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ABSTRACT

A STUDY OF FACTORS AFFECTING CARDIOVASCULAR REACVITY

by Andrew Gil Ventura

This pilot study was conducted to determine what affects the cardiovascular reactivity of different people. Variables such as the order of stress-inducing and relaxing activities, performing multiple tasks on different days, personality, familial history of diseases, and general health were investigated as to their effects, if any, on cardiovascular reactivity.

Ten healthy, normal male subjects, aged 18 – 33 years, volunteered to be subjected to studies on three experimental days, the first one being for gathering information. On the second and third days, the subjects performed the following activities: mental arithmetic, numeric repetition, 10 breaths-per-minute (bpm) pacedbreathing, and normal breathing, with 12-minute periods of rest before and after each activity. EKG, blood pressure, and respiration measurements were recorded from each subject throughout the entire 90-minute sessions.

Results from the statistical analysis showed that the vagal tone readings for the normal breathing activity were affected by the subject's depression state, the order of the activities, performing multiple tasks on different days (session), and the interaction of depression and order or session. Readings for both the mental arithmetic and numeric repetition activities were only affected by the subject's depression state. No factors affected the readings for the 10-bpm paced breathing activity. Other factors did not provide insight to the differences in vagal tone readings between subjects. Further study, preferably with more subjects, is needed to substantiate these results.

A STUDY OF FACTORS AFFECTING CARDIOVASCULAR REACTIVITY

by Andrew Gil Ventura

A Thesis Submitted to the Faculty of New Jersey Institute of Technology In Partial Fulfillment of the Requirements for the Degree of Master of Science in Biomedical Engineering

Biomedical Engineering Committee

January 2000

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APPROVAL PAGE

A STUDY OF FACTORS AFFECTING CARDIOVASCULAR REACTIVITY

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CHAPTER 1

INTRODUCTION

1.1 Objective

The objective of this pilot study was to investigate the cardiovascular reactivity of a person to stresses introduced in the subject's environment. The responses to these stresses were correlated to specific factors and variables. Variables such as personality, environment, familial history of diseases, and previous stress/non-stress events were used to determine if they have an impact on the cardiovascular reactivity in individuals. Variables such as heart rate change, blood pressure change, changes in catecholamine level, changes in immune parameters, family history of hypertension, and personality traits have been shown to be related with cardiovascular reactivity [1] [16] [17]. Even if levels of cardiovascular reactivity do not directly influence heart rate variability, cardiovascular reactivity may need to be accounted for in heart rate variability studies that measure any of the above variables. The results of this study will hopefully aid in the more accurate recording and interpretation of cardiovascular data by realizing the different factors, if any exist, that affect cardiovascular readings.

1.2 Background Information

1.2.1 Cardiovascular and Heart Rate Reactivity

For the purposes of this study, reactivity will be defined as how much change, if any, a set of physiological variables experience when exposed to stress and non-stress situations. Cardiovascular reactivity is defined as the change in heart rate, blood pressure, and vagal tone in response to a perceived stressful situation. Reactivity can be measured by calculating the changes in the above parameters before and after an activity. The difference between the before and after measurements gives an idea of how well a person reacts to that situation. For this study, cardiovascular reactivity can be observed from the different vagal tone readings of each subject during the different activities.

In contrast, heart rate (HR) reactivity refers to the change in heart rate only in response to a perceived stressful situation. HR reactivity is measured by taking the arithmetic difference between the normal heart rate and the heart rate measured during the stressful situation. Cardiovascular reactivity is more involved and provides more information than HR reactivity. Heart rate reactivity, however, can be a tool in realizing the objectives of this study.

1.2.2 Parasympathetic Nervous System

This study focused on the parasympathetic division of the central nervous system (CNS). The parasympathetic region is a subdivision of the autonomic nervous system, which is in turn a subdivision of the CNS. The neurons that can be classified as belonging to the autonomic nervous system innervates smooth muscles, glands, the neurons in the gastrointestinal tract, and most importantly for this study, the cardiac muscles. The autonomic nervous system is controlled by the hypothalamus, which controls emotions, reinforces certain behaviors, i.e., motivational behaviors such as the need to win because winning feels good, and is the single most important control area for homeostatic regulation of the body's internal environment, or simply keeping the body from getting too hot, too cold, and/or too dry. It is also responsible for behaviors dealing with the preservation of the individual, i.e., eating and drinking, and preservation of the species,

i.e. need for reproduction. In short, the hypothalamus is the part of the nervous system that controls "unconscious" functions of the body. The autonomic system is further subdivided into two areas: the nerve fibers of the parasympathetic region leave the CNS from the brain and sacral portion of the spinal cord, or the lower back, while the sympathetic region's nerve fibers leave the CNS from the thoracic, or chest, and lumbar regions. Each of these regions responds to different behaviors: the sympathetic region responds to "Fight or Flight," i.e., periods of anger and competition, while the parasympathetic region responds to "Rest and Recuperation," i.e., periods of meditation and relaxation [21].

Since the present study deals with the physiological effects of stress, the question arises as to why the parasympathetic region is emphasized and not the sympathetic. The answer relates to the use of a heart rate variability spectrum. Heart rate variability (HRV), the varying period of the heart beat, can be detected by noting the differences in time between successive R waves in the EKG. By using this information, the HRV power spectrum can be extracted. This method will be further explained in Chapter 2.

