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TEST OF PEAK VALUES IN PHYSIOPATHOLOGIC TIME SERIES [a/] [b/]

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Introduction

Physiologic or pathologic measurements often are made as a function of time. In statistical terms, such data constitute time series. Among the characteristic features of physiologic time series are certain roughly repetitive events, the so-called rhythms. The ensemble of physiologic rhythms constitutes a broad spectrum of nearly periodic phenomena (1-2). The periods involved vary from short ones, such as some of those encountered in the electrocardiogram or electroencephalogram, to longer ones, such as those of about one day (circadian; from L. circa=about and L. dies=day) (3-4), one month (menstrual), or yet longer duration.

Study of circadian rhythms prompted the presentation of this paper. These rhythms are readily synchronized with some environmental 24-hour cycles, such as the lighting regimen or social routine, in mice and men, respectively. If so synchronized, these rhythms show a predictable external timing (5). Thus, on a given specified environmental regimen, called the synchronizer (6), Zeitgeber (7) or entraining agent (3), the clock hour at which a given peak value is found represents an adaptive characteristic of the organism under study.

Locating such peaks in biologic data is of considerable interest, especially to the experimental pathologist, since the organism undergoes along a 24-hour scale substantial changes in susceptibility to injury. The effect of a variety of potentially harmful agents depends indeed critically upon the physiologic state of the organism at the time of their administration. Thus a given agent and organism can be characterized by a circadian susceptibility rhythm. The net effect of such rhythms may be as drastic as the difference between death and survival, following a mammal's exposure to agents varying from noise (8-10) or drugs (11-15) to bacterial (16) and other (17) poisons.

Data on mammalian susceptibility rhythms, revealing as they do dramatic differences under controlled conditions, must be viewed as statistical entities.

Thus, apart from a given rhythm under study, physiopathologic time series usually are complicated by other effects, ranging from rhythms with periods longer (18) or shorter than that under study to random effects. The latter may obscure the former or may altogether mask a given rhythm, unless experimental animals or subjects of study are appropriately homogeneous and environmental conditions as well as sampling procedures sufficiently controlled.

Consequently, the problem of locating in physiopathologic time series a suspected peak, e.g., in the susceptibility of a mammal to some harmful agent, usually constitutes a statistical problem, i.e., the need arises to test the significance of a given peak. A quick procedure for this purpose discussed in this note serves to introduce certain pertinent statistical considerations to students of physiologic rhythms. The method is applicable if (a) the location of a peak in a time series or other observations can already be suspected from data obtained earlier and if (b) the number of observations available for verifying the location of the peak is reasonably large.

1. Procedure for Validating a Suspected Peak in a Time Series

Observations are made on several individuals at each of k time points. The property observed depends, of course, upon the objective of a given set of studies, but the following kinds of data may serve as illustrative examples:

a. The proportion of individuals showing a pathologic response, such as death, as a function of the time when a given potentially harmful agent is administered.

b. The proportion of animals at different times with a physiologic measurement, such as a respiratory rate above a specified level.

The types of data cited above have in common that they represent proportions. Such proportions, computed from a sample, constitute a "statistic", unless one is able to test all organisms of a given kind in a specified test

situation, and thus to derive a proportion from a population as a whole. In a statistical sense the latter proportion constitutes a "parameter". Usually, we confront the task to estimate a parameter from a statistic. Conventionally, the statistician employs Greek letters to denote parameters--derived from populations--and Roman letters to denote statistics--based upon sample characteristics.

The analysis of the types of data here under discussion becomes more manageable when the sequence of observations can be regarded as serially independent. Serial independence for these kinds of experiments is intended to mean that the procedures and data obtained at a given time do not influence results obtained at other times.

Let us visualize an experimental situation in which the same organism is repeatedly studied. In this case, we can assume serial independence, if we know that the possession of a specified property at a given time and the procedures necessary for observing this property, in particular, do not affect the possibility of having the same characteristic at any other time. Even more pertinent to the types of observations discussed in this paper, however, is the serial independence of observations made on separate but comparable organisms used at each of several time points. In this case it would appear at first that the condition of serial independence is fully satisfied. A more thorough analysis of certain experimental situations may reveal, however, that it is solely the assumption of serial independence as to animals that holds without qualification.

The disturbances associated with a set of measurements on one group of mice can affect another group kept in the same room, although the latter group has not been previously used for sampling (19). These effects can be highly significant statistically when conditions are standardized for periodicity analysis.

