SHORT-TERM HEART RATE VARIABILITY AND CARDIAC
NOREPINEPHRINE SPILLOVER IN PATIENTS WITH DEPRESSION AND
PANIC DISORDER

Running title: Baumert M et al. Heart rate variability and cardiac norepinephrine

1Mathias Baumert, 2Gavin W Lambert, 2Tye Dawood, 2Elisabeth A Lambert,
2Murray D Esler, 2Mariee McGrane, 2David Barton and Prashanthan Sanders1,
3Eugene Nalivaiko

1Cardiovascular Research Centre, School of Medicine, University of Adelaide, Adelaide, Australia; 2Human Neurotransmitters Laboratory, Baker Heart Research Institute, Melbourne, Australia; and 3School of Biological Sciences, University of Newcastle, Newcastle Australia

Address for correspondence:
Dr Mathias Baumert
School of Medicine
University of Adelaide
Adelaide, AUSTRALIA
Tel: +61 8 8303 4115
Fax: +61 8 8303 4360
Email: mathias.baumert@adelaide.edu.au
Changes in measures of heart rate variability have been associated with an increased risk for sudden cardiac death. The mechanisms underlying this association are not known. The objective of this study was to assess the relationship between the amount of norepinephrine released from the cardiac sympathetic terminals and short-term heart rate variability.

The study comprised 8 healthy subjects, 12 patients with major depression and 7 patients with panic disorder. Cardiac norepinephrine (NE) spillover was determined using direct coronary sinus blood sampling coupled with NE isotope dilution methodology. Short-term heart rate variability was quantified using detrended fluctuation analysis, symbolic dynamics, sample entropy and standard time and frequency domain measures.

Neither HRV nor cardiac NE spillover was significantly different between the analysed groups. None of the standard HRV metrics was significantly correlated with cardiac NE spillover, but there was a moderate correlation between two complexity measures of HRV (symbolic dynamics) and cardiac NE spillover (2LV: $\rho = -0.37 \ p = 0.05$; 0V: $\rho = 0.34 \ p = 0.06$).

In conclusion, there is no correlation between standard HRV measures and cardiac NE spillover in humans. Short-term complexity of heart rate is only moderately affected by sympathetic neural outflow. 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.

Key words: Heart rate variability; complexity; sympathetic; norepinephrine; spillover.
INTRODUCTION

Heart rate variability (HRV) has been long recognized as a risk predictor of cardiac death after acute myocardial infarction (11) and has been increasingly analysed 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 non-invasively, using simple HRV indices, is therefore of great interest. Besides vagally mediated high frequency (HF) oscillations, which are related to respiration (respiratory sinus arrhythmia), power spectrum analysis of HRV reveals distinct low frequency (LF) oscillations that may or may 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 indices 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 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 Tab. 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 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: Ham D > 18; BDI > 18; positivity for MDD/PD on MINI and CIDI; and assessment as having a significant major depression or panic disorder as the primary illness on interview by a psychiatrist. Exclusion criteria included coexistence of any of the following: heart disease, diabetes, medicated hypertension, alcohol/drug abuse, infectious diseases, co-morbid psychotic disorders, eating disorders, mental
retardation, high suicide risk, personality disorders and epilepsy. All subjects provided written informed consent to the study protocol that was approved by the institutional Clinical Research and Ethics Committee.

Study Protocol

All investigations were performed in a catheter laboratory with subjects in the supine position. Studies were conducted in the morning, and caffeinated beverages and tobacco smoking were prohibited for 12 h prior to the study. The patients rested for at least for 20 minutes before lead III of the surface ECG was recorded for 5 minutes at a sampling rate of 1 kHz, using PowerLab(R) and the Chart(R) software (ADI Instruments, Australia). Immediately after terminating the ECG recording, cardiac NE spillover was measured. This procedure has been previously described in detail (1). Briefly, a coronary sinus angiographic catheter (Cordis Europa, Roden, Netherlands) was introduced via the antecubital venous sheath and placed under fluoroscopic control in the coronary sinus (CS) for blood sampling. CS blood flow was estimated from the double product (systolic blood pressure x heart rate), using the relation: CS blood flow = 18 + (0.02 x double product) as described previously (2). During the catheter study participants received a tracer infusion of ³H-labeled NE for the measurement of NE kinetics by isotope dilution.

