Spectral Analysis of Multiunit Action Potential Trains of Muscle Sympathetic Nerve Activity in Humans

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Abstract

The application of conventional signal processing methods used to obtain an integrated signal from muscle sympathetic nerve activity (MSNA) reduces the amount of information and may confound the spectral characteristics. We present a novel alternative method of processing the raw MSNA signal using a wavelet transform denoising technique that enables detection of individual action potentials and facilitates spectral analysis. A spike density function (SDF) is generated from the denoised signal by replacing the detected action potentials with delta functions and convolving with a 3 Hz Gaussian filter. This method was validated using data from a sinusoidal neck suction (NS) experiment in humans. The results of the analysis indicate that the oscillations of sympathetic nerve firings closely followed the NS frequency. In conclusion, the SDF representation allows for a novel and insightful analysis of spectral components of action potential trains in raw MSNA.

1. Introduction

For years scientists have scrutinized various cardiovascular signals with hopes of unearthing the etiologies underlying common pathological states. Muscle sympathetic nerve activity (MSNA) measured from multiple sympathetic axons innervating vessels in the muscle represents one such signal. The oscillations found in the sympathetic outflow have been shown to incorporate patterns similar to those found in cardiovascular signals [1-3] and may help to further define various pathologies, such as hypertension [1,4]. However, because of the low signal amplitude and the monopolar recording arrangement used during MSNA measurement, a number of environmental and bioelectric noise sources contaminate the raw neurogram. The conventional MSNA noise reduction and quantification strategy involves analog band-pass filtering (from 700 to 2,000 Hz), rectification, and integration (0.1 second time constant) of the raw signal to create the integrated or mean voltage neurogram [5,6]. Unfortunately, this procedure integrates band-limited noise and sympathetic action potentials indiscriminately. Furthermore, because the raw signal is composed of input from multiple sympathetic neurons, activity from individual axons cannot be identified and the amplitudes of the integrated bursts are highly sensitive to electrode position relative to groups of axons [7]. Therefore, spectral estimates of MSNA are computationally challenging, limited to multiunit activity, and consist of arbitrary or normalized units. Most investigators model the integrated neurogram using auto-regressive (AR) techniques. The power spectral density (PSD) of the neurogram is then approximated using the AR model coefficients [1-3]. The statistical uncertainty of both the AR model order and coefficients, however, have been called into question because of their sensitivity to additive noise [8,9]. Additionally, the spectra produced by current AR methods are normally band-limited to one-half the inverse of the mean RR-interval, eliminating potentially valuable information at higher frequencies. Although several fast Fourier Transform (fft) based algorithms have been employed to estimate integrated neurogram spectral density, these methods have been shown to produce inconsistent spectra [1].

Recently, a novel alternative to the integrated method of raw MSNA signal processing has been introduced [10]. This new algorithm incorporates digital band-pass filtering (from 700 to 2,000 Hz), wavelet denoising, and action potential peak detection. The denoising step is advantageous for PSD analysis and action potential classification in that it significantly reduces the pass-band noise while preserving the structure of the sympathetic action potentials [10]. This processing approach has been

applied here to analyze the spectral density of the MSNA response to sinusoidal neck suction stimulation of the baroreceptors in healthy subjects.

2. Methods

2.1. Data acquisition

Sympathetic outflow was measured from 7 healthy subjects (4 female, 32±3 years) in an upright-seated position. The MSNA signal was recorded using a tapered insulated tungsten electrode inserted percutaneously into the sympathetic bundle of the peroneal nerve at the fibular head. The electrode had a 200 μm diameter tip with an un-insulated portion approximately 1-2 μm in length (Fredrick Haer & Co., Bowdolnham, ME). A stainless steel reference electrode was placed subcutaneously about 2 cm from the recording site. Satisfactory placement of the electrode for good recordings of MSNA was insured by adhering to generally accepted criteria described by Sundlof and Wallin [11]. The neurograms were measured at 10kHz with a nerve traffic analysis system (662C-3 Bioengineering, University of Iowa, Iowa) and digitally recorded (14-bit, 10kHz) using the Windaq data acquisition software package (Dataq Instruments Inc., Akron, OH).

