Beyond Traditional HRV: Frequency-Band Analysis of Entropy and Tone Under Pharmacological Intervention

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Abstract

Traditional time and frequency-based heart rate variability (HRV) analysis assumes linearity and may fail to capture the complex, nonlinear dynamics and adaptive capacity of cardiac control. Tone and entropy are nonlinear measures of HRV and may offer a more comprehensive assessment of the autonomic nervous system (ANS) activity. However, current methods are predominantly time-based. Here, we propose a novel frequency-based method to estimate tone and entropy within the same frequency bands commonly used in HRV analysis which are the very low frequency (VLF), LF, and high frequency (HF).

Electrocardiogram (ECG) data were collected from 6 healthy male participants (31 ± 4) years old during rest and following intravenous administration of propranolol ($0.2\ mg/kg$) and atropine ($0.04\ mg/kg$). Tone and entropy were computed using both the time-based conventional method and the proposed frequency-based approach for comparison.

Frequency-based entropy significantly decreased after atropine across all frequency bands ($p < 0.05$), with no significant changes under propranolol. Tone differed significantly between control and propranolol in LF and VLF bands ($p < 0.05$), but not after atropine. The effect of propranolol on tone was not apparent in the conventional tone assessment, suggesting that our method may provide a complementary evaluation of entropy and tone.

1. Introduction

Heart rate variability (HRV) is a non-invasive index for the autonomic nervous system (ANS) regulation of cardiac function. HRV analysis can be done by deriving time-based metrics [1, 2], frequency-based metrics [1, 2] and symbolic-based metrics [3–5]. Traditional frequency-domain HRV analysis involves decomposing the RR interval (RRI) signal into three frequency bands: very low frequency (VLF): 0.0033 - 0.04 Hz, LF: 0.04 - 0.15 Hz, and high frequency HF: 0.15 - 0.4 Hz. Each band is believed to reflect specific activity of the ANS, for example, the HF is believed to be linked to the parasympathetic activity, the LF band is believed to reflect both the parasympathetic and sympathetic activities, the VLF, though less investigated, is believed to be related to thermoregulation and hormonal factors [1, 2].

While frequency-based HRV metrics have potential clinical values [2, 6–9], they are limited by the underlying assumptions of stationarity and linearity [10]. These limitations can limit the detection of nonlinear changes in autonomic modulation of cardiac function, especially under dynamic or pharmacologically altered conditions. Hence, there is a need for more adaptive and nonlinear measures to characterize the complexity and dynamical structure of HR fluctuations [10]. Entropy-based metrics offer a promising alternative by quantifying the unpredictability or irregularity of time series data, which reflects system adaptability and complexity. Various entropy measures, such as sample entropy and multiscale entropy, have been employed to analyse the nonlinear behaviour of HRV signals [12, 13]. However, these approaches are often not frequency-resolved and may miss frequency-specific autonomic signatures.

To address these limitations, we propose a frequency-based entropy and tone analysis approach that retains frequency band resolution while capturing both the dynamical complexity (entropy) and directional bias (tone) of RRI. Frequency-based entropy and tone were calculated by resampling RRI across time windows matching the HF, LF, and VLF bands.

2. Methods

2.1. Participant Recruitment and Data Collection

The dataset used in this study is discussed in detail in the previous literature [14]. The experiment was approved by the institutional review process in the graduate school, Kyoto University, Japan, and written informed consent was

collected from the participants. Eight healthy male participants (31 $\pm$ 4) years old were asked to seat in a recumbent position in a comfortable chair for 40 minutes in the morning and resting ECG (sampling frequency: 1000 Hz), was collected throughout the 40 minutes. After 10 minutes, Propranolol (0.2 mg/kg) was intravenously injected to the subjects. After another 15 minutes, atropine (0.04 mg/kg) was injected.

2.2. Entropy-Tone Analysis

Of the eight originally collected ECG recordings, only six were used due to missing data. Tone and Entropy were calculated by using Eq.1 and Eq.2, respectively [14]. Tone measures acceleration and deceleration periods and is calculated for RRI(i) $\neq 0$. When the number of accelerations is higher than the number of decelerations, the tone is positive and vice versa. Entropy was calculated by using the Shannon formula [15].

$$Tone = \frac{1}{N-1} \sum_{i=1}^{N-1} \left(\frac{RRI(i) - RRI(i+1)}{RRI(i)} \times 100 \right) \tag{1}$$

N is the serial number of the RR interval (RRI)

$$Entropy = -\sum_{i=1}^{n} p(i) \log_2 p(i) \tag{2}$$

$p(i)$ represents the probability that the RRI falls within the interval $i <$ RRI $< i+1$, where i is an integer and n is the number of bins for which $p(i) \neq 0$. The corresponding entropy quantifies the overall acceleration–inhibition activity, or the total variation in heart period, and is expressed in bits.