Standard heart rate variability analysis

Standard HRV measures in the time domain were computed based on 300 R-R intervals according to task force standards:

- meanNN—the mean normal-to-normal R-R interval, in ms;
• SDNN–the standard deviation of normal-to-normal R-R interval, reflecting the overall heart rate variability, in ms;
• RMSSD–root-mean-square of the beat-to-beat differences, in ms; reflecting the average magnitude of heart rate changes between consecutive beats (a marker of vagal heart rate modulation).

For HRV analysis in the frequency domain, 5-minute long R-R time series were interpolated at 250 ms to obtain equidistant values. Subsequently, Fast Fourier transform was applied. We quantified power in the 3 frequency bands that have been associated with different physiological rhythms.

• VLF–very low frequency power in the range of 0.003-0.04 Hz, in ms²; the origin of these oscillations is largely unknown but has been associated with thermoregulation and vagal modulations.
• LF–low frequency power in the range of 0.04-0.15 Hz, in ms²; mainly reflecting a ~10 s rhythm associated with Mayer waves in blood pressure and is influenced by both vagal and sympathetic tone.
• HF–high frequency power in the range 0.15-0.4 Hz, in ms²; mainly reflecting the magnitude of respiratory sinus arrhythmia; vagally mediated.
• LF/HF–ratio of low-to-high frequency power. A unitless measure often and perhaps erroneously referred to as a marker of ‘sympatho-vagal’ balance.
• LFn–low frequency power divided by the sum of high and low frequency power. This normalized measure is also used as a marker of ‘sympatho-vagal’ balance.

Detrended fluctuation analysis
Detrended fluctuation analysis (DFA) enables the detection of long-range correlations in non-stationary data (17). The DFA algorithm involves the break-down of the R-R time series into windows of length \( n \). Subsequently, for each of those windows the linear trend is removed and root-mean-square of the residual computed as a function of \( n \) (17). R-R interval time series usually comprise two distinct areas of scale invariance, covering short-term fluctuations (\( \alpha_1 \): computed for \( n = 4–11 \) R-R intervals) and long-term fluctuations (\( \alpha_2 \): computed for \( n = 12–64 \) R-R intervals). We limited our analysis to \( \alpha_1 \) as it has shown to be of most predictive value and furthermore can be reliably estimated from short recordings.

**Symbolic dynamics analysis**

According to the symbolic dynamics approach described by Porta et al. (23), the series of R-R intervals was transformed into an alphabet of 6 symbols (0, 1, 2, 3, 4, 5) that covers 6 equally spaced bins ranging from the minimum to maximum R-R interval. The sequence of symbols is subdivided into patterns of three consecutive symbols and grouped into 4 pattern families, according to the number and types of variations from one symbol to the next. These pattern families are: 1) patterns with no variation (0V, all three symbols are equal); 2) patterns with one variation (1V, two consecutive symbols are equal and the remaining one is different); 3) patterns with two like variations (2LV, the three symbols form an ascending or descending ramp), 4) patterns with two unlike variations (2UV, the three symbols form a peak or a valley).

**Entropy analysis**
In HRV analysis, entropy measures are used to quantify the complexity/regularity of heart rate fluctuations. Sample entropy (SampEn) 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 a 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 $r = 0.15 \times$ standard deviation of the R-R time series.

**Statistics**

To compare cardiac NE spillover levels and HRV between controls (CON), patients with major depressive disorder (MDD) and patients with panic disorder (PD), respectively, we computed non-parametric statistics including group medians, interquartile ranges and Kruskal-Wallis-tests. To investigate the relationship between cardiac NE spillover and HRV we computed non-parametric Spearman correlation coefficients based on the complete dataset.

**RESULTS**

The healthy subjects had median cardiac NE spillover levels of 11.9 ng/min (interquartil 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 $\leq$ 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, patients with major depression and patients with panic disorder (Figures 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 (Figure 1, Table 3). However, there was a negative and a positive correlation between symbolic dynamics based HRV metrics and cardiac NE spillover and 2LV ($\rho = -0.37 \ p = 0.05$) and 0V ($\rho = 0.34 \ p = 0.06$), respectively (Figure 2, 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 indices. 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 indices are often confounded by the non-stationary nature of heart rate that undergoes slow non-cyclical 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 NA 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, as at physiological conditions, the cardiac baroreflex is predominantly mediated by the vagus nerve (13).

