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* Permanent address: A. N. Belozersky Laboratory of Molecular Biology and Bio-organic Chemistry, Moscow State University, Moscow 117234, U.S.S.R.

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Ultradian Cortisol Rhythms in Monkeys: Synchronized or Not Synchronized?

Holaday *et al.* (*1*) reported that ultradian rhythms in plasma cortisol concentration in rhesus monkeys were "highly synchronized" between animals and with the light-dark cycle. They suggested that "the rhythms may stem from the light-dark circadian cycle, since the 90-minute period is a harmonic of 24 hours." However, an examination of their analytical procedures and a reanalysis of some of their raw data (*2*) give us reasons to conclude that these ultradian rhythms are not synchronized.

First, from the raw data (*2*) we estimate that, on average, the individually detrended data records showed a standard deviation about zero of 2.2 μg of cortisol per 100 ml of venous blood. From figure 1B in (*1*) we estimate the standard deviation of the group data to be approximately 0.7 μg/100 ml. Since these estimates are in the ratio of 3.14, which is close to 2.83 (the square root of the number of records in the group average), there is a strong suggestion that the individual records are statistically independent (*3*). Had the rhythms really been synchronized the strength of the oscillations in figure 1B would have been two to three times as large.

A second reason can be found in a comparison of figure 2A with figure 2C. Since a difference between the average of the spectra (figure 2A) and the spectrum of the average (figure 2C) will be found only if there is a phase consistency in the spectral components of the individual records, the magnitude of the difference gives an estimate of the degree of phase consistency. Unfortunately, both figure 2A and figure 2C are normalized, whereas the comparison should be made on the absolute spectra. Assuming that figure 2A and figure 2C represent roughly equal absolute power, we find the spectral components of 80 to 90 minutes per cycle are virtually identical under the two computations.

Third, a visual examination, and reanalysis (*4*), of the data on the cover (*2*) indicates that the phases are sufficiently varied to have come from a random population. Table 1 shows that we detected periodicities in the range of 63 to 123 minutes in each monkey's plasma cortisol pattern, but the phases were highly variable, ranging from 0° to 300° (*5*).

Thus we conclude there is no synchronization of these ultradian cortisol rhythms either with the light-dark cycle or between monkeys. Studies by Weitzman *et al.* (6) similarly revealed no consistent phase relationships between ultradian cortisol rhythms and other similar periodicities, such as REM-nonREM (*7*) sleep-stage cycles. Thus, the beguiling regularity of the group data in figure 1B are actually the result of combining a limited number of similar frequency rhythms with essentially random phases. This conclusion obviates the necessity of searching for physiological mechanisms which could account for an improbable subharmonic entrainment with circadian oscillators via the 16th harmonic (*8*).

Table 1. Independent period spectral analyses of monkey plasma cortisol periodicities in the ultradian range.

Monkey	Period (minutes)	Amplitude (μg/100 ml)	Phase* (phase delay of first acrophase)	Time of first† acrophase (hours:minutes)
H-927	81	1.46	80°	08:18
K-113	87	1.05	99°	08:24
L-071	84	1.54	231°	08:54
L-071	123‡	3.26	158°	08:54
K-787	81‡	2.05	240°	08:54
K-742	93‡	1.53	255°	09:06
L-822	54	1.24	200°	08:30
L-822	93‡	2.24	116°	08:30
L-968	63	1.61	103°	08:18
L-968	102‡	2.60	127°	08:36
L-959	78‡	2.04	0°	08:00
L-959	108	1.68	300°	09:30

*The phase delay in degrees of the first fitted maximum from the start of the data, where 360° equals the period of the detected periodicity. †Clock time that the first fitted maximum of the detected periodicity occurred. ‡Significant at $P < .05$.

