TIME AND FREQUENCY DOMAIN AND WAVELET ANALYSIS OF CARDIAC INTERBEAT INTERVALS IN THE LABORATORY RAT

S. D. FILIP TO*, PAUL A. DABISCH**, EDMUND K. KERUT***, JACQUELINE A SCOTTO**, JILL R. JARVIS****, ROBERT J. MIODUSZEWSKI**, XIAOYING TAN*

*Agricultural and Biological Engineering/Mississippi State University, USA
**Edgewood Chemical Biological Center, U.S. Army Research and Development Engineering Command, USA
***Cardiovascular Research Laboratory, Louisiana State University Health Science Center, USA
****Geo-Centers Inc., USA

Abstract: The effects of the parasympathetic antagonist atropine and the beta-adrenergic receptor antagonist propranolol on the cardiac inter-beat-interval were investigated in conscious rats. Transmitters were implanted into rats and lead II electrocardiogram (ECG) signals were transmitted and recorded. Rats were injected with atropine (1 mg/kg i.m.), propranolol (1 mg/kg i.m.), or combination of atropine (1 mg/kg i.m.) and propranolol (1 mg/kg i.m.). Both pre-injection and post-injection ECG recordings (5000 Hz) were made. Sixty-second inter-beat interval (IBI) records were generated and then re-sampled using a cubic spline interpolation function. The IBI data were analyzed in the time and frequency domains using both Fourier and wavelet techniques to determine the effects of the antagonists on autonomic function. Validation of the Fourier and wavelet algorithms was done by using mathematically generated multi-frequency data files and rediscovering the frequency components and the ratios of energies between the various frequency components. The results of the present study demonstrate that the wavelet transform appears to be superior to the standard Fourier transform in that it may be able to differentiate between the various treatments. However, additional data from additional animals are necessary to confirm these findings.

Introduction

This paper presents preliminary results of an analysis of the cardiac inter-beat interval (IBI) of conscious rats. The study was conducted to determine the effects of parasympathetic antagonist atropine and the beta-adrenergic receptor antagonist propranolol on in heart beat variability in concious rats. Time domain, frequency domain, and wavelet analysis techniques were employed to facilitate multi-perspective observations of the IBI information.

Materials and Methods

Lead II electrocardiogram (ECG) telemetric transmitters were surgically implanted into rats and recordings of the ECG data were made for a duration of up to three hours per session. The recordings were done before and after administration of the various antagonists. The recordings were done digitally with a sampling rate of 5000 samples per second at a 16- bit resolution. The treatments were as follows: after the recording of baseline data (no treatment) the rats were injected with atropine (1 mg/kg i.m.) Propranolol (1 mg/kg i.m) was administered 24 to 48 hours later. A combination of atropine (1 mg/kg i.m.) and propranolol (1 mg/kg i.m.) was administered 72 to 96 hours later. The pre-injection and post-injection recordings were made with exactly the same instrument settings and under laboratory conditions. Post-injection recordings were done within one hour after the injection. Hereafter the atropine treatment will be referred to as ATR, the propranolol treatment will be referred to as PRO, and the combination treatment of atropine and propranolol will be referred to as ATR+PRO. One-minute segments of IBI data were extracted from the ECG recordings using software provided by the equipment manufacturer (Data Science International, St. Paul, MN, USA). A total of thirty six segments of data from two rats (eighteen segments each) were selected for analysis using the methodology described in this paper.

The IBI data were analyzed in the time domain and in the frequency domain using Fourier and wavelet transform techniques. The results from each of the analyses were then statistically analyzed to determine the degree of correlation between rats and between treatments. The influences of outliers and common mode (DC component) data were also examined statistically.

The IBI data were analyzed in the time domain by examining the mean and standard deviation values pre and post-treatment for each rat (baseline, ATR, PRO, aTR+PRO). The changes in mean and standard deviation values were then tabulated and discussed.

