Short-term heart rate variability and cardiac norepinephrine spillover in patients with depression and panic disorder

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HEART RATE VARIABILITY (HRV) has been long recognized as a risk predictor of cardiac death after acute myocardial infarction (11) and has been increasingly analyzed in a wide range of research and clinical settings. However, the underlying physiological mechanisms underpinning HRV measures remain incompletely understood.

Elevated sympathetic cardiac activity is a potential cause of sudden cardiac death (8) and presumably the major contributor to arrhythmic events (12). Assessing sympathetic outflow to the heart noninvasively, using simple HRV indexes, is therefore of great interest. Besides vagally mediated high-frequency (HF) oscillations, which are related to respiration (respiratory sinus arrhythmia), a power spectrum analysis of HRV reveals distinct low-frequency (LF) oscillations that may or not be caused by sympathetic efferents (11). While the currently dominating view is that LF power is at least in part caused by the sympathetic cardiac tone, several recent studies, in which cardiac sympathetic outflow was directly assessed, demonstrated that the magnitude of those LF oscillations is a rather poor marker of sympathetic outflow (9, 13).

Since the original publication of the HRV Task Force standards in 1996 (11), a variety of new techniques has been proposed to quantify HRV, borrowing concepts from information theory and nonlinear systems theory such as entropy (19), scale invariance (16), and symbolic dynamics (28). Those novel indexes quantify the structure/patterns embedded in HRV independent of the average magnitude of variation. However, the physiological meaning of those measures is not well understood. Yet, there is clinical evidence that such techniques are superior in capturing HRV features predictive of sudden cardiac death (5, 6, 10, 20, 28) and therefore might carry information on excessive sympathetic outflow to the heart. The aim of this study was thus to investigate the possible link between HRV complexity and sympathetic outflow to the heart. We evaluated cardiac norepinephrine (NE) spillover (the gold standard of measuring cardiac sympathetic outflow) and HRV in healthy subjects and patients with a diagnosis of major depression or panic disorder, conditions known to be associated with an altered cardiac autonomic function (4). These patients encompass a wide range of sympathetic outflow levels, from normal to abnormal, while their cardiac substrate remains preserved.

METHODS

Study population. The study comprised 27 subjects: 8 healthy subjects, 12 patients with major depressive disorder, and 7 patients with panic disorder (for details see Table 1). The ECG records were drawn from a previously published study, in which QT variability and cardiac NE spillover were investigated (1). HRV complexity has never been described in that set of subjects. All patients were screened for inclusion using two diagnostic instruments: the Mini International Neuropsychiatric Interview (MINI) and the Composite International Diagnostic Interview (CIDI). The Hamilton Depression Rating Scale and Hamilton Anxiety Rating Scale (HamD and HamA, respectively), the Clinical Global Impressions scale (CGI), and the Beck Depression Inventory (BDI-1) were used. Patients who met all of the following criteria were eligible for entry: a HamD > 18, a BDI > 18, a positivity for major depressive disorder and panic disorder on MINI and CIDI, and an assessment as having a significant major depression or panic disorder as the primary illness on an interview by a psychiatrist. The exclusion criteria included the coexistence of any of the following: heart disease, diabetes, medicated hypertension, alcohol/drug abuse, infectious diseases, comorbid psychiatric disorders, eating

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Table 1. Group characteristics of the healthy subjects, patients with MDD, and patients with PD

<table>
<thead>
<tr>
<th></th>
<th>Healthy</th>
<th>MDD</th>
<th>PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>8</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>Sex, m/f</td>
<td>6/2</td>
<td>4/8</td>
<td>2/5</td>
</tr>
<tr>
<td>Age, yr</td>
<td>37±4</td>
<td>48±13</td>
<td>42±13</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24±4</td>
<td>27±4</td>
<td>25±3</td>
</tr>
<tr>
<td>Anxiety trait</td>
<td>—</td>
<td>51±13</td>
<td>39±18</td>
</tr>
<tr>
<td>Anxiety state</td>
<td>—</td>
<td>59±12</td>
<td>44±12</td>
</tr>
<tr>
<td>HamD</td>
<td>—</td>
<td>26±4</td>
<td>—</td>
</tr>
<tr>
<td>BDI</td>
<td>—</td>
<td>26±6</td>
<td>—</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, number of patients. MDD, major depressive disorder; PD, panic disorder; HamD, Hamilton Depression Scale; BDI, Beck Depression Inventory.