In such an experimental situation we are thus confronted with some degree of serial dependence derived from our manipulation of the experimental environment. Apart from such serial dependence "among time-periods" (or "time-points") demonstrated for a 4-hourly sampling schedule on separate groups of mice kept in the same room, there is probably "within-time-period" (within-time-point) serial dependence as well, derived from the effect exerted by our sampling from the first animal at a given time-point upon the immediately following observations on other animals in the same series. Hence, serial independence applies only as a first approximation to results from separate groups of animals removed at fixed intervals from the same mouse room at each of several times.

Let n_i, for $i = 1,\ldots,k$, be the number of experimental animals studied at the ith time-point. We note that we have selected our sample of animals to be what empirically is denoted as 'representative' of the conceptual population Ω_i of all possibly available animals of a given kind that could be studied at the ith time-point for some specified property. Let π_i, $i = 1, 2,\ldots,k$, denote the proportion of all experimental animals constituting the population Ω_i that possess a specified property. π_i is a constant and constitutes a parameter for the population Ω_i in the statistical sense. This parameter is not known for obvious reasons, but it can be estimated on the basis of sample proportion $p_i = r_i/n_i$, $i = 1,\ldots,k$ where r_i is the number of animals found to possess the specified property in the sample of n_i animals at the ith time-point. It is to be noted that the p_i's are not constants, having different values in separate experiments. We may call them "estimates".

In the situation under discussion, it is suspected, from previous data or otherwise, that the largest of the π_i is at a given time-point I. The purpose of inquiry, based on a given experiment, is to verify the validity

of this supposition. If the supposition is incorrect, π_I is not the largest. As an alternative, it might be near the average of the other values of π_i.

The procedure about to be proposed will actually test the so-called null hypothesis that the suspected π_I is equal to the average of the other values, against the alternative hypothesis that the suspected π_I is larger than this average. Rejection of the null hypothesis supports the original assumption that 'I' is a peak-time with the following qualification. The test of the data might indeed strongly suggest that π_I is larger than the average of the other π_i's, the latter to be denoted as π^*, yet there can be a particular π_i, say $\pi_{I'}$, such that $\pi_{I'} > \pi_I$. This circumstance must be kept in mind when inferences are made from the test outlined below, irrespective of whether the $\pi_{I'}$ is located at one of the studied time-points and detected. This qualification, however, does not render the test useless. The procedure to be introduced allows us to describe a time-point, such as I, as one associated with a proportion that is significantly above the average value for the response-parameter investigated. A time-point thus defined will be called a "peak" for our present purpose and it is not necessarily associated with the largest proportion.

It must be emphasized that prior information on a suspected peak is required if the technique discussed in this section is to be applied. Thus the time-point 'I' must be selected on the basis of previous data or other pertinent information. Techniques discussed below (cf. Section 5) differing from that discussed in this section, have to be used when, in the absence of prior information, the time-point of the peak proportion is chosen on the basis of no more than the experiment being analyzed.

The testing procedure is formulated below:

Null hypothesis: $H_o : \pi_I = \pi^*$

Alternative hypothesis: $H_1 : \pi_I > \pi^*$

where

$$\pi^* = (\pi_1 + \ldots + \pi_{I-1} + \pi_{I+1} + \ldots + \pi_k)/(k-1)$$

$$p_i = r_i/n_i \ , \ i=1,\ldots,k.$$

Let $p^* = (p_1 + \ldots + p_{I-1} + p_{I+1} + \ldots + p_k)/(k-1)$

Define $q_i = 1-p_i$, $d_I = p_I - p^*$, $V_k^2 = p_I q_I/n_I + \Sigma' p_i q_i/[n_i(k-1)^2]$

In the above Σ' stands for the sum in which the term i=I is omitted. If the null hypothesis $\pi_I = \pi^*$ is true, we expect that the difference of the estimates, i.e., d_I will be fairly small. In repeated sampling the value of d_I will fluctuate around zero value. A large deviation of d_I from the zero value in the positive direction, as we are dealing with the one-sided alternative $\pi_I > \pi^*$ will reject the null hypothesis and will support the alternative hypothesis. How large a deviation of d_I from the zero value arising due to sampling fluctuations can be considered insignificant (favoring null hypothesis $\pi_I = \pi^*$) depends on the distribution of the statistic d_I.

