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# The Statistical Analysis of Heart Rate: A Review Emphasizing Infancy Data

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#### ABSTRACT

Heart rate is a dependent variable used widely in psychological and psychophysiological research. Several statistical problems arise in the analysis of heart rate data, many of them specific to infancy research. The present paper discusses the problems of a statistically appropriate cardiac measure, the Law of Initial Values, the problem of differential variability in heart rate scores, and the use of multivariate statistical methods in analyzing heart rate data. Special attention is given to those problems and solutions which have potential application to the analysis of infant heart rate data. A flowchart is presented which may guide the researcher in the appropriate use of the several statistical techniques reviewed in this paper.

DESCRIPTORS: Heart rate, Heart rate statistical analysis, Infant heart rate, Statistical analysis, Infancy.

The statistical analysis of heart rate data is a problem which has been discussed in relation to adult heart rate research (e.g., Benjamin, 1963, 1967; Lacey, 1956; Wilson, 1967). Although mentioned in some reviews of infant heart rate experimentation (e.g., Crowell, Blurton, Kobayashi, McFarland, & Yang, 1976; Graham & Jackson, 1970), a systematic presentation of the range of statistical problems for infancy researchers does not exist. This paper summarizes some statistical difficulties which may confront the developmental psychologist or developmental psychophysiologist interested in heart rate research. The areas which are discussed are the Law of Initial Values, an appropriate cardiac measure, the problem of differential variability, time series analysis, and multivariate statistical methods. Special attention is given to the application of these problems to potential experiments using infant subjects, although much of the discussion might apply equally well to adult heart rate research.

The reader should be familiar with the analysis of

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Address requests for reprints to: John E. Richards, Department of Psychology, University of California, Los Angeles, CA 90024. variance and covariance, linear regression, and know of the extension of the analysis of variance to the case of multiple dependent measures. Throughout the paper the variable X will refer to prestimulus heart rate measurements, Y to poststimulus heart rate, Y<sub>i</sub> to poststimulus heart rate in the *i*th measurement interval, and D to the difference between pre- and poststimulus heart rate. Raw heart rate scores are represented by capital letters, while mean deviation scores are represented by lowercase letters, e.g.,  $x = X - M_X$ , where  $M_X$  is the mean of X scores.

The use of a computer and packaged statistical programs is helpful (if not necessary) in the analysis of heart rate data. The author has found the SAS (Barr, Goodnight, Sull, & Helwig, 1976; Helwig & Council, 1979), BMD (Dixon, 1970; Dixon, P-Series, 1977), and the SPSS (Nie, Hull, Jenkins, Steinbrenner, & Bent, 1975) statistical packages to be useful general purpose data analysis systems, and these will be referred to frequently as analysis techniques since they are so widely available. Ronald Wilson (Note 1) has prepared a set of computer programs especially for heart rate data, and the present author has compiled a set of computer programs which perform many of the statistical techniques presented in this paper (Richards, Note 2).

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# **Appropriate** Cardiac Measure

Two cardiac scores are commonly used in heart rate research, based on different units of analysis. The most common is heart rate, which is defined as the inverse of the heart period, usually measured by the length of the R-R interval in the electrocardiogram. Heart rate is measured in real-time units such as seconds, and standardized in terms of beatsper-minute (bpm). The other cardiac score which may be used is heart period, defined as the length of the R-R interval, and it is measured in cardiac-time units (beat-by-beat). Graham and Jackson (1970) and Graham (in press) discuss the relative merits of the different units of analysis. They favor real-time units, mainly because they are easier to coordinate with experimental events. Lewis, Bartels, and Goldberg (1967) and Lewis and Spaulding (1967) give some reasons for preferring the beat-by-beat units of analysis, since they provide a sort of "biological clock" against which cardiac responses can be evaluated. A method of making the cardiac score the average of a certain number of extreme (high or low) beats in a certain interval (e.g., Campos, Langer, & Krowitz, 1970; Kagan, 1971; Kagan & Lewis, 1965; Kinney & Kagan, 1976; Lewis, Kagan, Campbell, & Kalafat, 1966; McCall & Kagan, 1967) is not a good unit of analysis, though popular in infancy research, because changes in variance of the poststimulus period favor finding results in the hypothesized direction when such effects are not present in the data (Graham & Jackson, 1970).

One consideration in the choice of a cardiac measure and units of analysis has been demonstrated by Graham (1978b). Real-time units should be used with heart rate, and cardiac-time units should be used with heart period. Otherwise, the arithmetic mean of the unit estimates is not equal to the definitional estimate of the mean based on all units. In the past it has been assumed that the choice of time units was independent of the choice of heart rate or heart period (Graham & Jackson, 1970; Jennings, Stringfellow, & M. Graham, 1974; Khachaturian, Kerr, Kruger, & Schachter, 1972). Graham (1978a) also correctly asserts that arguments by Khachaturian et al. (1972) that heart period is the "raw data" and heart rate only a transformation of the raw data are incorrect. The units of analysis determine which is the raw data, being heart period for cardiac-time units and heart rate for real-time units.

