Modified Sequence Method to Assess Baroreflex Sensitivity in Rats

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Abstract— **Baroreceptors respond to fluctuations in blood pressure (BP) by modifying physiology in order to maintain a homeostatic set point. Baroreflex sensitivity (BRS) is used to quantify baroreceptor function and is a useful metric for tracking cardiovascular disease state and treatment effects. Pathological conditions such as hypertension (HTN) alter baroreflex function and reduce BRS. Traditionally, the sequence method is used to measure BRS, in which the linear slope of concomitant changes in BP and RR intervals are assessed. However, in rats, a high respiratory rate reduces the reliability of the sequence method. Here, we present a modified sequence method that captures BRS at lower frequencies and decreases the variability of the BRS estimate. This method was demonstrated using ECG and BP data from two groups of HTN rats: Sham rats and rats treated with vagus nerve stimulation. The modified sequence method resulted in lower BRS estimates than the traditional sequence technique when applied to the same data sets. Additionally, the modified sequence method resulted in lower BRS estimate variability.**

I. Introduction

The baroreflex is one of the key mechanisms that regulates blood pressure (BP). Arterial baroreceptors located in the aortic and carotid sinuses increase their firing rate in response to stretching of vessel walls and relay information to the medullary brainstem [1]. Changes in afferent firing mediate a reflex response that acts to buffer the original change in BP by altering physiology such as heart rate (HR), contractility, and level of vasoconstriction. The magnitude of the acute HR change that occurs in response to a given change in BP can be measured as baroreflex sensitivity (BRS).

BRS has proven to be a valuable clinical index used to evaluate the autonomic nervous system and holds significant prognostic value in numerous pathologies. In the case of hypertension (HTN), the baroreflex resets to a higher BP operating point and has reduced sensitivity [1, 2]. The inappropriate increase in sympathetic nervous system activity present in HTN plays a role in chronically altering the baroreflex through maladaptive changes in cardiovascular physiology, such as fibrosis and hypertrophy [3, 4]. In addition, the impairment in baroreflex function has been associated with the severity of HTN, with the BRS index significantly decreasing with worsening condition. Restoration of BRS values can be indicative of efficacy of therapeutic interventions [1, 5, 6].

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A. Measuring BRS

Traditional methods of BRS measurement have been used since the 1960s, and include applying an external stimulus, such as a vasoactive drug, to the patient to artificially raise or lower BP [7]. Due to the invasiveness of these techniques, and the fact that the introduction of external stimuli can confound BRS measurements by artificially moving BP outside its normal operating range, several techniques have been developed to measure the "spontaneous baroreflex," with the assumption that BP and HR fluctuations involving the baroreflex occur throughout the course of daily life.

In the time domain, spontaneous BRS is measured by the "sequence method," which consists of the identification of sequences of a minimum number of consecutive beats characterized by either a progressive rise in systolic BP (SBP) and lengthening of RR interval (RRI), i.e. an *increasing sequence*; or a progressive decrease in SBP and shortening of RRI, i.e. a *decreasing sequence* [8]. An analysis that can separate increasing and decreasing sequences is useful because an increasing sequence may involve a different autonomic response than a decreasing sequence. When the sequence technique is applied to physiological data collected in rats, the high respiratory rate of rats restricts the use of the sequence method to measure BRS [9]. Furthermore, not all of the fluctuations in BP and RR interval at the respiratory rate are baroreflex-mediated, and capturing these non-baroreflex oscillations may lead to an overestimation and increased variability in the sequence domain BRS estimate [10].

The goal of this paper is to modify the traditional BRS sequence method to provide an alternative BRS estimate that is able to capture lower frequency oscillations. In addition, this paper will use this technique to investigate physiological changes in the baroreflex in HTN rats treated with novel device-based therapy, vagus nerve stimulation (VNS).

II. Methods

A. HTN Rat Data Description

All experiments conform to the Guidelines for the Care and Use of Laboratory Animals (NIH publication No. 85-23, revised 1996) and the University of Minnesota guidelines for the care and use of animals. HTN was induced in Dahl salt-sensitive male rats (29-35 days old) using a high salt diet (S10001, Research Diets, Inc., New Brunswick, NJ, USA). At Week 6 rats were divided into VNS (n = 6, functional VNS stimulator), and Sham (n = 6, nonfunctional VNS stimulator)) groups and implanted with telemetry systems (HD-S11, Data Sciences, Inc. (DSI), Minneapolis, MN, USA) as described in [11], which allows for simultaneous continuous recording of BP and ECG at 500Hz. Low-level, intermittent VNS therapy was provided for four weeks.