RICHARD E. KRONAUER
Division of Engineering and Applied Physics, Harvard University, Cambridge, Massachusetts 02138
MARTIN C. MOORE-EDE
MARK S. MENSER
Department of Physiology, Harvard Medical School, Boston, Massachusetts 02115

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1. J. W. Holaday, H. M. Martinez, B. H. Natelson, *Science* **198**, 56 (1977).
2. Spontaneous cortisol patterns for individual monkeys were shown on the cover of the issue of *Science* in which (*1*) appeared.
3. Furthermore, a two-way analysis of variance performed on the raw data on the cover (*2*) confirmed that the time of day effect was insignificant ($P >> .05$), and that the apparent rhythm in the group data (figure 1B) was not a significant contributor to the total variance.
4. The data were digitized from the graphs on the cover (*2*), detrended, and then analyzed separately for each monkey in 3-minute intervals from 24 to 360 minutes according to a linear-nonlinear simultaneous multiple least squares regression period analysis [J. A. Rummel, J. K. Lee, F. Halberg, in *Biorhythms and Human Reproduction*, M. Ferin, F. Halberg, R. N. Richart, R. L. VandeWiele, Eds. (Wiley, New York, 1974), p. 53]. In Table 1 the determined phase for each periodicity in each monkey is expressed as the delay in occurrence of the first acrophase of the best fitted periodicity after the start of the data (with 360° being the interval between acrophases).
5. If one examines only the periodicities determined to be statistically significant ($P < .05$) contributors to variance these still have phases varying between 0° and 255°, and consequently the times of the first fitted maxima to the significant cortisol rhythmicities in each monkey were dispersed between 08:00 and 09:06 hours local time.
6. E. D. Weitzman and L. Hellman, in *Biorhythms and Human Reproduction*, M. Ferin *et al.*, Eds. (Wiley, New York, 1974), p. 371.
7. The abbreviation REM refers to the sleep stage of rapid eye movement.

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8. The 16th harmonic of a fundamental 24-hour periodicity is 90 minutes (1440 minutes/16 = 90) according to standard usage [*McGraw-Hill Encyclopedia of Science and Technology* (McGraw-Hill, New York, ed. 3, 1971), vol. 14, pp. 490–492]. The authors' reference to a 90-minute period as a "fourth harmonic" of a 24-hour rhythm (1440 minutes/2^4 = 90) is totally unacceptable in period analysis and can only lead to confusion.

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Kronauer *et al.* use an independent mathematical analysis to conclude that there is no synchrony among the control ultradian cortisol rhythms that we reported (*1*). Their comments fail to address the issue of the exact concurrence of peaks and troughs of these rhythms when compared across both grouped control data and values collected during infusion of adrenocorticotropin (ACTH) [figure 1, B and D in (*1*)]. If no synchrony existed, it would be highly unlikely that these two series of data collected over 18 months would show such close temporal correspondence. Thus, a major focus of our original report which demonstrated the persistence of an ultradian cortisol rhythm during continuous ACTH infusion and its apparent synchrony with control oscillations remains undisputed.

Two other observations support the apparent validity of these grouped data as an indication of synchrony across real time. First, the same predominant 85 to 90 minute periodicity was observed when we compared the individual power spectra [figure 2A in (*1*)] with the power spectrum from these control data grouped across real time [figure 2C in (*1*)] as well as with the grouped data from ACTH-infused animals [figure 2D in (*1*)]. Second, as mentioned (*1*), the pharmacokinetic estimates of the duration of cortisol secretion and clearance from the grouped data correspond well with published values for individual monkeys (*2*, *3*).

Essentially, there are two perspectives from which one can evaluate the possibility of synchrony in such time-series studies. The approach taken by Kronauer *et al.* represents one reasonable way to analyze this problem. Their cosinor analysis of individual data shows individual variability in period, amplitude, phase, and time of first acrophase among monkeys. We have employed an analysis using multiple complex demodulation (MCD). This technique synthesizes a series of digital filters which may be used to examine nonstationarities in time series. Our results in estimating time of the first acrophase as well as individual periodicities with this method agreed remarkably well with those of Kronauer *et al.* in their table 1. In addition, the individual filtered outputs from the MCD analyses were entered into a pairwise covariance analysis. Of all of the possible pairs of individual animals only two showed significant covariance.