Statistical considerations concerning the choice of a cardiac score favor using heart rate as the cardiac score for infancy research. Many parametric statistical techniques, including the analysis of variance, assume that the dependent variable is normally distributed and that variances are homogene-

ous across treatment conditions. Although many of the statistical techniques are robust to violations of these assumptions, a dependent variable which meets these assumptions is preferable to one which does not, methodological considerations being equal. Jennings et al. (1974), Khachaturian et al. (1972), and Graham (1978a) have studied the distributions of heart period and heart rate for infant and adult subjects, and the latter two studies also looked at the homogeneity of variance assumption. The data for adult subjects (Jennings et al., 1974; Graham, 1978a) are inconsistent, perhaps due to differences in obtaining the cardiac response. For infant subjects (Khachaturian et al., 1972; Graham, 1978a) it is clear that heart rate meets the assumptions better than heart period. In both studies, homogeneity of variances was more likely to be found with heart rate than with heart period. Khachaturian et al. report that the degree of skewness (left-right asymmetry) was greater for heart rate than for heart period, but distributions were more often skewed for heart period than for heart rate. Graham, reporting data over a wide range of studies with stimulated and unstimulated heart rate, found that skewness occurred more often in infant heart period than in infant heart rate. Kurtosis (excess or deficit in the distribution center) was equal in the two measures. These data clearly show the statistical superiority of heart rate over heart . period for the infant. Methodological considerations may lead the researcher to choose heart period. but in general heart rate should be the preferred cardiac score. In the remaining sections of the paper heart rate will almost exclusively be discussed, although heart period could be used in many of the statistical techniques as well.

#### Analysis of Variance of Heart Rate Data

Since the reader of this paper is presumed to be familiar with the analysis of variance, only a few comments will be included. Analysis of variance is the appropriate statistical technique for most experimental research, and heart rate research is not an exception. The dependent variable for the analysis may be heart rate or heart period, whichever the researcher chooses to be appropriate. Wilson (1967) has given some suggestions specific to the analysis of variance of heart rate data, and the reader is referred there for further details. Wilson has also prepared several computer programs for the statistical analysis of heart rate (Wilson, 1967, 1974, Note 1; Wilson & Scott, 1970). Benjamin (1967) provides statistical rationale for using the analysis of variance and covariance for heart rate data. For the general technique of the analysis of variance, the interested reader may consult one of several texts (Hays, 1973; Keppel, 1973; Winer, 1971).

# Law of Initial Values

One of the most prominent issues in the statistical analysis of heart rate data is the problem of the initial value of the prestimulus heart rate during experimental situations. The Law of Initial Values (Wilder, 1950, 1956, 1958), which applies to a wide range of physiological systems, states that the poststimulus heart rate level is partially determined by the prestimulus heart rate level. At high prestimulus levels the heart rate acceleratory response is smaller than it is at low prestimulus levels, and the heart rate deceleratory response is larger than it is at low prestimulus levels. Poststimulus heart rates, therefore, contain variance due not only to the experimental manipulations but due also to the level of the prestimulus cardiac rate. The statistical problem here is to remove the variance due to the lawful dependence in the heart rate, making the poststimulus heart rate scores independent of the prestimulus cardiac level.

The Law of Initial Values is important for the infancy researcher to consider. Heart rate level varies considerably over a range of behavioral state conditions, and it changes in the first few months of life. Harper, Hoppenbrouwers, Sterman, McGinty, and Hodgman (1976) found an overall linear decline in heart rate over the first six months of the infant as well as a cubic trend (Fig. 1). Fig. 1 also shows that heart rate occurs at different levels in the four behavioral states monitored. Harper, Hoppenbrouwers, Bennet, Hodgman, Sterman, and McGinty (1977) found that feeding was followed by higher heart rate in sleeping periods than in those sleeping periods not preceded by feeding. It has been suggested (Schmidt, Rose, & Bridger, 1974) that the different heart rates in behavioral conditions may be the cause of the observed relationship between behavioral state and the cardiac response to stimulation (e.g., K. M. Berg, W. K. Berg, &



Fig. 1. Averaged median rates for all individuals over six age periods during four different behavioral states. AW: awake: TR: transitional state; REM: rapid eye movement; QS: quiet sleep. (From Harper et al., 1976, used with permission)

Graham, 1971; Jackson, Kantowitz, & Graham, 1971; Lewis et al., 1967; Pomerleau-Malcuit & Clifton, 1973). Therefore, when different ages or different behavioral states are used in an experiment, different heart rates are likely to be found. Additionally, the normal amount of variability associated with heart rate implies that the cardiac levels on different prestimulus trials will be different.

At least three procedures can be followed to handle the problem of the Law of Initial Values. An experimental procedure would be to withhold presentation of the experimental stimulus until the heart rate level is within a certain range. Of course, this is not possible for all experiments, and ignores the possibility of mean heart rate level differences among subjects or conditions. A second strategy to control for significant mean heart rate differences among subjects or between conditions is the use of a mean deviation cardiac score such as the autonomic lability score suggested by various researchers (Crowell et al., 1976; Lacey, 1956; Wilson, 1967), and discussed in a later section of this paper. However, even with deviation scores, it is likely that there will be a relationship between pre- and poststimulus scores since not all prestimulus deviation scores will be identical. The analysis of covariance is the third procedure that can be used to control for effects due to the Law of Initial Values. It has the widest acceptance by cardiac researchers as the answer to the problem (Benjamin, 1963, 1967; Crowell et al., 1976; Graham & Jackson, 1970; Wilson, 1967).