Data analysis was performed on 4-hour windows of ECG and BP data during day (10AM to 2PM) and night (10PM to 2 AM) intervals at Week 6 (baseline, n=6 for both VNS and Sham) and at Week 10 (n=4 for both VNS and Sham). The results from day and night data were averaged together since negligible differences were found between them.

B. Traditional Sequence Method

The traditional sequence method was implemented as described in Henze et al. [9]. Briefly, sequences of 3 or more consecutive beats with either progressively decreasing or increasing changes in both SBP and RRI were identified. No minimum changes in SBP or PI were required. Each identified sequence was then fit with a regression line, and the slopes of the regression lines with an R^2 >0.85 were averaged together to represent the BRS. The coefficient of variance for each data segment was calculated as the ratio of standard deviation to mean of the detected slopes. An example of sequences identified using the traditional sequence method are shown in Figure 1A, with the linear regressions (PI vs SBP) of increasing (blue) and decreasing (red) sequences shown in Figure 1B.

C. Modified Sequence Method

After signal pre-processing (see section III A), the SBP and RRI signals were resampled at 10 Hz, using spline interpolation. Next, the signals were detrended, and low pass filtered using "designfilt" function in MATLAB with a cutoff frequency of 0.25 Hz in order to remove high frequency oscillations including the respiratory signal. The filtered signal was then resampled at the non-uniform sampling rate of the original RRI data to preserve the original interval times. Baroreflex-mediated sequences were identified in the same manner used for the traditional sequence technique, with the following modified thresholds: minimum sequence length of 16 consecutive beats, minimum change in BP of 5 mmHg, and a R^2 > 0.85. These threshold criteria were increased to ensure the robustness of the modified sequence method, so that small, alterations in BP or PI, such as those due to noise or respiration, did not affect the performance of the algorithm. No minimum change in RRI was required; since RRI is the "output" to the baroreflex, stipulating a minimum value here would bias BRS to higher values. The final BRS estimate and the coefficient of variation were calculated as described previously. These modified thresholds enabled consistent detection of the larger, low-frequency oscillations which is demonstrated in Figure 1C-D. Figure 1C shows an example of both increasing (blue) and decreasing (red) low frequency sequences identified using the modified thresholds that were not previously identified using the traditional sequence method.

D. Statistical Analysis

Data are presented as mean ± standard error. One-way ANOVA was performed to compare BRS measures and the coefficient of variance between Sham and VNS rats between methods. Paired t-tests were performed to compare measures at different time points (Week 6 and Week 10). P<0.05 was considered statistically significant.

III. Results

A. Data Pre-Processing

The data sets were pre-processed using custom written MATLAB code. Noise and arrhythmias were removed according to the following protocol.

1) Remove Saturation and Splice Data

Large sections of saturated values and non-physiological noise were first identified in the ECG and BP data. These segments were removed and the data on either side of the segments were then spliced together.

2) Detect SBP and Pulse Interval (PI)

Since ECG signals typically contained more noise than BP signals, PI was used as a surrogate for RRI. To validate the use of PI in this analysis, PI was compared to RRI in a clean data set. PI was defined in two ways: as the time interval between two consecutive SBP peaks or the time interval between two consecutive maximums of the derivative (dP/dt_{max}) of the BP traces. Figure 2 shows the correlation of RRI and PI from a sample set of data. Note that the PIs estimation from dP/dt_{max} resulted in a much higher correlation to RRI (R^2 = 0.97) compared to SBP peaks method (R^2 = 0.25), and therefore the former was used for subsequent analyses.

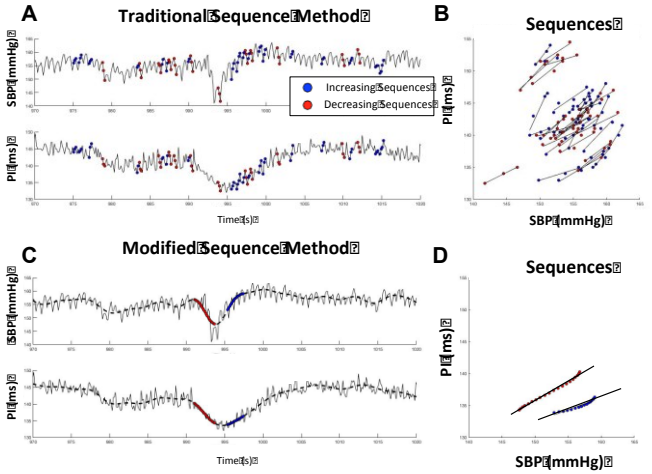

Figure 1. (A) Traditional sequence method captures high frequency oscillations both in SBP and PI. (B) Decreasing (red) and Increasing (blue) sequences from Panel A plotted as PI vs SBP, along with regression lines. (C) Modified sequence method captures low frequency oscillations in the same data set as Panel A. (D) Decreasing (red) and Increasing (blue) sequences from Panel C plotted as PI vs SBP, along with regression lines.

