

Two Methods for Using Period Length to Study Rhythmic Phenomena

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Summary. We have developed two distinct methods of biological rhythm analysis. The procedures are based on existing techniques for analysis of time series, Enright's periodogram and autocorrelation, and both of the new methods use the parameter, period length (τ), for defining oscillatory phenomena. We empirically evaluated the two types of analyses using real biological data from circadian rhythm studies in salamanders and sparrows.

The first method permits us to make a statistical comparison of period lengths between groups of animals in given treatments. This method is useful for data where the signal-to-noise ratio of the suspected rhythm is very low; and the method is not adequate for making a definitive judgment from single animals. It can best be applied to the question of whether a signal is entraining a rhythm or not and to questions of group differences in period length.

With the second method, we determined period length versus time. Using this procedure, we took into consideration the observation that the period length of many biological oscillations changes with time. The method is applicable to records from individual animals, and it can be used to compare treatment effects in individual animals. The technique can also be used to answer the common question of whether periodicity *per se* exists within a defined range in a time series.

Introduction

Often in studying biological rhythms, one encounters variables where the rhythmic signal is small in relation to the fluctuations, or noise, in the whole record. This occurs when a rhythm has a low amplitude, or when the data gathering technique is inadequate for the rhythm to be apparent in classical plots (Fig. 1, 2). We have developed a mathematical tool for filtering noise from biological oscillations which is empirically satisfying to us, and it does not require a great deal of mathematical sophistication. The method is based on a previously developed technique for studying rhythms, Enright's periodogram (Enright, 1965). Our procedure relies on measures of period length and is called "grouped-tau-comparisons". For empirical verification, we have applied our method to the question of whether a light signal entrained the locomotor activity oscillations of groups of particular organisms, tiger salamanders and house sparrows.

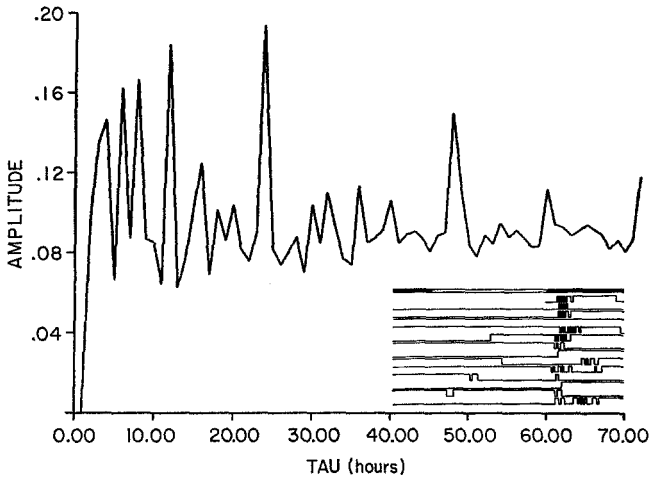


Fig. 1. Enright periodogram and event record from a salamander (*Ambystoma tigrinum*) with a clearcut rhythm in a light cycle (LD 12:12). The peak which is seen at 48 hours in the periodogram is due to the presence of the peak at 24 hours; this is an artifact of the periodogram technique. The bar over the event record indicates the relationship of the light cycle to the animal's activity record. The activity records have been cut into 24-hour segments at 0300 hours and aligned vertically in chronological order. The periodogram was calculated and plotted with a computer

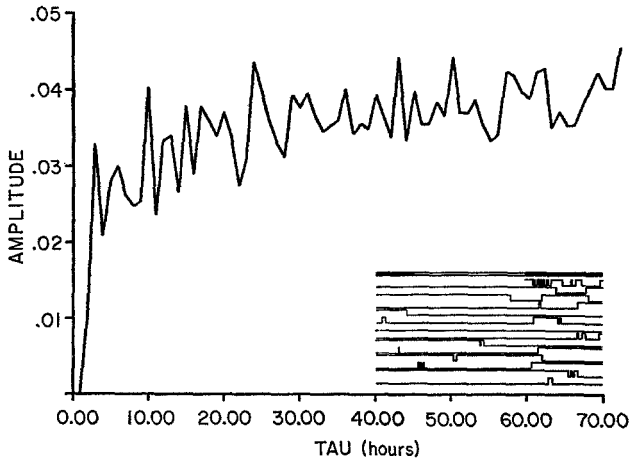


Fig. 2. Enright periodogram and event record from a salamander with considerable "noise" in its record. A peak does, however, occur at 24 hours though it does not stand above the noise as in Fig. 1. Other features as in Fig. 1

Since the grouped-tau-comparisons method depends on period length, it seems suitable to discuss it together with a second procedure, determination of "tau-versus-time". We developed tau-versus-time because biological oscillations change in period length with respect to time (Eskin, 1971; Aschoff, 1963; Aschoff *et al.*, 1972; DeCoursey and DeCoursey, 1964; Palmer, 1964; Roberts, 1960). Existing procedures for mathematical analysis of rhythms do not reveal the inherent changes in period length (τ), but instead yield one overall value for the collective period lengths in a time series. Our method for tau-versus-time is based on determination of successive period lengths by autocorrelation functions. It turns out that the procedure is not only useful as a way to measure changes in period length through time, but also as a means of detection *per se* of a rhythm in a given period range. We verified this method with artificial data and with locomotor activity records from sparrows.

