MULTIPLE CIRCADIAN PERIODICITIES IN HAMSTER MOTOR ACTIVITY AS DETERMINED BY TIME SERIES ANALYSIS

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#### ABSTRACT

MULTIPLE CIRCADIAN PERIODICITIES IN HAMSTER MOTOR ACTIVITY AS DETERMINED BY TIME SERIES ANALYSIS

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Hamster activity data recorded with a capacitance-type activity monitor under constant light (LL) and constant darkness (DD) were subjected to vigorous time-series analysis to determine if multiple periodicities were detectable from such records and, if so, to quantify their parameters. It has been shown that such records of gross motor activity contain information regarding the output from several "motor sub-sets" (such as eating, drinking, running wheel activity, etc.) which may exhibit independent rhythmicities when freed from light-dark synchronization (Wolterink et al., It was therefore supposed that as the commonly 1973). observed circadian rhythms of gross motor activity are best seen when the constituent "motor sub-sets" are synchronized, these "partial activities" would be seen best under conditions which might desynchronize the ensemble such as in constant light or constant darkness. Since classical "strip-chart" methodologies are inadequate to an investigation of multiple periodic components, more detailed

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statistical procedures need to be applied to the biological time series in order to provide the analytical basis for model building. The potential of three such methodologies (spectral analyses, autocorrelation functions, and periodograms) are examined in this dissertation.

Application of time series analysis to entrained and "dissociated" hamster activity data revealed the presence of multiple periodic components in the dissociated (but not the entrained) data. In particular, spectral analyses of 3-day non-overlapping data sub-sets offered evidence for the existence of multiple circadian components as well as for the existence of higher frequency components. Autocorrelation analyses confirmed the observation of several circadian components but the presence of higher frequency oscillations was difficult to establish. Periodograms, in general, appear to lack the resolution necessary for consistent detection.

The spectral analysis program (Program Waver) presented in this study offers an alternative to the "Halberg-cosinor" and the strict classical Fourier methodologies for the analysis of time structured observations.

# MULTIPLE CIRCADIAN PERIODICITIES IN HAMSTER MOTOR ACTIVITY AS DETERMINED BY TIME SERIES ANALYSIS

By David L. Norton

# A DISSERTATION

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# DEDICATION

To my mother and my wife who give me strength. And to my father, *in memoriam*; may I be as fine a man as he.

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#### INTRODUCTION

A vast amount of evidence has been accumulated to support the existence of endogenous oscillators in several species. Of the numerous reports concerning circadian rhythms and their sensitivity to changing environmental photoperiod, gross motor activity, owing to the relative ease of data collection, is most commonly measured (Lowe et al., 1967; Suter and Rawson, 1968; Aschoff et al., 1971; Kramm, 1973; Brown and Chow, 1974). Long time series can be obtained for a single animal, making the data more amenable to complex mathematical analyses and leading to an objective description of the biologic time structure. Activity/rest ratios (Aschoff, 1971) or fluctuations in activity onset (DeCoursey, 1961; Kramm, 1973) are generally emphasized, but most notable in the response of activity data to "constant conditions" is the relative stability of frequency in the face of easily affected phasing (Sollberger, 1965).

The existence of many other biorhythmic phenomena has been well documented; eosinophil counts (Halberg et al., 1957), body temperature (Folk and Schellinger, 1954), and urinary ketosteroid excretion (Pincus, 1943) to name a few.

It is evident that studies of biorhythms using specific physiological end points, such as urinary ketosteroids, can give important conclusions with respect to the detailed model which seeks to explain certain endocrine systems. On the other hand, gross motor activity measurements recorded in the classical strip-chart manner, may contain information regarding many neural control systems. This is especially true in animals like the hamster (Norton, 1974), whose sleepwakefulness cycles are strongly circadian. In these animals, feeding activity occurs only during twelve hours of animal activity (under LD<sub>12:12</sub> entrainment), hence the true periodicity of this "motor sub-set" may be masked if periodic sleep acts as a forcing function.

Entrainment of circadian rhythms is defined by Bruce (1960) as the phenomenon whereby a periodically repeated stimulus, such as a light cycle, causes an overt persistent rhythm to become periodic with the same frequency as the entraining cycle. There is thus a fixed phase relationship between the entrained rhythm and the entraining cycle. Since the commonly observed circadian rhythms in locomotor activity are best seen when the constituent motor sub-sets are synchronized, it was assumed that the partial activities might be seen best under conditions which tend to desynchronize the ensemble, such as constant light or constant darkness. Each variety of motor output might then be more or less free-running and, if sufficiently phase shifted,

produce detectable perturbations in the analyses. To the physiologist, this might allow the testing of physiological models which may describe the sub-systems responsible for each particular identifiable activity. Because the measurement techniques are non-invasive, such analyses of intact animals would avoid criticisms based on acute methodologies.

Periodogram analysis, autocorrelation functions, and power spectra are potential tools for determining the length and stability of the circadian period. Calculation of the best fitting cosine function by the least squares method is a useful technique to display other rhythm characteristics, i.e., amplitude, phase, and wave level. It was the object of this research, therefore, to determine which analyses might best reveal the presence of multiple periodic components in a given set of activity data. Such exhaustive analyses might then lead to a better understanding of the neural networks responsible for those complex motor behaviors collectively called circadian activity.

## REVIEW OF THE LITERATURE

Although the existence of biological rhythms has long been established, the possible existence of innate biological oscillators has only recently been accepted. A prominent view, and one still held by some researchers (Brown, 1960), was that the rhythms were purely exogenous; an overt expression of the periodic environment. This was particularly true for that class of low-frequency oscillations with an obvious external correlate in the daily light-dark cycle. These rhythms have since been termed *circadian* (Halberg et al., 1959), referring to those endogenous rhythms which have a period length of about (circa) a day (diem). An organism exhibiting a day-night periodicity, therefore, does not necessarily possess an endogenous circadian one. An environmental period may be the real and only cause of the rhythm, particularly if it decays in artificial constant conditions (Aschoff, 1960). A circadian system, however, is characterized by its capacity to oscillate in the absence of periodic factors in the environment (Aschoff, 1973). Differentiation is thus made between systems whose oscillations decay following the removal of exogenous periodic factors and systems which are capable of self-sustained oscillations. As Aschoff points out, an oscillating system

can be entrained "by another periodic source of energy" resulting in a forced oscillation with the same frequency as the driving agent. In contrast to exogenous rhythms, endogenous circadian frequencies become overt when there is no periodic driving agent. Such overt oscillations, occurring in the absence of environmental cues, have been called free-running (Pittendrigh, 1958) or spontaneous (Aschoff, 1958), and the periodic factors of the environment to which they can be synchronized have been designated as entraining agents (Bruce, 1960), synchronizers (Halberg et al., 1959) or Zeitgebers (Aschoff, 1960; 1965a).

Endogenous rhythms for a wide variety of daily physiological functions have now been demonstrated in both vertebrates and invertebrates. The review papers of Welsh (1938), Kleitman (1949), and Aschoff (1954,1963) give extensive summaries. Much investigation has been done in rodents, since a clear expression of "clock-controlled" locomotor activity is present in this group (DeCoursey, 1972). It is now fairly well established that daily activity rhythms in most animals are not passive responses to periodic environmental changes and probably depend on persistent endogenous oscillators (Aschoff, 1965b; Kramm, 1973). In favor of this theory has been the demonstration of persistent free-running activity rhythms having relatively stable period lengths of approximately 24 hours (DeCoursey, 1972; Pavlidis, 1973). However, DeCoursey (1961) has shown that animals free-running

in constant conditions, while exhibiting rhythmic activity patterns, drift out of phase from each other and from sidereal time. They are therefore dependent on environmental cues for synchronization to a precise 24-hour period and a distinctive phase.

The mechanism of circadian activity has usually been investigated by measuring the periodic course of a single parameter, typically activity onset (Rawson, 1959; Aschoff, **1965c).** Strip-chart recordings of the activity-rest cycle are made and activity onset is linearly regressed on time for a determination of period length (Richter, 1965). However, in view of the current hypothesis that the circadian system consists of a multiplicity of individual oscillators which, although normally coupled to each other, may become uncoupled to produce independent rhythmicities in the steady-state, more detailed statistical procedures for the detection of multiple periodicities need to be applied to biological time series. Three procedures which may prove useful to such investigations are examined in this review preceded by a brief research summary of typical circadian activity studies.

#### Entrainment Studies

#### Ranges of Entrainment

Studies on the mechanism of entrainment of circadian rhythms by light and temperature have shown that

synchronization of the rhythm to the period and phase of an external Zeitgeber (Aschoff, 1960) is possible only if the period of the exogenous cycle is close to that of the endogenous one (Bruce, 1960; Rawson, 1959). Tribukait (1954, 1956), for example, entrained mice (Mus) to a 24-hour cycle then gradually lengthened or shortened the period until the animals no longer entrained. This occurred with periods shorter than 21 hours or longer than 27 hours. He failed to get entrainment to 16, 20, 22 and 28 hours when these were established suddenly. The findings of Bruce (1960) on hamsters (Mesocricetus) and mice (Peromyscus) suggest that entrainment ranges may be species specific. Running wheel activity in these species entrains within narrow limits on either side of 24 hours, 23-25 hours, compared with Mus. DeCoursey (1972) compared circadian entrainment in a diurnal (Tamias striatus) and nocturnal (Glaucomys volans) rodent. The mechanism of entrainment appeared to be different for the two species. A rough generalization indicates that the more complex the organism the more difficult it becomes to entrain the rhythm to period lengths considerably different from 24 hours (Bruce, 1960). The work of Kleitman and Kleitman (1953), and of Lewis and Lobban (1954), illustrates the difficulties of entraining human subjects to artificial days shorter or longer than 24 hours.

As the frequency of a light-dark cycle to which an organism is entrained increases, the phase of the organism lags further behind the Zeitgeber phase until the limits of entrainment are reached and the organism free-runs. Leading phases of the entrained organism are seen when the frequency of the Zeitgeber is lowered. Aschoff (1964) observed the circadian activity pattern of mice in light-dark cycles of varying lengths and showed that in a 26-hour day, activity onset was advanced relative to its position in a 24-hour In a 22-hour day, activity onsets were delayed comday. pared with 24-hour controls. Aschoff views entrainment **limits** to be an index of the strength of self-excitation in the entrained oscillator. If the strength of a Zeitgeber is constant, a narrow range may indicate a strong, and a wide range a weak capacity for self-excitation.

Entrainment of activity rhythms to light-dark cycles may also depend on the photofraction. DeCoursey (1972) has measured the limits of entrainment for a wide spectrum of LD (light-dark) ratios on a 24-hour day schedule in the nocturnal flying squirrel (*Glaucomys volans*) and the diurnal chipmunk (*Tamias striatus*). Photoperiods for *Glaucomys* were varied from 1 second L:24 hours D to 18L:6D; those for *Tamias* ranged from 1/4L:23 3/4D to 23 3/4L:1/4D. Under these conditions, *Glaucomys* was able to entrain to all schedules from 1 second of light per day to 18 hours of light per day. Stable entrainment for *Tamias* occurred only

between 6L:18D and 21L:3D. Beyond these limits, oscillatory entrainment occurred prior to free-runs suggesting the beginning of synchronization breakdown. Aschoff (1965b) has examined the effect of varying LD ratios on entrainment for a wide variety of species. Within a 24-hour period, large changes in the photofraction may be made without disturbing entrainment. If the frequency of the Zeitgeber is altered, variation of the photofraction becomes more restrictive. Bruce (1960) noted further that the minimum amount of light required to entrain a rhythm is generally much less than the minimum amount of dark needed.

## Phase Response Studies

Rawson (1959) has shown that light may have quite a different phase controlling action if it occurs near the beginning of an active period (subjective early night) from its action if it occurs near the end of an active period (subjective late night). Presenting 12 hours of light to mice free-running in constant darkness when they were active, produced a delay in activity onset. When light of the same intensity was presented during an inactive period, no phase delay occurred. Pittendrigh and Bruce (1957) and Pittendrigh (1958, 1960, 1965) have published a systematic study of the effects of single perturbations to a freerunning rhythm and showed that they effect phase advances or phase delays depending on the phase of the rhythm at

which they are administered. Similar studies by DeCoursey (1961, 1964) and Wever (1965) have now led to a number of phase response curves for several species which Aschoff (1965a) reviewed. DeCoursey (1960a, 1960b) interprets such results in terms of a daily rhythm of light sensitivity in which early and late subjective night periods are sensitive to phase-shifting in opposite directions.

## Free-running Rhythms

Hemmingsen and Krarup (1937) reported that in the white rat, the period of spontaneous locomotor activity was lengthened in constant light. Johnson (1926, 1939) confirmed these findings for *Peromyscus* and also noted that the period increased with increasing intensity of constant illumination. Certain generalizations concerning the character of free-running activity rhythms have since been established.

Free-running periods are close to 24 hours, in general varying between 22 and 26 hours (Bruce, 1960; Pavlidis, 1973), and depend only slightly on the temperature at which they are measured (Rawson, 1959). Tabulations of some ranges of period lengths for different organisms have been compiled by Aschoff (1958), Hoffmann (1965), Folk (1966), and in Table 1.

Under constant experimental conditions, the rhythm of gross motor activity is generally maintained with a

Table 1. Free-running circadian periods under LL and DD.

Species	Rhythm	Range of Intensities (lux)	Free-running Period (hours)	Reference
Peromyscus leucopus	Running Total activity	0 - 1.4 0 - 258	23.17 - 24.50 24.00 - 24.30	Rawson, 1959 Johnson, 1939
Glaucomys volans	Running Running	0 - 5 0 - 10	23.80 - 24.40 23.50 - 25.00	DeCoursey, 1971 Aschoff, 1964
Mus musculus	Total activity Total activity	0 - 200 0 - 120	23.00 - 25.90 23.50 - 26.10	Aschoff, 1952 Aschoff, 1962
Mesocricetus auratus	Running	0	23.95	Pittendrigh- Bruce 1957
	Running	0.1 - 20	24.50 - 25.00	Aschoff, 1960
Rattus norvegicus	Running	0 - 11	24.00 - 25.25	Brown et al., 1956
Rattus rattus	Running	0 - 51	24.75 - 25.50	Folk, 1959
Sigmodon hispidus	Total activity	0 - 250	23.70 - 24.50	Calhoun, 1945
Tamias striatus	Running	0 - 5	24.70 - 23.00	Rawson, 1959
Microtus orghaster	Total activity	0 - 250	23.90 - 24.20	Calhoun, 1945
Passer domesticus	Total activity	10	24.15	Palmer, 1964
Sturnus vulgaris	Total activity	0.6 - 65	24.30 - 22.50	Hoffmann, 1960
Fringilla coelebs	Total activity	0.4 - 120	24.80 - 22.00	Aschoff, 1960

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relatively constant period characteristic of the individual animal. Deviations in any one animal usually range from less than one hour to less than fifteen minutes (Bunning, 1967; Pittendrigh and Bruce, 1957), whereas between animal differences may range from one to several hours. Aschoff (1955), for example, found the following specific period lengths for five mice in constant light: 25.0, 25.1, 25.3, 25.4, and 25.5 hours. DeCoursey (1960, 1961) reported a range of 23.0 to 24.5 hours for the activity of 16 flying squirrels in constant dark. For this reason, Aschoff and Honma (1959) refer to "individual patterns."

The value of the free-running period in constant dark generally differs from its value in constant light (Bruce, 1960; see Table 1). Bullfinches kept in continuous darkness exhibit a frequency of 24 hours; in constant light it changes to 22 hours. In mice (Mus), the period increases to 24 or 26 hours under continuous light and decreases to 23 or 23.5 hours in constant darkness (Aschoff, 1953, 1955; Meyer-Lohmann, 1955). Reductions in the activity-rest ratio of dark-active animals by constant light have also been reported (DeCoursey, 1961; Aschoff et al., 1971). Aschoff (1952a, 1958) expressed a general rule (circadian rule) concerning free-running activity patterns which states that the length of the period of animals active in light decreases with increasing light intensity; and in dark-active species it increases with increasing light

intensity. In all cases, however, frequencies change by
only 5-10% (Bruce, 1960). Exceptions to Aschoff's rule have
been noted by Hoffmann (1965).

## Dissociation of Circadian Rhythms

The circadian system of an organism consists of a number of rhythms which are normally entrained to the same frequency by a synchronizing Zeitgeber (Aschoff, 1973). The temporal relationships of such rhythms have been illustrated graphically in phase-maps (Halberg, 1960b; Halberg et al., 1959, 1967). When not entrained by a Zeitgeber, all rhythms within an organism may remain synchronized with each other, showing one free-running rhythm of the entire system (Aschoff, 1973), or they may become desynchronized to show different frequencies in the steady-state (Wever, 1973). This has led some investigators (Wever, 1971; 1972) to suggest that the circadian system is controlled by a multiplicity of individual oscillators which are normally entrained with each other but which may become uncoupled to oscillate at different speeds. Evidence for this hypothesis has been demonstrated by following the temporal course of different biological variables like activity and rectal temperature (Wever and Lund, 1973) or of a single variable which exhibits multiple components (Hoffmann, 1971). Wever and Lund (1973) illustrated desynchronization of several

physiological rhythms in humans living under constant light. Fourier analysis of activity and rectal temperature cycles resulted in a "two-peaked" spectrum suggesting the presence of two oscillations having significantly different period lengths. The predominant component for rectal temperature was a 25.1-hour period while that for activity was a 33.4hour period.

Pittendrigh (1960) and Swade (1971) have reported that after prolonged constant illumination the rhythm of locomotor activity in nocturnal rodents Mesocricetus and Peromyscus split into two components. These components showed distinctly different frequencies for some time but eventually resynchronized to produce one free-running pattern at a new phase relation. Evidence for the occurrence of multiple components in the activity rhythm of a lightactive animal (Tupais) as a function of light intensity has been presented by Hoffmann (1971). If light intensity was reduced below a certain level (usually 5 lux) the activity rhythm split into two and sometimes three components which oscillated at different frequencies. Eventually the components were observed to run parallel with identical frequencies. Resynchronization occurred when light intensity was elevated.

Dissociation of the circadian drinking pattern from eating, two "motor sub-sets" which comprise gross activity, has been demonstrated in rats by Oatley (1971).

Richter (1965) has reported free-running circadian rhythms in activity, eating and drinking in blinded rats. In addition, non-circadian periodicities for some "motor sub-sets" have been reported. In rats, urination produces a periodicity of about 3-4 hours while defecation occurs at slightly longer intervals (Richter, 1965).

#### Time Series Analysis

#### Introduction

The application of time series analysis to physiological data provides a method whereby a rhythmic signal, if present, can be detected, apart from superimposed random noise, and its parameters objectively quantified. Classical procedures for investigating circadian activity, using stripchart recorders and chronograms (DeCoursey, 1961), seem inadequate to a total understanding of the time series since they utilize a single (often subjective) estimate to evaluate it (typically activity onset). On the other hand, time series analyses which utilize the total length of record, can provide in depth statements regarding its biological time structure, which Halberg and Katinas (1973) define as the sum total of non-random and thus predictable aspects of organismic behavior including bioperiodicities.

