of Graph A in Figure 3 are 0.11 (at 36.8°C) and 0.04 (at 38.5°C). Consequently, the modal amplitude is 1.7°C (i.e., $38.5^\circ - 36.8^\circ = 1.7^\circ$ C). The mean level can be calculated from the weighted modal values: $\{[(36.8^\circ \times 0.11) + (38.5^\circ \times 0.04)] \div (0.11 + 0.04)\} = 37.2^\circ$ C.

Empirical Data

Empirical body-temperature data from a tau-mutant (top left panel) and a normal hamster (bottom left panel) are shown in Figure 4. The mutant clearly has a circadian period shorter than 24 h, since six of its cycles can be seen in only 5 days. The period of the normal hamster seems to be very close to 24 h. The periodograms shown on the right side identify significant periods in the range of 18–21 h for the mutant and 23–26 h for the normal hamster. The shapes of the peaks suggest that the mutant has a period of 19.6 h and the normal hamster has a period of 24.3 h. Previous data from this laboratory indicate that the 99% confidence interval for the circadian period of normal golden hamsters ranges from 23.7 to 24.3 h. Therefore, the mutant hamster shown in Figure 4 has a signifi-

cantly short period ($\tau = 19.6$ h), whereas the normal hamster is within the normal range ($\tau = 24.3$ h).

Typical circadian rhythms of body temperature plotted as hourly means for 3 different species are shown on the left side of Figure 5. In the hamster, body temperature is at about 37.2°C for about two thirds of the day and rises to 38.4°C in the remaining hours. In the human subject, the amplitude is also about 1°C; however, the lower temperature (down to 36.1°C) prevails for only one third of the day, whereas the higher temperature (about 37.3°C) prevails during most of the day. In this respect, the rhythms of hamsters and humans have opposite shapes. Accordingly, the histograms on the right side of Figure 5 have a higher left mode for the hamster but a higher right mode for the human subject. It is also evident in the histograms that the overall level of body temperature is lower in the human subject than in the hamster (i.e., the human histogram is farther left on the x-axis). Also shown in Figure 5 is the body-temperature rhythm of a tree shrew, which starts at a lower level, rises to a first peak, falls to an intermediate level, and rises again to a second peak before falling to the initial low level. This unusual shape of the circadian rhythm, which is extremely consistent from day to day (data not shown), is reflected in the unusual histogram that has a distinct right mode but a poorly defined left mode. The histogram also emphasizes the very large amplitude of the rhythm, with hourly means ranging from 36.5° to 39.7° C (and individual readings ranging from 35.6° to 40.3° C). The insets in Figure 5 show the mean, amplitude, and shape of the rhythms, as calculated using the modes of the histograms. This laboratory's 99% confidence intervals for the three parameters in the hamster are 36.7°–38.3° C, 1.0°–1.6° C, and 0.8–3.4. Thus, the shape of the hamster's rhythm shown in Figure 5 does not differ significantly from the normal population. On the other hand, the shape of the human subject's rhythm ($\Omega = 0.6$) is significantly different from the shape of the hamster's rhythm. Also, the tree shrew's modal amplitude (2.6°C) is significantly larger than that of the hamster.

A particularly interesting characteristic of the body-temperature histogram is that it provides a very simple and natural way of eliminating spurious data points. Original data points for 2 days (480 points) of body-temperature recording in a tau-mutant hamster are shown in Figure 6 (top panel). Although some points clearly seem to be spurious (e.g., the 35.1°C reading at 10 h and the 38.8°C reading at 15 h), others are more difficult to classify (e.g., the 36.4°C reading at 42 h). In the histogram, however, these low-frequency points either disappear at the tails of the distribution or add insignificantly to the bulk of it. Consequently, calculations of mean temperature level and mean amplitude of the rhythm can be performed without contamination by spurious points. Naturally, less conservative procedures may be used when all that is needed is presentation graphics. The bottom graph in Figure 6 is derived from the top graph by omitting points that differ by more than 1°C from the preceding point. This simple procedure might unduly alter calculations based on the

Figure 6. Body-temperature measurements of a tau-mutant hamster over 2 days plotted either as raw data (top) or as filtered data points connected by lines (bottom). The filtering process consisted of omitting any point that differed from the preceding point by more than 1.0°C.

data but is perfectly acceptable to enhance visibility of the rhythm in a graphic form.