# Analysis of Covariance

The analysis of covariance removes effects due to the Law of Initial Values by a linear regression adjustment of the poststimulus heart rate. Poststimulus scores are regressed on prestimulus scores, giving the linear regression coefficient,  $b_{Y,X}$ . The linear regression coefficient may be used to compute scores,  $\hat{\mathbf{Y}}$ , which are statistically independent of X. In many cases, actual Y scores are not computed and used in statistical significance tests. For example, in the analysis of variance, a covariance analysis would proceed by subtracting a term from the sums of squares which represents variance accounted for by the sum of deviation crossproduct scores relative to the deviation sum of squares of the prestimulus heart rate. Benjamin (1963, 1967) gives the statistical rationale for using the analysis of covariance with heart rate data, while one may consult Winer (1971), Hays (1973), Keppel (1973), or Kerlinger and Pedhazur (1973) for the general method of the analysis of covariance. The SAS, SPSS, and BMD computer programs have analysis of covariance routines.

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The analysis of covariance just mentioned only adjusts for linear relationships between pre- and poststimulus heart rate scores. It is possible, however, that other relationships exist among heart rate scores, which are curvilinear in nature. Graham and Jackson (1970) state that in heart rate data there will be few cases in which a curvilinear regression adjustment will be necessary, and in the cases in which one is done the increase in variance accounted for will be slight. The statistical significance of suspected curvilinear relationships should be tested, and any important curvilinear terms used to adjust poststimulus scores. One may do this adjustment on the BMD computer program OSR (P-Series, P5R).

A special problem arises in the adjustment of heart rate scores by the covariance methodology. Most researchers assume homogeneous regression coefficients and use a regression coefficient pooled across all groups, including experimental groups, repeated trials within each experimental treatment, and each measurement made in multiple poststimulus intervals (beats or seconds). The assumption of homogeneity of linear regression coefficients is necessary for the use of a pooled regression coefficient (Graham & Jackson, 1970; Lacey, 1956). Generally this assumption is warranted, since experimental conditions should not contribute to the Law of Initial Values. However, in some cases when covariances are not equal across all groups, this assumption may be violated. The assumption of homogeneity of regression coefficients should be tested before adjusting scores, or using the analysis of covariance. Details of the test can be found in Hays (1973), Keppel (1973), Winer (1971), Kerlinger and Pedhazur (1973), among others. This assumption is routinely tested in the analysis of covariance routine of BMD (PIV and P2V). If there is heterogeneity among regression coefficients one should adjust the Y values for each group by the regression coefficient for that group of scores. Further statistical analysis must be done separately for each group. Differences in adjusted heart rate scores will include treatment effects and effects due to heterogeneous regression coefficients.

An assumption of the analysis of covariance is that the covariate is reliably measured. It may not be the case that heart rate scores are a reliable measure, which would underestimate the appropriate value of  $b_{Y,X}$ , and thus not correct fully for the effect of the initial prestimulus level. If this underestimation is acceptable to the researcher, one may use the analysis of covariance with the uncorrected prestimulus heart rate. The value of the alternatives to the uncorrected heart rate is uncertain. For example, measurements based on several scores are generally more reliable than measurements based on single

scores. This suggests that a mean value over several prestimulus intervals should be a better covariate than the first second preceding the stimulus. However, if the sample of prestimulus scores is large enough the value will approach the population mean and the covariance adjustment will not be sensitive to deviations temporally contiguous to the stimulus presentation. Secondly, if the reliability of the prestimulus score is known, one may adjust the  $b_{\rm YX}$ upward by an amount proportional to the reliability. However, it is not usually the case that the value of the reliability of heart rate scores is known or can be estimated. A third approach which one could use to correct for this problem is to employ a time series smoothing function (Blackman & Tukey, 1959; Box & Jenkins, 1970; Fuller, 1976) which removes sampling error from the scores, leaving the first prestimulus heart rate value acceptable.

### **Difference** Scores

Difference scores are also subject to the Law of Initial Values. Graham and Jackson (1970) point out that many researchers use difference scores because it is felt that the correlation between the prestimulus score and the difference score is lower than the correlation between the pre- and poststimulus scores. Difference scores may also be used because the interpretation of heart rate *change* is of primary concern. Since, D = Y - X, a small derivation will show that,

$$b_{\rm D.X} = b_{\rm Y.X} - 1.0$$
 (1)

If the linear regression coefficient,  $b_{Y,X}$ , represents a significant relationship between Y and X, the regression coefficient,  $b_{D,X}$ , will also be of some value. Only when  $b_{Y,X} = 1.0$  is the dependency between D and X nonexistent and adjustment of difference scores unnecessary. The adjustment of difference scores, like the adjustment of actual Y scores, can be accomplished by adjusting the actual D scores or by adjusting the error and effect terms in the analysis of variance. Similarly, the problems of curvilinear regressions, homogeneity of regression coefficients, and reliability of the measures apply to difference scores.