Periodic functions are functions whose values recur at regular temporal intervals called the period (Halberg

and Katinas, 1973), and may be expressed as

$$f(t) = f(t + \tau)$$
 (2.1)

where  $\tau$  is the period of the rhythm under study (Halberg, 1969). It should be noted that under this definition, an organism need not generate activity which is sinusoidal to exhibit periodic behavior. Time series analyses which isolate periodic functions from random noise without specific assumptions regarding waveform, autocorrelation and periodogram analyses, for example, are especially useful in this regard. However, the non-sinusoidal nature of any given time series does not necessarily limit the usefulness of fitting cosines to the data by the method of least squares (Halberg et al., 1972). As illustrated in Figure 9, the circadian nature of a "24-hour" square wave can be detected by least squares cosine fitting given a time series which includes several repetitions of the cycle. Further, such a procedure is necessary for an objective quantification of detected periodicities in terms of an average period length ( $\tau$ ), average acrophase or crest-time ( $\phi$ ), and an average amplitude (C) demonstrated to be significantly different from the mean level  $(C_0)$  by statistical means.

The remainder of this chapter examines three analytical methods for the study of periodic functions obscured by random disturbances. Two of these, periodogram and autocorrelation analysis, have been used sparingly in physiological studies but are potential tools for determining the length and stability of oscillatory components (Sasaki, 1972), as well as for detecting the presence of multiple periodicities. The computational equations for the least squares fit of a cosine have been reserved for the following chapter, as they have been related to the operation of Program Waver used in this study.

## Cosine Curve Fitting

## Usage

Cosine curve fitting has as its objective the quantification of amplitude, phase and wave level of the rhythm under study. Smolensky et al. (1972) used the technique for the identification of circadian and circannual rhythms of birth and death. Estimations of amplitude and phase for a number of circadian functions in mice have been presented in phase-maps (Halberg et al., 1959; Halberg, 1960b). The computational procedure utilizes the method of least squares and has been extensively developed by Halberg (1960a, 1967; Halberg et al., 1967) as part of the cosinor technique. Recently, modified computational methods have been published (Halberg et al., 1972; Dewey, 1973).

## Terminology and Rationale

Sine-cosine curves are basic periodic functions and, unlike other trigonometric functions, are continuous in the time domain. They are therefore useful as approximating

functions in periodic regression analysis. In addition, a periodic function which is non-sinusoidal may be Fourier transformed to a constituent set of cosine waves and its waveform described by the summation of appropriate harmonics (see Sollberger, 1965, for methodology).

A rhythm detected by the least squares fit of a cosine can be described on the basis of several endpoints obtained from approximating functions of the form:

 $Y(t) = C_0 + C \cdot \cos(\omega t + \phi) \pm E_i \qquad (2.2)$ For data recorded at 0.1 hour intervals,  $C_0$  is comparable to a mean 6-minute average. The amplitude, C, measures the degree of variability existing over a time interval,  $\tau$ , called the period and is, in fact, equivalent to the halfamplitude of the fitted cosine. Since  $\omega$  denotes the (fixed) angular frequency of the fitted curve, equation (2.2) may be expressed as:

$$Y_{i} = C_{0} + C \cdot \cos\left(\frac{2\pi}{\tau}t_{i} + \phi\right) \pm E_{i} \qquad (2.3)$$

where each data point is represented as some fraction  $(t_i/\tau)$  of a complete  $(2\pi)$  cycle. The concept of least squares fitting of a cosine to a time series is illustrated in Figure 1 (after Halberg et al., 1972). As the figure indicates, the computative acrophase ( $\phi$ ) delineates, in time, the peak of the best fitting cosine function. For the C<sub>0</sub>, C, and  $\phi$  values shown, the function Y(t) results in a minimized sum of squares for error  $(\Sigma E_i^2)$ . Calculation of these parameters is outlined in the following section.

Estimation of rhythm parameters by least squares fitting of a cosine function.  $C_0$  = mean level; C = half amplitude;  $\phi$  = phase angle in degrees from midnight. Figure 1.

$$Y(t) = C_0 + C \cdot \cos(\omega t + \phi) \pm E_i$$

Reproduced with permission of Dr. Franz Halberg and the American Physiological Society from: The Physiology Teacher, Vol. 1, No. 4, January, 1972 (Figure 4, Society from: page 9).


Figure l

#### Periodogram Analysis

#### Usage

The application of periodogram analysis to biological data has been limited. Halberg (1960a, 1965) utilized periodograms for the analysis of rectal temperature cycles in blinded mice. Pochobradský (1970) recently investigated the usefulness of periodograms in the determination of menstrual cycles, and Binkley et al. (1973) compared periodogram analysis with autocorrelation techniques on freerunning activity data in sparrows. Enright (1965a) subjected previously published data which suggested the presence of lunar-tidal rhythmicities in activity, to a reexamination by periodograms and found such conclusions to be unwarranted.

Currently, two periodogram techniques have been investigated. One is that of Koehler et al. (1956) which involves the sequential use of periodic regression analysis (i.e., least squares cosine fitting) and the second that of Enright (1965a, 1965b). The method discussed here will follow that of Enright although the basic rationale is applicable to both techniques.

#### Periodogram Rationale

Periodogram analysis, as described by Enright (1965b), involves a posteriori evaluation of a given set of frequencies as a function of their relative amplitudes.

The procedure is a generalization of standard statistical methods for the form estimation of a periodic function. For example, if in a continuous time series of some biological variable, there is a stable oscillation with a period of 24.0 hours which is subject to randomly occurring disturbances ("noise"), then by classical statistical arguments, the mean value of all observations recorded at 1 a.m. becomes an unbiased estimator of the value of the underlying periodic function at 1 a.m., and the calculation of 24 such averages would lead to an unbiased estimate of its form. Likewise, in a 25-hour form estimate, the 1 a.m. value for the first day of record would be averaged with the 2 a.m. value for the second day, the 3 a.m. value for the third day, etc. Enright (1965a) has generalized this averaging procedure for any other integral period using the Buys-Ballot Table of Kendall (1946; Appendix A).

In frequency analysis, the significance of a given oscillation is generally associated with the magnitude of its amplitude. For periodogram analysis, the test statistic normally utilized is the root-mean-square amplitude  $(A_p)$  defined by Enright (1965b) as

$$A_{p} = \left[\frac{1}{P}\sum_{h=1}^{P} (Y_{p,h} - \overline{Y}_{p})^{2}\right]^{\frac{1}{2}} \text{ where } \overline{Y}_{p} = \frac{1}{P}\sum_{h=1}^{P} Y_{p,h} \qquad (2.4)$$

and all other symbols are as described in Appendix A. Plots of these amplitude estimates against a sequence of

assumed period lengths are called periodograms (Whittaker and Robinson, 1927) and, as equation (2.4) illustrates, these A values are essentially the sums of squared deviates from the mean, indicating that periodograms are variancetype spectrums.

**Examination** of equation (2.4) reveals that if a time series contains a stable oscillation with a period of 22 +  $\varepsilon$  hours ( $\varepsilon$  is a very small non-rational number) then the amplitude estimate for a mistakenly assumed 24.0-hour periodicity would equal zero over an infinite series of data. Even for a finite series, however, including several cycles of the real component, the estimate of amplitude for the assumed 24.0-hour period would be less than the estimate obtained for a 22.0-hour period (Enright, 1965b). As a result, periodogram analysis assumes the presence of no periodicity a priori, and instead consists of a comparison of amplitudes calculated from a series of form estimates, each of which is based on a different value of assumed period. By estimating amplitudes for all values of period within a range presumed to include the periods of major oscillatory components, differentiation can then be made between unusually large amplitude form estimates and those form estimates which have amplitudes no greater than background.

## Peaks Due to Sub- and Supermultiples

Enright (1965b) has noted that for any oscillation which does not show an appropriate symmetry, periodogram analysis may produce peaks at submultiples of the true period. When two symmetrical disturbances of 9 hours duration and an interval of 368 hours were added to a set of 1000 random numbers, periodogram peaks occurred at all major submultiples of 368 hours. As illustrated in Figure 2(b), the periodogram for a sine function exhibits no such peaks at its harmonic points (i.e., at 1/2, 1/3, 1/4 of its period length, etc.). To remedy this situation, Enright suggests a re-analysis of non-overlapping data subsets or replicate series. Any periodogram feature which appears in the total series and by all replicate subsets, implies a persistent rhythmic component.

A second complication following the use of periodograms is that the analysis cannot distinguish between peaks resulting from real periodic components and those which arise due to components having period lengths which are submultiples (harmonics) of the apparent value. The periodogram of Figure 2(a) was based on input data consisting of twenty cycles of a 12-hour sine function ( $p = \pi$  radians) while that of Figure 2(b) from ten cycles of a 24-hour sine function ( $p = 2\pi$  radians). As the figure illustrates, both oscillations produce a peak on the periodogram corresponding to a 24-hour cycle. Had the analyses covered a range of

Figure 2. Periodograms based on artificial, stable input data. Figure 2a was based on twenty cycles of a 12-hour sine function ( $p = \pi$ radians); Figure 2b on ten cycles of a 24hour sine function ( $p = 2\pi$  radians), and Figure 2c on ten cycles of an artificial square pulse ( $p = 2\pi$  radians). A<sub>p</sub> = root-mean-square amplitude (Appendix D); N = 2400;  $\Delta T = 0.1$  hr.



Figure 2

20-30 hours (because of an *a priori* assumption that the data contained a circadian oscillation), the conclusion might have been made that such an assumption was correct for both series. Extension of the analysis to include the first harmonic (12-hour period), however, indicates that for the data of Figure 2(a), such a conclusion is decidedly unwarranted. Further, Figure 2(a) would also have produced a peak at 36, 48, 60 hours, etc., and Figure 2(b) peaks at 48, 72, 96 hours, etc. had the analysis been extended to include these periods. Enright (1965b) suggests examination of the respective form estimates prior to any conclusions regarding periodicities inferred from components of the periodogram. If the form estimates show one, two, or three complete cycles etc. within a 24-hour period, the periodogram peak may be assigned to an appropriate harmonic.

### Form Assumptions

In contrast to procedures which assume the presence of sinusoidal functions, the detection of periodic components by periodogram analysis is possible without assumptions regarding form. Although the periodograms of Figure 2(b and c) were produced using input data of contrasting form (Figure 2(b) from a "24-hour" sine function and Figure 2(c) from a "24-hour" square wave), both reveal peaks corresponding to a 24-hour periodicity.

## Unstable Data

The data used to produce the periodograms of Figure 2 differ from most biological data in that the oscillatory components persisted with constant amplitude and period length. Enright (1965b) examined the properties of periodograms derived from non-stable input data and found that minor instabilities in the oscillatory components did not eliminate the usefulness of the method. Linear increases or decreases in either amplitude, frequency, or a combination of both, resulted in periodograms which still provided meaningful information about average properties of the It should be noted, however, that Enright's oscillation. test functions had period lengths in the circadian domain, indicating that, at least for low-frequency oscillations, the procedure has only limited sensitivity to minor shifts in either phase or period. For high frequency oscillations, such shifts could easily obscure the presence of a periodic component using this method.

## Autocorrelation Analysis

## Usage

Halberg (1960a), using autocorrelation procedures, found a 24-hour rectal temperature rhythm in data obtained at 4-hour intervals in mice. Sollberger (1970) applied the autocorrelation function to finch activity data and showed

clear circadian peaks through 26 days. Recently, Binkley et al. (1973) used a modified autocorrelation procedure to test the stability of free-running activity rhythms in sparrows. Theoretical considerations of the autocorrelation function have been extensively discussed by Yule (1921, 1927) and by Jenkins and Watts (1968).

### Rationale of the Autocorrelation Function

Sollberger (1965) has noted the usefulness of autocorrelation analysis in separating, from time series data, periodic components and random noise. The procedure was developed by Yule (1921) as a method of investigating periodicities in disturbed series and involves sequential calculation of the product-moment correlation coefficient,  $r_{\tau}$ . The observed series is duplicated and simultaneous values correlated with no time displacement ( $\tau = 0$ ) to yield an initial coefficient of r = +1. One of the series is repeatedly lagged an interval ( $\tau$ ) and each term of the original series correlated with the corresponding term of the lagged series. Mercer (1960) expressed the autocorrelation function,  $R(\tau)$ , of a time function, f(t), mathematically as

$$R(\tau) = \lim_{T \to \infty} \frac{1}{T} \int_{0}^{T} f(t) f(t + \tau) dt \qquad (2.5)$$

where  $\tau$  is the time delay and T the total length of record. In Kendall's (1945) notation, the coefficient of productmoment correlation between members of a series  $\tau$  intervals

apart is called the serial correlation of order  $\tau$ . For a finite series, the computational equation is given by

$$\mathbf{r}(\tau) = \frac{\Sigma(\mathbf{x}_{j} - \bar{\mathbf{x}}_{1}) (\mathbf{x}_{j+\tau} - \bar{\mathbf{x}}_{2})}{[\Sigma(\mathbf{x}_{j} - \bar{\mathbf{x}}_{1})^{2} (\Sigma(\mathbf{x}_{j+\tau} - \bar{\mathbf{x}}_{2})^{2})]^{\frac{1}{2}}}$$
(2.6)

where  $r(\tau)$  is the serial correlation coefficient of the series at time  $\tau$ ,  $x_{j} = f(t_{j})$ , and the summations run from j = 1 to  $j = N - \tau$ . It can be seen from equations (2.5) and (2.6) that at  $\tau = 0$ ,  $f(t) = f(t + \tau)$ ; hence correlation is maximal (r = +1) at zero lag. If f(t) represents a purely random function (with no periodic components), the autocorrelation function  $R(\tau)$  approaches zero for large values of  $\tau$ . This is so since in a random function the two ordinates to be multiplied, f(t) and  $f(t + \tau)$ , are as equally likely to be positive as negative (i.e., occurring below a mean base line) and the sum of a large number of them will tend to be zero (Murtha, 1961a, 1961b). Moreover, it is obvious from equation (2.6) that for a periodic function,  $R(\tau)$  will be repetitive, since a phase displacement of one period reproduces the condition at zero lag. For a sine wave with a period of  $2\pi$  radians, the "r" value will be +1 after displacements of  $2\pi$ ,  $4\pi$ ...  $n2\pi$  radians and -1 following displacements of  $\pi$ ,  $3\pi$ ... ( $\pi$  +  $2\pi$ ) radians. The autocorrelation function for this waveform is presented in Figure 3(a). As the figure illustrates, the periodic nature of the sine wave is preserved in the autocorrelation function as a

cosine and, further, that the frequency of oscillation is the same as that of the original time series.

If a time series contains a periodic component disturbed by random noise (a bioperiodicity),  $R(\tau)$  will again be repetitive. When the two curves are out of phase they will tend towards inverse values and the correlation coefficient towards -1; when in phase, the coefficient will approach +1 and produce a peak in the autocorrelation function equivalent in time to the period of the oscillation. In the process, random components will cancel. The effectiveness of the autocorrelation analysis in separating periodic components from noise will depend upon the magnitude of the random errors and the length of the time series. But, as Yule (1927) states, however large the errors, given a sufficient number of periods (i.e., a long enough time series), autocorrelation will provide a close approximation of the period of the underlying harmonic function. It should be noted, moreover, that correlograms yield no analysis into various components of the hidden periodicity or their phasing (Sollberger, 1965).

## Form Assumptions

Like periodogram analysis, autocorrelation procedures are valid without prior assumptions regarding waveform. In Figure 3, the autocorrelation function for three different sets of input data are presented. Figure 3(a) was obtained Figure 3. Autocorrelation functions derived from artificial input data. Figure 3a was based on ten cycles of a 24-hour sine function ( $p = 2\pi$  radians), Figure 3b on ten cycles of a 24-hour square pulse ( $p = 2\pi$  radians), and Figure 3c on a set of computer generated random numbers. Rho = serial correlation coefficient; N = 2400;  $\Delta T = 0.1$  hr.





from a 24-hour artificial sine wave while Figure 3(b) represents the autocorrelation function for a 24-hour artificial square wave. As the figures illustrate, the periodic nature of the input data is reproduced in the autocorrelation function though not necessarily with the same waveform. Figure 3(c) resulted from the autocorrelation of 2400 computer generated random numbers and exhibits no periodicity.

### MATERIALS AND METHODS

## Experimental Rationale

Recording gross motor activity from the isolated hamster with a capacitance activity monitor produces a printout in which the output from several "motor sub-sets" are confounded. If activity is recorded at the end of each 0.1 hr., each sum represents a variety of motor outputs (i.e., eating, drinking, running wheel activity, etc.) with varying durations and intensities. Since the commonly observed circadian rhythm of "gross motor activity" is best seen when the constituent motor sub-sets are synchronized (or observed separately as in running wheels; Rawson, 1959), it might be supposed that these "partial activities" would be seen best under conditions which would desynchronize the ensemble, such as constant light (LL) or constant darkness (DD). Each variety of motor output might then be more or less freerunning and dissociated from the others. Accordingly, hamster activity data recorded under LL and DD were subjected to rigorous time series analysis in order to determine if multiple periodicities were detectable from such records and, if so, to quantify their parameters. Such an

exhaustive analysis might then lead to a better understanding of the neural networks responsible for those complex motor behaviors collectively called circadian activity.

## Physical Setup

Adult male golden hamsters, *Mesocricetus auratus*, (Lakeview Hamster Colony, Newfield, NJ) were individually housed under a lighting regimen which consisted of fluorescent light (650 lux) from  $06^{00}$  to  $18^{00}$  hr. alternating with darkness from  $18^{00}$  to  $06^{00}$  hr. daily (LD<sub>12:12</sub>). Food and water were provided *ad libitum* and replenished at random or when needed.

Motor activity was measured using a capacitance-type activity monitor (Stoelting Co., Model #31400, Chicago, IL) equipped with a 6-digit printing counter (Stoelting Co., Model #22408), Figure 4. Movement of the animal resulted in changes in the capacitance field causing a "count." Counts were integrated over a 6-minute interval and a data printout obtained every 0.1 hr. After each print, the counters automatically reset to zero. Thus, 10 days of monitored activity (a time series) consisted of 2400 data points collected every 0.1 hr. of clock time. Activity counts were punched into IBM cards as four-digit numbers for computer analysis (Appendix B).

Instrumentation for the recording of gross motor activity data.
A = activity cage; B = nesting box; C = capacitance-type activity
monitor; D = 6-digit printout counter; E = activity record. Figure 4.