Our study is the first to investigate the relationship between cardiac NE spillover and 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 indices—was found to be reduced after physical or pharmacological interventions (eg. 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 co-activation, which has been reported to occur in the 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 relation between NE spillover
and the amount of 0V word types and an inverse relation between NE spillover and
the amount of 2LV words. Whether sympathetic cardiac activity was the sole cause
for alterations in these two symbolic dynamics indices or vagal withdrawal
contributed to these changes cannot be concluded from our results. The sensitivity of
symbolic dynamics indices to sympathetic cardiac activation could be explained by
the fact that those non-linear metrics reflect HRV features fundamentally different
from those reflected by linear indices. The categorization of R-R intervals into 6
different bins (symbols) allows assessing structure/patterns in RR interval changes
independent of the overall variability. Time and frequency domain indices, on the
other hand, all assess the magnitude of HRV. Porta et al. (21) showed that symbolic
analysis of HRV is superior to conventional spectral indices 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 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 existing data (14) indicates that pacemaker area and ventricular myocardium are controlled independently. Taking 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 indices (such as microvolt T-wave alternans (24) or T-wave loop morphology (18)) that could be more closely associated with the amount of NA 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 minute 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. Due to the invasive nature of our study the number of participating subjects was relatively small. To overcome this potential limitation we applied non-parametric 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).

Acknowledgement of Support:

This project was supported by a NHMRC project grant. MB is a holder of an early career fellowship from the Health faculty of the University of Adelaide; GWL, MDE, and EAL are supported by NHMRC fellowships, TD is supported by a NHF Australia postgraduate fellowship. PS is supported by the NHF of Australia. EN is a holder of the NHF fellowship (#CR06A2710).
Tables

Table 1: Group characteristics of the healthy subjects, patients with major depressive disorder and patients with panic disorder investigated.

<table>
<thead>
<tr>
<th></th>
<th>Healthy (n=8)</th>
<th>MDD (n = 12)</th>
<th>PD (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>6m, 2f</td>
<td>4m, 8f</td>
<td>2m, 5f</td>
</tr>
<tr>
<td>age, yrs</td>
<td>37 ± 14</td>
<td>48 ± 13</td>
<td>43 ± 13</td>
</tr>
<tr>
<td>BMI, kg*m⁻²</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>

Data are mean ± standard deviation. HamD – Hamilton depression score; BDI – Beck depression inventory, PD – panic disorder; MDD – major depressive disorder.
Table 2: Heart rate variability measures and cardiac norepinephrine spillover in normal subjects, patients with major depressive disorder and patients with panic disorder.