# When Should the Adjustment Be Made?

One final question in the statistical adjustment of the effect of the Law of Initial Values is when the adjustment is appropriate. Graham and Jackson (1970) state that when the Law of Initial Values is affecting the data,  $b_{Y,X}$  will be a positive number less than 1.0. This is based on the fact that the Law of Initial Values predicts a negative relationship between the level of the prestimulus heart rate and the difference score. Since  $1.0 = b_{Y,X} - b_{D,X}$  (Equation 1), a negative  $b_{D,X}$  will require that  $b_{Y,X}$  is

positive but less than 1.0 (Benjamin, 1963, 1967). Benjamin (1967) points out that  $b_{D,X}$ ,  $b_{Y,X}$ , and  $r_{XY}$ are not equivalent statements about the relationship between X and D. She suggests that a consideration of the methodology upon which these scores are based implies that  $r_{\rm XD}$  is the best indicator of the presence or absence of the Law of Initial Values. Thus when  $r_{\rm ND}$  is statistically significant, i.e., different from 0.0, covariance adjustments should be made. It may appear inconsistent to assess the existence of the Law of Initial Values by a score (ryp) which takes into account the two-way relationship between X and D, and then adjust scores by the linear regression coefficient, which includes only the variance of the prestimulus score as a standardizing function. However, the correlation coefficient may truly reflect the Law of Initial Values, and it still would be correct to remove variance due to X by the linear regression coefficient, since this procedure is the only one which results in a score, Y, which is statistically independent of X.

However, an easier approach would be to assess the amount of variance accounted for by the inclusion of the covariate, and if this is significant the adjustment should be made. No matter what the value of  $r_{\rm XD}$  is, if the adjustment does not result in a decrease in variance from Y to Y, the adjustment is superfluous. Statistical rationale for this test may be found in Kerlinger and Pedhazur (1973) and Tatsuoka (1971). The BMD and SPSS analysis of covariance routines provide a test for the significance of the covariate as standard output.

# **Differential Variability**

Three potential areas of difficulty face the researcher with regard to differential variability in heart rate. First, there is likely to be substantial intersubject variation in heart rate data in neonates and older infants (Porges, 1974). This differential variability implies that even though two scores are of the same magnitude they will have a different probability of occurrence because they are from different distributions. Secondly, infant subjects have been shown to have different levels of heart rate variation at different ages (Harper et al., 1976) and in different behavioral states (Harper et al., 1976, 1977). Finally, there are good reasons for suspecting that the assumption of homogeneity of the covariance matrix is violated with repeated measures designs using cardiac rate as the dependent variable.

#### Intersubject Heart Rate Variation

Porges and his associates have shown that infant heart rate variability is an important source of variance in infant research. For example, Porges, Arnold, and Forbes (1973) found that infants with high variability heart rates responded to an auditory stimulus in a pattern similiar to adult subjects, with greater acceleratory and deceleratory responses than infants with low variability heart rates. Stamps and Porges (1975) and Stamps (1977) report that infants with high variability in heart rate were more easily influenced by a conditioning procedure than infants with low variability in heart rate. This research demonstrates intersubject variability in heart rate, and suggests that such variability might be an important index of stimulus responsivity (Porges, 1974).

The problem of differential variability between subjects' heart rates has been addressed in a solution by Crowell et al. (1976) based on Lacey's (1956) autonomic lability score. A similiar score may be found in Program 3 of Wilson (Note 1). Individual heart rate lability and difference in mean tonic level are adjusted by transforming heart rate scores by a standardization procedure. Each heart rate score in the poststimulus period is posited as being a combination of the child's lability (reactivity, responsivity, variability), the mean heart rate level, effects of the Law of Initial Values, effects due to the cardiac response to the treatment condition, and error. The standardization procedure removes the effects of the child's lability and mean heart rate level, and since error is thought to be randomly distributed, only the Law of Initial Values and the response to the stimulus remain in the scores. If there is a significant covariance relationship among the standardized pre- and poststimulus scores, the procedures of the analysis of covariance discussed in the previous section will remove that effect. This leaves effects due only to the response to the experimental stimulus in the poststimulus score, which can then be analyzed by common statistical techniques. One may refer to Crowell et al. (1976) for a presentation of the technique, and an example of its use in a neonatal heart rate classical conditioning study. A slightly different form of the lability score is presented by Wilson (1967) and can be calculated from Program 3 of Wilson (Note 1; Wilson & Scott, 1970). A computer program for the Crowell et al. (1976) procedure is available from the present author (Richards, Note 2).

This deviation procedure adjusts for differences in the subjects' labilities and mean heart rate level. If means are normally distributed (or equal) and variances are not significantly different between subjects, the analysis of heart rate data should be done on unadjusted scores. Since the deviation procedure demands multiple measurement intervals in the prestimulus period, a repeated measures analysis of variance of prestimulus scores and variances would test intersubject differences in the prestimulus period. A groups main effect should not be significant since the experimental treatments should not affect prestimulus (pretreatment) scores. The subjects sum of squares tested against the groups by subjects interaction term, if significant, would reveal intersubject differences. If there are no mean or variance differences the deviation procedure should not be used.

# Differential Variability in Treatment Conditions

It is likely that the assumption of the analysis of variance that variances be homogeneous for all treatment groups will be violated for heart rate scores. The research of Harper et al. (1976, 1977) has shown that heart rate variability is affected by behavioral state and that variability changes in the first six months of life (Fig. 2). Differential variability in experimental groups may occur in developmental studies of heart rate when age is used as a factor, or in studies of heart rate when behavioral states vary. This problem occurs when the treatment factors contain independent subjects. In a repeated measures design a different problem may occur (next section).