Table 2. HRV and cardiac NE spillover in normal subjects, patients with MDD, and patients with PD

<table>
<thead>
<tr>
<th></th>
<th>Healthy</th>
<th>MDD</th>
<th>PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac NE spillover, ng/min</td>
<td>11.9</td>
<td>8.1–15.0</td>
<td>23.5</td>
</tr>
<tr>
<td>SDNN, ms</td>
<td>948</td>
<td>851–1,002</td>
<td>898</td>
</tr>
<tr>
<td>RMSSD, ms</td>
<td>58</td>
<td>50–71</td>
<td>49</td>
</tr>
<tr>
<td>VLF, ms²</td>
<td>1,234</td>
<td>731–2,198</td>
<td>1,551</td>
</tr>
<tr>
<td>LF, ms²</td>
<td>1,062</td>
<td>537–2,240</td>
<td>707</td>
</tr>
<tr>
<td>HF, ms²</td>
<td>581</td>
<td>490–1,275</td>
<td>293</td>
</tr>
<tr>
<td>LF/HF</td>
<td>1.60</td>
<td>1.14–2.13</td>
<td>2.22</td>
</tr>
<tr>
<td>LF/nu</td>
<td>0.62</td>
<td>0.53–0.68</td>
<td>0.69</td>
</tr>
<tr>
<td>α₁</td>
<td>1.17</td>
<td>1.05–1.37</td>
<td>1.10</td>
</tr>
<tr>
<td>α₂, 0%</td>
<td>34</td>
<td>29–35</td>
<td>29</td>
</tr>
<tr>
<td>1V, 0%</td>
<td>47</td>
<td>45–49</td>
<td>46</td>
</tr>
<tr>
<td>2UV, 0%</td>
<td>7</td>
<td>6–9</td>
<td>10</td>
</tr>
<tr>
<td>SampEn</td>
<td>1.77</td>
<td>1.71–1.90</td>
<td>1.92</td>
</tr>
</tbody>
</table>

Values are medians and interquartil ranges. HRV, heart rate variability; NE, norepinephrine; meanNN, mean normal-to-normal R-R interval; SDNN, SD of R-R intervals; RMSSD, root mean square of beat-to-beat differences in the R-R interval; LF/nu, low-frequency (LF) power normalized to the sum of LF and high-frequency (HF) power; VLF, very LF; α₁, short-term fractal scaling exponent; 0V, words with no variations; 1V, words with 1 variation; 2LV, words with 2 like variations; 2UV, words with 2 unlike variations; SampEn, sample entropy; nu, normalized units.
Entropy analysis. In HRV analysis, entropy measures are used to quantify the complexity and regularity of heart rate fluctuations. A sample entropy is a refined version of the traditionally used approximate entropy measure (19) and quantifies the irregularity and unpredictability of a time series. It represents the conditional probability that two sequences of $m$-consecutive R-R intervals, which are similar to each other (within given tolerance $r$), will remain similar when the consecutive R-R interval is included (25). According to previous studies, we have chosen $m = 2$ and $r = 0.15 \times SD$ of the R-R time series.

Statistics. To compare cardiac NE spillover levels and HRV between controls and patients with major depressive disorder and patients with panic disorder, respectively, we computed nonparametric statistics including group medians, interquartile ranges, and Kruskal-Wallis tests. To investigate the relationship between cardiac NE spillover and HRV, we computed nonparametric Spearman correlation coefficients based on the complete data set.