Figure 4

Recordings were obtained from individual animals maintained in Habitrail cages (Metaframe Corp., East Patterson, NJ) fitted with running wheels and an "isolated nesting box" which effectively positioned the animals above the electrical field and eliminated small extraneous counts during sleep. Cages were positioned on activity monitors and the entire unit (Figure 4) enclosed in a chamber covered with heavy black Visqueen. Hence, recorded data represents "total activity" including that associated with feeding, drinking, running wheel activity, etc. Four fluorescent lights (cage light intensity 650 lux) controlled by a time switch, imposed a 24-hour light-dark photoperiod (LD<sub>12.12</sub>; lights on  $06^{00} - 18^{00}$  hrs.) with step transitions from L to D and D to L. Daily temperatures averaged 27 + 2°C during the trial periods. (Note: some of the trials were conducted in a semi-soundproof room with overhead lighting for illumination.)

After 10 days of LD entrainment, animals were kept in constant illumination (LL) for 20 days followed by 20 days of constant darkness (DD; 0 lux). Thus, a complete time series for any one animal consisted of 50 continuous days of record. Although a transverse (between animal) profile was analyzed (see Table 3), emphasis in this thesis concerned longitudinal studies of time series data. Changes in the synchronized pattern of gross motor activity and the detection of multiple periodicities following alteration of

the lighting regimen were of particular interest. Hence, spectral amplitude analyses were performed on full 10-day data segments and on 3-day non-overlapping data subsets in order that the time course of these changes be more closely examined. In addition, the time series were subjected to autocorrelation and periodogram analyses for a comparison of findings. All calculations were performed with the aid of a CDC 6500 computer while all figures were plotted with the aid of a CDC Calcomp Plotter. The FORTRAN programs developed for each of the analyses employed in this study appear in Appendix B.

### Analysis of Data

# Least Squares Analysis: Program Waver (Appendix B)

Cosine functions were fitted to individual time series by the method of least squares. The following model was assumed:

$$Y_{i} = C_{0} + C[\cos(\omega t_{i} + \phi)] \pm \hat{E}_{i}; i = 1, 2, ... N$$
 (3.1)

where  $Y_i$  are measurements of the physiological variable (motor activity) at times  $t_i$ ; totalling N in number (for 10 days of data, N = 2400). The quantities  $C_0$ , C,  $\omega$ , and  $\phi$ represent the level (mean), amplitude, angular frequency, and acrophase of the fitted cosine. The least squares error estimate is given by  $\hat{E}_i$ . It was further assumed that if the data were aperiodic and random,  $C[\cos(\omega t_i + \phi)]$  could be eliminated and equation (3.1) reduced to:

$$Y_{j} = C_{0} \pm \hat{E}_{j}; j = 1, 2, ... N$$
 (3.2)

where  $\hat{E}_{j}$  represents the error associated with the mean. Elimination of the oscillating term might be due either to  $\omega$  assuming a value not statistically different from zero (i.e., because there is really no overall average periodicity), or by C assuming a value not statistically different from zero (i.e., the amplitude of the oscillation is hidden in the noise, or error term,  $\hat{E}_{i}$ ). Thus there is a real difference in logic between a truly aperiodic system and one that is not demonstrably periodic because of a low signal to noise ratio. In the usual case, however, periodicity is suggested if the error  $\hat{E}_{i}$  is minimized or reduced after fitting the data to equation (3.1) (i.e., when  $\hat{E}_{i} < \hat{E}_{i}$ ).

Expansion of equation (3.1) yields:

$$Y_i = C_0 + C \cdot \cos\phi \cos(\omega t_i) - C \cdot \sin\phi \sin(\omega t_i) \pm \hat{E}_i$$
 (3.3)

where  $(C \cdot \cos \phi)$  and  $(-C \cdot \sin \phi)$  represent "weighted amplitude" coefficients of the fitted cosine and sine functions. The values of these coefficients are given in the output to Program Waver (Appendix B) where they are denoted as HC and HS respectively. By substitution, equation (3.3) becomes:

$$Y_{i} = C_{0} + HC \cdot \cos(\omega t_{i}) + HS \cdot \sin(\omega t_{i}) \pm \hat{E}_{i}$$
(3.4)

Least squares regression theory defines a linear regression line as that straight line which results in  $\Sigma d_{y \cdot x}^2$  being at a minimum where  $\Sigma d_{y \cdot x}^2$  is equivalent to residual variation. In comparison, the minimizing equation for the least squares fit of a cosine is:

$$\sum_{i=1}^{N} \left[ \hat{E}_{i} \right]^{2} = \sum_{i=1}^{N} \left\{ Y_{i} - \left[ C_{0} + HC \cdot \cos(\omega t_{i}) + HS \cdot \sin(\omega t_{i}) \right] \right\}^{2}$$
(3.5)

For an illustrative example of the fitted equation and its variables, the reader is referred to Figure 1, from Halberg et al. (1972) and to the sample output of Appendix B.

All data sets were tested for the existence of statistically significant periodicities with period lengths ranging from  $\tau = 99.9$  hr. to  $\tau = 3.0$  hr. ( $\tau =$  period length in hours). Beginning with the first fitted frequency ( $\omega = 1$ cycle/99.9 hr.), Program Waver assembles a "weighted variance spectrum" where the amplitude of each period tested is associated with its own least squares error. Point estimates for the amplitudes of the fitted cosines  $\hat{C}$  (denoted as H in Waver output, Appendix B), were given by:

$$\hat{C} = \{(HC)^2 + (HS)^2\}^{\frac{1}{2}} = C \text{ (of equation 3.1)} (3.6)$$

since: HC =  $C\cos\phi$  and HS =  $-C\sin\phi$ 

$$\hat{C} = (C^2 \cos^2 \phi + C^2 \sin^2 \phi)^{\frac{1}{2}} = C$$
(3.7)

$$\hat{C} = [C^2 (\cos^2 \phi + \sin^2 \phi)]^{\frac{1}{2}} = C$$
 (3.8)

$$\hat{C} = (C^2)^{\frac{1}{2}} = C$$
 (3.9)

The ratio of H (for  $\omega = 1$  cycle/99.9 hr) to the standard

error of H (SE<sub>H</sub>) composed the first value of the "weighted variance spectrum." Successive frequencies fitted were determined by constant percentage (1%) decrementations of the preceding wavelength. Hence, the second fitted  $\omega$  would be <u>98.901</u> [99.9 - (99.9 X .01)], the third <u>97.912</u>, etc. Amplitude/standard error ratios for each wavelength fitted gave the amplitude-weighted variance spectrum. A theoretical example of one such spectrum is shown in Table 2.

	Period Length (hrs) (τ <sub>i</sub> )	Relative Deviate for the Cosine Amplitude (H/SE <sub>H</sub> )
τι	99.900	<u>1.8</u>
<sup>τ</sup> 2	98.901	2.6
τ3	97.912	3.0 printed sig.*
τ <b>4</b>	96.933	2.7
<sup>τ</sup> 5	95.964	2.6
<sup>τ</sup> 6	95.004	1.0
<sup>τ</sup> 7	94.054	0.8
<sup>τ</sup> 8	<u>93.113</u>	1.7 printed but NS
<sup>τ</sup> 9	92.182	1.6
τ10	91.260	0.5

Table 2. Example of a Typical Amplitude-Weighted Variance Spectrum

Only the peaks (i.e., 2.6 < 3.0 > 2.7) are printed out. All pertinent data for that peak are given as output (see Appendix B). It can be shown that the ratio  $H/SE_{\rm H}$  is comparable to the relative deviate of a "t" distribution. Hence, if the value of  $H/SE_{\rm H}$  for any  $\tau$  was greater than 2.58, that wavelength was said to have an amplitude which was significantly different from zero at p < 0.01 and N =  $\infty$ .

The final computer printout from Program Waver represents all periods for which the  $H/SE_{\rm H}$  ratios corresponded to spectral maxima. In Table 2, both  $\tau_3$  and  $\tau_8$  would appear as output but of the two only  $\tau_3$  is significant. Moreover,  $\tau_2$ ,  $\tau_4$  and  $\tau_5$  are also significant periodicities but since they do not represent spectral maxima, they do not appear as output. Decrementation of  $\tau_1$  continued until  $\tau_1 = 3.00$  hrs.

The point estimate,  $\hat{\phi}$ , for acrophase (in degrees from 00<sup>00</sup> hrs) is:

$$\tan\hat{\phi} = \frac{\text{sine coefficient (HS)}}{\text{cosine coefficient (HC)}} = \tan\phi \qquad (3.10)$$

$$\hat{\phi} = \arctan \frac{HS}{HC} = \phi$$
 (3.11)

and is given indirectly in terms of crest time (CT) of the fitted cosine, by the formula:

or

$$\hat{\phi} = \frac{C \cdot T \cdot}{\tau} (360^{\circ})$$

The basic rhythm parameters  $C_0$ , C,  $\tau$  and  $\phi$  were quantified using the method of least squares for a cosine function. The effect of changing photoperiod upon each of these variables was then examined. In addition, the time

series were analyzed longitudinally by examining the amplitude-frequency spectra produced by least squares analysis of full 10-day data segments or by 3-day nonoverlapping data subsets. Further, all data sets were evaluated in terms of an autocorrelation function, for examination of oscillatory stability in the time domain, and in terms of amplitude as a function of frequency in a periodogram.

# The Autocorrelation Function: Program Main (Appendix C)

Least squares analysis of a given set of data results in an average  $\tau$  value for any one periodicity within the time series. The stability of those detected periodicities in time and as lighting regimens were altered, was examined using sequential plots of the autocorrelation function applied to 10-day data segments of a complete 50-day time series.

The autocorrelation function consists of a series of correlation coefficients obtained by first correlating the entire time series with itself then lagging the data by 6-minute (or hourly) intervals and recorrelating. Calculation of the autocorrelation function for each data set required the use of Program Main (Appendix C) and a CDC 6500 computer, particularly for computation of the lagged sums of products (a maximum of 1200 computations is needed).

The estimate of r(k) for any lag, k, was computed as:

$$\mathbf{r}(\mathbf{k}) = \frac{\sum (\mathbf{x}_{t} \mathbf{x}_{t+k}) - (\sum \mathbf{x}_{t}) (\sum \mathbf{x}_{t+k})/n}{\left[ \sum (\mathbf{x}_{t}^{2}) - (\sum \mathbf{x}_{t})^{2}/n \right] \left[ \sum (\mathbf{x}_{t+k}^{2}) - (\sum \mathbf{x}_{t+k})^{2}/n \right]^{\frac{1}{2}}}$$
(3.13)

where n = N/2 (the number of pairs) and the summations run from t = 1 to t = n. Autocorrelation of each data segment continued until k = n (when the first half of the data was correlated with the last half). Detection of periodic components was aided by plotting the autocorrelation function for each time series as a correlogram with the aid of a CDC Calcomp Plotter and the plot routine of Appendix E.

# Periodogram Analysis: Program Spect (Appendix D)

Periodogram analysis, as described by Enright (1965a), represents a generalization of the Buys-Ballot form estimating technique, an averaging procedure for obtaining an unbiased estimate of the form of the underlying periodicity. This averaging procedure can be generalized for any integral period, p, in the form of a table (Appendix A).

The analysis involves an evaluation of a given set of frequencies as a function of their relative amplitudes. The test statistic computed in Program Spect (Appendix D) is the root-mean-square amplitude defined as:

$$A_{p} = \left[\frac{1}{P}\sum_{h=1}^{P} (Y_{p,h} - \bar{Y}_{p})^{2}\right]^{\frac{1}{2}}$$
(3.14)

All notations for equation (3.14) are defined in Appendix A.

Amplitude is estimated for all values of period length within a range presumed to include the period of the primary oscillation. Hence, the period value at which amplitude is maximal should represent the best estimater of the period of the primary oscillation. In this study, amplitude estimates were made at all values of period length from 20 hours to 30 hours for data sets of the kind described for autocorrelation analysis.

#### RESULTS

Longitudinal profiles of gross motor activity were obtained from a capacitance-type activity monitor as a fourdigit printout recorded every six minutes (0.1 hr.) for 50 days. Lighting regimens for the time series are described on page 39. Parameters defining the activity rhythms (i.e., amplitudes, frequencies, phase-angles and means) were estimated by periodic regression analysis.

The data were transformed to an assembly of cosines, each with its own period, amplitude, and phasing, using approximating functions of the form  $Y_i = C_0 + C[\cos(\omega t_i + \phi)] \pm E_i$ . The time series were then examined for the presence of multiple periodicities by plotting relative amplitudes of the cosine components  $(C/C_0)$  against frequencies (cycles/day) to yield a spectrum (after Sollberger, 1967). Peaks in such spectra represent dominant cosine components with large amplitudes.

## Transverse Profiles

Between-animal comparisons of the effects of constant light and constant dark on circadian activity parameters are presented below as a general orientation. When cosines were fitted to entire 10-day time series, a single dominant

component was found in the circadian frequency domain (22-27 hr.) representing the average and most significant periodicity for the time series. The characteristics of these components (i.e., period length, amplitude, phase angle, etc.) have been tabulated for five hamsters in Table 3.

The value found for period lengths  $(\tau)$  are in agreement with those cited for this species in the literature, Table 1. In general, changes in period length following constant illumination or constant darkness tend to follow Aschoff's rule; increasing slightly under constant light and decreasing slightly in constant darkness. However, period changes were, in all cases, small (1%) and because of the high between-animal variance and small sample size, no statistical significance could be attributed to treatment effects.

The specific lighting regimens imposed on each time series, twenty days of constant light followed by twenty days of constant dark, resulted in continuous phase drifts from an LD reference point which initially crested around midnight. The average phase angle during entrainment was -359.24 degrees with a between-animal variance of only 1.02 degrees (4.8 minutes). As expected, the absence of an <u>external</u> acrophase during constant light and constant dark resulted in a greater between-animal phase variance with the highest cumulative variation occurring after 20 days of constant dark (by this time the "clock" was nearly 16 hours

on circadian activity	
(DD)	
dark	
constant	
and	
(ILL)	
Effects of constant light	parameters† in hamsters.
Table 3.	

Treatment	<sup>LD</sup> 12:12 <sup>*</sup>	Consta	nt Light LL	Consta	unt Dark DD
Treatment days	10	10	20	10	20
Period length (T)	23.97	24.15	24.06	23.89	23.8 <b>4</b>
(hours)	± 0.13	± 0.14	± 0.08	± 0.20	± 0.25
<pre>Crest time (C.T.) (0.00 = midnight)</pre>	23.92	0.22	0.79**	7.74**	15.58 <b>**</b>
	± 0.19	± 0.21	± 0.32	± 0.36	± 2.15
Phase angle (¢)	-359.24	-3.24	-11.68**	-116.76**	-236.47**
(degrees from midnight)	± 1.02	± 3.78	± 1.06	± 6.28	± 34.21
Amplitude (C)	667.79	195.78**	701.55	545.46	341 <b>.99**</b>
(counts/0.1 hr.)	± 72.42	± 62.89	± 71.77	± 29.78	± 40.49
Mean activity level (C <sub>0</sub> )	<b>490.7</b> 6	159.72 <b>**</b>	<b>499.3</b> 3	560.71	325 <b>.35**</b>
	± 36.75	± 39.31	± 56.19	± 17.49	± 28 <b>.</b> 31
Normalized Amplitude $(C/C_0)$	<b>1.4</b> 0	<b>1.17**</b>	1.41	+ 0.08	<b>1.03**</b>
	± 0.02	± 0.09	± 0.03	+ 0.08	± 0.0 <b>4</b>

† = mean ± standard error (N = 5)
\* = control entrainment
\*\* = significant from LD control at p < 0.05; t > 2.306

slow, as well as 30 times more variable). As a result, a
statistically significant shift in phase was not demonstrable until the second half of the LL time series.

Significant changes in both mean activity levels (C<sub>0</sub>) and circadian amplitudes (C) were observed during the initial period of constant light and the final period of constant dark. Initial exposure to constant light resulted in a depression of mean activity from an average of 490.76 counts/0.1 hr. during entrainment to 159.72 counts/0.1 hr. during the first ten days of constant light. Circadian amplitudes during this period averaged 195.78 counts/0.1 hr. compared to a mean of 667.79 counts/0.1 hr. during entrainment. This depression of activity by constant light agrees with previously published findings for nocturnal rodents (DeCoursey, 1961). However, the effect appeared to be transitory since both parameters tended to recover to preconstant light (LD control) values by the end of the 20-day LL series.

Circadian amplitudes fell slightly while mean activity levels increased during initial exposure to constant darkness (DD). Neither parameter changed significantly, however, until 20 days of DD. Circadian amplitudes continued to fall throughout the time series while the initial rise in activity level was followed by an eventual depression. By the end of a 20-day DD series, circadian amplitudes averaged 341.99 counts/0.1 hr. while the average

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activity level was 325.35 counts/0.1 hr.

When circadian amplitudes were normalized to their respective means  $(C/C_0)$ , it was found that both constant light and constant dark caused a significant reduction in the ratio. Initially, under LL, the  $C/C_0$  ratio fell to an average value of 1.17  $\pm$  0.09 from a previous LD average of 1.40  $\pm$  0.02. Both amplitudes and mean levels fell during this period, however a disproportionate fall in circadian amplitudes (71%) compared to mean levels (67% fall) accounted for an overall reduction in the  $C/C_0$  ratio. The effect of LL again appeared to be transitory.

The C/C<sub>0</sub> ratio was significantly reduced throughout the entire DD period. The initial reduction (to a value of 0.98  $\pm$  0.08) resulted from both a fall in circadian amplitude and a rise in activity level. The combined effect of these changes was to greatly reduce the C/C<sub>0</sub> ratio. By the end of the DD period, the C/C<sub>0</sub> ratios showed partial recovery (mean = 1.03  $\pm$  0.04) but were still significantly depressed from their LD values.

In summary, analysis of 10-day time segments of hamster motor activity reveals the presence of a relatively stable and apparently synchronized circadian periodicity. While the period length  $(\tau)$  of this major component varies only slightly, its phase angle and amplitude are highly labile. Moreover, although transverse profiles are useful in the comparison of circadian parameters between individual

animals, longitudinal profiles allow for a more complete description of changes within the time series, including those which are transitory. Accordingly, the presence of additional periodicities was evaluated by examining the results obtained when spectral analyses were applied to individual time series.

## Spectral Analysis of Hamster Motor Activity

The spectral analysis procedure used in this study (Program Waver; Appendix B) produces a computer output of calculated amplitudes (with standard errors) and crest times for a large number of significant periodicities. Not all of these appear to be biologically meaningful when plotted in a line spectrum and are, in fact, introduced into the computations by the total length of record (T) and the interval between data points ( $\Delta$ T). It was apparent that an analysis of these "artifacts" (sidebands) was necessary prior to a meaningful description of the biological data. Consequently, computer simulations were performed on artificial input data of known waveform. Results of this analysis are presented in Figures 6-9.