In conclusion, the periodogram and histogram procedures provide an easy and simple way of analyzing the major parameters of the circadian rhythm of body temperature without making any assumptions about the raw data (such as absence of spurious points or sinusoidal shape of the rhythm). All parameters (period, amplitude, shape, and mean level) can be statistically evaluated to some degree. The program in Appendix 1 should be useful for a number of users, and other users can develop their own programs using the principles described here.

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APPENDIX 1

Program for the Analysis of the Circadian Rhythm of Body Temperature

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10 | * * * * *
20 | * ANALYSIS OF CIRCADIAN RHYTHM OF BODY TEMPERATURE *
30 | * by R. Refinetti 1990 *
40 | * SIMPLIFIED VERSION *
50 | * * * * *
60 |
70 | VARIABLE DECLARATIONS
80 CLEAR
90 DIM FILES$
100 DIM BIN%,BN%,I%,J%,K%,L%,R%,TN%
110 DIM BT,LP,N,NP,P,SM,SSP,SST,T,TEMP,TM
120 DIM D(2160) : DIM H(51) : DIM Q(21)
130 |
140 | MAIN MODULE

```

```

150 PRINT : '
160 INPUT "Name of data file: ",FILE$
170 INPUT "Bin size (min): ",BIN%
180 R%=60/BIN%
190 OPEN "i",1,FILE$ : '
200 WHILE NOT EOF(1)
210 TN%=TN%+1 : INPUT# 1,TEMP : '
220 IF TEMP<34 THEN TEMP=34 : BN%=BN%+1 : '
230 IF TEMP>41 THEN TEMP=41 : BN%=BN%+1
250 BT=349 : GOSUB 480 : '
260 GOSUB 690 : '
270 WEND : CLOSE : '
280 N=TN%/R%
290 NP=21 : LP=14 : GOSUB 330 : '
300 GOSUB 530 : '
310 GOTO 80 : '
320 '
330 ' PERIODOGRAM
340 FOR P=LP TO (LP+NP-1)
350 K%=INT(N/P) : SSP=0 : SST=0 : TM=0
352 FOR I%=1 TO (P*K%) : TM=TM+D(I%)
354 SST=SST+D(I%)^2 : NEXT I%
356 SST=SST-TM^2/(P*K%)
358 TM=TM/(P*K%)
360 FOR I%=1 TO P : SM=0
370 FOR J%=I% TO (P*K%) STEP P
390 SM=SM+D(J%)
400 NEXT J%
410 SSP=SSP+((SM/K%)-TM)^2
420 NEXT I%
440 Q(P-LP+1)=(K%*SSP)/(SST/N)
450 NEXT P
460 RETURN
470 '
480 ' HISTOGRAM
490 L%=INT(TEMP*10-BT)
500 H(L%)=H(L%)+1
510 RETURN
520 '
530 ' PRINTING
540 LPRINT "> ";FILE$;" < Mean temperature:";TM
550 LPRINT : LPRINT "PERIODOGRAM"
560 FOR I%=1 TO 21
570 LPRINT TAB(10) I%+13;TAB(20) Q(I%)
580 NEXT I%
590 LPRINT : LPRINT "HISTOGRAM"
600 FOR I%=1 TO 25 : T=BT+I%
610 LPRINT TAB(10) T/10;TAB(20) H(I%);
620 LPRINT TAB(35) (T+25)/10;TAB(45) H(I%+25)
630 NEXT I% : LPRINT
640 LPRINT TAB(10) "Total points:";TN%
650 LPRINT TAB(10) "Good points:";TN%-BN%
660 LPRINT CHR$(12);
670 RETURN
680 '
690 ' HOURLY MEANS
700 K%=INT((TN%-1)/R%)+1
710 J%=J%+1
720 D(K%)=D(K%)+TEMP
730 IF J%=R% THEN D(K%)=D(K%)/R% : J%=0
740 RETURN

```

(Continued on the next page)

APPENDIX 2
Critical Values of Chi Square for Simultaneous Testing
of 21 Periods (from 14 to 34 h)

Period	$\theta=0.05$	$\theta=0.01$	Period	$\theta=0.05$	$\theta=0.01$
14	31.88	36.46	25	48.03	53.46
15	33.42	38.09	26	49.43	54.92
16	34.95	39.69	27	50.83	56.37
17	36.45	41.29	28	52.21	57.84
18	37.95	42.86	29	53.59	59.26
19	39.42	44.41	30	54.96	60.70
20	40.88	45.95	31	56.33	62.13
21	42.33	47.47	32	57.69	63.55
22	43.77	48.98	33	59.04	64.96
23	45.20	50.48	34	60.38	66.36
24	46.62	51.97			

Note—Periods are expressed in hours.

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