RESULTS

The healthy subjects had median cardiac NE spillover levels of 11.9 ng/min (interquartile range, 8.1–15.0 ng/min). The patient cohort demonstrated two distinct patterns of cardiac NE spillover, as previously reported (1). Eleven patients (7 with panic disorder) had normal cardiac NE spillover values ≤ 10 ng/min, whereas eight patients had markedly elevated values (>20 ng/min).

Data for all HRV measures are presented in Table 2. There were no significant group differences in cardiac NE spillover and HRV measures between healthy subjects and patients with major depression and patients with panic disorder (Figs. 1 and 2). Thus all three groups were pooled for the correlation analysis.

None of the standard HRV Task Force measures was significantly correlated with cardiac NE spillover (Fig. 1, and Table 3).
However, there was a negative and a positive correlation between symbolic dynamics-based HRV metrics and cardiac NE spillover and 2LV (\(r = -0.37\), and \(P = 0.05\)) and 0V (\(r = 0.34\), and \(P = 0.06\)), respectively (Fig. 2, and Table 3).

**DISCUSSION**

The major finding of our study is that some novel measures of short-term heart rate complexity moderately correlate with cardiac sympathetic outflow assessed directly by cardiac NE spillover. We did not find, however, any significant association between cardiac NE spillover and standard HRV indexes. This result provides direct evidence that sympathetic neural outflow to the heart is a determinant of HRV, although its impact appears to be rather low.

While the sympathetic innervation of the heart has been implicated as a contributor to HRV in many studies, to date there has been a paucity of direct comparisons between cardiac sympathetic activity and HRV. Specifically, many previous studies attempted to prove the sympathetic nature of LF oscillations indirectly, by either pharmacological sympathetic blockade, administration of exogenous catecholamines, or autonomic reflex tests (11). On one hand, HRV spectral indexes are often confounded by the nonstationary nature of heart rate that undergoes slow noncyclical or abrupt changes (e.g., tachycardia associated with locomotion in animals or with alerting thoughts in humans are rarely controlled for), which might blur the association between LF oscillations and sympathetic activity. On the other hand, in several recent studies where cardiac sympathetic activity was assessed directly, by either recording cardiac nerves activity (7) or by sampling NE spillover from the myocardium (9), no association between these parameters and the LF power of the HRV was observed, similar to our findings. It may be that the LF component of HRV is related to baroreflex-related cardiac sympathetic activity; for these instances, however, relatively large (and cyclical) falls in arterial pressure are a prerequisite, since at physiological conditions, the cardiac baroreflex is predominantly mediated by the vagus nerve (13).

![Fig. 2](http://ajpheart.physiology.org/)  
**Fig. 2.** HRV complexity measures versus cardiac NE in healthy subjects (○) and patients with major depression (□) and panic disorder (△). A: short-term fractal scaling exponent (\(\alpha_1\)). B: symbolic dynamics: percentage of words with no variations (0V). C: symbolic dynamics: percentage of words with one variation (1V). D: sample entropy (SampEn). E: symbolic dynamics: percentage of word with 2 likewise variations (2LV). F: symbolic dynamics: percentage of words with 2 unlike variations (2UV).
Our study is the first to investigate the relationship between cardiac NE spillover and the complexity measures of HRV. Previous studies were limited to pharmacological autonomic activation/blockade and/or cardiovascular reflex response tests. In the discussion that follows, we briefly describe previously reported findings for each complexity index and compare them with our current observations.

The short-term scaling exponent $\alpha_1$, perhaps the most popular and best investigated metric among the novel HRV complexity indexes, was found to be reduced after physical or pharmacological interventions [e.g., facial cold water immersion (26) or NE infusion (27)], suggesting a direct relationship between sympathetic outflow to the heart and $\alpha_1$. Since we did not observe a correlation between cardiac NE spillover and $\alpha_1$, we presume that vagal coactivation, which has been reported to occur in both above-mentioned studies, might be largely responsible for the change in $\alpha_1$.