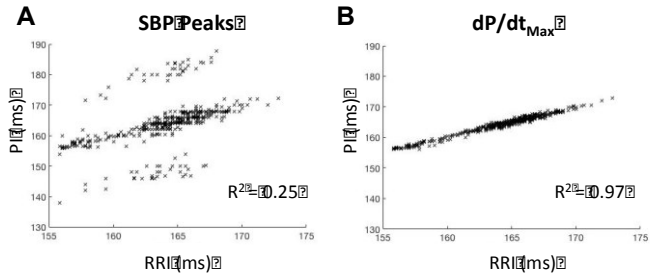

Figure 2. PI as a function of RRI. PI was calculated as the time interval either (A) between SBP peaks, or (B) between dP/dt_{max} .

3) Removal of Arrhythmic Events

The SBP and PI signals were then filtered to exclude arrhythmic episodes such as pre-ventricular contractions and any remaining noise, by removing and replacing values that deflected by more than a specified threshold (15 ms for PI and 20 mmHg for SBP) from the previous value. These spurious values were replaced using spline interpolation as opposed to deletion [12, 13].

B. The effect of VNS on BRS in HTN Rats

Figure 3 displays the BRS calculated using the modified and traditional sequence methods for Sham and VNS rats at baseline (Week 6) and after 4 weeks of VNS therapy (Week 10). BRS values calculated using the modified sequence method are lower than those from the traditional method. This decrease is significant at Week 6 both for the Sham and VNS rats (compared separately, $P < 0.05$ for both). In addition, BRS calculated using the modified sequence method, decreased from Week 6 to Week 10 in both groups, with the decrease only significant in Sham ($P<0.05$), but not VNS ($P=0.18$) rats. Traditional sequence method also resulted in a decrease for BRS between Weeks 6 and 10, with no statistical significance either in Sham ($P = 0.85$) or VNS ($P =0.16$) rats.

Further analysis was performed to compare BRS calculated from increasing vs. decreasing sequences using the two methods. Our results (Figure 4) indicate no significant differences in BRS between increasing and decreasing sequences, although for the traditional sequence method there was a trend toward higher BRS detected with increasing sequences. Figure 5 shows the coefficient of variance for the modified and traditional sequence method. Variability of the sequence method slopes detected were significantly smaller for the modified sequence method. This result is consistent for both Sham and VNS rats, at both Week 6 and Week 10 ($P < 0.05$). The traditional sequence method detected on average 538.2±309.89 sequences per hour, where as the modified sequence method detected 110.03±28.11 sequences per hour, ($p<0.05$), indicating that the modified sequence method is more selective than the traditional method, and that these two approaches evaluate different physiological responses.

IV. DISCUSSION

In this study, a modified sequence method is presented that can be used to isolate low frequency oscillations in rats by removing high frequency respiratory oscillations which confound traditional BRS measures. The major findings are (1) the traditional technique consistently records higher BRS than the modified technique, suggesting a large influence of respiration on the traditional BRS estimate, and (2) the modified technique has decreased variability of the BRS estimates.

A. Difference between Modified and Traditional Sequence Methods

There is a physiological difference between the types of sequences captured by the traditional and modified sequence methods, and therefore, the modified technique presented here should be viewed as complementary, rather than a replacement method. However, there are certain disadvantages to the traditional sequence method applied in rats that reduce its reliability. High frequency HR and BP oscillations are generally attributed to the act of respiration, and likely occur through multiple mechanisms, both baroreflex-mediated and non-baroreflex-mediated. Therefore, BRS estimates that capture sequences at the respiratory rate may overestimate BRS, and this claim is supported by data presented here, since BRS estimates using the traditional sequence technique were significantly higher.