Heterogeneity of variance in treatment conditions is not a serious problem for most experimental research. Monte Carlo studies of the violation of this assumption with both the F (Box, 1953, 1954; Norton, 1952) and the t ratios (Baker, Hardyck, & Petrinovich, 1966; Boneau, 1960) have shown that the violation of the homogeneity of variances assumption is not serious when equal sample sizes are used in treatment groups. Therefore, testing for homogeneity of variances is not necessary if the researcher assures equal sample sizes in the treatment conditions. If unequal sample sizes exist, however, the empirical probability of rejecting the



Fig. 2. Average interquartile ranges of heart rate variability for 8 normal infants from birth to 6 months of age in different behavioral states. AW: awake; TR: transitional states; REM: rapid eye movement; QS: quiet sleep. (From Harper et al., 1976, used with permission)

null hypothesis is greater than the nominal  $\alpha$  level. This may occur in clinical or naturalistic studies where equal sample sizes cannot be obtained. In this case, one should consult analysis of variance textbooks for the proper procedure.

# Differential Variability of Repeated Measures Treatment Conditions

Assumptions in the repeated measures analysis of variance are more stringent than in the independent groups analysis of variance. Repeated measures analysis of variance demands distribution normality and homogeneity of variance in any between-group factors. It also has the assumption that subjects retain their relative standing in the various conditions of the repeated measures factor. This is formally known as the homogeneity of covariance matrices assumption. It demands that: 1) within each level of the nonrepeated factors homogeneous covariances are found among the repeated factors, and 2) the covariance matrices are homogeneous across all factors.

The consequence of violating the covariance homogeneity assumption is that statistical tests are biased positively on the repeated measures factors (Box, 1950; Collier, Baker, Mandeville, & Haves, 1967; Keppel, 1973; Winer, 1971). This means that the researcher will reject the null hypothesis of no difference among repeated factors means more often than the nominal  $\alpha$  level implies. Collier et al. (1967) in a Monte Carlo study of the violation of the covariance homogeneity assumption used a design with one repeated and one nonrepeated factor. They found that the nonrepeated factor was not affected by the violations. The repeated factor, however, was almost always positively biased (including main effects and interactions). For example, with the  $\alpha = .05$  only 2 of 60 times was the empirical test size below .05, and ranged as high as .115. Empirical  $\alpha$  levels of this size are unacceptable to the researcher.

Heterogeneity in the covariance matrix may occur in infant heart rate research for several reasons. These reasons are based on a dictum that measurements made on the same individual adjacent in time are more highly correlated than measurements separated in time. This could occur in heart rate research when repeated measurements are made in several intervals on each trial. It may also occur in a longitudinal experimental design. One would expect that the correlation between occasions 1 and 2 might be higher than the correlation between occasions 1 and 3. A third potential design in which the homogeneity assumption is often violated is when heart rate measures are made in different treatment conditions separated by unequal time periods. Ot these three potential causes of covariance

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homogeneity, the first and last problems are most unique to heart rate research. This is especially true when multiple measures of heart rate are made in the poststimulus intervals and treated as a repeated measures factor in the analysis of variance.

One can test a set of repeated measures heart rate data for homogeneity of covariances by the Box (1949, 1950) test. The Box test is a generalization of the Bartlett (1937) test for homogeneity of variances in independent groups. One calculates a value which may be compared against  $\chi^2$  or against F distributions. The reader may refer to Box (1949, 1950), Cooley and Lohnes (1971), Morrison (1967), or Namboodiri, Carter, and Blalock (1975) for the statistical rationale of the test and details required for its calculation. The researcher is encouraged to use one of several computer programs to do the actual calculations (Nie et al., 1975, DISCRIMI-NANT, Statistic #7; Rawlings & Pautler, 1972a; Richards, Note 2; Wilson, Note 1, Program 12).

What should the researcher do if heterogeneity exists in the covariance matrix? The two most reasonable options are the Greenhouse and Geisser (Geisser & Greenhouse, 1958; Greenhouse & Geisser, 1959) correction of the degrees of freedom of the critical F ratio or the use of a multivariate test (Bock, 1975; Harris, 1975; McCall & Appelbaum, 1973; Morrison, 1967). Both Davidson (1972) and McCall and Appelbaum (1973) recommend the use of the multivariate method over the Greenhouse and Geisser correction, claiming the latter is too conservative. Wilson (1975) points out that their conclusion is based on the maximum conservative value of the Greenhouse and Geisser correction, rather than the correction value which is proportional to the degree of heterogeneity in the covariance matrix. Opposing the Davidson and the McCall and Appelbaum conclusion is the study of Collier et al. (1967) which found that the correction resulted in an empirical test size approximately equal to the nominal  $\alpha$ level. Wilson concludes that the repeated measures analysis of variance with the Greenhouse and Geisser correction is fully protected against bias resulting from violation of the homogeneity of covariance assumption.