Regarding HRV complexity measures based on symbolic dynamics, sympathetic activation and vagal withdrawal (induced pharmacologically or by autonomic reflex tests) increased the percentage of low-variability word types (0V) and, at the same time, reduced the percentage of high-variability word types (2LV) (5, 21). Mechanistically, this could be explained by the fact that fast fluctuations in the R-R interval are predominantly mediated by vagal efferents, so that vagal withdrawal by itself may lead to these reciprocal changes. Our current findings, however, suggest that sympathetic activation (NE release from cardiac neural endings) may play the leading role in such changes, as we observed a direct relationship between NE spillover and the amount of 0V word types and an inverse relationship between NE spillover and the amount of 2LV words. Whether sympathetic cardiac activity was the sole cause for alterations in these two symbolic dynamics indexes or vagal withdrawal contributed to these changes cannot be concluded from our results. The sensitivity of symbolic dynamics indexes to sympathetic cardiac activation could be explained by the fact that those nonlinear metrics reflect HRV features fundamentally different from those reflected by linear indexes. The categorization of R-R intervals into six different bins (symbols) allows to assess structure/patterns in R-R interval changes independent of the overall variability. Time and frequency domain indexes, on the other hand, all assess the magnitude of HRV. Porta et al. (21) showed that a symbolic analysis of HRV is superior to conventional spectral indexes in quantifying changes in cardiac autonomic modulation induced by graded head-up tilt, possibly due to its sensitivity to sympathetically mediated heart rate fluctuations.

Regarding the sample entropy of HRV, graded tilt testing, i.e., sympathetic activation paralleled by vagal withdrawal, has been shown to decrease entropy values with increasing tilt angles (22). In full agreement with a previous study on renal sympathetic nerve activity in sheep (2), we did not observe a correlation between cardiac NE spillover and the sample entropy of heart rate, suggesting that sample entropy is not reflective of sympathetic neural outflow to the heart.

The lack of strong correlations between cardiac NE spillover and HRV, independent of the metric used for its quantification, is not surprising when taking into consideration that HRV exclusively reflects neural influences on the pacemaker region of the heart and that existing data (14) indicate that pacemaker area and ventricular myocardium are controlled independently. When one takes further into account that the amount of NE released in the pacemaker area must be much smaller than the total release from the heart, it has to be seriously doubted that HRV can be used to derive indexes of sympathetic activation of the myocardium, as it is still commonly done. The predictive value of HRV for cardiac risk stratification might therefore lie predominantly in its ability to quantify vagal outflow to the sinus node (15). The lack of consistent sympathetic influence on HRV might also explain its limited clinical value for sudden cardiac death prediction.

**Clinical implications.** HRV as a sole marker does not provide a useful indication of cardiac sympathetic activity, most likely because it is based on measuring consequences of autonomic influences exclusively at the cardiac pacemaker area. Presumably, future ECG-based attempts of cardiac risk stratification should focus on developing metrics quantifying ventricular indexes [such as microvolt T-wave alternans (24) or T-wave loop morphology (18)] that could be more closely associated with the amount of NE release in the heart from sympathetic terminals. Disappointingly, the QT variability, an index developed to assess ventricular repolarization lability, did not show a significant correlation with cardiac NE spillover either (3).

**Limitations.** Our analyses were conducted on rather short data of 300 R-R intervals or 5-min recordings for frequency domain analysis, respectively. But all HRV methods applied in our study have been specifically designed to provide meaningful results on such short data segments. Because of the invasive nature of our study, the number of participating subjects was relatively small. To overcome this potential limitation, we applied nonparametric statistics.

**Conclusion.** Only HRV complexity measures based on symbolic dynamics are (moderately) correlated with sympathetic outflow to the heart. Therefore, the predictive value of most HRV measures for sudden cardiac death may predominantly result from their capacity to capture vagally mediated heart rate modulations (15).

**GRANTS**

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