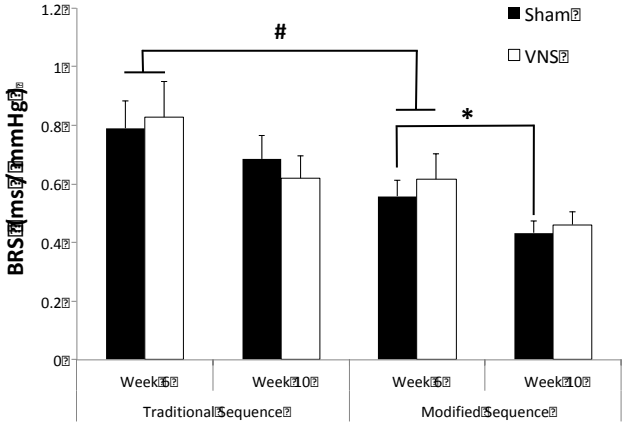

Figure 3. Comparison of BRS calculated using the traditional and modified sequence methods in Sham and VNS-treated hypertensive rats at Week 6 (baseline) and at Week 10 (after 4 weeks of therapy). # indicates statistical significance between traditional and modified sequence methods . * indicates statistical significance between Week 6 and Week 10.

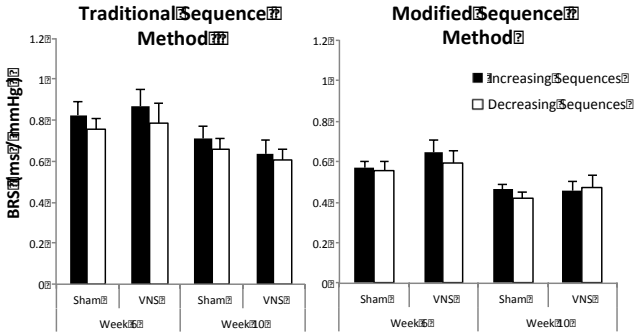

Figure 4. Comparison of BRS calculated from either increasing or decreasing sequences using the traditional and modified sequence methods in Sham and VNS-treated hypertensive rats at Week 6 (baseline) and at Week 10 (after 4 weeks of therapy).

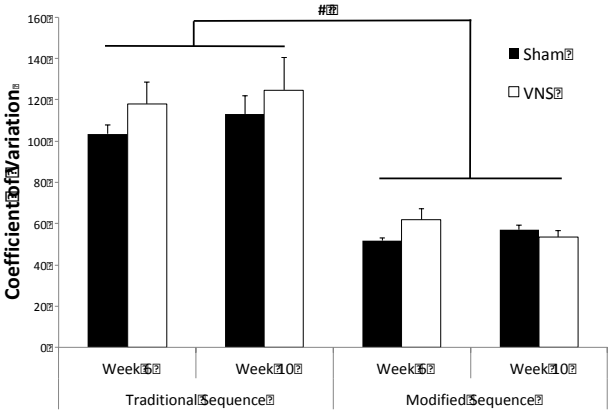

Figure 5. Coefficient of variation for BRS calculated using the traditional and modified sequence methods. # indicates statistical significance between traditional and modified sequence methods.

An advantage of the modified sequence method is the lower variability than the traditional method. The higher spreads seen with the traditional method likely have two causes in the data presented here. First, the traditional method may be more prone to capture random noise, as a result of the lower threshold requirements (minimum sequence count = 3). Second, variation in depth and rate of respiration over time may account for higher variability of the traditional sequence method. By eliminating high frequency oscillations, the BRS estimates of the modified method are less variable.

B. Assessing Therapeutic Effects in HTN Rats

BRS is a valuable clinical prognostic tool that allows for the evaluation of the autonomic nervous system. In the case of HTN, it is known that there is an autonomic imbalance, and VNS therapy aims to rebalance this system through direct stimulation of the parasympathetic nervous system. The HTN rats exhibited decreased BRS as high salt diet was maintained and BP continued to rise. When the BRS slope becomes flatter, as is often the case in pathological conditions such as HTN, this may be a result of abnormal vagal function or the inability to counteract the overactive sympathetic nervous system [14]. In HTN, chronic structural changes in the heart and vasculature alter the function of the arterial baroreceptors decreasing their sensitivity to fluctuations in BP [4].

Initially both Sham and VNS groups had higher BRS values (as calculated with the modified method): around 0.6 ms/mmHg, which dropped to approximately 0.45 ms/mmHg. However, through paired analysis, only the Sham rats had a significant decrease in BRS rats at Week 10 when compared to the baseline (Week 6). The VNS rats had a decrease in BRS, however this decrease was non-significant, which could be evident of the VNS therapy attenuating the alteration of baroreceptor function due to HTN. Longer durations of therapy and higher stimulation amplitudes may lead to a stronger effect of VNS therapy on BRS.