Wilson (1975) may be correct in stating that the Greenhouse and Geisser correction protects the analysis of variance framework against bias when compared to the uncorrected analysis of variance. However, direct comparison needs to be done between the multivariate procedure and the Greenhouse and Geisser correction to correctly assess the relative power and error protection of the two methods. There are other reasons that a researcher may prefer the multivariate method. It is not restricted by the homogeneity of covariance

assumption, and so evades the issue entirely. It is a unified statistical technique developed within the theory of classical statistical testing and is associated with several well-established and exact testing procedures. The multivariate method can be incorporated into many analysis routines, including mean comparisons and polynomial trend comparisons. In some cases, such as clinical studies in which one or more data points are missing for subjects, the multivariate method is the only appropriate one (Wilson, 1975). On the other hand, the Greenhouse and Geisser correction method appears to the present author to be an ad hoc technique with no relationship to the central statistical premises of the analysis of variance. The study of Collier et al. (1967) upon which the recommendation of Wilson (1975) is based studied the correction value only for some limited designs. It may or may not have the same success with other designs. Finally, the ease of computing the Greenhouse and Geisser correction and the associated repeated measures analysis of variance is no longer of concern due to the use of computer programs for multivariate research (e.g., Wilson, Note 1, Program 12), excellent discussions of the statistical rationale behind the test (Bock, 1975; Morrison, 1967), and its application to research (Davidson, 1972; McCall & Appelbaum, 1973). One may refer to Wilson (1975), McCall and Appelbaum (1973), and Davidson (1972), for a further discussion of this topic.

The multivariate test proceeds by transformation of the data by a matrix which represents several post hoc comparisons. One may test such hypotheses as equality among adjacent scores (Morrison, 1967), compare a criterion measurement interval with several other intervals, or use a set of polynomial trend contrasts (McCall & Appelbaum, 1973). Following only the significant multivariate tests (Hummel & Sligo, 1971; McCall & Appelbaum, 1973; Wilkinson, 1975) each univariate mean comparison is tested. McCall and Appelbaum's (1973) presentation of the multivariate method for repeated measures designs is an excellent guide for the conceptual and computational explication of this technique. The multivariate test can be done with Program 12 of Wilson (Note 1) or the program of Rawlings and Pautler (1972b). The SAS package (Barr et al., 1976; Helwig & Council, 1979) has good, flexible multivariate programs under the GLM procedure. The program of Finn (1973) is comprehensive, but requires some knowledge of design matrices.

Under the appropriate conditions, researchers may prefer the Greenhouse and Geisser correction. In this correction, a value is computed which is multiplied by the degrees of freedom of the critical F value. This value is always less than 1.0, and is proportional to the amount of heterogeneity in the covariance matrix. The greater the heterogeneity in general leads to more positively biased statistical tests. Thus, the greater the heterogeneity, the smaller the degrees of freedom of the critical F value after the correction, and the greater the obtained F value needed to reject the null hypothesis (McCall & Appelbaum, 1973). Program 12 of Wilson (Note 1) computes the correction value, and the complete analysis of variance with the Greenhouse and Geisser correction can be done with the computer program of Games (1975, 1976). Both Wilson (1975) and McCall and Appelbaum (1973) give examples of its usage.

Two cautions must be observed in the use of the Greenhouse and Geisser correction or the multivariate method. First, these methods are only alternatives to the univariate repeated measures analysis of variance when there is some indication of heterogeneity in the covariance matrices. The uncorrected test is more powerful than either of the two alternatives when its assumptions are met. Secondly, only those factors involving repeated measures (i.e., trials, intervals) should be tested by the alternative method. Collier et. al. (1967) demonstrated that heterogeneity of covariance matrices does not affect factors not involving the repeated measures, and the analysis of variance is robust to violations of the homogeneity of variances assumption for independent groups with equal sample sizes. Therefore in the Greenhouse and Geisser correction the correction is only applied to the main effects and interactions involving the repeated factors. In the multivariate strategy, the tests for nonrepeated factors are identical to conventional tests while those effects involving repeated measures employ the multivariate statistical techniques.

Graham (1970) shows another way in which the homogeneity of the covariance matrix is important to consider in heart rate statistical analysis. In many heart rate experiments one measures several intervals on each trial, has several treatment conditions, and may present each condition more than once to each subject. Recall again that measures made close in time will be correlated higher than measures separated in time. In the designs just mentioned, the measurement of intervals is closer in time than either the trials or the treatment factors. The homogeneity of covariance assumption is that the covariance matrices representing both the trials and the intervals be identical, which will not often be the case because of the different separations of time.

Graham (1970) states that this type of heterogeneity demands unpooled error terms in the repeated measures analysis of variance tests. Pooled error terms will inflate the F ratio for the interval variable since it usually has a small effects variance.

while the pooled error term has taken from it the much larger variance of the trials factor. Tests for significance should use error terms specific to that effect. This includes tests for trends, which should not be tested against the total interval error term, since it is pooled across all trends, but tested against the error term specific to that trend. Researchers should be cautious in the use of packaged statistical programs which do not allow specification of effect and error terms, since they often test every effect against a pooled error term. The SAS statistical package (Barr et al., 1976; Helwig & Council, 1979) is recommended for this purpose, since the procedure GLM allows the specification of error terms and therefore offers the greatest flexibility in the, analysis of repeated measures designs. The caveat against the use of pooled error terms is applicable to both univariate and multivariate methods of testing repeated measures designs.

#### **Time Series Analysis**

David Crowell and his associates (Crowell et al. 1976: Jones, Crowell, & Kapuniai, 1969; Jones, Crowell, Nakagawa, & Kapuniai, 1971) present an autoregression technique for the analysis of infant heart rate data. Heart rate is a time series in which each score contains lawful dependence on the previous score. The time series technique of autoregression removes the sequential dependency among heart rate scores and allows one to identify significant deviations from the time series.