Cubic Spline was used to reconstruct and resample the IBI data at a sampling rate of 20 Hz before Fourier and wavelet analyses were performed. The Fourier frequency domain analyses were done by performing

512-point power spectral density (PSD) computation followed by the calculation of energy ratios in three frequency bands against each other and against the total energy content of the data. The ratios were VLF/LF, VLF/HF, VLF/total, LF/HF, LF/total, and HF/total, where VLF (very low frequency) had a frequency range of 0.01 to 0.2 Hz, LF (low frequency) with a frequency range of 0.27 to 0.74 Hz, and HF (high frequency) with a frequency range of 0.74 to 3.85 Hz. The value of each frequency band was obtained by simply adding the PSD amplitudes within its frequency range, and the total is the simple sum of the amplitudes from 0.01 to 3.85 Hz. The Fourier frequency domain analysis method was validated by rediscovering the frequency components of a mathematically generated data set that had known frequency components using the algorithms developed to analyze the rat IBI data. Continuous Wavelet Transform (CWT) operations were done on the IBI data sets and the equivalent frequency components (pseudo frequencies) were calculated from the "level" values of the transformation results. The same ratios defined above were computed by summing the coefficients of the levels that fell inside the respective pseudo frequency band.

The statistical analyses were a 2×4 factorial arrangement of treatments in a completely randomized design with the treatment combination being Rat and Level. The design presented in this paper has two rats (Rats 2 and 5) and four levels (baseline, ATR, PRO, ATR+PRO), and only the analyses of balanced (same number of data segments for all rats) cases are presented. Two-way ANOVA was used to determine statistical significance. The results of the statistical analysis presented in this paper were based on eighteen segments of data of each rat and each level. One of the objectives of the statistical analysis was to find the ratio of energy of each frequency band that had the most consistent trend from level to level and from rat to rat.

Results

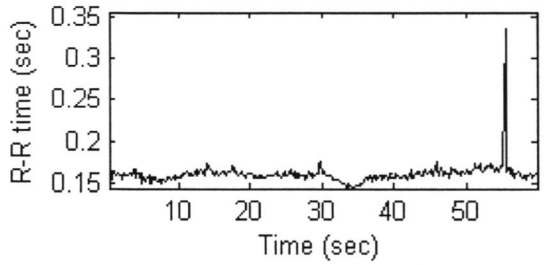

Figure 1. Example Baseline IBI Data with Outliers

An example of the baseline IBI data is shown in Figure 1. Note the presence of an outlier at about 55 seconds (the tall peak). The outlier is the result of either a pause in the cardiac rhythm or an error in the IBI extraction algorithm. In either case, depending on the number of outliers in the data set, the results of the time and the frequency analysis may be affected, necessitating the removal of the outliers prior to analysis. The removal of outliers, when performed, was done by replacing the data point with the moving average of the data points up to the current position. It was observed that the existence of outliers affected the standard deviation values, but did not affect the general trend of the mean values of the IBI throughout the treatments levels (baseline, ATR, PRO, ATR+PRO). Figure 2 depicts the mean of IBI values of two rats.

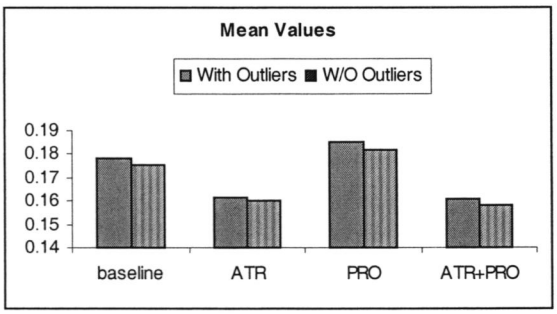

Figure 2. Outliers Did Not Affect the Overall Trend in Time Domain.

In frequency domain, most of the energy was located in the lower end of the frequency spectrum and only a small amount was located at the higher frequency range. Figure 3 shows the frequency contents of the same baseline IBI data from figure 1 with the common mode values (DC component) removed to highlight the other frequency components. The frequency contents are similar to those presented by Perlstein et.al.[1] with the exception that the 2.5 Hz component was not as prominent in the present study.

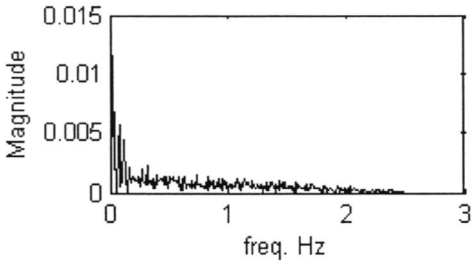

Figure 3. The Frequency Components of Figure 1.

There were six ratio quantities computed in frequency domain: VLF/LF, VLF/HF, VLF/total, LF/HF, LF/total, and HF/total. Statistical analyses were done to determine which of these ratios presented similar trends across rats. The statistical analyses performed to select ratios that had the most consistent trend were a complete randomized design with 2×4 factorial arrangement of treatments. The results presented in this paper are from balanced cases, where all rats had the same division sizes (same number of data segments). Two-way ANOVA procedure was used to get the f-test P-value results.