#### Computer Simulations

The typical 24-hour (LD) rhythm of the hamster was the conceptual starting point for the simulation. Figure 5 illustrates daily activity records of several hamsters

Figure 5. Daily activity records of several hamsters recorded at different times during a 10-day period of LD<sub>12:12</sub> entrainment. Horizontal bars below each graph indicate lights off. Amplitudes are in counts/hr. x 10<sup>2</sup>. Each figure represents a daily record from a single animal.






recorded at different times during a 10-day period of LD<sub>12:12</sub> entrainment. Activity (in counts/hr.) is indicated on the ordinate; time (in hours) appears on the abscissa. The dark band below each graph represents lights off.

As the figure illustrates, activity in these animals occurred during the 12-hour dark span. Exact times for activity onsets and offsets varied between animals with onsets appearing less variable than offsets. Moreover, the waveform of entrained hamster activity is frequently multimodal, closely resembling a "rippled" square wave. Accordingly, the effect of fitting cosines to a non-sinusoidal function was examined using ten cycles of an artificial square wave as input to Program Waver. For comparison, cosines were also fitted to ten cycles of a single sine function. Thus, both data decks simulated a ten-day time series with  $\Delta T = 0.1$  hr., N = 2400, and f = 1 cycle/day ( $\tau = 24.0$  hrs.). The results are shown in Figure 6.

Figure 6 (a and b) represents the amplitude-frequency spectra for the square wave and sine wave analysis respectively. (Note: For significance levels, the reader is referred to Figure 7.) Amplitudes are expressed relative to the mean and are indicated on the ordinate as a  $C/C_0$ ratio. Corresponding frequencies appear on the abscissa in cycles/day. The figure indicates a dominant spectral peak at the frequency of the fundamental component (1 cycle/ day) for both waveforms.

Figure 6. Amplitude-frequency spectra from spectral analysis of a 24-hour square pulse (a) and a 24-hour sine function (b). Amplitudes are normalized to the mean at  $C/C_0$ . N = 2400;  $\Delta T = 0.1$  hr.



For the square wave (Figure 6a), the fundamental frequency gave a  $C/C_0$  ratio of 1.27 while the ratio for the sine function was 1.00. Moreover, the analysis produced numerous sidebands and harmonics even though a single periodicity was present. It is interesting to note that, apart from the fundamental components and their immediate sidebands (which had identical frequency values), the spectral patterns produced by the contrasting waveforms are quite different.

The sine spectrum (Figure 6b) indicates that the original waveform can be described as a single oscillatory component (a sinusoid) having a frequency of 1 cycle/day. Sideband amplitudes approach zero as the frequencies examined by the analysis increase. No secondary peaks occur at harmonic periods.

In contrast, the square wave spectrum (Figure 6a) shows several secondary peaks which rise significantly above background noise at frequencies of 3.0, 5.0, 7.0 and 9.0 cycles/day. These represent cosine components with period lengths of 8.00, 4.80, 3.42, and 2.67 hrs. respectively, and correspond to the odd harmonics of the fundamental frequency. (Note: A square wave is generated by summating a sufficient number of the odd harmonics of a sinusoid; Fourier synthesis.)

In Figure 7 (a and c), these spectra are again reproduced but include only those components with amplitudes

after alteration of sampling interval ( $\Delta T$ ). Figure 7a: significant cosine components of a 24-hour square pulse; N = 2400;  $\Delta T$  = 0.1 hr. Figure 7b: significant cosine components of a 24-hour square pulse; N = 240;  $\Delta T$  = 1 hr. Figure 7c: significant cosine components of a 24-hour sine function; N = 2400;  $\Delta T$  = 0.1 hr. Figure 7d: significant cosine components of a 24-hour sine components of a 24-hour sine function; N = 240;  $\Delta T$  = 1 hr. Comparison of amplitude-frequency spectra for square and sine functions Figure 7.



significantly higher than the overall mean level when evaluated statistically. In addition, six-minute data values were summated in these simulations (reducing N by a factor of 10, from N = 2400 to N = 240) to give hourly totals. The resulting spectra are shown in Figure 7 (b and d). As these figures indicate, a reduction in the number of data points comprising a time series and an increase in the sampling interval ( $\Delta T$ ), results in a loss of information provided by the spectra. For example, in the hourly square spectrum, Figure 7b, only the first three odd harmonics are significant, whereas the first nineteen odd harmonics are significant when  $\Delta T = 0.1$  hr. and N = 2400 (Figure 7a). Precise values for the spectra of Figure 7 (a and c) have been tabulated in Appendix F. All significant periods ( $\tau$  values) which appeared as output are listed, as well as their frequencies,  $C/C_0$  ratios, and phase angles. In addition, the amplitude of each component relative to the circadian amplitude has been expressed in a ratio  $(C/C_{24})$ column 5).

In the final simulation, cosines were fitted to a single square wave cycle to determine this method's usefulness in analyzing daily activity records resembling square waves. The results are shown in Figure 8. The best fitting cosine had a period length of 29.00 hrs. (frequency 0.82 cycles/day) rather than the expected 24.00 hrs. However, all secondary peaks represent the odd harmonics of a

Spectral analysis of a single cycle of a 24-hour square pulse. Amplitudes are normalized to the mean as  $C/C_0$ . The value appearing next to the highest component is its period length in hours  $(\tau)$ . Figure 8.



Figure 9. Relationship between continuous days of record of a 24-hour square pulse and the frequency of the circadian spectral component. Numbers indicate period lengths (hrs.) converging on 24.0 hrs.





24-hour square wave with the lowest frequency component being an 8.01 hr. harmonic (3 cycles/day).

In Figure 9, the relationship between continuous days of record (T) and the frequency of the dominant oscillation is illustrated. As the simulation deck was increased from one day of record to ten days of record, the dominant spectral component approached the value of the true periodicity (24.00 hrs.) as an assymptote. As the figure shows, a minimum of 3 continuous days of record was needed in order to reduce the distortion of the true periodicity from 5.00 hrs. to 0.37 hrs. (22.2 minutes). Ten days of record reduced the error of  $\tau$  to 0.02 hrs. (1.2 minutes). A tabulation of values for the three-day square wave spectrum can also be found in Appendix F.

## Analysis of Hamster Data

The results of spectral analysis of a complete 50-day time series from a typical hamster are presented sequentially in Figures 10-14. Each figure represents the spectral pattern produced when cosines were fitted to a 10-day data segment of motor activity. Amplitudes of the cosine components are indicated on the ordinate as a normalized ratio  $(C/C_0)$ ; frequencies are indicated on the abscissa in cycles/ day (24.0 hr. = 1 cycle/day). Occasionally, the period length ( $\tau$ ), in hours, of a dominant component is indicated along with its phase angle ( $\phi$ ) and its amplitude relative to the circadian amplitude  $(C/C_{24})$ .

In general, the spectra produced by 10 days of hamster motor activity typically show a single major component in the circadian frequency domain. Unlike the spectra of the pure waveforms used in the simulations, however, secondary peaks having various amplitude and phase relationships to the circadian component, are usually found at all harmonic (and sometimes at non-harmonic) frequencies. The peaks which occur at odd harmonic frequencies, for example at 3 cycles/day ( $\tau$  = 8.00 hrs.), are predictable from the square wave spectrum, but the appearance of even harmonics represents a deviation from both the square wave and sine waye simulations. Moreover, changes in the relative amplitudes of these components seem to be related at least in part, to the degree of internal dissociation produced by either LL or DD. In the illustrative examples, dissociation of the circadian rhythm into its components is demonstrated by the differences between the LD spectrum of Figure 10, the spectrum representing the first 10 days of LL (Figure 11), and the spectra from the entire DD series (Figures 13 and 14). Under constant light, the dissociation is transient and missing from the second 10-day period (Figure 12). This is demonstrated by a transient increase in the relative amplitudes of non-circadian frequencies and a transient decrease in the amplitude of the major 24-hour periodicity. The spectra illustrating the effect of DD,

Spectral analysis of 10 days of entrained (LD12:12) hamster activity data from a 50-day time series. [Amplitudes are normalized to the mean as  $C/C_0$ . Values for period lengths (hrs.) and phase angles (degrees) appear next to major components. Numbers in parentheses indicate the amplitude relative to the circadian amplitude ( $C/C_{24}$ ).] Figure 10.



however, suggest a more complete dissociation of the synchronized activity rhythm, demonstrated by a progressive increase in the significance of non-circadian frequencies and a relatively long lasting depression of the amplitude of the fundamental circadian component.

In Figure 10, the spectrum produced by synchronized (LD) data is illustrated. As the figure indicates, a single circadian component with a period length of 23.95 hrs. (1 cycle/day) and a phase angle of 83.63 degrees was the dominant periodicity for this animal during entrainment. The normalized amplitude  $(C/C_0)$  of this component had a value of 1.36 ± 0.02. In addition, with the exception of harmonic  $\ddagger7$ , a component can be identified at all harmonic frequencies up to 8 ( $\tau$  = 3.00 hrs.). Of these components, the first three had relatively high amplitudes when compared to the circadian peak. The 12-hour component had an amplitude which was 27.5% of the circadian amplitudes which were 31 and 30.2% of the circadian amplitude respectively.

As mentioned above, Figure 11 extends the analysis to include the first 10 days of the LL period following LD. The spectrum shows a single dominant component in the circadian domain with a period length of 23.98 hrs. and a phase angle of 96.34 degrees. Secondary peaks were again found at harmonic frequencies, slightly shifted but not

Spectral analysis of hamster activity recorded during the first 10 days of a 20-day constant light (LL) time period in a 50-day time series. [Amplitudes are normalized to the mean as  $C/C_0$ . Values for period lengths (hrs.) and phase angles (degrees) appear next to major components. Numbers in parentheses indicate the amplitude relative to the circadian amplitude  $(C/C_{24})$ .] Figure 11.



significantly different in period lengths from those seen in the LD spectrum.

Amplitude changes for both the fundamental and harmonic components constitute the greatest deviation from the LD The  $C/C_n$  ratio of the circadian component was spectrum. 1.07  $\pm$  0.04 during this period compared to a value of 1.36  $\pm$ 0.02 during LD. Further, the spectrum suggests some degree of dissociation, as evidenced by the presence of highamplitude harmonics. The peak at a frequency of 3 cycles/ day (period length 7.90 hrs.) for example, now has an amplitude which is 66% of the amplitude of the circadian compon-Similarly, the amplitudes of all other harmonics, ent. relative to the circadian amplitude, showed an increase from their values in the LD spectrum. In addition, peaks were found at other non-harmonic frequencies (e.g., at 2.8 cycles/ day) suggesting the presence of additional periodicities.

The spectrum illustrating the second half of the LL time series is shown in Figure 12. The general pattern is similar to that seen during LD, which suggests the reoccurrence of synchronization. A single major circadian component was found to have a period length of 24.17 hours and a phase angle of 88.48 degrees. Its normalized amplitude of  $1.37 \pm 0.03$  was comparable to the value seen during LD. In addition, the relative amplitudes of harmonic components (i.e., 12, 8, and 6-hour periodicities), showed a return to LD values. The 12-hour component had an amplitude which was

ţ Spectral analysis of hamster activity recorded during the second 10 days of a 20-day constant light (LL) time period in a 50-day time series. [Amplitudes are normalized to the mean as  $C/C_0$ . Values for period lengths (hrs.) and phase angles (degrees) appear next t major components. Numbers in parentheses indicate the amplitude relative to the circadian amplitude  $(C/C_{24})$ .] Figure 12.



19.4% of the circadian amplitude while the amplitudes of the 8 and 6-hour components were 27.9 and 16.1% of the circadian amplitude respectively. Peaks at non-harmonic frequencies were not discernible.

The spectra produced by activity data recorded under constant darkness are shown in Figures 13 and 14. Figure 13 includes the first 10 days and Figure 14 the second 10 days of a 20-day series. Both figures suggest a dissociation of the data which lasted for the entire time series. Figure 13 shows a major circadian component with a period length of 24.00 hrs. and a phase angle of 205.43 degrees. Its normalized amplitude, as expected from Table 3, fell to a value of 0.930  $\pm$  0.02. Components representing 12.3 and 8.2 - hr. periodicities were found to have amplitudes which were 57.2 and 63.6% of the major circadian amplitude.

Data recorded during the second half of the DD series produced a circadian component with a period length of 23.53 hrs. and a 12.04-hour component whose amplitude was 81.2% of the circadian amplitude, Figure 14. The normalized amplitude of the circadian period remained at a value of 0.932 ± 0.03 while the relative amplitudes of secondary components increased from their previous DD values. Further, higher frequency components became more significant during this period as, for example, in the occurrence of a 4.00 hr. periodicity with an amplitude 42.6% of the circadian amplitude.

Spectral analysis of hamster activity recorded during the first 10 days of a 20-day constant dark (DD) time period in a 50-day time series. [Amplitudes are normalized to the mean as  $C/C_0$ . Values for period lengths (hrs.) and phase angles (degrees) appear next to major components. Numbers in parentheses indicate the amplitude relative to the circadian amplitude  $(C/C_{24})$ .] Figure 13.



10 ţ Spectral analysis of hamster activity recorded during the second days of a 20-day constant dark (DD) time period in a 50-day time series. [Amplitudes are normalized to the mean as  $C/C_0$ . Values for period lengths (hrs.) and phase angles (degrees) appear next major components. Numbers in parentheses indicate the amplitude relative to the circadian amplitude  $(C/C_{24})$ .] Figure 14.



In summary, as the above figures indicate, spectral analysis of ten-day data segments of hamster activity produce amplitude-frequency spectra containing a single major component in the circadian domain. It is possible, however, that high amplitude peaks represent the summation of several circadian (or non-circadian) periodicities which occurred either transiently (and then dropped out or were resynchronized) or whose phase relationships made them indistinguishable as separate components in the specific 10day analysis examined. Consequently, detection of these additional periodicities was attempted by analyzing 3-day non-overlapping data subsets. The results are shown in Figure 15.

## Analysis of Three-day Data Subsets

Figures 10-14 illustrate that a major circadian periodicity can be identified from both LL and DD data. During these periods, however, significant changes in the amplitude of the circadian spectral peak (relative to its value in the LD spectrum) suggest a possible interference from one or more additional periodicities. Higher-thanexpected peaks at other frequencies also suggest the presence of transient (but real) components which are normally hidden in the synchronized record. Accordingly, spectral analyses were obtained from 3-day non-overlapping data subsets in order that changes in the gross activity

patterns be more closely documented. For the data presented in Figures 10-14, the results of such an analysis are shown in Figure 15 (a-p). As above, normalized amplitudes are plotted on the ordinate as a ratio  $(C/C_0)$ ; frequencies are shown on the abscissa in cycles/day. (Note: Amplitudes may be compared directly between plots since ordinates are drawn to the same scale; frequency scales vary slightly.)

Figure 15 (a-c) represents the spectra obtained from nine days of entrained (LD) activity data. Each plot includes 3 consecutive days. A single circadian component was found in each data subset with period lengths of 24.2, 23.8 and 24.0 hrs. ( $\bar{x} = 24.0 \pm 0.11$  hrs.; 1 cycle/day) and normalized amplitudes of  $1.43 \pm 0.03$ ,  $1.42 \pm 0.04$ , and  $1.34 \pm 0.04$  ( $\bar{x} = 1.39 \pm 0.03$ ) respectively. The slightly lower value seen during days 7-9 was caused principally by a reduction in amplitude (C). Mean activity level  $(C_0)$ remained constant at 522.73 ± 16.2 counts/0.1 hr. throughout the series. As in Figure 10, secondary peaks were found at "harmonic" frequencies with greatly reduced amplitudes compared to the circadian peak. The similarity of each consecutive plot suggests that entrainment of hamster activity produces a relatively stationary (stable) time series with minimal dissociation or desynchronization.

Data recorded during the first 3 days of LL (Figure 15d) showed a significant reduction in mean activity level (119.93 ± 11.23 counts/0.1 hr.) from previous LD values

50-day time series. Respective time segments and lighting regimens are indicated by each plot. Amplitudes are normalized to the mean as  $C/C_0$  and drawn to scale. Numbers appearing next to major components represent period lengths (hrs.). LD = light-dark; LL = constant light; DD = constant dark. Spectral analysis of 3-day non-overlapping data subsets of a continuous 50-day time series. Respective time segments and lighting regimens are Figure 15.







(p < 0.01). Both the 25.7 and 24.0 hr. periodicities appear to represent independent rhythms, since their phase angles (and crest times) were quantitatively different (48.4 and 91.4 degrees respectively). The phase difference of these two components is equivalent to 3 hours of clock time. In addition, secondary components at frequencies of 5.3 and 4.7 cycles/day had amplitudes which were 91 and 94% of the circadian amplitudes. Hence, the spectrum for this time period indicates a splitting (into two or more components) of the strongly circadian sleep-wakefulness pattern demonstrated in the LD spectra.

The most significant periodicity found during days 4-6 of LL had a period length of 8.03 hrs. and a normalized amplitude of 1.05  $\pm$  0.07, which suggests additional data splitting. A circadian component with a period length of 24.05 hrs. and an amplitude of 0.921  $\pm$  0.07 was also found. The longer circadian component seen in the previous three days could not be separated during days 4-6 but reappeared in days 7-9 (Figure 15f) and days 10-12 (Figure 15g). Mean activity level remained depressed during this period at 111.87  $\pm$  5.69 counts/0.1 hr. but circadian amplitudes rose slightly to produce somewhat higher ratios.

Like Figure 15d, Figure 15h, for days 13-15 of LL, shows two circadian components with period lengths of 25.8 and 24.1 hrs. and amplitudes of 1.46  $\pm$  0.05 and 1.43  $\pm$  0.05 respectively. As in Figure 15d, the circadian components

represent independent rhythmicities with a phase angle difference of 45 degrees (51.8 and 96.0 degrees) equivalent to three hours of clock time. Moreover, the small phase angle difference of the "25.7-hour" component between days 1-3 and days 13-15 of LL (3.4 degrees) suggests either that considerable phase shifting occurred for this periodicity or that the estimate of period length has an inflated error and is actually closer to 24.0 hours. In addition, secondary peaks at harmonic frequencies had greatly depressed amplitudes suggesting data resynchronization. This is further supported by the pattern of Figure 15i (also from LL) which resembles those spectra produced by the entrained data.

The remaining spectra of Figure 15 (plots j-p) extend the time series to include the DD data. Analysis of the first 12 days, Figure 15 (j-m), revealed a single component in the circadian domain having an average period length of 24.4  $\pm$  0.34 hrs. Some degree of data dissociation is suggested by a highly significant 12.1 hr. component in Figure 15j (with a normalized amplitude greater than the circadian period for this time segment) and, likewise, by an 8.42 hr. periodicity in Figure 15k (whose normalized amplitude was 96.3% of the circadian peak). Moreover, Figure 15j, for the initial 3 days of DD, shows a 53% reduction in the C/C<sub>0</sub> ratio of the circadian period (0.68  $\pm$  0.04) which resulted from the combined increase in the overall mean activity

level  $(C_0)$  and a decrease in its circadian amplitude (C).

