

Validity Concerns of Common Heart-Rate Variability Indices

Addressing Quantification Issues in Time- and Frequency-Domain Measures of HRV

Heartrate variability (HRV) analysis is an extensively used tool in contemporary biomedical and psychophysiological research to assess cardiac autonomic control (see [1]-[3] for reviews). Low values in a variety of HRV measures have been linked with manifold pathophysiological and psychopathological conditions such as cardiovascular (CV) disease, diabetes [4], anxiety disorders [5], and attentional deficits [6]. HRV indices that reflect vagal control have been of particular value in such studies. Unfortunately, this research has been marked by validity concerns related to the use of certain common HRV measures and quantification procedures. In this article, several HRV data sets will be utilized to evaluate the impact of these issues, with the aim of appraising their gravity for investigators using HRV analysis.

The two basic forms of HRV analysis are often designated as *time-* and *frequency-domain* measures. Time-domain HRV indices are derived either directly from interbeat intervals (heart period; HP, (statistical differences exist between the use of HR and HP, but the issues addressed here apply to both; the term “HRV” is used in this article generically to refer to both types of measures [3]) or from differences between adjacent interbeat intervals in the HR time series [1]. Frequency-domain measures such as spectral analysis mathematically decompose the HR time series into its component frequencies [7]. Although each approach has its own quantification issues, frequency-domain techniques are more computationally complex and have generated a larger set of concerns. Time-domain indices are more straightforward to derive, but questions about their appropriateness for HRV research have also arisen. In this article, validity issues related to both spectral analysis of

HRV (a frequency-domain technique) and to mean successive differences of HR (MSD; a commonly used time-domain HRV measure) are evaluated in application to several real HRV data sets. An additional time-domain index, respiratory sinus arrhythmia (RSA), is also used in the service of addressing these concerns. RSA refers to rhythmic variations in HP synchronous with the respiratory cycle, believed to be regulated primarily by vagal activity [8].

Power in the so-called “high frequency” (HF; 0.15-0.4 Hz) band derived from spectral analysis of HR as well as RSA and MSD have all been used as putative measures of cardiac vagal control [1], [2]. This article will focus on the degree of commonality across these measures. Both RSA and HF spectral power reflect variability in HR at the frequency of respiration; MSD statistically filters out frequencies typically below that of respiration and so also closely estimates vagal modulation of HR [1], [2]. Data from two studies will be used to examine three specific issues related to these measures: 1) the importance of *stationarity* for spectral analysis of HR, 2) sampling biases in MSD, and [3] the degree of convergence among HF power, RSA, and MSD. It is hoped that this information will assist investigators in weighing the potential impact these quantification issues may have for their research.

The Effect of Nonstationarity on Spectral Analysis of Heart Period

One of the most frequently raised issues concerning spectral analysis on HRV data is the importance of *stationarity* in the time series [3], [7]. In general, the stationarity of a time series refers to the invariance of its distributional characteristics over time. *Weak* stationarity of a time series is said to be met when the first-

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and second-order moments (i.e., mean and variance) of a time series are constant [9]. This condition is a formal requirement for spectral analysis to be used on time-series data, including that of HR or HP [7], [10]. However, most physiological signals, including HR, routinely deviate from stationarity, so in practice this assumption is generally not met. As such, it is often contended that spectral estimates of RSA and cardiac vagal control may be biased [7], [10].

However, it is also possible that the consequences of violations of the stationarity assumption for spectral estimation of RSA may be exaggerated. Indeed, when spectral analysis is performed only on the stationary time segments from an essentially nonstationary time series, these results may be even more biased than spectral estimates obtained from the uncorrected time series [11]. A computer program has been developed to test whether a time-series data set meets the stationarity assumption (PSPAT) [10]. If the test criterion is not met, the program sequentially searches the time series for smaller sections of data that do meet the stationarity assumption. A point spectral analysis can then be performed on both stationary and nonstationary sets, rendering it possible to compare results obtained from both. A data set collected in a study of CV activity in premenopausal women will be utilized here to assess the relative impact of nonstationarity on spectral analysis of HRV (Study 1). Associations are calculated on HF power derived from both uncorrected and nonstationarity-adjusted ECG data.

Time Domain: Successive-Difference Filters

A group of time-domain techniques known as *successive-difference filters* act as statistical filters to remove slow trends from time-series data [7]. The remaining faster variability is often used to estimate HRV at the frequency of respiration and therefore cardiac vagal tone. There are several ways to quantify these measures, such as taking the absolute difference between successive HPs and averaging these absolute values over the time period (MSD) [12]. Alternatively, this measure can be calculated by squaring each successive HP difference, summing the squared differences, and taking the square root of the average of this sum (rMSSD) [1], [2]. Both MSD and rMSSD amplify rhythms due to faster sources, and as such,

should closely correspond with other HRV indices of cardiac vagal control that reflect the respiratory frequency [2]. In fact, this has typically been found to be the case, with correlations generally on the order of magnitude of 0.9 [13], [14]. Moreover, MSD has been shown to be stable across measurement occasions [14].