Methods

A. Animals and Data

The data used for empirical validation of the analytic procedures were obtained in experiments with amphibians (salamanders, *Ambystoma tigrinum*) and with birds (sparrows, *Passer domesticus*). The amphibian data were counted manually from individual, continuous, Rustrak event records (obtained by methods of Adler, 1969, Note 4) as 2-digit hourly totals, 24 numbers per day. The bird data were collected and summed automatically (by methods of Binkley, Kluth, and Menaker, 1972) from individuals as a continuous series of 2-digit numbers; each number was a 6-minute perching activity total; 240 such numbers were obtained per day per bird. Forty-eight salamander records were analyzed; the records from salamanders in light-dark cycles (LD 12:12) were 14 days long and the records from salamanders in constant darkness (DD) were 22 days long. Twelve sparrow records were used in the analyses; the individual records ranged from 15–28 days in length. The birds had been subjected to a light cycle (LD 12:12), to constant darkness (DD), to constant light (LL), and to darkness and pinealectomy (PINX). These treatments produce entrained, freerunning, freerunning or aperiodic, and aperiodic locomotor behavior, respectively (Gaston and Menaker, 1968; Gaston, 1971; Binkley, Kluth, and Menaker, 1972).

The data sets from the amphibians and birds were punched onto computer cards and computations were made with a Univac 1107 computer; a Calcomp Digital Paper Plotter was used for graphing results of analyses. Event records were reduced photographically.

B. Grouped-Tau-Comparisons: Method for Computation

In this procedure, a continuous time series from a single salamander was analyzed by the method described by Enright (1965), and a periodogram was obtained with points at one-hour intervals for periods ranging in length from 0 to 72 hours. Since a circadian rhythm was suspected, the periodogram was divided into regions containing $\tau = 24$ as the modal period value, and the periodogram maximum in the selected region was determined. This procedure was repeated for all 24 animals in

the study separately for LD and DD portions of the experiment. The period lengths at which the maxima occurred in the individual animals were then grouped by treatment (LD or DD) and compared using standard statistical methods. Similar periodograms and maxima were calculated for birds. Controls were calculations on a set of random numbers and tests of the amphibian data for a 33-hour rhythm (Tables 1 and 2).

C. Tau Versus Time: Method for Computation

In the second procedure, a continuous perching record from a bird was partitioned relative to solar time so that the first segment consisted of days 1, 2, and 3 of the record, a 72-hour section containing 720 data values. An autocorrelation function was calculated on this series for the periods 12.0 to 36.0 hours, or lags 120 to 360 (Blackman and Tukey, 1958). The maximum value of the autocorrelation function was determined, and the period length at which this maximum occurred constituted the first measure of period length. Then a segment including days 2, 3, and 4 was subjected to identical calculations, giving the second measure of period length. This was repeated to the end of the continuous data record yielding $N-2$ values of period length, where N = the number of sidereal days in the record. As a control, the calculation was also made on a set of pseudorandom numbers obtained from the computer's random number generator.

Results

A. Grouped-Tau-Comparisons

We used grouped-tau-comparisons to test the hypothesis that an imposed LD 12:12 cycle synchronized the activity of birds and salamanders. If such synchronization occurred, the activity rhythms of the animals should exhibit two detectable properties. First, the period length of the synchronized rhythms should better approximate the period of the entraining cycle (24 hours) than the period lengths of the unsynchronized rhythms; second, the amount of variation in period length should be less in the synchronized animals than in those exhibiting endogenous (and therefore more individual), unsynchronized rhythms.

Using the method of grouped-tau-comparisons, we found that, as expected, the sparrows in DD exhibited a greater variance in period length than the sparrows in LD (Table 1) and the period length of the LD sparrows was equal to precisely 24 hours, the period length of the entraining cycle. In the salamanders, where the rhythms were not as clear as those in the sparrows, the variances of the grouped-tau-comparisons support the hypothesis that the imposed light cycle synchronized the rhythms for the 19–29 and 14–34 hour ranges. When the whole periodogram was tested, as expected, the significance disappeared due to the large amount of noise in the salamander records. The average LD period length was closer to 24 than the DD average. When the 28–38 hour range of the same periodogram was tested for a hypothetical 33-hour rhythm, the LD and DD variances were not significantly different (Table 2).