Dissociation of the activity data into components is further evidenced in Figure 15n for days 13-15 of DD. With the exception of a 20.5 hr. component with a relatively low amplitude, no "circadian" period could be demonstrated. Instead, the most significant periodicity isolated during this segment had a period length of 12.19 hrs. and an amplitude of 0.95 ± 0.08.

Spectral splitting in the circadian domain occurred after 16 days of DD, Figure 150. Two high amplitude components were found to have period lengths of 23.9 and 22.7 hrs. and normalized amplitudes of 1.15 ± 0.05 and 1.14 ± 0.05 respectively. As was the case in the LL spectra (Figure 15 d and h), both components represent independent rhythms which crested 2 hours apart for an average phase angle difference of 30 degrees (352.5 and 22.5 degrees respectively). Finally, in contrast to LL, the last spectrum of the DD series (Figure 15p) offers no evidence for data resynchronization which would restore the spectrum to its LD pattern.

## Analysis of Hamster Data Using Autocorrelation Functions and Periodograms

Spectral analyses of hamster motor activity under LL and DD demonstrate the presence of multiple periodic components representing independent rhythmicities. To confirm
such findings, the time series were subjected to autocorrelation and periodogram analyses. Each technique emphasizes a different aspect of the data and, hence, the detection of multiple components by all methods would further support the conclusions of the spectral analyses. For the data presented in the previous section, the results of autocorrelation and periodogram analyses are shown in Figures 16-24.

## Results of Autocorrelation Analyses

The effect of lighting regimen on hamster activity patterns is illustrated in Figures 16-23. The five graphs of activity data (top) comprise a typical 50-day time series presented chronologically, with each figure encompassing a 10-day data segment. In order that the entire series be illustrated, only the hourly sums are reproduced (since the CDC Calcomp Plotter accepts only 500 data points as input to the plot routine). Nevertheless, analysis of 6-minute data produced patterns of the autocorrelation function similar to the hourly data (bottom). In some cases, for example the first 10 days of the LL series, a greater resolution of the autocorrelation pattern was needed. For this reason, detailed plots of the autocorrelation analyses from 6-minute data have also been included (Figures 18, 21 and 23).

In Figures 16-23, hamster activity (raw data) expressed in counts/hr. as recorded from the activity monitors, is

Figure 16. Ten-day time series of entrained  $(LD_{12:12})$ hamster activity (a) and corresponding autocorrelation function (b). Amplitude ordinate is in scientific notation in ranges of 1.857 x  $10^3 - 7.426 \times 10^3$  to 1.021 x  $10^4 - 1.578 \times 10^4$ counts/hr. Rho = serial correlation coefficient; lag = hourly shifts of the time series.





indicated on the ordinates in scientific notation (see Legends on facing pages). Time, in hours, is plotted on the abscissa. The corresponding autocorrelation functions for each data segment appear below the raw data plots for comparison. Each of these graphs represents a sequence of product-moment correlation coefficients, Rho, obtained by autocorrelation of the full ten-day segment. The values of the coefficients range from +1.0 to -1.0 on the ordinate, while the time lag extends to 120 hours on the abscissa. All analyses utilized Program Main (Appendix C) and a CDC 6500 computer; results were plotted with a CDC Calcomp Plotter.

Figure 16b presents the typical correlation sequence for entrained  $(LD_{12:12})$  hamster data. As the figure illustrates, photoperiodic entrainment of hamster activity produced an autocorrelation pattern in which a single periodic component was observed. Autocorrelation of ten days of activity data produced five highly significant peaks with an average interpeak interval of 24.0 ± 0.1 hrs. corresponding to an exogenous 24.0 hr. photoperiod. Peak correlation coefficients averaged a highly significant 0.82 ± 0.01 (p << 0.01) and occurred at 24.2, 48.2, 71.7, 96.0 and 120.0 hrs. The raw data, Figure 16a, from which the autocorrelation function was derived, are typical of entrained hamster activity, exhibiting sustained periods of activity (when the lights are off) followed by equally sustained

periods of sleep. This is evidenced by a high first-order serial correlation coefficient for the LD data of 0.81. It should be noted that the LD data, which are the controls for other lighting regimens, appear to be almost square waves (in the plot of raw data) or triangular waves (in the autocorrelation plot). The reader is referred to Figure 3 for similarities between the autocorrelation functions for a sine wave, a square pulse, and LD hamster activity data (page 33).

Figure 17a illustrates the initial effect of LL on the activity pattern. In the absence of an exogenous photoperiod, sustained periods of activity and sleep, like those previously discernible, are still present but with a more erratic pattern. The first-order serial correlation coefficient for this series was reduced to 0.42. In addition, LL caused a 78.5% reduction in the average intensity of activity from that seen under LD (note the difference in magnitude of the amplitude scales, Figure 16a vs. Figure 17a). Mean activity level under LD measured 5315 ± 421 counts/hr. compared with an initial level of 1143 ± 134 counts/hr. during LL.

Figure 17b illustrates the autocorrelation function for the data recorded during the first 10 days of the 20day LL series. A detailed analysis (using 6-minute data;  $\Delta T = 0.1$  hr.) is presented for the same time period in Figure 18. As the analysis suggests, peak correlation for

Figure 17. Ten-day time series of hamster activity
recorded during the first half of a 20-day
constant light (LL) time period (a) and corresponding autocorrelation function (b).
Amplitude ordinate is in scientific notation in
ranges of 875 - 7.002 x 10<sup>3</sup> counts/hr. Rho =
serial correlation coefficient; lag = hourly
shifts of the time series.



this time segment followed lags of 24.1, 48.4, 72.8, 96.2, and 119.5 hrs. for an average interpeak interval of 23.9  $\pm$ 0.23 hrs. Moreover, in contrast to the single oscillation observed for LD data, a second peak occurred in the autocorrelation function after a lag of 25.3 hrs. (and again at 50.4, 74.3, and 100.8 hrs.) suggesting the presence of a 25.2  $\pm$  0.53 hr. periodicity. (These peaks are better illustrated in Figure 18, although only every fifth point has been plotted.) In addition, autocorrelation produced secondary peaks which occurred at regular intervals as the data were lagged. They are illustrated in Figure 18 preceding the major components.

The first of these peaks occurred after lags of 6.5, 31.7, 57.0 and 82.6 hrs. and had an average interpeak interval of 25.36  $\pm$  0.12 hrs. Likewise, the second peak in the autocorrelation function reoccurred four times (at 17.4, 41.0, 64.7, and 88.2 hrs.) resulting in a periodicity with an interpeak interval of 23.6  $\pm$  0.05 hrs. Following a lag of 90 hrs., however, secondary peaks in the autocorrelation function are difficult to observe. This suggests that multiple periodicities produced by LL are transitory (at least initially), are best detected from the initial data segment, and that resynchronization is likely to occur after about 5 days, suggesting beat phenomena. It is interesting to note, furthermore, that peak correlation values for this ten day segment were significantly lower than those observed

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Six-minute autocorrelation function of hamster activity recorded during
the first half of a 20-day constant light (LL) time series. Rho =
serial correlation coefficient; lag = shift interval in 0.1 hr.
1. \overline{x} = 25.3 \pm 0.12 hrs.
2. \overline{x} = 23.6 \pm 0.05 hrs.
3. \overline{x} = 23.9 \pm 0.29 hrs.
4. \overline{x} = 25.2 \pm 0.75 hrs.
        Figure 18.
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for the LD data (p << 0.01). The most significant oscillation ( $\tau = 23.90 \pm 0.23$  hrs.) had an average peak correlation value of only 0.29  $\pm$  0.03 (Figure 17b) compared to an average value of 0.82  $\pm$  0.01 under LD. This drop in correlation was probably due to the presence of multiple periodicities in the data as well as their wide phase dispersion.

Figure 19a continues the data record to include the second 10 days of the LL series. As the figure illustrates, data splitting was less frequent than in the previous 10 days. Mean amplitudes during the first three days of this time period were comparable to those of the preceding series, but by day 14 daily amplitudes returned to LD levels. Mean activity intensity for the entire series was 4377 ± 397 counts/hr., representing a 73.9% increase from the previous ten days.

The clean delineation of sleep and activity cycles seen in the LD data returned after 13 days of LL producing an autocorrelation function similar in shape to that observed during entrainment (Figure 19b). This was further evidenced by an increase in the first-order serial correlation coefficient to a value of 0.69. It is apparent from the LL figures that the partial periodicities seen during the first 10 days of LL resynchronized by the end of 20 days of LL, presumably by shifts in phase. The resynchronization of these periodicities was followed, in turn, by a significant increase in the resultant amplitude vector.

Figure 19. Ten-day time series of hamster activity recorded during the second half of a 20-day constant light (LL) time period (a) and corresponding autocorrelation function (b). Amplitude ordinate is in scientific notation in ranges of  $1.752 \times 10^3 - 9.636 \times 10^3$  to  $1.226 \times 10^4 - 1.489 \times 10^4$  counts/hr. Rho = serial correlation coefficient; lag = hourly shifts of time series.





The autocorrelation pattern calculated for this second ten day segment had peaks at 23.8, 47.8, 72.3, 96.3 and 119.9 hrs., for an average period length of 23.98  $\pm$  0.14 hrs. In addition, peak correlation increased during this period to a mean of 0.64  $\pm$  0.03.

Dissociation of hamster activity by DD is illustrated in Figures 20a and 22a. Data splitting occurred but to a lesser degree than that seen during LL. The first-order serial correlation coefficient fell only slightly during the first 10 days of DD to a value of 0.67, and was still relatively high by the end of 20 days at a value of 0.55.

For the first 10 days (Figure 20a) the mean activity level was 5146  $\pm$  378 counts/hr. which was not significantly different from the mean levels of the LD and final LL time series (Figures 16a and 19a). Moreover, the autocorrelation function for this 10-day segment (Figure 20b) revealed three periodic components. A major peak occurred at 24.6, 49.2, 73.3 and 97.8 hrs. indicating a 24.45  $\pm$  0.12 hr. periodicity with a relatively high average correlation value of 0.55  $\pm$  0.03. Secondary peaks are illustrated in Figure 21 for the analysis of six-minute data. The peak at 7.6 hrs. occurred four additional times (at 33.5, 58.7, 81.0 and 104.9 hrs.) suggesting a rhythmicity with an average period length of 24.13  $\pm$  0.06 hrs. Likewise, the peak at 17.0 hrs. appeared four times to suggest the presence of a 24.77  $\pm$  0.30 hr. periodicity.

Figure 20. Ten-day time series of hamster activity recorded during the first half of a 20-day constant dark (DD) time period (a) and corresponding autocorrelation function (b). Amplitude ordinate is in scientific notation in ranges of  $1.865 \times 10^3 - 7.460 \times 10^3$  to  $1.026 \times 10^4 - 1.5854 \times 10^4$  counts/hr. Rho = serial correlation coefficient; lag = hourly shifts of time series.



Figure 20

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Six-minute autocorrelation function of hamster activity recorded during
the first half of a 20-day constant dark (DD) time series. Rho =
serial correlation coefficient; lag = shift interval in 0.1 hr.
1. \overline{x} = 24.4 \pm 0.15.
2. \overline{x} = 24.1 \pm 0.67.
3. \overline{x} = 24.7 \pm 0.30.
          Figure 21.
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In contrast to the LL time series, dissociation seemed to be more complete during the second ten days of DD, Figure 22b. In fact, it is difficult to identify multiple periodic components which possess relative stability other than a single, stable circadian period averaging 23.90 ± 0.18 hrs., with peaks at 23.6, 71.6, 95.9, and 119.5 hrs. Peak correlation values averaged 0.33 ± 0.03 and, although several secondary peaks occurred during the initial lags, their stability following subsequent lags was not easily discernible. The transitory nature of the secondary peaks is again suggestive of wide phase dispersal. Mean activity level was reduced 50.64% to 2623 ± 265 counts/hr. which was significantly lower than the LD value (p << 0.01).

## Periodogram Analysis

Figure 24 (a-e) offers examples of periodograms calculated from the hamster activity data of Figures 16-23 (top). The range of period lengths examined was permitted to vary in steps of 0.1 hr. from 20-30 hrs., limits which include the circadian domain of 24.0 hrs. Estimates of  $A_p$  (rootmean-square amplitude) for each of the 101 values of assumed period are plotted on the ordinates; corresponding period lengths are found on the abscissa.

The primary feature of each graph is an amplitude peak at a period value which corresponds closely to the value obtained by each of the autocorrelation analyses. For the

Figure 22. Ten-day time series of hamster activity recorded during the second half of a 20-day constant dark (DD) time series (a) and corresponding autocorrelation function (b). Amplitude ordinate is in scientific notation in ranges of  $1.570 \times 10^3 - 8.633 \times 10^3$  to  $1.099 \times 10^4 - 1.334 \times 10^4$  counts/hr. Rho = serial correlation coefficient; lag = hourly shifts of time series.





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Six-minute autocorrelation function of hamster activity recorded during the second half of a 20-day constant dark (DD) time series. Rho = serial correlation coefficient; lag = shift interval in 0.1 hr.
1. \overline{x} = 24.0 \pm 0.21.
           Figure 23.
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LD data, Figure 24a, maximum amplitude occurred at a period of 23.9 hrs., which is comparable to the 24.0 hr. periodicity seen in the autocorrelation function for this time segment. Likewise, periods of 23.9 and 24.0 hrs. are present in the periodograms for the first and last 10 days of the LL time series (Figure 24b and c), corresponding to autocorrelation values of 23.9 and 24.0 hrs. respectively. In addition, the results of Figure 24b, for the first 10 days of LL, agree with the findings of the autocorrelation analysis that at least two periodicities were present in this data segment. Besides the peak at 23.9 hrs. previously cited, a second, equally significant peak occurred for a period of 25.0 hrs. which is comparable to the 25.2 hr. periodicity identified by the autocorrelation function.

In contrast to the autocorrelation analysis, however, resolution of multiple periodicities from the DD data is not possible from the periodograms for these segments (Figure 24d and e). Instead, only a single peak occurred in each case at 24.5 and 24.0 hrs. for the first and second 10-day segments. These values, however, do correspond closely to the major periodicities detected by the autocorrelation analysis (24.4 and 24.0 hrs. respectively).

Figure 24. Periodograms of 10-day segments of hamster activity data from a continuous 50-day time series. Figure 24a: LD entrained data. Figure 24b: Initial 10 days of LL data. Figure 24c: Final 10 days of LL data. Figure 24d: Initial 10 days of DD data. Figure 24e: Final 10 days of DD data. A = root-mean-square amplitude.



Figure 24



Figure 24 continued

## DISCUSSION

Any time series, regardless of waveform, may be analvzed (by means of Fourier's theorem) into an additive assembly of cosines whose parameters (i.e., frequencies, amplitudes and phasing) can then be objectively quantified (Sollberger et al., 1967). The phase and amplitude of each of these cosine components plotted as a function of frequency constitute the spectrum. In general, the procedure involves converting the data from a function of time to a function of frequency. The spectrum then obtained will show prevalent periodic components (rhythmicities) having prominent amplitude peaks at their respective frequencies as opposed to the "flat" spectrum representing white noise. Strict Fourier procedures, however, make assumptions regarding the length of the fundamental component (f = k/T; frequency = k/total length of record) and, as a result, the calculated harmonic frequencies include the true basic periodicity of the time series only by coincidence. Accordingly, the amplitude-frequency spectra of standard Fourier analyses are discontinuous since they contain only one set of harmonics.

To avoid this criticism, the spectral analysis procedure of Program Waver uses a least squares format to fit

(by predetermined increments) cosines of sequentially decremented period lengths. Those cosines which show a greater amplitude/standard error ratio than that of functions fitted immediately preceding or following it, appear as output. The result is a quasi-continuous spectrum of peaks from a periodogram of best fitting cosine functions (see Table 2). Moreover, it should be noted here that the error estimate for values of period length can therefore be approximated by twice the decrementation interval. Hence, if the decrements occur as 1% of the previous wavelength, a conservative estimate of the error associated with any one periodicity can be given as ± 2% of the value of the respective wavelength.

The computer simulations on test input data having known waveforms, period lengths, amplitudes, and phase angles, have established certain criteria for the interpretation of spectra obtained from biological data. The first of these concerns the accuracy of period estimation given a periodic function that is not a sinusoid. As illustrated in Figures 8 and 9, the distortion produced by the fit of a cosine to a square pulse is inversely related to the length of the time series or, more accurately, to the number of repetitive pulses. Increasing the number of cycles of a "24-hour" square pulse from one cycle to ten cycles reduced the distortion of the true periodicity from 20% to 0.08%. For entrained hamster data, which are similar in shape to a square pulse, it appears (from Figure 9) that a minimum of

3 continuous days of record is needed for the production of meaningful spectra, in the sense of a low error in period length. There is still an error in absolute amplitude, however, as discussed below.

A second criterion established by the computer simulations relates the waveform to the pattern of harmonic peaks. As illustrated in Figure 6, a pure "24-hour" sine function with a crest time at  $06^{00}$  hrs. produces a single spectral peak (with its sidebands) at a frequency of 1 cycle/day and a phase angle (as indicated in Appendix F) of 90.76 degrees. In addition, no secondary peaks occur at harmonic frequencies. On the other hand, the spectrum produced by a "24hour" square pulse exhibits secondary peaks at all of the odd harmonic frequencies of the fundamental component. The square pulse had a step transition from 0-100 at 12<sup>00</sup> hrs. which resulted in a phase angle of 270 degrees (midpoint of the pulse) for the fitted cosine. Concurrently, the phase angles for all of the odd-harmonic components averaged 269.5 ± 1.8 degrees.

The relationship of the amplitude of a harmonic component to that of the fundamental frequency  $(C/C_{24})$ , as evidenced by the values for the square spectrum (Appendix F), is given by the reciprocal of the harmonic number (at least for functions approximating a square pulse). Thus, for a "24-hour" square wave the third harmonic ( $\tau = 8.00$  hrs.) has an expected amplitude of 1/3 or 33% of the amplitude of

the 24-hour period, while the fifth harmonic ( $\tau = 4.8$  hrs.) produces a C/C<sub>24</sub> ratio of 1/5 or 0.20, etc. Moreover, it should be noted in Appendix F that the least squares fit of a cosine to a 24-hour square pulse results in an "overshoot" of the circadian amplitude estimate by approximately 27% (as evidenced by a C/C<sub>0</sub> ratio of 1.27 for the square pulse as opposed to a value of 1.00 for the sine). It is evident that this error is easily compensated for by normalizing all amplitudes to the circadian peak.