To calculate the tone and entropy power spectrum, RRI signals were resampled by calculating average RRIs over specific time windows corresponding to each band: HF (3 – 6 seconds), LF (7 – 25 seconds), and VLF (26 – 100 seconds). For each resampled signal, tone and entropy were calculated by using Eq.1 and Eq.2, respectively [14]. After that, the mean values of entropy and tone were calculated per band. Figures 1 show examples of tone (Figure 1A) and entropy (Figure 1B).

2.3. Statistical Analysis

Comparison of the means was conducted using Mann-Whitney U test (Wilcoxon rank sum test) in MATLAB.

3. Results

The results of the conventional entropy and tone analysis across control, propranolol, and atropine conditions are summarized in Table 1. Entropy values showed a marked decrease following atropine administration (1.60 $\pm$ 0.53) bits compared to the control condition (3.7 $\pm$ 0.41) bits, with the difference reaching statistical significance (p = 0.0022). No significant difference in entropy was observed between the propranolol (3.9 $\pm$ 0.54) bits and control conditions (p = 0.48). Tone values were significantly different between the control and atropine conditions (- 0.058 $\pm$ 0.04 vs. - 0.01 $\pm$ 0.01, p = 0.004), indicating reduced directional fluctuations after atropine injection. A larger negative tone value was also observed under propranolol (- 0.13 $\pm$ 0.10), although the difference from control did not reach statistical significance (p = 0.18).

The results of the frequency-based tone and entropy analysis across control, propranolol, and atropine conditions are summarized in Table 2.

Frequency-based Entropy showed significant reductions after atropine across all three frequency bands. HF entropy dropped from (3.8 $\pm$ 0.55) bits to (1.2 $\pm$ 0.19) bits (p = 0.0022), LF entropy from (2.9 $\pm$ 0.24) bits to (1.1 $\pm$ 0.20) bits (P = 0.0022), VLF entropy from (1.8 $\pm$ 0.15) bits to (0.93 $\pm$ 0.22) bits (p = 0.0022). No significant differences were observed between propranolol and control in any frequency band (P $>$ 0.05), although there was a trend toward higher entropy under propranolol in LF and VLF bands.

Frequency-based tone analysis revealed that the LF band tone was significantly more negative with propranolol (- 0.65 $\pm$ 0.64) than control (- 0.03 $\pm$ 0.45, p = 0.026), reflecting increased decelerations. A significant difference was also observed in the VLF band (p = 0.041), where tone became more negative under propranolol (- 2.2 $\pm$ 2.2) compared to control (0.13 $\pm$ 0.94). No significant tone differences were found between control and atropine in any frequency band (all $p > 0.05$), nor in the HF tone between control and propranolol.

4. Discussion

In this study, we proposed a frequency-based approach to calculate entropy and tone from RRI dynamics, allowing for a more dynamic detailed evaluation of autonomic modulation across the HF, LF, and VLF bands opposed. The bands were identified based on the conventional frequency-based HRV bands. The proposed method offers a novel way to quantify entropy and tone based on frequency rather than time, as is conventionally done [16, 17].

To assess the diagnostic potential of our metrics, we compared frequency-based entropy and tone calculated across control, propranolol, and atropine conditions. Entropy, which quantifies the unpredictability of RRI was significantly reduced following atropine administration across all frequency bands (Table 2). This finding is consistent with the expected reduction in ANS complexity,