Table 1 shows the P-values of f-test from the two-way ANOVA and the average coefficients of variance (CV)

for frequency domain ratios with the outliers included. Table 2 shows the results with the outliers removed. The values in the second column of the table are the P-values indicating the interaction between Rat and Level. The P-values in the Rat×Level column that are less than 0.05 indicate a significant interaction between Rat and Level, which means the treatment Levels do not have similar magnitude of effect from rat to rat. Large Rat×Level P-values on the other hand indicate that the ratios have similar trend in treatment levels across different rats. The third column of the table contains the P-values of treatment Level, which indicate how different the treatment Levels from each other. Small P-values in this column are preferred in the analyses since they indicate large difference between the treatment levels. The last column in the table contains the average CV values of ratios, in which small CV values are preferred in the same treatment Levels for the same Rats. The highlighted entries in the table (bold print) are the ratios that had the most consistent trend across rats and across treatments. Observing the values in Table 1 and Table 2, the VLF/total ratio seems to have the best overall values to indicate the influence of the injections to the rats. The highlighted Rat×Level value in Table 2 is larger than the value in Table 1 suggests an influence of the outliers to the computation of VLF/total). Also, with the removal of outliers, CV values are decreased as shown in Table 2 compared to values in Table 1.

Table 1. The P-values of Frequency Domain Ratios with Outliers Included

	Rat×Level	Level	CV
VLF/LF	0.0434	0.0130	50.5
VLF/HF	0.1501	0.3333	81.2
VLF/total	0.1659	0.0392	14.6
LF/HF	0.2727	0.6218	49.0
LF/total	0.4101	0.5464	126.3
HF/total	0.1516	0.6987	113.5

Table 2. The P-values of Frequency Domain Ratios with Outliers Removed

	Rat×Level	Level	CV
VLF/LF	0.2607	0.4876	15.2
VLF/HF	0.0510	0.0014	32.5
VLF/total	**0.9641**	**0.0014**	**4.7**
LF/HF	0.0302	0.0002	24.9
LF/total	0.4130	0.5820	41.1
HF/total	0.3397	0.7072	104.4

When compared to the baseline value, the amount of VLF energy decreased when atropine, propranolol, or the combination of atropine and propranolol were injected (Figure 4)

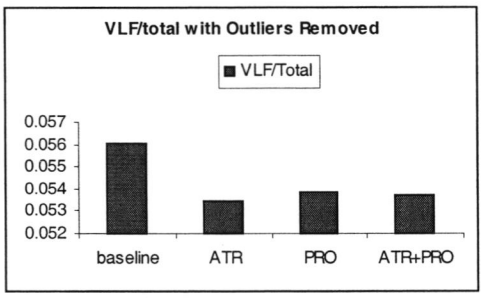

Figure 4. Frequency Domain VLF/total Trend with Outliers Removed

The Continuous Wavelet Transform (CWT) analyses were carried out using three different wavelets: Haar, Daubachies-7 (db7), and Mexican Hat (MexH). The same statistical analyses as done in the Fourier frequency domain analysis were carried out. The results from the three wavelets techniques were similar. Only the results using Haar wavelet are presented here.

The results of statistical analyses for identifying which ratio presented the most consistent trend between rats and between treatments are shown in tables 4 and 5. Table 4 shows the results with outliers included and Table 5 shows the results with the outliers removed.

Table 4. P-value of Ratios with Outliers Included, Haar Wavelet

	Rat×Level	Level	CV
VLF/LF	0.3009	0.0277	38.4
VLF/HF	0.2491	0.1327	71.0
VLF/total	0.5111	0.4442	0.9
LF/HF	0.2295	0.1402	39.6
LF/total	0.5533	0.4729	90.1
HF/total	0.5068	0.7024	122.3

Table 5. P-value of Ratios with Outliers Removed, Haar Wavelet

	Rat×Level	Level	CV
VLF/LF	0.0949	0.1781	8.8
VLF/HF	**0.1956**	**0.1585**	18.9
VLF/total	0.0896	0.1882	<0.1
LF/HF	0.2009	0.2819	16.8
LF/total	0.1491	0.2657	12.5
HF/total	0.2946	0.4692	58.1

Based on the results shown in Table 5, the ratio of VLF/HF seemed to be the strongest indicator of the effects of antagonists on the IBI of the rats. The overall trend of the VLF/HF of the Haar wavelet analysis

indicated that the value increased following atropine administration and decreased following propranolol administration. The combination of atropine and propranolol resulted in a value of VLF/total that was similar to the baseline value.