The term "harmonic" in the above description indicates frequencies in the spectrum (detectable by the least squares fit of a cosine) which are integer multiples of the fundamental. For "pure functions," their expected amplitudes and phase angles will vary in a manner consistent with the waveform of the major periodicity. In the case of a sine wave, for example, peaks in the spectrum will be absent at harmonic frequencies (expected amplitude 0% of the amplitude of the fundamental), whereas periodic functions which approximate a square pulse would be expected to produce spectral peaks at odd harmonic frequencies, with predictable relative amplitudes and phase angles. However, biological oscillators would rarely (if ever) be expected to produce symmetrical periodicities. Hence, peaks at harmonic frequencies in the biological spectra, while referred to as "harmonic" components in this text, may in fact represent real and independent periodicities since their amplitude and phase

characteristics (under LL and DD) are often inconsistent with either the square wave spectrum or the spectrum of LD control data. It is hard to imagine, for example, a spectral peak at a frequency of 2 cycles/day ( $\tau = 12.0$  hrs.) which has an amplitude that is 81.2% of the amplitude of the circadian period as being anything less than an indicator for some real component (see Figure 14) rather than the simple second harmonic of the 24.0-hr. period, in the strict sense. Moreover, if the 12.0-hr. periodicity does represent a "real" component, then the analytical power of Program Waver is such that multiple periodic components may be identified as coexisting in the same time series. To test this, Kasiske (1972) applied the least squares analysis (Program Waver) to an artificial 24-hour sine function and, in addition, to a function formed from the summation of four sinusoids (period lengths 23.9, 8.0, 6.0 and 4.0 hrs.) with different amplitudes and phase angles. The function can be expressed as:

$$(t) = 142 + [127 \cos(\frac{2\pi}{23.9}t - \frac{2\pi}{24} 21.8) + \\ 84 \cos(\frac{2\pi}{8}t - \frac{2\pi}{8} 5.7) + 73 \cos(5.1) \\ (\frac{2\pi}{6}t - \frac{2\pi}{6} 4.8) + 60 \cos(\frac{2\pi}{4}t - \frac{2\pi}{4} 3.1)]/4$$

As expected, for the 24-hour pure sine simulation, the analysis illustrated a single peak in the amplitude spectrum

f

at a frequency of 1 cycle/day. However, for the function described by equation (5.1), amplitude peaks were detectable at all component frequencies (i.e., at 1, 3, 4, and 6 cycles/day) and at phase positions identical to those of the input curves. As evidenced by equation (5.1), their amplitude values were equivalent to  $C_i/4$  (where  $C_1 = 127...$  $C_4 = 60$ ). Moreover, no significant shifts in these parameters occurred after the addition of random noise. In other words, the output spectrum of f(t) revealed the presence of 4 periodicities of predictable amplitude and phase which were known a priori to be independent.

Finally, it should be noted that, as Figure 7 illustrates, the resolution of any given spectrum is inversely related to the length of the sampling interval ( $\Delta$ T) and directly related to the length of the time series (T). The analytical power of least squares analysis will thus be increased by small sampling intervals and long time series.

As illustrated in Figure 5, the general waveform of entrained hamster activity bears closer resemblence to a "rippled" square wave than to a sinusoid. This is due principally to the sharp rises in activity onset and, usually by equally sharp falls in offsets. (The reader is not to infer, however, that such patterns are reproduced in the natural state, since they may in fact, be produced by the step transitions of the artificial LD photoperiod in the laboratory). Consequently, the output spectra produced

by least squares analysis of LD data were expected to exhibit patterns similar to the square pulse simulation (Figure 6a). However, as shown in Figure 10, entrained hamster data show characteristic deviations from both of the previously mentioned simulations. (The reader is here referred to Appendix G for numerical values of the five biological spectra presented earlier.) Unlike the sine wave simulation (Figure 6b), the amplitude spectra typically produced by LD data exhibit components at all harmonic frequencies with harmonics 2, 3 and 4 ( $\tau = 12.0$ , 8.0, and 6.0 hrs. respectively) generally possessing "substantial" amplitude values relative to the higher frequency harmonics. Moreover, the appearance of the "even harmonics" represents a deviation from the square wave spectrum and, as alluded to earlier, cannot be attributed to the presence of random noise.

Like the square wave simulation, the 8.0-hr. component illustrated in Figure 10 had an amplitude and phase value predictable by square wave analysis (see Appendix G for  $\tau = 8.0$  hr.). Consequently, the LD spectra typically offer little evidence regarding the "authenticity" of this component as being anything other than the third harmonic of the circadian period. In principal, of course, this alone war rants a closer examination of the 8.0-hr. oscillation (and other significant harmonics) since the actual waveform of the data may reflect important features of the neural

system which produces circadian behavior. In the case of a square pulse, for example, these higher frequency components correspond to the fine ripples and sharp corners of the waveform. It may be, however, that these secondary components correspond to rhythmicities in the output data which reflect interactive sub-systems of the neural networks. This is evidenced by the fact that when the system is disturbed, as with constant light (see Figures 11 and 12), the amplitudes and phase angles of these harmonic components appear to be modulated independently from the fundamental. In Figure 11, for example, the 8-hour component (actually 7.9 hrs.) showed a phase angle of -267°, constituting a phase shift of approximately 180° from its value under LD, while the circadian component shifted by only 14°. In addition, its amplitude relative to that of the circadian period was twice its predicted value. When the data showed resynchronization (Figure 12), the relative amplitude of the 8hour component returned to a value compatible with the simulation but its phase angle (-137°) remained inconsistent with the expected value. Evidence for a "real" 8-hour component is further suggested, though not completely demonstrated, by the fact that data dissociation produced an 8.33-hr. component (Figure 11) with a phase angle of 61.3° and a relative amplitude which was 62.8% of the circadian amplitude. Since the amplitudes of these components (and therefore their significance in the spectrum) do not differ
significantly, there is no criteria for accepting one as a "real" component and the other as merely a sideband. It should be noted, moreover, that the ability of the least squares spectra to illustrate "additional" independent rhythmicities will, of course, depend in large part on the "natural strength" of any given periodicity as well as the degree to which it can be dissociated from the synchronized cycle by either LL or DD. Nevertheless, at this point, lacking more precise measures of data collection, Figure 11 suggests a periodicity around 8 hours possibly representing an independent "motor sub-set." Peaks at higher component frequencies, previously observed, may do the same.

That the waveform of entrained hamster activity data resembles the square pulse of the simulation, aside from producing odd-harmonic components in the amplitude spectrum, is further evidenced by the fact that spectral analysis of the LD data, which produced the ten-day spectrum of Figure 10, on a day-to-day basis resulted in an average periodicity of  $30.3 \pm 0.34$  hrs. as a fundamental frequency. When these daily records were analyzed as a ten-day time series, however, the spectrum produced a highly significant circadian period of 23.95 hrs. corresponding to the 24.0 hr. photoperiod (Figure 10). This relationship follows the square wave simulation illustrated in Figure 9.

A deviation from the square wave spectrum, which has yet to be explained, is the appearance of secondary

components at even-harmonic frequencies in the spectra of LD data. Simulations which include the addition of random noise, as mentioned earlier, have yet to demonstrate the production of these unexpected components. Moreover, they are not produced as artifacts to the LD<sub>12:12</sub> photoperiod. It might be argued, for example, that if the waveform of entrained data is, on the average, a "bimodal" function, with peaks at onset and offset separated by 12 hours, the fit of a 12-hour cosine might produce a lower least squares error estimate than surrounding frequencies and, as a result, appear in the spectrum. Recently, however, data recorded under LD<sub>16:8</sub> entrainment, where activity occurred only during the 8 hours of darkness, produced spectra with highly significant peaks at 12.0 and 6.0 hrs. In the LD<sub>16.8</sub> data, no combination of peaks resulted in either a 12 or 6-hour interval to which these components might then have been related. Moreover, no waveforms thus far simulated, including triangular waves, can account for the presence of 6 and 12-hour periodicities. Whether these components are real or artifactual (and certainly all waveforms have not been tested), the evidence to date characterizes them as peculiar only to the biological spectra. Thus there is no apparent way, as yet, to question their validity as reflections of true biological activity.

Aschoff (1973) defines the transitory state whereby rhythmicities within an organism change their mutual phase

relations from one steady state to another steady state as "internal dissociation." Internal desynchronization, on the other hand, occurs when different rhythms show different frequencies in the steady state. It appears from this study, that complete desynchronization of the strongly circadian activity cycle of the hamster is difficult to demonstrate in a 20-day time series. In general, as the activity graphs of Figure 16-22 (top) exemplify, LL animals tended to show a transient dissociation initially after which resynchronization occurred in the absence of an external periodicity. Further, although DD data also dissociated, it generally took longer than LL data (compare Figures 20 and 22) and hence, the 20-day time series was too short to reveal complete desynchronization. Nevertheless, the spectra of Figures 10-14 offer evidence for the presence of multiple periodicities in circadian activity in dissociated data.

Aside from the "non-circadian" spectral components previously considered, the 10-day spectra suggest that several periodicities may be present whose period lengths are near 24 hours. This is evidenced, not by the appearance of several circadian peaks, but by the reduction in the  $C/C_0$  ratio of the single peak seen in the LL and DD spectra (see also Table 3). Recent simulations have shown that a time series containing three "24-hour" cosines separated by 90° produces a spectrum with a single peak in the circadian

domain but with a greatly reduced normalized amplitude  $(C/C_{o})$  and a phase angle representing the average of all three. However, because of the specific operating format of Program Waver, the fitting of the significant 24.0-hr. cosine ( $\tau = 1$  cycle/day) is followed by a 1% frequency decrementation and the fitting of additional cosines with smaller wavelengths. Consequently, the presence of multiple periodicities having identical period lengths would not be directly detected by the Waver program (that is by the appearance of several circadian peaks in the spectrum). In fact it is unlikely that additional circadian peaks will appear in a 10-day spectrum unless the real periodicities which they would represent had period lengths different enough to allow for several decrementations to occur. In this case, the presumption is that at least one of the relative deviates of the separating decrements might produce a trough in the amplitude spectrum (between the periodicities) and allow peaks to occur at the appropriate frequencies. All circadian components, differing by at least 2%, might then appear in the output spectrum. Nevertheless, regardless of the number of peaks which appear in the circadian domain, any time series whose spectra indicate significant reductions in the  $C/C_0$  ratio of the circadian peak must be viewed as potentially possessing more than one circadian periodicity. It is appropriate to suggest for future study that, since several circadian components may be "visible"

in dissociated or desynchronized data, the decrementation interval in the circadian domain be reduced (in Program Waver) to increase the probability of finding multiple circadian peaks in 10-day spectra. Comparisons with LD spectra from time series of equal length (using the same decrementation schema) would of course have to be made.

An additional note concerning the occurrence of only a single circadian peak in the 10-day spectra has to do with the stability (in time) of the various periodicities. In this case, spectral analysis of the full 10-day time series might be able to isolate only a single component whose period length, amplitude, and phase angle represent an averaging of various unknown circadian components. Analysis of shorter time series, however, might uncover these "transient" components provided that the "strength" of any one periodicity is sufficient enough to allow for its detection and also that an appropriate data sub-set is chosen. Moreover, if any given sub-set contains more than one periodicity, their period lengths should be sufficiently separated to allow for the occurrence of several decrementations according to the rationale stated above. The presumption of these remarks relates of course, to the analytical requirement for stationarity in the basic data set. Detectability is thus seen to be a balance between the length of record and the time structure of the biological output data set.

Figure 15 illustrates the results of spectral analyses on shorter time series. Three-day non-overlapping data subsets of hamster activity were chosen in accordance with the criteria established in Figure 9, namely, that there be at least three repetitive cycles for periodic functions resembling a square pulse. For a comparison with a 3-day square pulse spectrum, the reader is referred to Appendix F.

Typically, the spectra from LD sub-sets (Figure 15 a-c) indicate that entrainment of hamster activity produces relatively stationary (stable) time series having a single circadian component and hence, are in general agreement with the 10-day LD spectra. In contrast to the 10-day LL spectra, however, 3-day analysis of LL data offer evidence for the presence of additional circadian components when the activity data are dissociated. Data splitting is evidenced in Figure 15(d and h) by the simultaneous occurrence of two circadian components of equal significance (amplitude) but phase shifted by about 45° (3 hours). Moreover, the spectra following Figure 15d indicate that the stability of these components is such that they appear transiently (and then fade out) until, as in this example, the data resynchronize (Figure 15i). In addition, two circadian components were "uncovered" from the DD data of the example (Figure 150) although not until 16 days of constant darkness. However, the spectra preceding Figure 150, for example Figure 15n, indicate that some dissociation began prior to the

occurrence of the "split-spectrum." In days 13-15 of this time series (Figure 15n) the most significant periodicity, according to spectral analysis, had a period length of 12.1 hrs. In fact, no circadian component appeared, with the possible exception of a low amplitude 20.6-hr. component, during this time segment. Since many of these higher frequency components appear in the 3-day analyses, and with greater significance levels than the "true" circadian frequencies [see Figure 15 (e, j, m and n)], it is difficult to call them either harmonics or sidebands of a circadian period. The possibility that they are correlates of real biological rhythmicities must therefore be considered.

It should be noted here, in accordance with a previous discussion, that the appearance of two circadian components in two of the LL and one of the DD spectra probably occurred because they were sufficiently different in period length to allow for several decrementations to occur. For the LL spectra, at least 7 decrementations occurred between the fitting of the 25.7 and 24.0-hr. components; at least 5 occurred in the DD spectrum between 23.9 and 22.7 hrs. This does not suggest, however, that when several peaks are found in the circadian domain of least squares spectra they must therefore have significantly different period <u>lengths</u> when evaluated statistically. It must be emphasized that if such components appear with "equal" amplitudes but with crest times which are 3 hours apart (as in this example),

the possibility that they represent independent rhythmicities must be considered even if statistical differences in period lengths cannot be demonstrated.

Before leaving the 3-day spectra, attention is drawn to the difference in the splitting patterns observed in the LL and DD spectra. Under LL, the split occurred between a 24.0-hr. component and a slightly longer component of 25.7 hrs. In the DD spectrum, the split occurred between a 23.9hr. component and a slightly shorter component of 22.7 hrs. Whether this pattern bears any relationship to Aschoff's rule is not known but it is pointed out here as a possible question for future study.

Two alternative methods for finding the frequency content of periodic time series are autocorrelation analysis and the periodogram methodology of Enright (1965a). The general impression of this study regarding the periodogram technique, however, is that it lacks the resolution necessary for the detection of multiple periodicities. As illustrated in Figure 24b for LL data, two periodogram peaks representing periodicities of 23.9 and 24.8 hrs. were detected. However, the DD periodograms (Figure 24 d and e) failed to clearly resolve any periodicities other than a single 24-hr. component even though the spectral and autocorrelation analyses (both of which represent more powerful techniques) offer evidence for the presence of multiple components in the DD data. Autocorrelation analysis, on the

other hand, is a useful technique for the detection of periodic signals although, since it does not offer information regarding the phase of a rhythmicity, it is best used in conjunction with spectral analysis.

A comparison of findings between the spectral analysis procedure used in this study and autocorrelation analysis reveals that the two are in general agreement. For LD data, both autocorrelation and spectral analysis indicate that entrainment results in a single observable periodicity. However, the number of circadian components resolved from dissociated data is often different for the two techniques. For the example presented here, spectral analysis of LL data showed two circadian components whereas autocorrelation of the same time series resulted in four. This is probably because the decrementation criterion seen in the spectral program is not identical to the lag sequence required for the autocorrelation analysis. Examination of the four periodicities indicated by the autocorrelation function of Figure 18 (average period lengths are  $25.3 \pm 0.12$ ,  $23.6 \pm$  $0.05, 23.9 \pm 0.29$ , and  $25.2 \pm 0.75$ ) reveals that their proximity probably accounts for the appearance of only two of them in the spectrum. A sufficient number of decrementations does not occur between 25.3 and 25.2 hrs. nor between 23.9 and 23.6 hrs. Consequently the spectrum revealed only two circadian components but their period lengths are in general agreement with those of the autocorrelation

function. The same argument may be used for the three circadian components found by autocorrelation of the DD time series (Figure 16) where the spectra revealed only two.

The correlograms of Figures 18 and 21 suggest that multiple circadian periodicities can be detected in both LL and DD activity data. Moreover, the fact that the LL data in this example resynchronized after 14 days, suggesting a dissociated rather than a completely desynchronized time series, illustrates that autocorrelation functions may detect periodicities even when they are "transients." It should also be noted that detection of any one periodicity by autocorrelation is probably contingent on the number of "visible" cycles which present themselves in the complete time series and of course, on the number of actual periodicities present. The latter is so because the presence of additional periodicities may reduce the value of the particular correlation coefficient for any one component (as opposed to the case where only one was present) and hence, certain periodicities may not be discernible above the "noise." This would also be true for periodicities which exhibit phase shifts or frequency changes. In this case, they would appear in the correlogram at their average length with reduced amplitude or, if the shifts were large enough, possibly not at all.

Finally, the degree to which activity data are dissociated (or desynchronized) may be identified in the first data

lag by the value of the first-order serial correlation coefficient; a measure of whether high values tend to be followed by high values or low values by low values. In general, both LL and DD data show lower first-order serial correlation coefficients than LD controls. Moreover, as in the example of Figure 19, an increase in the serial correlation coefficient from data recorded under either LL or DD is an indication of resynchronization. The LL data of Figure 17 for example, had a first-order serial correlation coefficient of 0.42 (compared to 0.81 under LD) which is reflected in the raw data plot, Figure 17a, by a high degree of "data splitting." The data of Figure 19a, however, which appear to be relatively synchronized under LL, reflect an increase in the first-order serial correlation coefficient of 0.69.

The phenomena of beats in frequency analysis has only been alluded to above by the reference to frequencies which fade in and out. Perfectly stationary output time series will of course show beats (i.e., difference frequencies) whose measurable amplitudes go through maxima and minima. These may, in the long run, be the most revealing of all for the construction of those physiological models for gross motor activity toward which this field of investigation is ultimately directed.

The current hypothesis which proposes a multiplicity of individual oscillators in circadian organization may be

applicable to activity data. It has been shown that such records contain information regarding the output from several "motor sub-sets" (such as eating, drinking, running wheel activity, etc.) which may exhibit independent rhythmicities when freed from light-dark synchronization (Wolterink et al., 1973). Classical strip-chart recordings of motor activity which measure (often subjectively) the periodic course of a single parameter (typically activity onset) are therefore inadequate to a total understanding of the neural networks which contribute to circadian behavior. Recording gross motor activity, however, with a capacitance-type activity monitor produces a numerical printout which is easily amenable to complex time series analysis. Moreover, it is obvious that recording techniques which separate (from the total time series) the output from different motor sub-sets will further this understanding.

The above considerations have almost been a defense of a "modified-Halberg-cosinor" methodology, guided by autocorrelation and simulation, despite recent attacks upon its basic validity. By the use of 1% decrementations, looking at first-order coefficients becomes a reasonable first approximation to a "multiple-Fourier" periodogram. It fails, however, to do true second, third and n-order coefficients, and it is only by simulation, not by analysis, that the results can be shown to be biologically meaningful. Nonetheless, such analyses are necessary to an understanding

of those motor mechanisms responsible for circadian activity as they allow for a more detailed description of the biological time structure and provide the analytical basis for model building.