Subjects were allowed to stabilize over a 10 minute period following insertion of the electrode. Two padded, defornable neck suction cuffs joined by an elastic band were affixed to either side of the neck directly over the region of the carotid barorecptors after a 3-minute period of baseline MSNA measurement. The pressure within the cuff was created by a vacuum pump controlled by 12-bit digital-to-analog board which was passed through a phase-control power unit. This system allowed for sinusoidal intra-cuff presure modulation between atmospheric pressure and –60 mmHg. Sympathetic activity was measured during separate 3-minute intervals of NS modulation at frequencies of 0.1 and 0.2 Hz.

2.2. Data analysis

The raw MSNA signal was processed using Matlab analysis environment (The MathWorks Inc., Natick, MA) according to the wavelet denoising procedure described in detail by Diedrich, et al [10]. Briefly, the raw signal was band-pass filtered from 700 to 2,000 Hz using a digital filter, the variance of the noise (σ) was estimated from the slope of the linear region of the Quantile-Quantile plot, and a hard threshold, T, was established using:

$$T = 0.8\sigma\sqrt{2\ln(N)} \qquad (1)$$

where N is the number of data points in the signal. The signal was decomposed into low frequency approximation coefficients and five levels of high frequency detail coefficients using the wavelet Symmlet 7 in the Mallat algorithm for discrete wavelet transformation. The detail coefficients smaller than T were eliminated and the signal with reduced noise level was reconstructed. A peak detection scheme was then employed to locate the positions of individual action potentials. Each action potential was then replaced with a delta function while all other signal values were set to zero to construct a spike train of the original signal. The spike train was then convolved with a Gaussian kernel of unity area and 3 Hz cutoff in the frequency domain. This step creates a spike density function (SDF), a common estimator of spike rate [12-14]. The cutoff frequency of Gaussian kernel was chosen to match an estimated maximum heart rate of 180 beats per minute. The PSD of the SDF was estimated using the Welch Periodogram with 90 sec segments, 50% overlap, and the Hanning window [15]. Power within specific bands was then calculated using the trapezoidal rule for integration.

Statistical differences were measured using a paired Student's t-test with an alpha level of 0.5.

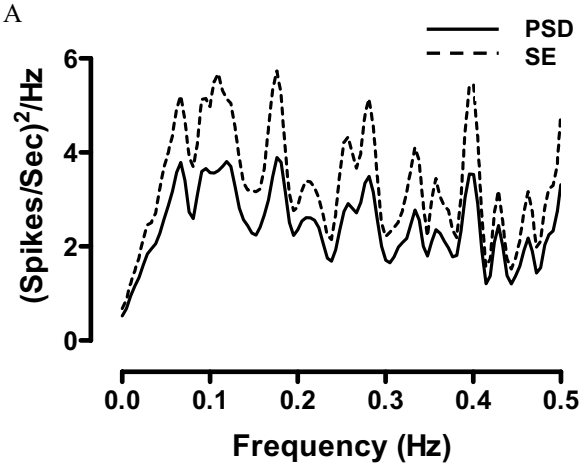
3. Results

Nerve activity was successfully recorded from all subjects during baseline and 0.2 Hz neck suction (NS) states, however, technical complication prevented accurate recording at 0.1 Hz NS frequency in one subject. Figure 1 displays the average PSD across subjects from 0.0-0.5 Hz for baseline (a), 0.1 (b) and 0.2 Hz (c) NS states. Since the PSD estimates were based on spike rate rather than integrated burst amplitudes, absolute, tangible units of spectral density ((spikes/sec)^2/Hz) could be used instead of normalized arbitrary units. Most subjects exhibited a noticeable increase in power in the 0.0-0.5 Hz range in response to both NS conditions. The prominent spectral peaks found at the respective NS frequencies in Fig. 1 (b) and (c) were apparent in a majority of subjects. However, common baseline spectral components, such as the 0.1 Hz peak thought to be associated with blood pressure Mayer waves, were not distinguishable under resting conditions. These peaks are usually noted in studies employing AR methods of spectral estimation [1-3].