Table 1. Grouped-tau-comparisons in sparrows (obtained by three methods). The individual overall taus (determined for 4 individuals, A-D), the averages, and the variances are listed. The entrained birds (those in *LD 12:12*) exhibit less variation than the freerunning birds (those in *DD*). Note that the three methods do not always yield identical overall tau values for the same individual

	Individual tau (period length)				Average	
	A	B	C	D	Tau	Variance
Freerunning						
Autocorrelation	25.0	25.2	23.9	22.2	24.07	1.4
Enright's periodogram	24.5	25.4	23.9	22.3	24.04	1.3
Tau-versus-time (ave.)	24.5	25.6	23.3	22.2	23.9	1.5
LD entrained						
Autocorrelation	24.00	24.00	24.00	24.00	24.00	0
Enright's periodogram	24.00	24.00	24.00	24.00	24.00	0
Tau-versus-time (ave.)	24.04	24.00	24.07	24.00	24.02	0.03

Table 2. Grouped-tau comparisons in amphibians (obtained with the Enright periodogram method). The taus were taken where periodogram maxima occurred in the indicated range; 24 individuals constitute each point. The four regions are based on the same group of periodograms. As the range is widened, the noise in the records obscures the circadian peak

Interval	Light	Mean	Variance	Significance
(24-hour rhythm test)				
19-29	LD	23.91	2.77	Significant
	DD	23.58	10.34	
14-34	LD	23.80	8.84	Significant
	DD	25.16	39.44	
3-72	LD	32.12	409.50	Not significant
	DD	38.75	571.23	
(33-hour rhythm test)				
28-38	LD	33.04	11.00	Not significant
	DD	33.08	9.34	

B. Tau-Versus-Time

Tau-versus-time has two uses. First, taus can be measured and plotted versus time (Fig. 3 and 4) for an individual. Such curves, or parts of them, can be compared within or between individuals. Second, the variability in tau values obtained in animals in a given condition provides a measure of the stability of the period of the cycling function, and indeed, an indication as to whether the examined period appears at all in the data.

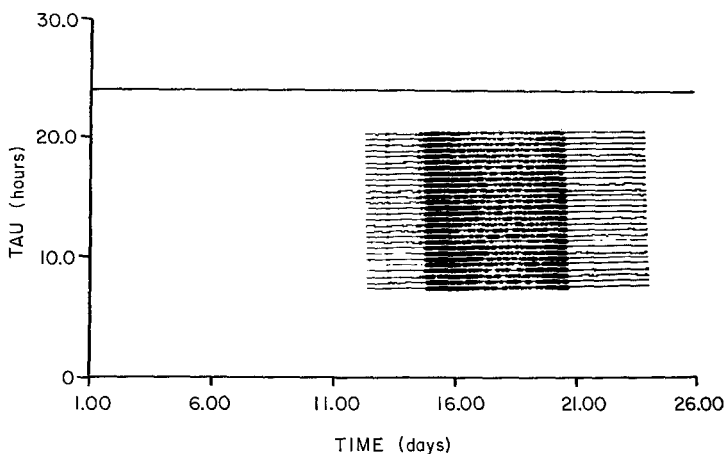


Fig. 3. Plot of tau-versus-time and activity record from a sparrow whose perching activity was synchronized with the light portion of its lighting regime. (LD 12:12). The event records has been arranged as described for the salamanders in Fig. 1 and 2 except that it was cut at 2400 hours. As expected, $\tau = 24$ at all points

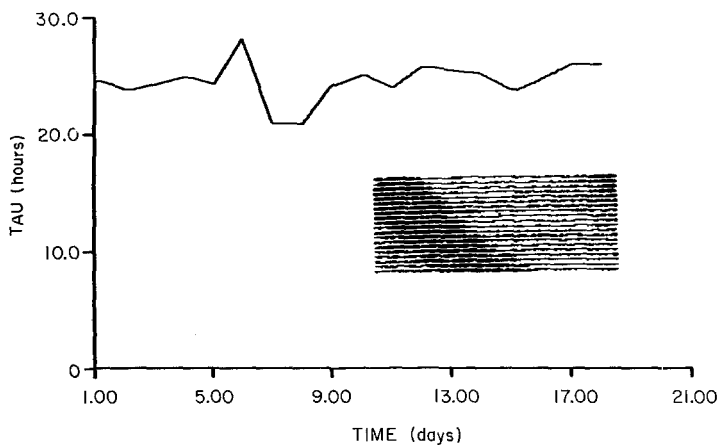


Fig. 4. Plot of tau-versus-time and activity record from a sparrow whose perching activity was freerunning in constant darkness (*DD*) with a circadian rhythm. The period length fluctuates with time, displaying greater variation than the period length of the entrained rhythm in Fig. 3