## SUMMARY AND CONCLUSIONS

 Circadian activity data recorded in constant light or constant darkness exhibit minor shifts in frequency but major shifts in amplitude and phase.

2. Both constant light and constant darkness cause a general depression of mean activity levels, although constant darkness may cause a transient increase initially. This is usually accompanied by a reduction in circadian amplitude thus reducing the  $C/C_0$  ratio.

3. Spectral analysis of activity data involves a conversion of the time series from a function of time to a function of frequency. Prevalent periodic components are then represented by peaks in an amplitude-frequency spectrum.

4. The spectral analysis program developed for this study (Program Waver) produces a modified-Fourier periodogram which results in a more complete amplitude-frequency spectrum than either classical Fourier analysis or the simpler cosinor program of Halberg.

5. Computer simulations (spectral analyses) on test input data of known waveform, period length, amplitude and phase angle are useful in establishing criteria for the interpretation of spectra obtained from biological data.

6. In general, the spectral pattern of a periodic time series depends in part on the overall shape and stationarity of the periodicity.

7. For periodic functions that are not sinusoids, such as for square waves, the distortion in period length produced by the fitting of a cosine can be reduced by increasing the number of repetitive cycles.

8. The resolution of any given spectrum is inversely related to the length of the sampling interval ( $\Delta T$ ) and directly related to the length of the time series (T).

9. Spectral analysis of daily entrained hamster activity records containing as many as 240 points in one day may result in an error in period length estimation of as high as 20%. For this reason, at least 3 continuous days of record are needed for the production of meaningful spectra.

10. Since entrained hamster activity closely resembles a square pulse, the estimate of amplitude even in a 10-day record may result in an "overshoot" of about 27% in the estimation of a circadian amplitude.

11. The time series methodology examined in this dissertation (spectral analyses, autocorrelation functions, and periodograms) indicate the presence of multiple periodicities in "dissociated" hamster activity.

12. In addition to the detection of multiple-periodic components in time series data, spectral analysis results in quantification of rhythm parameters in terms of period length, phase, and amplitude.

13. Although multiple periodicities are easily detected in "dissociated" data by autocorrelation functions, they are best used in conjunction with spectral analysis since they offer no determination of phase and only indirect estimation of amplitude.

14. The periodogram procedure of Enright (1965a) appears to lack the resolution necessary for the detection of several circadian periodicities.

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## LITERATURE CITED

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APPENDICES

## APPENDIX A

## BUYS-BALLOT TABLE\*

Row 1	x <sub>1</sub>	×2	•••	x p
Row 2	x <sub>p+1</sub>	x <sub>p+2</sub>	•••	x <sub>2p</sub>
•••	•••	• • •	• • •	• • •
Row m	X _p(m-1)+1	X_p(m-1)+2	• • •	x pm
Totals	U <sub>p,1</sub>	U <sub>p,2</sub>	•••	U <sub>p,p</sub>
Averages	Y <sub>p,1</sub>	<sup>Y</sup> p,2	•••	Y <sub>p,p</sub>
$N = tota$ $X_{i} = hour$ $p = peri$ $an i$ $P = larg$ $h = hour$ $inte$ $m = numb$	I number of ly observati od for which nteger); est integer for which a eger (0 < h < p per of measur	hourly observa on for the <i>i</i> th form is to be < p; e.g. if p n average is t ); rements enterin an integer:	hour (0 < i e estimated b = 24.7, P to be calculat:	<pre>integer;** i &lt; N);  (need not  = 24; lated, an ion of an</pre>
y <sub>p,h</sub> = mean esti	hourly valu mate for ass	the for the <i>h</i> th sumed period, p	hour of the	e form
j = an i numb	nteger, esse per in the Bu	entially the equiverse of the equiverse of the second second second second second second second second second s	uivalent of .e.	f the row
For each w less than	value of h an or equal to	d p, let m be	the larges	t integer

be

$$1 + \frac{N - h}{p} .$$

Then

$$Y_{p,h} = \frac{1}{m} \sum_{j=1}^{m} X_{(h+(j-1)p)}$$

<sup>\*</sup> from Enright (1965b) ΔT may equal 0.1 hr.

	ບັບ	PROGRAM WAVER (INPUT, OUTPUT, TAPE60=INPUT, TAPE61=0UTPUT) CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
ഹ	000	THIS PROGRAM IS DESIGNED TO TAKE TEN DATA NUMBERS PER CARD AND USE A SIN AND COSINE WAVE TO DEVELOP AN APPROXIMATE FIT TO THE DATA. THIS PARTICUI
	U U U U	SETUP IS FOR THE HAMSTER DATA. CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
10	с С	REAL LENGT, LENGTH, LENGTHQ
	ບບ	READ IN CONTROL VALUES.
15		<pre>1 READ (60,300) DTIME, DLENGTH, NL, LENGT 300 FORMAT (10X,2F10.5,110,F10.5) DL =DLENGTH</pre>
	ບບ	DTIME IS THE SEPARATION . IN TIME, BETWEEN THE DATA POINTS.
20	000	DLENGTH IS THE DELTA LENGTH (IN PER CENT OF THE WAVELENGTH) USED IN THE DECREMENTS.
	ບບບ	NL IS THE NUMBER OF TIMES THE DECREMENTATION IS TO BE DONE LENGT IS THE STARTING WAVELENGTH.
	)	PI=3.1415926536
25	ບບ	
	υυυ	READ IN DATA, SAVING DO LOOP INDEX AND OUTPUT DATA
06	ر	10 CALL DATA (N, FLAG)
) )	υ	SET THE DATA POINT COUNTER TO THE NUMBER OF DATA POINTS.

APPENDIX B

Spectral Analysis Program for 6500 CDC Computer\*

```
H
                                                                                                                                                                                                                                           = ATQ
                                                                                                                         WRITE (61,401) N, DTIME, DLENGTH, NL, LENGTH
401 FORMAT (* N =*,15,10X,*DTIME =*,F5.2,10X,*DLENGTH =*,F5.1,10X,
1 *NL =*,15,10X,*LENGT =*,F5.2/)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  COMPUTE THE DIFFERENCE FROM THE AVERAGE OF EACH DATA POINT.
                                                                                                                                                                                                                                          = SEQ2
                                                                                                                                                                                                                                        LENGTHQ = ERSQ = HQ = SEQ = YQ = CTQ = PSQ = HSEQ
1 HSQ = HCQ = HD = TOTAL = 0.0
                                                                                                                                                                                                                                                                                                                       TOTAL IS THE SUMMATION OF ALL THE DATA POINTS.
                                                                                                                                                                                                                                                                                                                                                                                                                                                  WRITE (61,402) AVERAGE
FORMAT (* AVERAGE OF THE DATA = *,F15.10/)
DO 102 I = 1,N
                                                                                                                                                                                                                                                                                                                                                                                                    COMPUTE THE AVERAGE OF THE DATA.
                                                                                                                                                                                          SET VARIOUS STUFF TO ZERO.
                                                                                            OUTPUT THE CONTROL VALUES.
                                                                                                                                                                                                                                                                                                                                                     TOTAL = TOTAL + X(I)
                                                                                                                                                                                                                                                                                                                                                                                                                                    AVERAGE=TOTAL/REALN
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  XA(I) = X(I) - AVERAGE
                                                                                                                                                                                                                                                                                      DO 101 I = 1,N
                              LENGTH = LENGT
                                                                                                                                                                                                                                                                       LENGTH=LENGT
                                           DLENGTH = DLTFLAG = 0
              200 \text{ REALN} = \text{N}
                                                                                                                                                                                                                                                                                                                                                                      101 CONTINUE
                                                                                                                                                                                                                        IDHQ=0
                                                                                                                                                                                                                                                                                                                                                                                                                                                                    400
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```
CALCULATION OF THE RESPECTIVE SINES AND COSINES OF VARIOUS ANGLES.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                              XAS IS THE SUMMATION OF XA TIMES THE ASSOCIATED SINE.
ERSQ IS THE SUMMATION OF THE DIFFERENCES SQUARED.
                                                                                                                                     SEQ2 IS THE STANDARDED ERROR SQUARED OF THE DATA.
                                                                                                                                                                                                             SED=SQRT (SED2)
WRITE (61,403) SED
FORMAT (* STANDARD ERROR OF THE DATA*,F15.10)
DO 103 M = 1,NL
                                                                                                                                                                                   SEQ IS THE STANDARD ERROR OF THE DATA.
                                                                         AVERSQ IS THE AVERAGE OF ERSQ.
                             XA(I) + ERSQ
                                                                                                                                                                                                                                                                                                                        ANGL(I) = (I - 0.5) * DANGLE
                                                                                                                                                                                                                                                                           IF (LENGTH.LE.1.0) GO TO 980
                                                                                                                                                                                                                                                                                          DANGLE= 2.0*PI*DTIME/LENGTH
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          XAS = XA(I) * SEN(I) + XAS
                                                                                                                                                                                                                                                                                                                                                        = 0.0
                                                                                                                                                   SED2=AVERSQ/(REALN - 1.0)
                                                                                                                                                                                                                                                                                                                                                                                                                                 SEN(I) = SIN (ANGL(I))CES(I) = COS (ANGL(I))
                                                                                                                                                                                                                                                                                                                                                       XAS = XAC = SS = CC
DO 105 I = 1,N
                                                                                                       AVERSQ=ERSQ/REALN
                             ERSQ = XA(I) *
                                                                                                                                                                                                                                                                                                       DO 104 I = 1,N
                                             102 CONTINUE
                                                                                                                                                                                                                                                                                                                                        CONTINUE
                                                                                                                                                                                                                                              403
                                                                                                                                                                                                                                                                                                                                        104
                                                                                                                                                                                                                                                                                                                                                                                     υυυ
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```
IF ONE DESIRES THESE VALUES OUTPUT (LEAST SQUARES ERROR AND ITS SQUARE)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  DUPLICATE THE NEXT TWO FORTRAN STATEMENTS WITHOUT THE C IN COLUMN ONE.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   450 FORMAT (* LEAST SQUARES ERROR =*,F10.5,*LEAST SQUARES ERROR SQUARE
              XAC IS THE SUMMATION OF XA TIMES THE ASSOCIATED COSINE.
                                                                                                                                                                                                 HC IS THE MAXIMUM AMPLITUDE OF THE FITTING COSINE WAVE.
                                                                                                                                HS IS THE MAXIMUM AMPLITUDE OF THE FITTING SINE WAVE.
                                                                                                                                                                                                                                                                 H IS THE MAXIMUM AMPLITUDE OF THE RESULTANT WAVE.
                                                                                                                                                                                                                                                                                                                                                                  CALCULATION OF THE LEAST SQUARES ERROR.
                                                                                                                                                                                                                                                                                                                                                                                                  Z = XA(I) - HS * SEN(I) - HC * CES(I)
                                             XAC = XA (0) * CES (I) + XAC
SS = SEN (I) *SEN (I) + SS
CC + CES (I) * CES (I) + CC
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  WRITE (61,450) SE, SE2
                                                                                                                                                                                                                                                                                                                                                                                                                                                                    SE=SQRT (Y/ (REALN-1.0))
                                                                                                                                                                                                                                                                                                 H=SQRT (HS *HS+HC+HC)
                                                                                                                                                                                                                                                                                                                                DO 106 I = 1,N
                                                                                                                                                                                                                                                                                                                                                                                                                   א
+
                                                                                                                                                                                                                                HC = XAC / CC
                                                                                                                                                               HS = XAS / SS
                                                                                                                                                                                                                                                                                                                                                                                                                  Σ * Σ = Σ
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    SE2=SE*SE
                                                                                                                                                                                                                                                                                                                                                                                                                                  CONTINUE
                                                                                             105 CONTINUE
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    HSE=H/SE
                                                                                                                                                                                                                                                                                                                                                                                                                                                   X = X/N
                                                                                                                                                                                                                                                                                                                   Y=0.0
                                                                                                                                                                                                                                                                                                                                                                                                                                    106
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                                                                                                                                                                                                                                                                                 120
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                               105
```

1D = \*, F10.5/00000 140

AT IS THE TANGENT OF THE PHASE ANGLE.

AT=HS/HC

145

PS IS THE PHASE ANGLE. υυυ

PS=ATAN (AT)

DETERMINE IF THE PHASE ANGLE IS IN THE PROPER QUADRANT, IF NOT, NORMALIZE. υυυ 150

```
IF (PS.GE.0.0) GO TO 201
PS=PS+PI
```

```
IF (HS.GE.0.0) GO TO 202
PS=PS+PI
201
          155
```

```
υυυ
```

```
CT IS THE CREST TIME OF THE RESULTANT WAVE.
```

```
202 CT = PS * LENGTH / (2.0 * PI)
IF (HSE-HSEQ) 203,204,205
                       160
```

```
GO TO 206
IDH = 0
GO TO 206
IDH = -1
203
```

```
204
```

```
IDH = 1
205
206
```

```
HD = H
IF (IDH.GT.0.0.0R.IDHQ.LT.0.) GO TO 209
IF (IFLAG.NE.0)GO TO 208
                                                      IFLAG=1
```

```
DLENGTH = DL / 10.0
LENGTH = LENGTH + 2.0 * DL * LENGTH / 100.0
  170
```

```
GO TO 209
DLENGTH =DL
         208
```

```
IFLAG=0
```

```
WRITE (61,404) LENGTHQ,HQ,SEQ,HSEQ,SEQ2,CTQ,ATQ,HSQ,HCQ
404 FORMAT (/,4X,*LENGTH*,7X,*H*,11X,*SE*,9X,*HSE*,10X,*SE2*,9X,*CT*,10
1X,*AT*,10X,*HS*,10X,*HC*,/,1X,F9.4,8(2X,F10.6))
                                                                                                                                                                                                                                                                                                                                                                                                        FLAG WAS PASSED FROM SUBROUTINE. THE VALUE OF -1 INDICATES
THERE ARE ADDITIONAL SETS OF DATA TO BE READ AND -2
                                                                                                                                                                                                                                                                                                                                                                                                                                       INDICATES THAT NO MORE DATA SETS ARE AVAILABLE
                                                                                                                                                                                                                                                                                                              COMPUTE THE NEXT DECREMENT OF THE WAVELENGTH.
                                                                                                                                                                                                                                                                                                                                                                                          TEST IF ADDITIONAL DATA AVAILABLE
                                                                                                                                                                                                                                                                                                                                            LENGTH = LENGTH - LENGTH * DLENGTH / 100.
                                                                            SAVE THE PRESENT VALUES OF THE VARIABLES.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     FORMAT (*-*15X*END OF RUN*)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                     980 IF (FLAG.EQ.-1.0) GO TO 10
                                                                                                                                                                                                                                                                                LENGTHQ=LENGTH
                                                                                                            IDH
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     PRINT 985
                                                                                                                                                                                                     HSEQ=HSE
                                                                                                                                                                                                                                                                                                                                                            CONTINUE
                                                                                                                                                                                     SEQ2=SE2
                                                                                                             H
                                                                                                                                                                                                                                  ATQ=AT
                                              N4=N-4
                                                                                                                                                       CTQ=CT
                                                                                                                                                                       SEQ=SE
                                                                                                                                                                                                                    PSQ=PS
                                                                                                                                                                                                                                                  SH=QSH
                                                                                                                                                                                                                                                                 HCQ=HC
                                                                                                         СНОІ
                                                                                                                                         х=ох
                                                                                                                           H=QH
                                                                                                            209
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     985
                                                                                                                                                                                                                                                                                                                                                            103
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    END
                                                                                                                                                                                                                                                                                                                                                                            0000000
                                                              υυυ
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                      205
                                                                                                                                                                                                                                                                                                               195
                                                                                                                                                                                                                                                                                                                                                                                           200
                                                                           180
                                                                                                                                                       185
                                                                                                                                                                                                                                   190
 175
```

SUBROUTINE DATA(N,FLAG) COMMON /INP/ X(3000).AW(3000)	C THIS WILL READ IN RAW DATA AND KEEP AS IT IS C READ IN DATA UNTIL YOU FIND -SIGN BUT SAVE DO INDEX	DO 10 I=1,1000,10 N=1+9 READ(60,20) (X(J),J=*,N),(AW(J),J=I,N) 20 FORMAT(10F4.0, 9X,A2,1X,A3,1X,A2,1X,A2,1X,A3,1X,A4,1X,A3,1X,A3,A1, 1A2)	<pre>IF (EOF (60)) 60,55 55 IF (X(I).GE.010) GO TO 10 N=L-1 FLAG=X(I) GO TO 30 10 CONTINUE </pre>	C 60 CONTINUE C PRINT OUT RAW DATA	<pre>30 WRITE(61,25) 25 FORMAT(*1*40X*RAW DATA*) D0 40 J=1,N,10 K=J+9 WRITE(61,50) (X(L),L+J,K),(AW(L),L=J,K) 50 FORMAT(* *10(2X,F5.1),10X,A1,1X,A3,2 (1X,A2),1X,A3,1X,A3,1X,</pre>	IA3,AI,A2) 40 CONTINUE RETURN END
	ĿО	0	Ю	0	ъ	0
	.,	1(	Ä	2(	5	ЭС
\*Partial Output Sample From Program Waver

LENGT = 99.90	
NL = 997	
DLENGTH = 1.0	087500000
DTIME = .10	THE DATA = 561.
N = 240	AVERAGE OF

STANDARD ERROR OF THE DATA 52.3156108386

LENGTH	Н	SE	HSE	СT	АТ	HS	НС
54.6471	778.22	39.98	<b>19.4</b> 6	53.19	1680	28.9	767.46
23.9811	820.20	36.47	22.48	5.44	6.8995	811.7	117.64
12.7540	310.53	50.34	6.16	5.45	4905	136.7	78.80
7.5532	190.03	51.58	3.68	2.22	-3.4754	182.6	-52.54
5.5913	343.84	49.94	6.88	2.91	.1308	-44.6	40.94

\*Data Deck Format

INPUT. 789 Binary Deck 789 Control Card Data Deck -002 6789 APPENDIX C

Autocorrelation Program for 6500 CDC Computer\*

PROGRAM MAIN (INPUT, OUTPUT, TAPE60=INPUT, TAPE61=OUTPUT, PUNCH) CALL DATA (N, FLAG) COMMON RHO (1300) 502

COMPUTE THE NUMBER OF LAGS TO TAKE. M=0.5\*N

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- CALL SPEC(N,M) CHECK FLAG FOR EOF 0 IF(FLAG.EQ.-1) GO TO 502 PUNCH 26,(I,RHO(I),I=1,M) 6 FORMAT(5(I5,F10.5)) WRITE(61,605) 500
  - 26
- FORMAT (\*-\*20X\*END OF RUN\*) END 605

```
FORMAT (*1*40X*RAW DATA OF PRODUCT MOMENT CORRELATION*)
DO 10 K=1,3700,10
N=K+9
SUBROUTINE DATA (N, FLAG)
                     COMMON /INP/ X (2500)
DIMENSION AW (4)
                                                                WRITE(61,5)
```