C. Limitations

There are several limitations of the current analysis. The main limitation of this study is that there was no gold-standard used in comparisons. In a subsequent study, this method may be compared to an “invasive” measure of BRS (such as those measures that require injection of a vasoactive substance to artificially raise or lower BP). Secondly, the sample sizes for this study were small with only 6 rats in each group at Week 6 and 4 rats per group at Week 10. Thirdly, the parameters chosen for the modified BRS technique, including filtering and sequence identification, were developed for this data set acquired from HTN rats, and may change based on the species and condition of the subjects. In addition, the data analyzed was only a subset of the data acquired. Finally, spontaneous BRS quantifies only baroreceptor function at the center of the curve with small deviations in BP. Evaluating only these small changes provides some information regarding baroreceptor function and how it is altered in a disease state, but it does not give a full picture of the sigmoidal function of the baroreceptors that can be investigated using classical, invasive methods which induce changes in BP to evaluate the baroreflex at both high and low BP levels.

V. Conclusion

Quantifying BRS provides a tool to indirectly measure autonomic tone and provides prognostic value in assessing disease progression and therapeutic efficacy. Modifying the traditional sequence method allows for high frequency oscillations, including respiration, to be removed from the BRS estimate and isolates low frequency baroreflex-mediated oscillations in rat data. By removing the respiration-driven oscillations, the quantification of BRS avoids overestimation and increased variability as respiration frequency can vary over time. Isolating the low frequency oscillations in the time domain can provide additional physiological information, and assess both increasing and decreasing sequences, to better understand baroreceptor function in health and diseased states.

VI. References

[1] H. Mussalo et al., "Baroreflex sensitivity in essential and secondary hypertension," Clinical Autonomic Research, vol. 12, no. 6, pp. 465-471, 2002.

[2] O. Ormezzano, J.-L. Cracowski, J.-L. Quesada, H. Pierre, J.-M. Mallion, and J.-P. Baguet, "EVAluation of the prognostic value of BARoreflex sensitivity in hypertensive patients: the EVABAR study," Journal of hypertension, vol. 26, no. 7, pp. 1373-1378, 2008.

[3] J. L. Izzo and A. A. Taylor, "The sympathetic nervous system and baroreflexes in hypertension and hypotension," Current hypertension reports, vol. 1, no. 3, pp. 254-263, 1999.

[4] M. W. Chapleau, "Baroreceptor Reflexes-Chapter 33," 2012.

[5] T. Kishi, Y. Hirooka, S. Konno, and K. Sunagawa, "Cilnidipine inhibits the sympathetic nerve activity and improves baroreflex sensitivity in patients with hypertension," Clinical and experimental hypertension, vol. 31, no. 3, pp. 241-249, 2009.

[6] M. S. Botelho-Ono, H. V. Pina, K. H. Sousa, F. C. Nunes, I. A. Medeiros, and V. A. Braga, "Acute superoxide scavenging restores depressed baroreflex sensitivity in renovascular hypertensive rats," Autonomic Neuroscience, vol. 159, no. 1, pp. 38-44, 2011.

[7] G. Parati, M. Di Rienzo, and G. Mancia, "How to measure baroreflex sensitivity: from the cardiovascular laboratory to daily life," Journal of hypertension, vol. 18, no. 1, pp. 7-19, 2000.

[8] G. Bertinieri, M. Di Rienzo, A. Cavallazzi, A. Ferrari, A. Pedotti, and G. Mancia, "A new approach to analysis of the arterial baroreflex," Journal of hypertension. Supplement: official journal of the International Society of Hypertension, vol. 3, no. 3, pp. S79-81, 1985.

[9] M. Henze, D. Hart, A. Samarel, J. Barakat, L. Eckert, and K. Scrogin, "Persistent alterations in heart rate variability, baroreflex sensitivity, and anxiety-like behaviors during development of heart failure in the rat," American Journal of Physiology-Heart and Circulatory Physiology, vol. 295, no. 1, pp. H29-H38, 2008.

[10] J. Frederiks et al., "The importance of high- frequency paced breathing in spectral baroreflex sensitivity assessment," Journal of hypertension, vol. 18, no. 11, pp. 1635-1644, 2000.

[11] E. M. Annoni et al., "Intermittent electrical stimulation of the right cervical vagus nerve in salt- sensitive hypertensive rats: effects on blood pressure, arrhythmias, and ventricular electrophysiology," Physiological reports, vol. 3, no. 8, p. e12476, 2015.

[12] G. G. Berntson et al., "Heart rate variability: origins, methods, and interpretive caveats," Psychophysiology, vol. 34, no. 6, pp. 623-648, 1997.

[13] M. A. Peltola, "Role of editing of R–R intervals in the analysis of heart rate variability," Frontiers in physiology, vol. 3, 2012.

[14] M. T. La Rovere, G. D. Pinna, and G. Raczak, "Baroreflex sensitivity: measurement and clinical implications," Annals of Noninvasive Electrocardiology, vol. 13, no. 2, pp. 191-207, 2008.