The characteristics of reliability and computational simplicity make successive difference filters an appealing surrogate for more mathematically complex and less accessible frequency-domain measures such as HF power [14]. Furthermore, they afford the opportunity to estimate cardiac vagal activity without respiratory measurement, as is required for assessment of RSA.

Nevertheless, MSD has been criticized in the HRV literature on various grounds. For example, it has been asserted that unless the data are sampled at equal intervals, MSD will not be comparable across individuals and conditions [7]. That is, when sampling is a function of beats, rather than seconds, the sampling rate will lack interindividual and intertask consistency, due to differences in HR. Resampling the data using equal intervals has been recommended to avoid this possible bias. MSD has also been criticized on more general grounds for its lack of precision and sophistication [3]. To scrutinize these criticisms, MSD is quantified in two different ways in Study 1: raw (uncorrected) and corrected for potential sampling biases. These measures are then compared to HF power derived from the same data. The sensitivity of MSD vis-à-vis RSA is then examined in terms of their respective abilities to detect differences in laboratory autonomic CV manipulations (Study 2).

Study 1: Method and Results

Seven minutes of resting HP data were collected from 21 healthy, premenopausal women during two laboratory sessions. On each occasion, subjects were seated in a comfortable lounge chair, and Ag-AgCl electrodes were attached to the chest in a modified ECG Lead II placement configuration. The ECG signal was amplified and filtered by a Colbourn Instruments bioamplifier Model #S75-11 (Allentown, Pennsylvania). Customized software and an R-wave detector produced a signal that was sampled at the rate of 100 Hz.

Estimates of HF spectral power (0.15-0.35 Hz) were derived from these data using the PSPAT program. These

values were calculated both with (STAT) and without (NONSTAT) the stationarity test; STAT refers to spectral estimates based only on stationary epochs, and NONSTAT refers to power values calculated on the entire data set. Pearson correlations were computed between these measures for both laboratory sessions to assess the relative impact of nonstationarity on HF spectral estimates of cardiac vagal control. One subject's data from session two was not used due to missing data.

MSD was calculated using the raw HP data; i.e., sampled by beats (nonequal intervals), and after transformation into a series sampled with equal 500 ms windows (MSDTRANS) [15]. Mean HP was also calculated. Correlations among all measures are displayed in Table 1.

Study 2: Method and Results

Six college-aged females participated in three experimental sessions spaced one week apart. Each session consisted of ten 3-min laboratory manipulations selected to induce a range of autonomic activity (see [16] for methodological details). Two measures of cardiac vagal control—RSA and MSSD—were obtained during each task, both derived from ambulatory monitoring of the ECG and impedance cardiogram (AMS, Vrije Universiteit, Amsterdam, the Netherlands). Impedance cardiography has been shown also to be an effective method to quantify respiratory parameters [17]. AMS software uses this technique (“impedance pneumography”) to derive RSA estimates.

Five tasks were chosen from this set on the basis of their ability to elicit distinct cardiac ANS responses [18], [19]. A strongly sympathetic task (squeezing a hand-grip dynamometer) was used as a contrast reference with three predominantly vagal manipulations (supine rest in reclining chair, seated rest, and seated paced breathing at eight breaths per minute, all with eyes closed) and a mixed sympathovagal condition (playing a video game with cash bonus incentive (sympathetic) while a chilled gel facemask was held on the forehead (vagal)). The purpose of using these criteria was to compare the relative abilities of MSD and RSA to distinguish conditions with contrasting cardiac ANS responses.

Measures were standardized within-subjects and averaged across tasks and sessions, as has been advocated for studies of dynamic functioning in individ-

uals over time and diverse situations [20], [21]. It was predicted that both RSA and MSD would distinguish the sympathetic and vagal tasks; i.e., both RSA and MSD would be less during hand grip than in supine rest, quiet sitting, or paced breathing. The latter three tasks were not predicted to differ from each other on RSA or MSD, and the combined sympathovagal task was predicted to fall in between the hand-grip task and the three vagal tasks on these measures.

One-tailed contrast analyses showed both MSD and RSA to be significantly less in hand grip than in supine rest (MSD: $t(5) = 5.61, p < 0.005$; RSA: $t(5) = 4.34, p < 0.01$), sitting rest (MSD: $t(5) = 4.15, p < 0.01$; RSA: $t(5) = 4.08, p < 0.01$), and paced breathing (MSD: $t(5) = 4.91, p < 0.005$; RSA: $t(5) = 3.64, p < 0.05$). RSA was significantly greater in the mixed sympathovagal video game task than in hand grip, ($t(5) = 2.17, p < 0.05$) but MSD did not differ in the two tasks ($t = 0.1, n.s.$). All condition means are displayed in Figure 1.