A second perspective from which one can evaluate synchrony is to analyze simultaneously the collective rhythms of all monkeys using grouped data. That was our approach in (*1*). In response to our request for a further evaluation Cleveland (*4*) devised a simplified method of statistically evaluating these data by an ensemble technique, and found that there was some, but not totally convincing, evidence that these control cortisol data contain synchronized rhythms. Although such evaluations of grouped data may be suitable in predicting their collective responses, changes in time series parameters for individual animals become obscured.

We suggest that the rather larger variability in our individual control animal data presented by Kronauer *et al.* should be expected because of the low signal-to-noise ratio and short data series in our original data. Halberg (*5*) suggested that daily rhythms in individual biological systems are not exactly 24 hours but instead are approximate (*circa*dian), thus it is probable that individual variability in ultradian rhythms should also be expected (*circ*ultradian?).

The strongest evidence for or against synchrony in biological ultradian oscillators would be the demonstration of phasic consistency in other studies of rhythmic behaviors. Tannenbaum and Martin (*6*) have reported a light-entrained, synchronized ultradian growth harmonic rhythm in rats. One of us (B.H.N.) has recently published evidence which suggests synchrony of plasma norepinephrine ultradian oscillations in monkeys (*7*) as well as in ultradian patterns of feeding and drinking in this species (*8*). Unlike Kronauer *et al.*, we believe that the collective evidence discussed herein suggests the existence of some synchrony in biological ultradian rhythms which, according to Cleveland, "is a possibility which ought to be looked for in future experimentation and after which it seems reasonable to try to find an explanation in theoretical terms."

JOHN W. HOLADAY
Department of Medical Neurosciences, Division of Neuropsychiatry, Walter Reed Army Institute of Research, Washington, D.C. 20012

BENJAMIN H. NATELSON
New Jersey Medical School, Primate Unit, East Orange 07019

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4. W. S. Cleveland (personal communication) describes what he terms an "incomplete and not totally satisfactory analysis of the data." Initially, a least-squares, straight-line fit was subtracted from each of the eight monkey control series. Ignoring for statistical purposes the effects of this detrending, the following simplified model was hypothesized:

$$y_{it} = \alpha \cos 2\pi\omega + \beta \sin 2\pi\omega t + \epsilon_{it},$$

for $i = 1, \ldots, 8$ and $t = 0, \ldots, 18$, where y_{it} is the detrended value for monkey i at times t; α, β, and ω are unknown parameters; and ϵ_{it} are independent normal variables with mean 0 and variance σ^2. "The estimates of the parameters, using least squares, and their standard errors, using a linearization technique in which it is supposed that the mean function is nearly linear in a neighborhood of the true value, are $\hat{\sigma} = 2.23$, $\hat{\alpha} = -.55 \pm .26$, $\hat{\beta} = -40 \pm .30$, $\hat{\omega} = .235 \pm .007$. In a complete analysis the exact standard errors would be computed and an analysis of residuals would be carried out to see if a small fraction of the data was substantially affecting the results. The F statistic for the regression is

$$\frac{(TSS\text{-}RSS)/3}{RSS/(136\text{-}3)} = 2.14,$$

where TSS is the total sum of squares of the y_{it} and RSS is the residual sum of squares. Again, we can approximate this, using the linearization assumption, by an F distribution with 3 and 133 degrees of freedom. The .05 and .1 percentage points for an F with 3 and ∞ degrees of freedom are 2.68 and 2.08."
5. F. Halberg, in *Biological Rhythms and Endocrine Function*, L. W. Hedlund, J. M. Franz, A. D. Kenny, Eds. (Plenum, New York, 1973), p. 1.
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9. We thank W. Cleveland (Bell Laboratories) for evaluating our data, and F. W. Hegge and H. C. Sing for the multiple complex demodulation and covariance analyses.

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