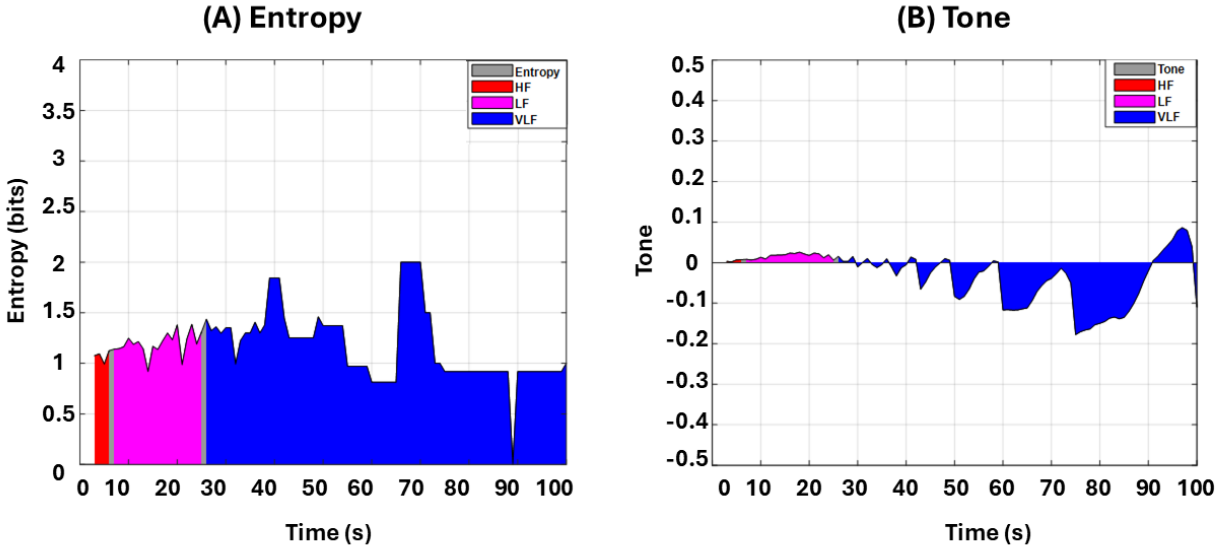

Figure 1: Frequency-based entropy and tone analysis. (A) Entropy and (B) tone were estimated by resampling RR interval (RRI) over three frequency bands, very low frequency (VLF) (blue), LF (magenta), and high frequency (HF) (red). A. Entropy quantifies the unpredictability of RRI fluctuations, while tone (B) reflects the directional bias of RRI changes. The transition across frequency bands corresponds to increasing time windows used in the resampling process (e.g., HF: 3–6 s, LF: 7–25 s, VLF: 26–100 s).

Table 1: Conventional Tone and Entropy Analysis

Feature	**Control**	**Propranolol**	**Atropine**	***p*-value (Control - Propranolol)**	***p*-value (Control - Atropine)**
Entropy (bits)	3.7 ± 0.41	3.9 ± 0.54	1.60 ± 0.53	0.48	0.0022
Tone	-0.058 ± 0.04	-0.13 ± 0.10	-0.01 ± 0.01	0.18	0.004

Table 2: Frequency-Based Tone and Entropy Analysis

Feature	**Control**	**Propranolol**	**Atropine**	***p*-value (Control-Propranolol)**	***p*-value (Control-Atropine)**
Mean RRI (ms)	913 ± 125	996 ± 97	677 ± 90	0.31	0.0087
Entropy					
HF (Ln)	3.8 ± 0.55	3.8 ± 0.56	1.2 ± 0.19	0.94	0.0022
LF (Ln)	2.9 ± 0.24	3.1 ± 0.16	1.1 ± 0.20	0.064	0.0022
VLF (Ln)	1.8 ± 0.15	1.9 ± 0.79	0.93 ± 0.22	0.0931	0.0022
Tone					
HF (Ln)	-0.10 ± 0.15	-0.28 ± 0.29	-0.01 ± 0.04	0.24	0.18
LF (Ln)	-0.03 ± 0.45	-0.65 ± 0.64	-0.01 ± 0.12	0.026	0.39
VLF (Ln)	0.13 ± 0.94	-2.2 ± 2.2	-0.02 ± 0.39	0.041	0.70

RI: RR interval, VLF: very low-frequency power, LF: low-frequency power, HF: high-frequency power.

manifested as a suppression of parasympathetic input to the heart [14]. This change reflects a shift toward more deterministic and less adaptive cardiac control in the absence of vagal modulation. Propranolol, on the other hand, did not significantly alter entropy in any band. This implies that entropy is more sensitive to parasympathetic rather than sympathetic withdrawal, mostly under resting conditions.

While atropine did not significantly alter tone values in any frequency band (Table 2), propranolol produced significantly more negative tone values in the LF and VLF bands. This suggests an increased decelerations in HRs in the absence of sympathetic drive.

Comparison between the conventional (time-based) tone

and entropy analysis and our frequency-based approach reveals that each method offers different measurements of ANS activity, particularly in tone values. The difference between the control and atropine conditions was significant in Table 1 but not in Table 2. In contrast, tone was not significantly different between the control and propranolol conditions in Table 1, but it was significantly different in the LF band in Table 2. These findings suggest that the two methods provide complementary insights into ANS effects on cardiac activity.

5. Limitation

The small sample size ($n = 6$), constitutes a major limitation of this study. In addition, the study involved healthy male subjects and this may not reflect the broader population or other physiological states (e.g., disease). Future studies should apply this method in larger and more diverse cohorts, and investigate its clinical relevance.

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