In following this procedure, two kinds of errors can arise. The first kind of error is that of rejecting the null hypothesis when in fact it is true--by observing a 'large' deviation of d_I from the zero value. The second kind of error is that of accepting the null hypothesis when in fact the alternative is true--by observing a 'small' deviation of d_I from the zero value. The usual practice for obtaining the largest deviation allowable in favor of the null hypothesis is to hold the error of the first kind at a specified level and to minimize the error of the second kind.

It is found that for reasonably large value of n_i, and under the null

hypothesis $\pi_I = \pi^*$, the quantity d_I is nearly normally distributed with a mean of zero and variance V_k^2. This statement holds for the variance if all the animals studied are kept in such a way that there is no effect of any one of them upon the others, i.e., there is serial independence. The quantity d_I/V_k will have a variance of one. Thus $z_I = d_I/V_k$ can be used as a standard normal variable, i.e., a normal variable whose mean is zero and variance is unity if the n_i are reasonably large (e.g., 25 or more).

A normal variable has a bell-shaped distribution symmetrical about the mean with frequency tapering off rather quickly to zero as we move away from the mean in either direction. Thus, an interval equal to six times the standard deviation centered at the mean covers about 99% of the entire population.

If the magnitude of the error of the first kind is fixed at .05 (a 5% one-sided test)[1/], it is found that rejecting the null hypothesis if $z_I > 1.64$ minimizes the magnitude of the error of the second kind. For preliminary work, samples with n=10 may be satisfactory, but for the application here suggested one may require conservatively that the n in each sample be at least 25.

2. Confidence Limits

Associated with a two-sided test at a probability level of significance

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1/ Since interest is concentrated on the question as to whether π_I is larger than π^*, and there is no particular concern if it should be smaller, there is a one-sided testing situation. If one were only concerned with whether or not π_I differed from π^*, then either large or small (large negative) values of z_I would be of interest and a two-sided test should be used. If so, one rejects the null hypothesis if z_I is either less than -1.96 or greater than +1.96.

denoted as α there is a two-sided confidence interval, with a confidence coefficient equal to $1-\alpha$. Such an interval, based on the observations, has probability equal to $(1-\alpha)$ of including the true value of the parameter of interest, e.g., $\pi_I - \pi^*$ of Section 1. The larger the value of $(1-\alpha)$, the more confident the experimenter will feel that the interval includes the true parameter value. Choosing $(1-\alpha)$ large, however, will make the interval long, i.e., the confidence interval will not sharply delineate the reasonable values of the parameter. Collecting more data, on the other hand, will tend to decrease the length of the interval.

Corresponding to the discussion of Section 1, if the n_i are assumed to be reasonable large, the $(1-\alpha)$ level confidence limits for the population difference $\pi_I - \pi^*$ can be stated as follows:

$$(2) \quad (p_I - p^*) - \lambda_{\alpha/2} V_k < \pi_I - \pi^* < (p_I - p^*) + \lambda_{\alpha/2} V_k \ .$$

$\lambda_{\alpha/2}$ is the $100\alpha/2$ percent point of the standard normal distribution. For instance, if a 95% confidence interval is desired, $\lambda_{\alpha/2} = \lambda_{.025} = +1.96$. This means that in repeated sampling, on an average, the interval $((p_I - p^*) - 1.96\ V_k\ ,\ (p_I - p^*) + 1.96\ V_k)$ will cover the population difference, $\pi_I - \pi^*$, in 95 out of 100 experimental applications.

3. Several Peaks in a Time Series ($k > 2$)

The above test may be insensitive when one suspects more than a single peak in the data. If so, the following modification is indicated. Let one of two suspected peaks be at the Ith time-point and another at some subsequent Jth time-point. Then the hypotheses are:

$$H_{01} : \quad \pi_I = \pi^{**}, \quad \text{vs.} \quad H_{11} : \quad \pi_I > \pi^{**}$$

$$H_{02} : \quad \pi_J = \pi^{**}, \quad \text{vs.} \quad H_{12} : \quad \pi_J > \pi^{**}$$

where $\pi^{**} = (\pi_1 + \ldots + \pi_{I-1} + \pi_{I+1} + \ldots + \pi_{J-1} + \pi_{J+1} + \ldots + \pi_k)/(k-2)$.