In the autoregression technique, one calculates an autoregression coefficient,  $\alpha_1$ , which is based on the prestimulus heart rate scores:

$$\alpha_{1} = \frac{\sum x_{1} x_{1+1}}{\sum x_{1}^{2}} = \frac{CoV_{x_{1}x_{1+1}}}{VAR_{x_{1}}}$$
(2)

where  $x_i$  and  $x_{i+1}$  are from prestimulus heart rate, and are deviation scores at adjacent intervals.  $\Sigma x_i x_{i+1}$  and  $\Sigma x_i^2$  are the sum of deviation crossproducts and the sum of squared deviations of  $x_i$ , respectively, and  $CoV_{x_i x_{i+1}}$  and  $VAR_{x_i}$  are the covariance and variance associated with these scores. The autoregression coefficient is actually the linear regression coefficient between adjacent heart rate scores. It contains information about the sequential dependency among adjacent heart rate scores. Given the autoregression coefficient and the mean of the prestimulus heart rate scores,  $M_x$ , the autoregression formula,

$$Y_{i+1} = M_X + \alpha_1 (Y_i - M_X),$$
 (3)

is used to predict poststimulus scores given the immediately preceding heart rate score. The prediction of the poststimulus scores given the unstimulated sequential relationship can be compared to the actual score following presentation of the stimulus.

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Given the standard deviation of the prestimulus scores,  $SD_x$ , one may compute a *t* value based on predicted and actual  $Y_i$ ,

$$t = \frac{\mathbf{Y} - \hat{\mathbf{Y}}}{SD_{\mathbf{X}}},\tag{4}$$

which is distributed as Student's t with df = d - 2. where d is the number of measurements in the prestimulus interval upon which the autoregression coefficient is based. One can thus identify significant deviations from the time series. Fig. 3 presents graphically both a significant and a nonsignificant response to an auditory stimulus in a newborn subject, with 95% confidence intervals around the predicted heart rate scores. Jones et al. (1969) present a Fortran subroutine which may be used for this entire process. Jones et al. (1971) extend this basic analysis to smooth irregularities in heart rate data due to rapid fluctuations, and develop a model which adaptively updates parameters of Equations 2, 3, and 4, weighting prestimulus data of the most recent stimulus more heavily than past prestimulus data. Both programs are available from the present author (Richards, Note 2). Examples of the use of the autoregression technique can be found in Jones et al. (1969) and Crowell et al. (1976).

The autoregression technique has met with little usage in infant heart rate research, but is worth considering for several types of experimental designs. Jones et al. (1969) and Crowell et al. (1976) use it to determine the pattern of response in the

poststimulus period. If one is interested in comparing the effects of several stimuli, this technique may be preferred over factorial design analysis for detecting significant responses to stimulation. It may be used in single-subject experiments or in studies where the number of subjects is not as large as the number of variables the researcher is interested in measuring. The autoregression technique, as well as several other types of time series analyses, have proven beneficial in clinical applications when only one subject is available and isolation is needed of single critical events such as atrial fibrillation with PVC's (Gersch, Lilly, & Dong, 1975) or cardiac arrhythmias (Gersch, Eddy, & Dong, 1970). Perhaps it may be used in conjunction with discriminant analysis for a pattern of responses or events which occur in conjunction with heart rate deviations. Finally, this technique should prove useful in programming computers for on-line decision making, since the critical parameters can be estimated adaptively as data is collected.

# **Recent Statistical Applications**

A brief mention will be made of some statistical techniques which have recently been applied to the analysis of heart rate data. They will not be extensively discussed because for the most part the applications to date have been with adult subjects, and little discussion exists about their problems or their applicability to infant heart rate data. They deserve mention, however, because they are potentially



Fig. 3. Observed and predicted heart rate responses of a 30-hr-old female newborn to two 2000 Hz modulated pure tones at 50 dB ( $S_1$ ) and 80 dB ( $S_2$ ) with 95% confidence intervals; the overall change at the 80 dB level ( $S_2$ ) was highly significant. (From Jones et al., 1969, used with permission).

valuable tools for the analysis of infant heart rate data. Such a discussion in a review of this type might encourage their usage and supply researchers with a better understanding of the techniques and of their application to heart rate data analysis.

As mentioned in the preceding section, heart rate can be thought of as a time series. One property of a time series is the frequency spectrum which underlies variations of the data (Blackman & Tukey, 1959: Box & Jenkins, 1970; Fuller, 1976; Jenkins & Watts, 1968). Spectral analysis of heart rate data has been used recently to examine variability in heart rate data caused by respiration activity. Porges (1976) and Porges and Humphrey (1977) have used the cross-spectral correlation between heart rate and respiration as an indicator of parasympathetic nervous system integrity. Porges (1976) speculates that the lack of coherence between heart rate and respiration in retarded and infant populations may be an index of the inability of those subjects to engage in sustained attention. Harper, Walter, Leake, Hoffman, Sieck, Sterman, Hoppenbrouwers, and Hodgman (1978) have found that respiratoryinduced variation in heart rate is different in behavioral states which differ, and increases over the first six months of life. These changes may be caused by the increasing influence of the parasympathetic branch of the autonomic nervous system in the first few months of life.