Across all tasks and sessions, the correlation between MSD and RSA was $r(28) = 0.75, p < 0.001$. If the mixed sympathovagal task is excluded (the one task in which MSD and RSA seemed to diverge, relative to the sympathetic hand-grip task), the correlation increases to $r(22) = 0.89, p < 0.001$.

Discussion

In Study 1, the relationship among the various measures was relatively unaffected by the stationarity of the time series or the alleged sampling bias of MSD. The average correlation between STAT and NONSTAT across the two sessions was 0.96, and the average correlation between MSD and MSDTRANS across the sessions was 0.98. Furthermore, although the average correlation of HF spectral power values (STAT and NONSTAT across both session) with MSD was somewhat higher when using transformed MSD ($r = 0.84$) rather than untransformed ($r = 0.75$) MSD values, this difference was small, amounting to 0.0081% improvement in variance explained. Thus, the issues of stationarity and equal sampling of MSD did not appear to substantially effect the relationships among these measures of cardiac vagal control.

In the second study, both MSSD and RSA were roughly equivalent in distinguishing a strongly sympathetic task from several tasks in which vagal activation

dominates. Only under conditions of mixed sympathetic-parasympathetic coactivation did MSD and RSA diverge. In particular, MSD seemed to be more sensitive to the sympathetic manipulation (playing the video game), showing a level similar to the sympathetic hand-grip task. In contrast, RSA appeared to be more responsive to the vagal component of the task (facial cooling), displaying a quantity comparable to those obtained in the "pure" vagal tasks. These differences reinforce the proposition that autonomic substrates should be considered when comparing the use of MSD as a vagal indicator vis-à-vis RSA or HF spectral power. Furthermore, instances when the measures diverge suggest that it may be a good strategy to employ both kinds of measures to achieve greater sensitivity.

If examined across both studies, the correlation between MSD and HF power (Study 1) or MSD and RSA (study 2) ranged from $r = 0.70$ (between stationary HF and uncorrected MSD in study 1) to $r = 0.89$ (in Study 2, when the mixed task was excluded). If one considers only unaltered data—i.e., NONSTAT and untransformed MSD in study 1, and all tasks included in study 2, the average correlation between MSD and HF or RSA is $r = 0.75$. This value is slightly lower than associations reported in some other studies, which tend to show a correlation on the order of magnitude of 0.9 [13], [14]. However, the present data are in line with other correlations reported on 24-h ambulatory monitoring of the ECG, which showed a range of correlations between MSD and HF of 0.39 and 0.95 (median = 0.89) [22]. The latter study showed that the magnitude of the correlation varied with time of day, perhaps as a function of circadian rhythms in autonomic activity. Our finding that the relationship between these measures varied under different autonomic conditions supports this notion. In general, these data suggest that one might consider factors such as time of day or the nature of the experimental manipulation when using MSD as a surrogate vagal indicator for HF, since this relationship may depend somewhat on the autonomic milieu in which cardiac activity is recorded. This recommendation is in accord with general guidelines that HRV measures of autonomic activity should be validated across a broad range of conditions [3].

An argument that should be considered concerns the interpretation of degrees of association among these

measures of HRV. It has been contended that "high correlations among various methods of measuring heart rate variability do not confirm that the measures behave the same..." ([7], p. 747). This assertion was supported by showing that RSA and HP standard deviation diverged as a function of stage of sleep, purportedly demonstrating that "high within-condition correlations do not provide information regarding the behavior of the derived variables" (p. 750). In contrast, the data in Study 2 showed high correlations between MSD and RSA not only within but also across conditions, and largely similar condition effects in these variables. The latter finding replicates previous research by the primary author in which MSD and HF power showed analogous directional effects in distinguishing among both tasks and subject groups that differed in degree of cardiac vagal control [23]-[26]. Of course, the standard deviation of HP does not discriminate among sources of variability and so should not necessarily be expected to behave like a component of that variability which reflects primarily vagal activity [7]. It may be that the combination of highly shared variance and similar behavior across conditions or subject groups collectively indicate that the two variables are tapping into similar underlying constructs or regulatory processes.

Various additional points should be considered when appraising the impact these quantification issues had upon the data in these studies. First, it is possible that the data in study 1 were in fact largely stationary, and thus stationary and nonstationary sets were very comparable. Indeed, the extremely high correlation between the two sets across both studies confirms this was so. Yet, this evidence strengthens the notion that stationarity was not a substantive concern in this sample (which of course was not selected with the criterion of stationarity in mind). Though it is difficult to verify how representative this sample is in terms of stationarity, the relatively trivial effect this issue had on spectral estimates of HF power is supported by other similar findings [8], [10].