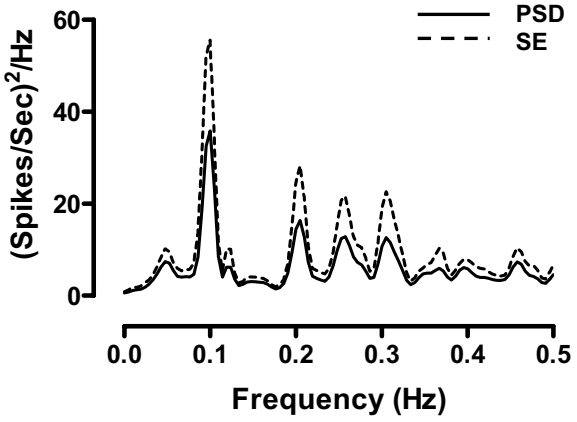
Because the wavelet based algorithm was not limited to sampling the MSNA once per cardiac cycle, the frequency information garnered by this approach extended well beyond the typical range of half inverse of the mean RR-interval (approximately 0.5 Hz). Table 1 presents the power (area under the PSD) within defined frequency ranges. Here, we define low frequency (LF), high frequency (HF), and cardiogenic frequency (CF) as 0.04-0.15 Hz, 0.15-0.4 Hz, and 0.4-3.0 Hz, respectively.

While most subjects exhibited increased power within these ranges during both NS conditions, the increases were not found to be statistically significant.

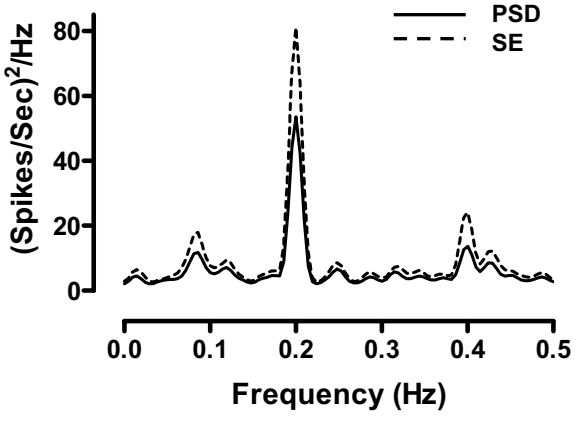
A



(a)



(b)



(c)

Figure 1. Average PSDs across all subjects during baseline (a), 0.1 Hz (b), and 0.2 Hz (c) NS frequencies.

Table 1. Power ((spike/sec)^2) in defined frequency ranges.

	LF	HF	CF
Baseline	0.35 ± 0.11	0.63 ± 0.18	4.94 ± 1.49
0.1 Hz	0.99 ± 0.45	1.56 ± 0.77	9.46 ± 4.35
0.2 Hz	0.54 ± 0.18	1.67 ± 0.69	9.10 ± 3.40

4. Conclusions

Traditionally, the study of the neural sympathetic response to various cardiovascular stimuli has been limited to analysis of the integrated neurogram. While this processing method produces a visually appealing signal, it also eliminates potentially valuable information and complicates spectral analysis of MSNA. Here we presented a novel method of spectral estimation that takes advantage of a wavelet-based denoising algorithm [10]. Where integration may conceal single, solitary action potentials not incorporated into a burst of sympathetic activity, the wavelet-based technique may identify such isolated features. The detection of these individual spikes may be important in estimating the spectral content of the signal, especially at lower frequencies. Furthermore, the spectral estimation technique used here did not employ common AR methods, the parameters of which may have a large degree of statistical uncertainty [8,9].

The analysis of data acquired during neck suction (NS) stimulation of the carotid baroreceptors indicated that the sympathetic activity was able to follow the frequency of NS at both 0.1 and 0.2 Hz. Prominent peaks in the PSD of the MSNA at the NS frequencies was observed in most subjects and are reflected in the average data displayed in Fig. 1 (b) and (c). Additionally, because the PSD calculation was based on spike rate rather than integrated burst parameters, power was independent of needle position and could be presented in non-normalized, non-arbitrary units. The calculation of power was also extended beyond the common LF (0.04-1.5 Hz) and HF (1.5-0.4 Hz) regions to include the cardiogenic (CF; 0.4-3.0 Hz) region (Table 1). The analysis of this higher frequency region may reveal additional information about the physiological importance of the oscillations in sympathetic activity. In the current study, several subjects exhibited an increase in LF, HF, and CF power during NS stimulation at both frequencies, however, the increases were not found to be statistically significant.

In conclusion, the algorithm proposed here for computing the power spectral density of MSNA allows for a more detailed and insightful analysis sympathetic activity than previously used methods. Additional studies must be performed for a more direct comparison between this newly proposed procedure and current methods of MSNA spectral estimation.

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