We tested tau-versus-time on a variety of known types of data from sparrows and on random numbers. We obtained predictable results based on previous knowledge of event records. Entrained birds had tau values very close to the entraining period (24.0) with very small variances (av-

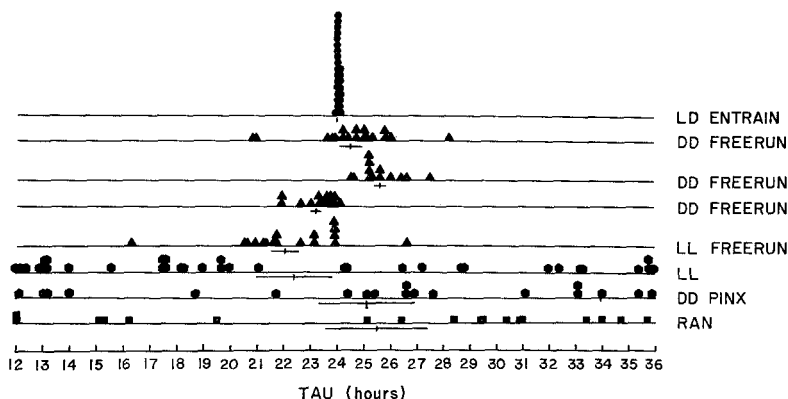


Fig. 5. Histograms of the taus obtained in tau-versus-time calculations for sparrows in three conditions and for random numbers (*RAN*). Four records were analyzed for each condition, but not all are shown where results were very similar (for *LD* and aperiodic birds). *LD* Entrain is a histogram from a bird that was subjected to a light cycle (*LD 12:12*); the four freeruns were obtained in *DD* or *LL* (note the individual differences in tau); and aperiodic records were analyzed from a normal bird in constant light (*LL*) and from three birds which were pinealectomized and in constant darkness (*DD PINX*). The mean and two variances are indicated for each individual just below its histogram

erage $s^2 = 0.03$, $N = 4$). The freerunning birds exhibited a somewhat greater variance in keeping with their changing period lengths (average $s^2 = 2.22$, $N = 4$). The aperiodic birds had very high variances corresponding to their failure to exhibit a detectable rhythm (average $s^2 = 49.60$, $N = 4$). These tendencies are readily seen in sample frequency distributions of the taus obtained from individual birds (Fig. 5). Random numbers were not unlike the aperiodic birds in their frequency distribution and amount of variation.

Discussion

The sophisticated reader will quickly see that we have not mapped all of the many ways of travelling to the same destination. For example, grouped-tau-comparisons can be made with taus determined by auto-correlation, tau-versus-time, or with the cosinor (Blackman and Tukey, 1958; Halberg, Tong, and Johnson, 1967). Further, tau-versus-time could make use of other mathematical ways of finding the taus. We have chosen methods for their computational simplicity. Each method we have presented is especially applicable to certain types of questions.

The method of grouped-tau-comparisons is limited to experiments where entrainment is being tested or to Aschoff's Circadian Rule ex-

periments (Hoffmann, 1965). The individual taus may also be used as an objective criterion for guidance in reinspection of the original data.

The technique, tau-versus-time, has much broader application. Since it measures period length objectively, it has potential value for studies of biological rhythms where period length is the experimental variable. A curve of taus graphed against time is a better measure of this period length than a single overall measure because progressive changes are taken into account. Indeed, overall taus obtained by autocorrelation, power spectra, and Enright's periodogram are not quantitatively identical for the same data set because the techniques tend to emphasize different characteristics of the data (Table 1; Strumwasser, Schlechte, and Stree-ter, 1967). The risk in tau-versus-time determinations, rather than single overall tau calculation, is a loss in resolution—that is, the fewer cycles contributing to a tau determination, the greater the possible error of that determination. However, where tau is known to change with time, statistical resolution of tau becomes a less meaningful distinction.

Period lengths have previously been determined by setting up criteria for onsets, graphical filtering, and use of special data collection techniques according to the individual experiment being analyzed (Aschoff *et al.*, 1971; Eskin, 1971; DeCoursey and DeCoursey, 1964; Palmer, 1964; Roberts, 1960). A strength of tau-versus-time is that it avoids setting up arbitrary criteria for determining onsets. The method should be applicable to a wide variety of data types. We hope that tau-versus-time may provide measures of period length suitable to experiments with many types of biological oscillations.

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