Another statistical technique which may be fruitfully applied to infant heart rate analysis is factor analysis. Many readers will be aware of the application of factor analysis to the structural analysis of psychometric tests. In factor analysis, one determines the underlying structure of a set of multivariate dependent variables (Cooley & Lohnes, 1971; Harman, 1960; Tucker, 1958, 1966; Van Egeren, 1973). Given several dependent variables which are observed by the researcher, one uses factor analysis to reduce these into a few unobserved latent constructs which are thought to be the true variables underlying the observed variables. Since heart rate when measured over several intervals may be conceived of as a set of multiple dependent measures, factor analysis may be relevant in its statistical analysis. A study by Van Egeren, Headrick, and Hein (1972) is a good example of the application of factor analysis to heart rate scores. They analyzed the factor structure of a set of heart rate scores in intervals 10 sec prior to and 3 sec after the presentation of a conditioned stimulus. After determining the factors, scores of each individual on the factors were calculated and the group averages were plotted. They determined three response patterns to exist in the data, including an acceleratory-deceleratory shift which may represent both an unconditioned and a conditioned response,

a monophasic response which may be based on orienting (deceleration) and defensive (acceleration) reactions, and a deceleratory-acceleratory shift.

One should be cautious in using factor analysis on heart rate scores. Cronbach (1967) shows that the factor analysis of data in which scores close in time have higher correlation than scores far apart in time may result in arbitrary factors determined by the amount of separation of the measurement intervals rather than by an underlying theoretical construct. One should check the validity of the factors by comparing the individual subjects' raw data with the obtained factor pattern (e.g., Van Egeren et al., 1972, Fig. 3). Another technique to avoid this problem would be to cluster subjects according to the similarity of the factor score patterns, and use the resulting groups as empirically defined response pattern groups, doing quantitative analyses on the raw scores of the defined groups (e.g., McCall, Appelbaum, & Hogarty, 1973, 43ff.).

Factor analysis in most cases has been exploratory, i.e., it is an a posteriori analysis of the structure inherent in the observed response system. On the other hand, confirmatory factor analysis (Jöreskog, 1969) is a model testing system which can be used to test specific structural models against observed data. For example, Porges and his associates (Porges, 1976; Porges & Humphrey, 1977; Walter & Porges, 1976) have speculated that a two-component attentional system exists which includes reactive and sustained attention, and which can be indexed by patterns of heart rate responding. Presumably the first few poststimulus seconds represent reactive or reflexive attention which is stimulus dependent, and the next poststimulus seconds while attention is occurring is sustained attention which is cognitively determined. This prediction could be tested by specifying two factors underlying heart rate responding in the poststimulus intervals, one reflected by immediate response patterns and one reflected by long-term responses. The computer program COFAMM (Sörbom & Jöreskog, 1976) can be used to do confirmatory factor analysis. Jöreskog has recently extended this type of analysis to a general structural model testing system which includes confirmatory factor analysis and causal modeling (path analysis) in one system (Jöreskog, 1970, 1973, 1974, 1977), and a computer program is available for computational aid (Jöreskog & Sörbom, 1978).

Factor analysis methodologies are restricted to analyzing single populations. With more than one group discriminant analysis can be used in a similiar fashion. In discriminant analysis a function is estimated which is a weighted linear combination of the multiple dependent variables which maximally dis-

criminates between multiple groups by maximizing the variance of the scores of the predefined groups on the discriminant function (Bock, 1975; Cooley & Lohnes, 1971; Tatsuoka, 1971; Van Egeren, 1973). The discriminant function can then be used to classify a new set of individuals into one of the previously defined response groups. Multivariate analysis of variance estimates a discriminant function for each effect being tested and may be described as a univariate analysis of variance on the scores of the groups on the linear discriminant function. Given multiple intervals of heart rate in two or more groups, or in the case of an experimental factorial design, one may use discriminant analysis to combine heart rate patterns for the best discrimination among the experimental groups. This technique has an important clinical application. One may be able to classify persons belonging to pathological populations by comparing the score on a discriminant function which was obtained by discriminating between criterion normal and pathological groups. This technique is especially important to the heart rate researcher interested in those diseases which are known to produce characteristic heart rate patterns, such as respiratory distress syndrome.

# **Conclusion and Summary**

The present paper has been devoted to the presentation of several statistical problems encountered by the heart rate researcher, emphasizing those which are encountered in infancy data. Table 1 presents a series of steps based on this discussion which the researcher might follow in choosing the appropriate statistical techniques for analyzing heart rate data.

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#### TABLE 1

#### Steps for choosing statistical techniques

- I. Choose the appropriate cardiac measure.
- II. Use a lability score if there are differences in variances and means of each subject. If no difference, or if means are normally distributed, use raw scores.
- III. Choose the appropriate statistical analysis for the main experimental questions. Analysis of variance is usually appropriate for factorial experimental designs. Use autoregression techniques if individual responses or on-line decisions are of interest. Could the heart rate response pattern be profitably analyzed with a multivariate statistical technique?
- IV. Should covariance adjustments be made for the Law of Initial Values? If so, are curvilinear relationships important? Are linear regression coefficients homogeneous? Are the cardiac scores reliable? Adjust raw scores or use analysis of covariance.
- V. If repeated measures analysis of variance is used, check the homogeneity of covariance assumption. If heterogeneity is present, use the multivariate test or the Greenhouse and Geisser correction. Do not use pooled error terms in repeated measures factors.

As with all statistical procedures, the indiscriminate application of techniques is unwarranted. Statistical methods each have assumptions underlying their usage and characteristics which define their range of application. The researcher is advised to proceed with caution in the application of these techniques. Hopefully, the existence of this paper will help eliminate some of these problems and provide the researcher with the knowledge necessary to apply these techniques.

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