Procedures similar to that in Section 1 may be adopted for testing these hypotheses. Define,

$$\pi^{**} = (p_1+\ldots+p_{I-1} + p_{I+1}+\ldots+p_{J-1} + p_{J+1}+\ldots+p_k)/(k-2),$$

$$d_I = p_I - p^{**}, \qquad d_J = p_J - p^{**},$$

$$V_{Ik}^2 = p_I q_I/n_I + \Sigma'' p_j q_j/[n_j(k-1)^2]$$

$$V_{Jk}^2 = p_J q_J/n_J + \Sigma'' p_j q_j/[n_j(k-1)^2]$$

in which Σ'' stands for the sum with $j \neq I$ and $j \neq J$. Then let

$$z_I = d_I/V_{Ik}, \qquad z_J = d_J/V_{Jk}.$$

z_I and z_J can be used as standard normal variables if the n_i are reasonably large (cf., e.g., Section 1).

4. Illustrative Examples:

The convulsive response to noise was tested by methods described elsewhere (8-10), in two groups of inbred mice, here identified merely as the Ce and D groups, since the main purpose of this section is the presentation of a statistical procedure. The purpose of the tests, however, was to evaluate the significance of a suspected peak in susceptibility to audiogenic convulsions at 20 hours (8 p.m.), predicted on the basis of several earlier reports (8-10). These experiments were done in the laboratory of the Cambridge State School and Hospital and were analyzed in the Department of Pathology at the University of Minnesota. The data analyzed are given in Tables I and II.

Table I: Ce group susceptibility to fatal audiogenic convulsion

Clock hour	:	12	16	20	24	04	08
n_i (No. tested)	:	47	48	49	48	45	48
r_i (No. of deaths)	:	10	10	13	12	8	5

[Tables I and II are from mice standardized in light from 06 to 18, alternating with darkness (cf. 19).]

Table II: D group susceptibility to fatal audiogenic convulsion

Clock hour	12	16	20	24	04	08
n_i (No. tested)	30	30	29	28	28	28
r_i (No. of deaths)	1	1	8	6	5	1

For the Ce group, the peak is suspected at 20 hours. The computations for formula [1] are given as follows[2]: $p_{20} = .265$, $p^* = .191$, $n_{20} = 49$, $q_{20} = .735$, $k = 6$, $p_{20}q_{20}/n_{20} = .00398$, $V_k^2 = .00462$ or, $V_k = .0680$, $d_{20} = .0742$. Hence $z_{20} = d_{20}/V_k = 1.10$ significant only at the 13.5% level.

Instead of p_{20}, next p_{24} was tested ignoring p_{20} and it was not found to be significant. However, the presence of a large proportion at 24 hours might have rendered the test insensitive. Hence, using formula [2] above, ($I = 20$, $J = 24$), the corresponding test was carried out. The computations are as follows: $p_{20} = .265$, $p^{**} = .176$, $V_{20,k}^2 = .00474$ or, $V_{20,k} = .0688$, $d_{20} = .0895$, $z_{20} = d_{20}/V_{20,k} = 1.30$ significant only at the 9.7% level. A similar test for p_{24} also indicated non-significance. Thus the data on Ce mice in Table I do not suffice to demonstrate the suspected occurrence of peaks[3].

2/ The subscripts in this example are the time-points expressed as clock hours.

3/ The analysis performed on the Ce group data does not rule out the occurrence of a rhythm in susceptibility to convulsions, in the individual Ce mice tested. Such rhythms may have been free-running in individual Ce mice and, if so, they were not identifiable by the experimental procedure employed. The physiopathologic data are presented solely for illustrating the statistical procedures.

The evidence for the D group, however, is different. A peak at 20 hours is suspected. The computations are as follows: $p_{20} = .276$, $p^* = .0990$, $n_{20} = 29$, $q_{20} = .724$, $V_k^2 = .00747$, $V_k = .0865$, $d_{20} = .177$. Hence, $z_{20} = d_{20}/V_k = 2.05$ which is significant at the 2% level.

The 95% confidence interval in this case (i.e., for $\pi_{20} - \pi^*$) is found to be (.008, .346).

5. Additional Remarks

Information on rhythms in physiopathologic time series serves, inter alia, for the choice of an appropriate experimental zero-time. In any bioassay, the stage of a susceptibility rhythms in which a given test is made must be specified as are other characteristics of a given organism studied, such as genetic background, sex or age. It must be remembered, however, that from a physiopathologic viewpoint, peak susceptibility to different agents might not be the same. Thus, the peak susceptibility of certain inbred mice to ouabain differs from that to other agents such as ethanol (11,5)12, 17; cf. also 4,5).