<table>
<thead>
<tr>
<th></th>
<th>healthy</th>
<th>MDD</th>
<th>PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>cardiac NE [ng/min]</td>
<td>11.9 8.1-15.0</td>
<td>7.6-31.0</td>
<td>7.9 5.8-10.4</td>
</tr>
<tr>
<td>meanNN [ms]</td>
<td>948 851-1002</td>
<td>898 864-1001</td>
<td>891 877-1028</td>
</tr>
<tr>
<td>sdNN [ms]</td>
<td>58 50-71</td>
<td>49 36-64</td>
<td>50 47-71</td>
</tr>
<tr>
<td>Rmssd [ms]</td>
<td>33 26-48</td>
<td>26 19-41</td>
<td>32 26-57</td>
</tr>
<tr>
<td>VLF [ms²]</td>
<td>1234 731-2198</td>
<td>1551 634-2406</td>
<td>1031 720-2157</td>
</tr>
<tr>
<td>LF [ms²]</td>
<td>1062 537-2240</td>
<td>707 515-2045</td>
<td>1230 793-2792</td>
</tr>
<tr>
<td>HF [ms²]</td>
<td>581 490-1275</td>
<td>293 149-926</td>
<td>832 275-1679</td>
</tr>
<tr>
<td>LF/HF [nu]</td>
<td>1.60 1.14-2.13</td>
<td>2.22 1.65-3.22</td>
<td>1.99 1.53-3.43</td>
</tr>
<tr>
<td>LFn [nu]</td>
<td>0.62 0.53-0.68</td>
<td>0.69 0.62-0.75</td>
<td>0.67 0.60-0.77</td>
</tr>
<tr>
<td>α₁ [nu]</td>
<td>1.17 1.05-1.37</td>
<td>1.10 0.85-1.28</td>
<td>0.92 0.89-0.99</td>
</tr>
<tr>
<td>0V [%]</td>
<td>34 29-35</td>
<td>29 20-42</td>
<td>25 20-37</td>
</tr>
<tr>
<td>1V [%]</td>
<td>47 45-49</td>
<td>46 42-51</td>
<td>47 42-50</td>
</tr>
<tr>
<td>2LV [%]</td>
<td>7 6-9</td>
<td>10 6-12</td>
<td>12 8-14</td>
</tr>
<tr>
<td>2ULV [%]</td>
<td>11 10-14</td>
<td>12 9-15</td>
<td>11 8-14</td>
</tr>
<tr>
<td>SampEn [nu]</td>
<td>1.77 1.71-1.90</td>
<td>1.92 1.66-2.02</td>
<td>1.90 1.71-2.00</td>
</tr>
</tbody>
</table>

Data are medians, interquartil ranges.

Table 3: Correlation analysis between cardiac norepinephrine spillover and heart rate variability (HRV) measures.

<table>
<thead>
<tr>
<th>HRV measure</th>
<th>ρ</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>meanNN [ms]</td>
<td>-0.06</td>
<td>0.77</td>
</tr>
<tr>
<td>sdNN [ms]</td>
<td>-0.26</td>
<td>0.19</td>
</tr>
<tr>
<td>rmssd [ms]</td>
<td>-0.30</td>
<td>0.13</td>
</tr>
<tr>
<td>VLF [ms²]</td>
<td>-0.14</td>
<td>0.47</td>
</tr>
<tr>
<td>LF [ms²]</td>
<td>-0.34</td>
<td>0.09</td>
</tr>
<tr>
<td>HF [ms²]</td>
<td>-0.32</td>
<td>0.10</td>
</tr>
<tr>
<td>LF/HF [nu]</td>
<td>0.23</td>
<td>0.26</td>
</tr>
<tr>
<td>LFn [nu]</td>
<td>0.24</td>
<td>0.24</td>
</tr>
<tr>
<td>α₁ [nu]</td>
<td>0.23</td>
<td>0.26</td>
</tr>
<tr>
<td>0V [%]</td>
<td>0.37</td>
<td><strong>0.06</strong></td>
</tr>
<tr>
<td>1V [%]</td>
<td>-0.25</td>
<td>0.21</td>
</tr>
<tr>
<td>2LV [%]</td>
<td>-0.38</td>
<td><strong>0.05</strong></td>
</tr>
<tr>
<td>2ULV [%]</td>
<td>-0.15</td>
<td>0.45</td>
</tr>
<tr>
<td>SampEn [nu]</td>
<td>-0.24</td>
<td>0.24</td>
</tr>
</tbody>
</table>

ρ - Spearman correlation coefficient, p – significance.
Figures

Figure 1: Time and frequency domain HRV measures versus cardiac norepinephrine in healthy subjects (squares) patients with major depression (triangles) and panic disorder (circles). A: mean R-R interval, B: standard deviation of R-R intervals, C: root-mean-square of beat-to-beat differences in the R-R interval, D: low frequency power normalized to the sum of low and high frequency power, E: very low frequency power F: low frequency power, G: high frequency power, H: low-to-high frequency power ratio.

Figure 2: HRV complexity measures versus cardiac norepinephrine in healthy subjects (squares) patients with major depression (triangles) and panic disorder (circles). A: short-term fractal scaling exponent; B: Sample entropy; C: Symbolic dynamics: Percentage of words with no variations; D: Symbolic dynamics: percentage of word with 2 likewise variations; E: Symbolic dynamics: percentage of words with one variation F: Symbolic dynamics: percentage of words with two opposing variations.
References


