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Parameter estimation or hypothesis testing in the statistical analysis of biological rhythms?

ERNST PÖPPEL
Max-Planck-Institut für Psychiatrie, 8 Munich 40, F. R. Germany

The "microscopic" techniques in biological rhythm research which are used to estimate period, phase, and amplitude of physiological and psychological functions on the basis of sinusoidal approximations are criticized. It is suggested that one confine oneself to hypothesis testing and evaluation of the data with the "naked eye."

In the description of biological rhythms, two different statistical procedures may be considered. In one procedure, the statistical dependence of the time series data is tested. Such a procedure can be called *hypothesis testing* because the statistical analysis leads to the decision as to whether the time series consists of independent random variables (null hypothesis) or whether there is some deviation from such a model (alternative hypothesis). If these data are not independent, one possible cause of the dependence is a biological rhythm. The statistical analysis stops with hypothesis testing, and period, amplitude, phase, and wave form of the biological rhythm may be defined by using other knowledge that is not based on special statistical techniques.

The other procedure for analysis of time series data has been called *microscopic* (Halberg, 1969). The aim of this procedure is to estimate the parameters of a biological rhythm statistically. This is done by formulating a theoretical model which adequately represents the raw data. In the microscopic analysis of circadian rhythms, this *parameter estimation* has been done by using a sinusoidal function for the approximation of the raw data. "The acrophase estimates are determined objectively from all available data, rather than by subjectively inspecting the temporal location of crests in zigzags representing time plots of original data—the chronograms. Of course, the so-called 'microscopic' evaluation of the circadian component *does not contradict the 'macroscopic' impression* [italics by Pöppel] from the chronogram; rather it serves for objective quantification (Halberg et al., 1969)."

This last statement is misleading as can be seen by comparing raw data that show a clear rhythm on macroscopic inspection and the computed estimates. In Figure 1, four examples are given that demonstrate this fact. The curve at the top shows the raw data of intraperitoneal temperature of an adult female rat as a function of time of day (Halberg, 1969); the next curve shows the raw data of human body temperature (Halberg, Tong, & Johnson, 1967). The two following curves represent electromyographic activity and presence of the Sleep Stages 3 and 4 in rhesus monkeys (Crowley, Kripke, Halberg, Pegram, & Schildkraut, 1972). The arrow indicates in each case at what point in time the maximum (acrophase) is estimated using a cosine approximation of the data. It is quite obvious that by using this cosine approximation the paramter estimation of the maximum deviates in all cases considerably from the maximum of the raw data.

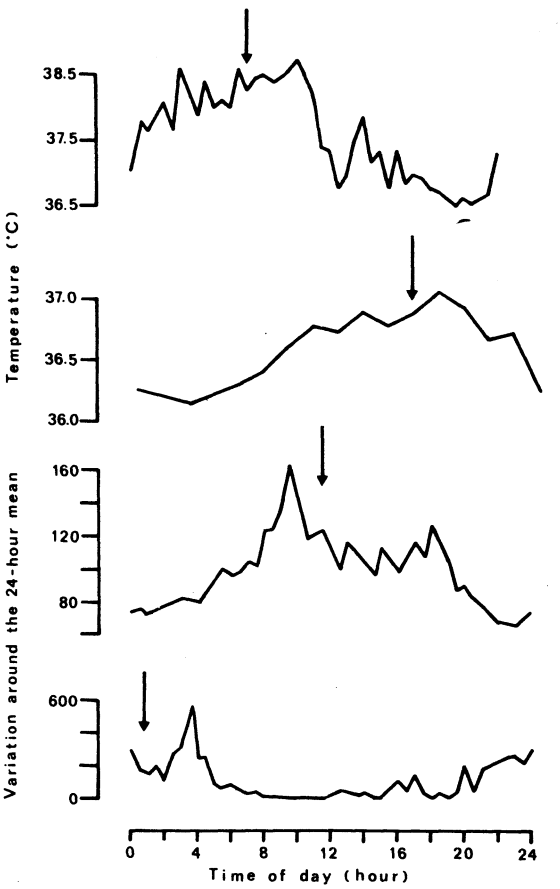

Figure 1. Four examples of the 24-h variability of physiological functions (top to bottom: body temperature in rats and men; EMG activity and presence of Sleep Stages 3 and 4 in rhesus monkeys). Arrows indicate the location of the estimated maxima using a cosine approximation.

The deviation from a sinusoidal oscillation of the

functions shown in Figure 1 are not isolated cases; in fact, these deviations are often even greater. In many published illustrations, it is evident to what extent the 24-h variation of various functions deviates more or less from a sinusoidal oscillation. A few additional examples besides those shown in Figure 1 are: locomotor activity of mammals and birds (Aschoff, 1964); electrolyte concentration and volume of urine (Halberg & Reinberg, 1967; Pöppel, 1968); orthostatic cardiovascular response (Aschoff & Aschoff, 1969); eosinophil level (Halberg & Reinberg, 1967); psychomotor performance like reaction time or time estimation (Aschoff, Giedke, Pöppel, & Wever, 1972; Pöppel, Aschoff, & Giedke, 1970; Pöppel & Giedke, 1970). This fact, the deviation of the circadian functions from sinusoidal oscillations, has also been predicted from theoretical considerations (Wever, 1966).

Therefore, the conclusion must be drawn that as a cosine approximation to raw data can lead to biased parameter estimations, the microscopic procedure has to be limited in its applications. It should apply only to those cases where the theoretical model and the empirical curve correspond exactly except for deviations due to random noise; i.e., that the raw data actually are distributed sinusoidally. One can see that in unfavorable cases as in sawtooth-like oscillations (cf. Figure 1), the comparison of the maxima can differ by several hours compared to the actual position of the maxima.

If a cosine approximation is not a reasonable choice, the question remains: which approximation is to be used? The raw data give the impression that it is impossible to find a unique theoretical model for the statistical approximation of all the different variables. Sometimes it is even impossible to formulate such a model for *one* variable because it has been shown that a function may change its pattern systematically with a change in the experimental conditions (Aschoff, Gerecke, & Wever, 1967); in fact, the whole model may be changed, not just the parameters, in such cases.

One must conclude that appropriate statistical models for the approximation of each oscillating function can only be found if in each case the underlying physiological mechanism of the periodic process is understood. The knowledge of the fundamental characteristics of the observed oscillation is a prerequisite for defining its appropriate statistical model and then for estimating the parameters on a statistical basis.

It follows that by using one particular statistical model for describing the data, one is unlikely to get reasonable insight into the basic mechanisms of biological rhythms because the microscopic approach necessarily leads to a biased estimation of the parameters; thus, the important features of the oscillating system may remain hidden, or may even be misrepresented.

As the knowledge of the properties of biological rhythms is still limited and the adequate theoretical models for the statistical analysis cannot yet be formulated, one should not ignore the problems of parameter estimation by using one of the simplest approximations, the sinusoidal fit. One should rather confine oneself to *hypothesis testing* (e.g., Miescke, 1972; Pöppel, 1970) and to other knowledge of the system that is not derived from statistical parameter estimation. It seems reasonable to keep as much as possible to the raw data and not to work on these data with inappropriate methods. Much information about biological rhythms has already been obtained on the macroscopic level simply through observation of the raw data with the naked eye (e.g., Pittendrigh, 1974).

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