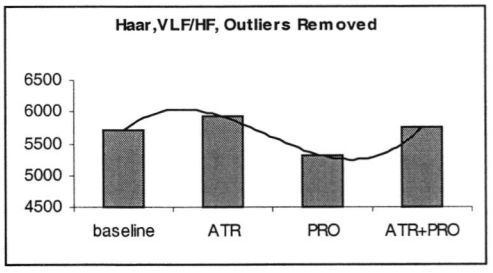

Figure 5. VLF/HF Trend of Haar Wavelet Analysis.

Discussion

From the results presented in figure 2, it is clear that outliers did not affect significantly the mean values of the IBI in the time domain. The general trend of the mean values showed that the value decreased below baseline following administration of atropine (ATR) indicating an increase in heart rate. Propranolol administration resulted in an increase in the mean value of the IBI, indicating a decrease in heart rate. These results are consistent with the known pharmacologic actions of these compounds, and were expected.

The statistical results shown in Table 2 indicate that the frequency domain ratio of VLF/total is more consistent from rat to rat than the other ratios. In addition, VLF/total has a Level P-value less than significant level 0.05, indicating a significant difference between treatment levels. The average CV value of VLF/total is also small enough to ensure the reproducibility of the analysis. Hence, the results suggest that VLF/total is appropriate to use for determining the effect of the various antagonists on IBI of conscious rats in the frequency domain.

The amount of VLF energy decreased when atropine, propranolol, or the combination of atropine and propranolol were injected, when compared to the baseline value. A decrease in VLF energy suggests more energy in higher frequency band. While all three treatments produced changes in the VLF power, it may not be possible to distinguish between the treatments based on VLF power since the change was similar following each treatment. However, data from additional animals are necessary to confirm this. The statistical analyses of the ratios produced by the computations in the frequency domain suggest that the presence of outliers affects the results significantly, particularly the VLF/total (see Tables 1 and 2). The removal of outliers from the data sets resulted in greater consistency in the values from rat to rat and also reduced the CV values of the results.

Relating the results of CWT analysis to this discussion is more complex because the values used in the statistical analyses were the coefficients of the wavelet series. While it was possible to compute the pseudo/equivalent frequency from the "levels" values, deriving a quantity that was similar to PSD in the frequency domain was not attempted. Based on the CWT computation done in this experiment, the ratio of VLF/HF (Figure 5) increased when atropine was administered. Propranolol administration resulted in a decrease in the ratio of VLF/HF power, and following the administration of the combination of atropine and pranolol there was a slight increase in the ratio of VLF/HF. Unlike Fourier PSD analysis, the results of CWT analysis demonstrate the atropine and propranolol produce opposite changes in the ratio of VLF/HF. Thus, it may be possible to determine which antagonist was administered based on the ratio of VLF/HF determined using Haar wavelet analysis. However, data from additional animals are necessary to confirm this.

Conclusions

This aim of the present study was to determine the effects of the parasympathetic antagonist atropine and the sympathetic antagonist propranolol on the IBI of conscious rats. The results clearly show that atropine and propranolol affected the heart rate of rats. Atropine increased the heart rate, and propranolol decreased the heart rate, as indicated by the decrease and increase in the IBI mean values, respectively. Frequency domain analysis facilitated a more versatile view of the information in that it was possible to "slice" the IBI information into bands of frequency. The Fourier frequency domain analysis demonstrated that the antagonists used produced consistent changes in the PSD. However, the changes produced by all three treatments were similar and it was, therefore, not possible to determine which antagonist had been used in which case. The wavelet transform also demonstrated that the antagonists used produced consistent changes in the frequency ranges examined. However, the changes observed using wavelet transform analysis were dissimilar for atropine and propranolol. Thus, the wavelet transform appears to be superior to the standard Fourier transform in that it may be able to differentiate between the various treatments. However, additional data from additional animals are necessary to confirm these findings. Additionally, the additional information contained in the results of wavelet transform, although it was not utilized in the present study, may be useful for other applications in the future.

References

1. I. Perlstein and A. Hoffman, "Cumulative plot of heart rate variability spectrum assesses kinetics of action of cholinergic drugs in rats", Am. J. Physiol Heart Circ Physiol. 279: H110–H115, 2000.