Circadian rhythms in susceptibility to agents acting upon the same physiologic substrate, in turn, are likely to have a similar timing. Pertinent in this connection are changes in convulsive susceptibility with time, in response to electro-shock, as compared to auditory stimulation. Thus, Woolley and Timiras tested rats for their electro-shock thresholds and found that 71% of all rats tested between 2100 and 2300 at a current strength of 22 ma or at a lower strength showed convulsions, whereas only 13% of the animals tested between 1300 and 1500 convulsed at the same stimulus intensities (18). These tests served primarily for investigating changes in convulsive susceptibility during the estrus cycle. Accordingly the authors

demonstrated statistically significant differences, the threshold for minimal seizures being highest during diestrus and lowest during estrus. A circadian rhythm in threshold also was apparent at all stages of estrus cycle from spot-checks restricted to two time-points along the 24-hour scale. Moreover, the differences recorded as a function of circadian system-phase were substantially larger than the changes related to the stage of estrus cycle.

The important study by Woolley and Timiras demonstrates for the same physiopathologic function, convulsive susceptibility, the operation of two periodic components, circadian and estral. Finally, as far as spot-checks at only two time-points permit (18), the data of Woolley and Timiras agree well with the results on peak convulsive susceptibility documented herein and earlier (8-10) for the D group of mice rather than rats and in response to auditory stimulation rather than electro-shock.

From a statistical point of view it must be emphasized that the method here suggested for evaluating a suspected peak in a series of observations made as a function of time is applicable to data other than time series as well. This method applies to any analysis of the behavior of several groups of individuals if one suspects that one of these groups has a larger proportion of responses than the others.

There may not be previous information concerning I, i.e., the experimenter cannot predict or suspect which of a given set of proportions to be obtained experimentally might be larger than others. One may proceed as follows: The largest of the observed proportions will be denoted as p_{max}. A statistic like z_I is formed but the p_{max} plays the role of p_I. Tests of significance and confidence intervals can then be formed, again with the restraint of using moderately large samples. Now, however, one must not use the ordinary tables of the normal distribution. Instead, one turns to

a table of standardized extreme deviates, such as Table 25 of H. O. Hartley and E. S. Pearson (20). By this procedure, one can test whether or not the most discrepant proportion is different from the average of the others, and we can do so without assuming beforehand that one knows which proportion is largest. Naturally, since one is not using prior knowledge, the critical values and the confidence intervals will be larger than those for the case of a suspected peak, discussed above in detail.

If one has no prior information on whether any one proportion is larger than others and one cannot even assume that there will be a particularly large p_i one can analyze the difference between the largest and smallest proportions, denoted as p_{max} and p_{min}. For this "contrast" procedure one forms the following statistic:

$$\frac{[p_{max} - p_{min}]\,[n_{max}^{-1} + n_{min}^{-1}]^{\frac{1}{2}}}{\sqrt{\frac{p_{max}(1-p_{max})}{n_{max}} + \frac{p_{min}(1-p_{min})}{n_{min}}}}$$

The appropriate critical values of the above statistic can be found in Table 29 of Hartley and Pearson (20). The interested reader should examine the introduction to these tables (pp. 52-53) prior to their use. Significant results obtained on this basis would indicate that not all of the population proportions are equal, and thus for future experiments the proportion associated with p_{max} or p_{min} could be suspected to be the largest or the smallest proportion, respectively.

In the cases discussed above and, in particular, for the original problem, revolving around the verification of a suspected peak in a time series, one should note the "diluting effect" exerted by high values other than the peak. Values adjoining a peak, for instance, can be expected to be larger than others. Nonetheless, such values raise the overall average of the mean

computed by excluding the suspected peak and the effectiveness of the procedure is thus reduced. An effort to eliminate this effect is made in the illustrative example given above.

Circadian susceptibility rhythms are broad in scope, in plants (e.g., 21) as well as animals (4, cf. also 19). Recently, and what seems important, under controlled conditions Davis has extended indirect periodicity analysis on rodents to pharmacodynamic problems (15), with a particular view of interactions among synchronizers (22). Cancer research along similar lines has also begun (23). The above statistical procedures serve as useful and quick tests for analyzing such a variety of findings, if these are available from data that, as a first approximation, may be regarded as serially independent.

Finally, analogous procedures can be found for the case of quantitative measurements, but they remain beyond the scope of this paper.

Summary

A simply applied statistical procedure for testing a suspected peak in a time series is presented and applied to data on circadian periodic aspects of audiogenic physiopathology in certain inbred mice. Also discussed are methods for locating unsuspected peaks, and for locating either peaks or troughs in sets of responses obtained from several groups of individuals. These methods are applicable when the data consist of proportions.

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