Another possibility is that there was not a wide range of resting HRs among subjects in study 1, and so interindividual differences in sampling rates were small. However, the range of resting HPs was rather wide: at time 1 it was 684-1159 ms, and at time 2 it was 661-1170 ms. Hence,

restricted range of HP cannot explain the similarity between the time- and event-sampling results for MSD. It should be emphasized that empirical realities such as the level of nonstationarity in a data set, or differences in HR across subjects or conditions, should bear upon the applicability of correction procedures such as the removal of nonstationary data in spectral analysis or resampling HR data in calculating MSD. Such attempts may be unnecessary and at worst distort the veracity of the data. It should also be noted that, in regard to spectral analysis of HRV, the distinction between an event-based and a sampling rate (time)-based time series is essentially irrelevant; both methods produce similar results [27]. By analogy, it is possible the same applies to the use of successive-difference time-domain methods, and so MSD was relatively unchanged in Study 1 when it was recalculated as if sampled over an equal time interval series.

One potential limitation to Study 1 might be the relatively slow ECG sampling rate (100 Hz), which may not have been optimal for spectral analysis. However, recent data showed that correlations among measures of HF ECG spectral power were virtually unaffected by systematic sampling rate reductions from 1000 Hz to as slow as 20 Hz [28]. This finding applied particularly to resting ECG data, as were the data in Study 1. Additionally, the trend in [28] was toward a slight decrease in magnitude of the correlations as sampling rate decreased. Hence, if any bias might have been caused by the sampling rate in study 1, it would be toward underestimation of the reported correlations and would not affect the conclusions drawn from these data.

Collectively, the data from these studies suggest that certain HRV quantification issues that have been raised in a theoretical context may not be of major practical import when applied to real data sets. These results should not be construed as encouragement to disregard methodological rigor in the quantification and analysis of HRV. Rather, a number of more prudent directives can be inferred. First, the similarity among indices of association found in these studies, together with the comparable abilities of MSD and RSA in discriminating autonomic tasks revealed in study 2, argue against the discounting of past research on grounds of nonstationarity or MSD sampling bias. Rigid application of such criteria to prior

HRV studies can lead to the discrediting of valid findings, with consequent damage to the wealth of information and continuity provided by the extant HRV literature. Neither should reviewers dismiss significant findings purely on the basis of these issues. Rather, stationarity and the appropriateness of MSD use should be weighed among the many factors present in a study, since in fact these concerns may have had a trivial impact on the results. Reflexive invocation of the stationarity issue, or automatic outright rejection of MSD as a vagal indicator, can lead to a spuriously high "Type II error" rate for journals and so deprive the scientific community of important results.

Conclusions

Time- and frequency-domain analyses of HRV have provided researchers with important measures of cardiac vagal activity. Stationarity is of theoretical importance for such analyses in the frequency domain but may not be of practical significance in any particular data set. It has been argued that if a stationarity test is available, it should be used [29]. On the other hand, it is also possible that the RSA is quite robust to nonstationarity [10]; the spectral data from study 1 support that contention. Furthermore, procedures that correct for nonstationary data segments may compromise the representativeness of the data set. With regard to the time domain, MSD is advantageous in that it is conceptually and computationally simple, does not require respiration data, and under many conditions may be a reasonable alternative to HF spectral power or RSA. All told, the selection of cardiac vagal control indices and use of correction procedures should be based upon experimental situation and availability to the researcher, rather than orthodox adherence to idealized standards.

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Table 1. Correlations among cardiac measures in Study 1.

| Study 1 | | | | |
|--------------------------|-------|-------|----------|-------|
| | STAT | MSD | MSDTRANS | HP |
| NONSTAT | 0.99* | 0.71* | 0.81* | 0.32* |
| STAT | | 0.70* | 0.80* | 0.32 |
| MSD | | | 0.98* | 0.83* |
| MSDtrans | | | | 0.72* |
| * $p < 0.01$, $df = 19$ | | | | |
| Study 2 | | | | |
| NONSTAT | 0.93* | 0.79* | 0.89* | 0.28 |
| STAT | | 0.75* | 0.85* | 0.26 |
| MSD | | | 0.98* | 0.77* |
| MSDtrans | | | | 0.63* |
| * $p < 0.01$, $df = 18$ | | | | |

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1. RSA and MSD means by condition in Study 2.

CALL-OUTS

It is often contended that spectral estimates of RSA and cardiac vagal control may be biased

Certain HRV quantification issues that have been raised in a theoretical context may not be of major practical import when applied to real data sets

The selection of cardiac vagal control indices and use of correction procedures should be based upon experimental situation and availability to the researcher,