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- CHECK FOR OVERWRITING ARRAY IF (N.LE.2500) GO TO 15 FORMAT (\* ARRAY TOO SMALL FOR INPUT\*) WRITE (61,20) 20
  - STOP
- 15 READ(60,25) (X(J),J=K,N), (AW(MK),MK=1,4) 25 FORMAT(10F4.0,4A10)

```
DIMENSION SSX2 (1300), SPX1X2 (1300), RHO (1300)
                                                                         WRITE(61,40) (X(J),J=K,N), (AW(MK),MK=1,4)
                                                                                                                                                                                                                                                                                                    SUMX1=SUMX2=SUMX1SQ=SUMX2SQ=SUMX1X2=0.0
                                                                                                                                                   = *I4
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        SSX2 (LAG)=SUMX2SQ-(SUMX2**2)/XN
                                                                                                                                                FORMAT (*ONUMBER OF DATA POINTS
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                SUMX2SQ=SUMX2SQ+ (X (K) **2)
SUMX1X2=SUMX1X2+ (X (K-LAG) *X (K) )
                                                                                         FORMAT (* *10 (1X, F7.2), 3X, 4A10)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             SPX1X2 (LAG)=SUMX1X2-PRODX12
                                                                                                                                                                                                                                                                                                                                                                                                                  SSX1=SUMX1SQ-(SUMX1**2)/XN)
                                                                                                                                                                                                                                                                                                                                                            SUMX1SQ=SUMX1SQ+ (X(K) **2)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                SUMX2=SUMX2SQ=SUMX1X2=0.0
IF (X (K).GE.0.0) GO TO 30
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         PRODX12=SUMX1 *SUMX2/XN
                                                                                                                                                                                                                           SUBROUTINE SPEC (N, M)
                                                                                                                                                                                                                                                               COMMON /INP/ X (2500)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               SUMX2=SUMX2+ X(K)
                                                                                                                                                                                                                                                                                                                                        SUMX1=SUMX1+ X (K)
                                                                                                                                                                                                                                             COMMON RHO (1300)
                                                                                                                               WRITE(61,60) N
                                                                                                                                                                                                                                                                                                                                                                                                                                    DO 15 LAG=1,M
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            DO 10 K=I,J
                                                                                                                                                                                                                                                                                                                      DO 5 K=1,M
                                    FLAG=X(K)
                                                     GO TO 35
                                                                                                             CONTINUE
                                                                                                                                                                                                                                                                                                                                                                               CONTINUE
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      CONTINUE
                                                                                                                                                                                                                                                                                                                                                                                                                                                        J=M+LAG
                                                                                                                                                                                                                                                                                                                                                                                                                                                                           I=LAG+1
                                                                                                                                                                    RETURN
                 N=K-1
                                                                                                                                                                                                                                                                                                                                                                                                W=NX
                                                                                                                                                                                       END
                                                                                        40
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15 CONTINUE
    PRINT 30
30 FORMAT(*1*5X* K *10X*RHO*//)
D0 20 K=1,M
RHO(K)=SPX1X2(K)/(SQRT(SSX1*SSX2(K)))
RHO(K)=SPX1X2(K),SSX1,SSX2(K)
PRINT 25,K,RHO(K),SPX1X2(K),SSX1,SSX2(K)
25 FORMAT(* *4X,I4,5X,F10.5,3(2X,F16.3)
20 CONTINUE
END
```

The program provides the user with a punched output deck containing five X,Y coordinates If all points per card. The autocorrelation plot routine plots every fifth coordinate. are desired, a change in the punch format statement is needed (line 10).

Output: K = Time lag RHO = correlation coefficient

APPENDIX D

Periodogram Program for 6500 CDC Computer\*

PROGRAM SPECT DIMENSION IARAY (30,500),IDAT (10,1000) REAL MEAN (500), MEAN2 INTEGER FINPER IWRT=1

- READ VALUE FIRST TIME THRU SUBROUTINE
- ບບບ
- 20 IF(IWRT.NE.1) GO TO 60
   PRINT 4002
  4002 FORMAT (\*1\*)
   READ 3002, INTPER, FINPER
  3002 FORMAT (214)
   J=INTPER-1
   DO 50 IHOUR = 1,1000
   READ (60,1002) (IDAT(I,IHOUR
   READ (60,1002) (IDAT(I,HOUR
   R
- NEW DATA \*\*\*\*\* USE 1012 FOR OLD DATA READ (60,1002) (IDAT (I, IHOUR), I=1,10) FORMAT (1012) FORMAT (1014) NEW DATA \*\*\*\*\* USF IF (EOF,60 ) 60,50 (1014) CONTINUE 1002 50 1002 ບ
- \_

GO TO 70

- ບບ
- \*` II WRITE(61,2002) AP,J,MEAN2,(MEAN(I),I=1,J) PUNCH 2003, P,AP 2002 FORMAT (\*0 AP = \*,F10.5,\* FOR PERIOD = \*,I4,\* WITH GRAND MEAN IF10.5,/,\* TABLE OF COLUMN MEANS \*,/, 50(/,3X,10F13.5)) IF (IWRT.NE.2) GO TO 70 IWRT=3 60

PUT DATA INTO CORRECT ARRAY TO PERFORM SUMS, MEANS FOR COLUMNS GO TO 280 CALCULATE SUMS AND MEANS. J = J + IIF (J, GT, FINPER) GO TO 600 (N.LT.IHOUR) GO TO 200 ICOUNT (IARAY (NN, MN).EQ.-1) SUM = IARAY (NN, MN) + SUM = IDAT (M,N) IF (M.LE.10) GO TO 200 GO TO 270 + LJ=LJ J \* IJ ICOUNT = ICOUNT + / WNS SUM = ICOUNT = 0DO 280 NN = 1, IJ1 1,1J DO 300 MN = 1, JĽ, II Ъ (KJ.NE.0) 11 (K,I) 11 IF (I.LE.J) IARAY (K,I) IJ = 2400= 2400 W = N = IN = N + 1GO TO 270 I = I + 1 DO 210 K + W = W CONTINUE CONTINUE MEAN (MN) CONTINUE CONTINUE DO 200 LARAY M = 1 Ц Ч Η KJ 70 200 210 270 280 300

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```
IF (IWRT.EQ.1) GO TO 500
WRITE(61,2002) AP.J,MEAN2,(MEAN(I),I=1,J)
PUNCH 2003, P,AP
FORMAT (2F10.5)
GO TO 20
IWRT=2
                                                         DO 400 L = 1,J
SUM3 = SUM3 + (MEAN(L) - MEAN2)**2
AP = SQRT (SUM3 / J)
P=J
                       + SUM2
/ J
SUM2 =0
DO 350 L = 1,J
SUM2 = MEAN(L)
MEAN2 = SUM2 / 1
                                                                                                                                                                                   CONTINUE
PRINT 4002
END
                                                                                                                                                                          GO TO 20
                                                                                                                                                                500
                         350
                                                                      400
                                                                                                                                          2003
                                                                                                                                                                                      600
```

```
A_p = Root Mean Square
Amplitude for Period p = A_p = \left[\frac{1}{P}\sum_{h=1}^{P}(y_{p,h} - \overline{Y}_p)^2\right]^{4}(Enright, 1965b)
               *Output:
```

#### APPENDIX E

#### Basic Plot Routine

ATTACH (STAT, STAT3) STAT. FILEBUILD, DATOUT=DATA1 L1=LAG, RHO FILEBUILD, DATOUT=DATA2 L1=LAG,RHO FILEBUILD, DATOUT=DATA3 (etc.) PLOT (1,2) PD, NODS, PLOTJECT, DATIN=DATA1 HSIZE=10,VSIZE=6, DOT, NHINT=24, VSEP=2, SPECIAL15 PLOT(1,2) PD, NODS, PLOTJECT, DATIN=DATA2 HSIZE=10, VSIZE=6, DOT, NHINT=24, VSEP=2, SPECIAL15 (etc.) FORMAT (VARIABLE) (DATADECK) END OF DATA FORMAT (VARIABLE) (DATADECK) END OF DATA (etc.)

### APPENDIX F

## I. Ten-day Square Wave Spectrum

τ (hrs.)	f(cycles/ day)	¢ c∕c₀	(degrees)	c/c <sub>24</sub>	Harmonic #
68.99	0.347	0.112	176.32	0.088	
53.48	0.448	0.121	177.80	0.095	
43.64	0.549	0.136	178.88	0.107	
36.89	0.650	0.159	181.07	0.125	
31.91	0.752	0.205	183.77	0.161	
28.05	0.855	0.315	190.12	0.247	
24.00	1.000	1.273	268.21	1.000	1
21.01	1.142	0.239	346.03	0.188	
19.29	1.244	0.125	349.82	0.098	
17.83	1.346	0.077	350.66	0.061	
16.60	1.446	0.058	352.92	0.040	
8.40	2.858	0.094	194.02	0.074	
8.00	3.000	0.424	269.60	0.333	3
7.64	3.143	0.086	346.68	0.068	
4.94	4.855	0.055	189.00	0.044	
4.80	5.000	0.251	269.75	0.200	5
4.67	5.114	0.052	349.06	0.042	
3.43	7.000	0.182	271.01	0.143	7
2.67	8.999	0.142	268.10	0.111	9
2.18	11.000	0.116	270.43	0.091	11
1.85	12.999	0.098	268.18	0.077	13
1.60	14.994	0.085	259.95	0.067	15
1.41	16.998	0.076	267.54	0.059	17
1.26	19.007	0.067	281.95	0.059	19

τ	f(cycles/		φ			
(hrs.)	day)	c/c <sub>0</sub>	(degrees)	C/C <sub>24</sub>	Harmonic #	
48.82	0.490	0.426	175.06	.335		
24.37	0.985	1.271	261.57	1.000	1	
16.43	1.461	0.155	339.40	.122		
9.48	2.530	0.088	197.09	.069		
8.00	3.000	0.425	270.00	.334	3	
6.93	3.460	0.065	340.78	.051		
4.80	5.000	0.255	270.00	.201	5	
3.43	7.000	0.182	271.84	.143	7	
2.67	8.999	0.142	268.31	.112	9	
2.18	11.000	0.116	270.83	.091	11	
1.85	12.999	0.098	270.49	.077	13	
1.60	14.994	0.085	268.20	.067	15	

II. Three-day Square Wave Spectrum

III. Ten-day Sine Wave Spectrum

τ (hrš.)	f(cycles/ day)	C/C <sub>0</sub> =C/C <sub>24</sub>	φ (degrees)	Harmonic #
68.99	0.347	0.073	3.42	
53.48	0.448	0.080	1.86	
43.64	0.549	0.091	0.27	
36.89	0.650	0.110	1.56	
31.91	0.752	0.146	4.34	
28.05	0.855	0.233	10.77	
24.00	1.000	1.000	90.76	1
21.01	1.142	0.204	168.88	
19.29	1.244	0.115	170.75	
17.83	1.346	0.078	174.06	
16.60	1.446	0.058	174.00	

#### APPENDIX G

Computer Output From Spectral Analysis of Hamster Data

## I. LD Data (Figure 10)

 $C_0 = 531.52 \pm 15.26$   $C_{24} + 727.45 \pm 11.08$ 

τ (hrs.)	frequency (cycles/day)	c/c	τ (hrs)	frequency (cycles/day)	c/c
67.54	0.355	0.152	6.53	3.673	0.101
53.05	0.452	0.140	6.00	4.001	0.413
44.17	0.543	0.083	5.77	4.162	0.148
37.19	0.645	0.265	5.44	4.409	0.087
31.75	0.756	0.283	5.30	4.531	0.098
27.83	0.863	0.398	4.90	4.893	0.092
23.95	1.002	1.369	4.78	5.024	0.123
21.03	1.141	0.339	4.65	5.158	0.100
19.25	1.247	0.212	4.56	5.254	0.096
17.61	1.363	0.166	4.43	5.418	0.135
15.66	1.533	0.116	4.18	5.739	0.076
14.72	1.630	0.139	4.00	6.001	0.154
13.62	1.762	0.167	3.74	6.422	0.149
12.74	1.883	0.154	3.60	6.660	0.079
11.90	2.024	0.377	3.59	6.676	0.077
10.68	2.247	0.192	3.56	6.738	0.085
10.05	2.387	0.142	3.35	7.163	0.095
9.45	2.539	0.146	3.24	7.406	0.121
8.56	2.803	0.094	3.13	7.658	0.080
8.00	3.154	0.426	2.99	8.034	0.188
7.61	3.552	0.131	2.90	8.266	0.113
6.76	3.552	0.159			

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II.	$\mathbf{L}\mathbf{L}$	Data	(Figure	11)
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	$C_0 = 113.85 \pm$	5.48	C <sub>24</sub> =	121.78 ± 5.19	
τ (hrs.)	frequency (cycles/day)	c/c <sub>0</sub>	τ (hrs.)	frequency (cycles/day)	c/c <sub>0</sub>
74.75	0.321	0.175	5.89	4.077	0.425
54.23	0.442	0.207	5.70	4.212	0.459
43.33	0.554	0.208	5.35	4.484	0.253
35.86	0.669	0.220	5.19	4.627	0.350
31.97	0.750	0.171	5.12	4.684	0.169
28.39	0.845	0.281	5.07	4.733	0.209
23.98	1.002	1.066	4.93	4.859	0.481
20.78	1.155	0.240	4.80	5.004	0.361
18.49	1.298	0.337	4.61	5.207	0.452
15.20	1.579	0.294	4.36	5.499	0.234
13.78	1.741	0.308	4.22	5.686	0.216
12.31	1.938	0.480	4.07	5.891	0.288
10.44	2.298	0.164	3.85	6.233	0.247
9.93	2.417	0.162	3.71	6.471	0.208
9.21	2.607	0.290	3.63	6.618	0.322
8.79	2.731	0.259	3.56	6.734	0.286
8.33	2.888	0.669	3.41	7.033	0.388
7.90	3.055	0.705	3.29	7.286	0.220
7.49	3.206	0.203	3.21	7.473	0.245
7.25	3.309	0.312	3.15	7.612	0.355
6.98	3.438	0.256	2.98	8.055	0.402
6.43	3.727	0.366	2.90	8.278	0.321
6.21	3.862	0.529			

# III. LL Data (Figure 12)

	$C_0 = 437.75 \pm$	14.34	° <sub>24</sub> ≖	= 601.77 ± 11.3	8
τ (hrs.)	frequency (cycles/day)	c/c <sub>0</sub>	τ (hrs)	frequency (cycles/day)	c/c <sub>0</sub>
46.03	0.521	0.168	5.80	4.138	0.108
37.45	0.640	0.102	5.54	4.331	0.116
30.26	0.793	0.249	5.16	4.647	0.095
24.17	0.993	1.374	5.03	4.767	0.087
19.75	1.215	0.162	4.91	4.884	0.125
17.86	1.344	0.088	4.80	5.005	0.228
16.41	1.462	0.102	4.66	5.149	0.107
13.79	1.740	0.138	4.51	5.324	0.182
11.87	2.023	0.267	4.17	5.754	0.087
10.04	2.391	0.098	4.02	5.967	0.165
9.30	2.582	0.089	3.91	6.145	0.091
8.86	2.707	0.160	3.81	6.303	0.148
8.47	2.833	0.188	3.62	6.623	0.093
8.02	2.992	0.384	3.42	7.008	0.168
7.46	3.217	0.111	3.36	7.152	0.122
7.08	3.387	0.121	3.27	7.329	0.085
6.49	3.698	0.120	3.14	7.631	0.132
6.28	3.820	0.116	3.00	7.986	0.258
6.05	3.966	0.222	2.92	8.208	0.118

IV. DD Data (F	'igure 13)
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	$C_0 = 567.42 \pm$	15.24	$C_{24} = $	528.09 ± 13.20	
τ (hrs.)	frequency (cycles/day)	c/c <sub>0</sub>	τ (hrs.)	frequency (cycles/day)	c/c <sub>0</sub>
74.30	0.323	0.135	6.74	3.562	0.204
69.45	0.346	0.136	6.52	3.680	0.176
47.48	0.505	0.243	6.11	3.925	0.197
37.67	0.627	0.258	5.90	4.067	0.177
31.69	0.757	0.189	5.72	4.197	0.190
28.02	0.856	0.140	5.53	4.339	0.118
24.00	1.000	0.930	5.23	4.573	0.099
20.82	1.251	0.231	5.09	4.715	0.117
19.17	1.450	0.105	4.90	4.099	0.192
16.54	1.694	0.253	4.51	5.324	0.099
14.16	1.702	0.223	4.32	5.554	0.125
13.22	1.815	0.190	4.20	5.709	0.144
12.30	1.951	0.532	3.96	6.055	0.156
11.63	2.064	0.260	3.87	6.198	0.155
11.01	2.180	0.185	3.79	6.326	0.137
10.53	2.279	0.130	3.68	6.521	0.135
10.01	2.397	0.291	3.50	6.852	0.205
9.64	2.491	0.182	3.35	7.163	0.117
9.23	2.602	0.172	3.10	7.740	0.099
8.89	2.701	0.260	3.09	7.758	0.098
8.69	2.762	0.115	3.09	7.776	0.095
8.58	2.796	0.134	3.05	7.891	0.067
8.18	2.932	0.592	3.01	7.964	0.107
7.45	3.224	0.196	2.83	8.455	0.089
7.15	3.357	0.216	2.75	8.734	0.058

# V. DD Data (Figure 14)

	$C_0 = 262.32 \pm$	10.51	$C_{24} = 2$	44.53 ± 9.90	
τ (hrs.)	frequency (cycles/day)	c/c <sub>0</sub>	τ (hrs.)	frequency (cycles/day)	c/c <sub>0</sub>
61.33	0.391	0.199	5.76	4.164	0.354
44.99	0.534	0-197	5.33	4.505	0.299
36.53	0.657	0.185	5.14	4.672	0.410
30.66	0.783	0.283	4.84	4.954	0.223
23.53	1.020	0.932	4.68	5.132	0.251
19.97	1.202	0.480	4.50	5.328	0.321
16.90	1.420	0.328	4.34	5.520	0.317
14.62	1.642	0.214	4.22	5.691	0.351
13.06	1.838	0.300	4.11	5.843	0.264
12.04	1.992	0.757	4.00	6.001	0.397
10.78	2.226	0.366	3.89	6.174	0.202
9.86	2.435	0.332	3.79	6.327	0.213
8.57	2.800	0.109	3.65	6.568	0.128
7.92	3.029	0.431	3.56	6.751	0.124
7.41	3.240	0.204	3.37	7.115	0.186
7.11	3.374	0.298	3.28	7.327	0.243
6.90	3.478	0.233	3.21	7.480	0.125
6.65	3.607	0.142	3.02	7.957	0.293
6.27	3.829	0.313	2.89	8.310	0.233
6.04	3.975	0.323			