The HRV spectrum has three regions, as shown in Figure 1.1, which are the high frequency, the low frequency, and very low frequency regions [18]. The HF region ranges from 0.15 to 0.4 Hz, the LF region ranges from 0.05 to 0.15 Hz, and the VLF region frequency below 0.05 Hz. A noninvasive index of parasympathetic activity, or the vagal tone, can be found by measuring the area under the HF region of the spectrum. This is because the HF region responds to only parasympathetic activity. The LF region is affected by both the sympathetic and parasympathetic nervous systems. Therefore, to have a clearer understanding of the physiological effects of stress, it is easier to

concentrate on the HF region, which is affected by only parasympathetic signals. If parasympathetic signals were blocked, i.e., through drug administration, then studying the LF region would be more advantageous.

1.3 Previous Studies

There have been other studies done on the physiological effects of stress on the body. Variables that were studied included gender, personality, and familial history of hypertension. Results that were analyzed included cardiovascular response, cardiovascular reactivity, and respiratory sinus arrhythmia response [1] [16] [17].

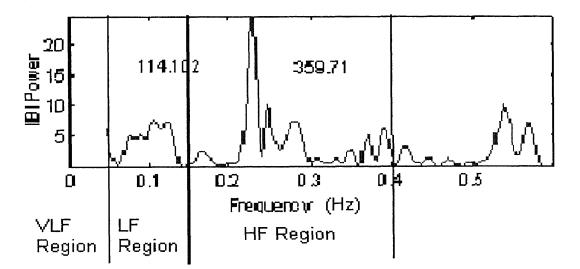


Figure 1.1 The regions of the Heart Rate Variability Spectrum

In [16], the effects of a perceived stressful situation on the cardiovascular, endocrine, and cellular immune systems were studied in 22 males ranging in age from 18 to 31 years. Forty subjects volunteered in the initial pre-screening study. Of those 40, twelve men who displayed the highest HR reactivity in response to a speech stressor and twelve men who displayed the lowest HR reactivity were chosen (1 subject in each group did not participate in the main study accounting for the 11 subjects in each group). The main study consisted of an introductory period, a 30-minute supine period (resting, no measurements), a 5-minute baseline-measurements period, and a 12-minute mental arithmetic (MA) exercise with random 100-dB noise blasts. Blood was drawn after the supine period and after the mental arithmetic exercise for endocrine and immune assays. Heart rate and blood pressure measurements were continuously recorded during the baseline and the MA periods. Results revealed that the stressor caused an increase in natural killer cell numbers and cytoxicity in the blood, epinephrine levels, and heart rate and blood pressure responses during the MA exercise. These results suggest that the interactions among the autonomic nervous system, endocrine system, and immune system are not only amenable to psychophysiological analysis but that such analyses may play an important role in illuminating underlying mechanisms.

Two studies have dealt with how other factors may affect cardiovascular reactivity. The studies in [1] and [17] focus on the influences of gender, personality, and familial history of hypertension on cardiovascular reactivity. These two studies are similar to the study being presented in this paper. The study in [1] had 22 female / 20 male ratio in subjects with a mean age of 22 years; in [17] the ratio was 103 female / 106 male with an age range of 17 - 33 years, 90% of whom are between 18 and 23. Each study consisted of multiple tasks; mathematics and handgrip exercises were common to both. All subjects were given personality tests to determine certain aspects of their behavior, i.e., hostility, aggression, and general well being. The Cook-Medley Hostility test was used on both to determine the level of each subject's cynical and mistrusting attitude. Other tests included a defensiveness test (the Marlowe-Crowne Social

Desirability Scale), an anger expression scale, an empathy test, and a Personal Attribute Questionnaire. These tests were used to quantify personality characteristics in order to evaluate their ability to explain potential gender differences in cardiovascular responses. Heart rate and blood pressure measurements were taken from all subjects. Results from the studies contradict each other.

In [17], it was found that women who attained a low score on the anger expression scale (as compared to those who attained a high score) and attained a low score in the defensiveness test had greater diastolic blood pressure reactivity. Men who had a high hostility rating and a low defensive rating were associated with greater heart rate reactivity; the opposite effect was observed in high-defensive men. Conclusions from this study state that individual differences in anger, hostility, and defensiveness have implications for cardiovascular reactivity and a positive familial history of hypertension seems to be an amplifier of reactivity. Moreover, predictions of reactivity can be improved by taking into account gender and family history.

In [1], contradicting results in comparison to [17] were obtained: men exhibited greater systolic and diastolic responses while women showed larger increases in heart rate. It is also stated that personality differences in men and women did not explain significant variance in the gender differences in cardiovascular responses, another contradictory observation from [17] which stated that "gender, family history, and defensiveness each affected differential DBP {diastolic blood pressure} reactivity to the three tasks."

CHAPTER 2

METHODS

2.1 Discussion of Present Study

The present study was organized and conducted by Melissa DePrince, candidate for a Ph.D. degree in Electrical Engineering at New Jersey Institute of Technology, Newark, New Jersey, under the advisement of Dr. Stanley Reisman, Director of Biomedical Engineering. The study was performed at the Kessler Institute for Rehabilitation, West Orange, NJ. Author Andrew Gil M. Ventura completed the analysis and interpretation of the data and the resulting report.

This pilot study was created to determine the relationships between heart rate variability and cardiovascular reactivity. In this study, differences in measurement, functions and interactions of the autonomic nervous system were explored by exposing healthy subjects to both a relaxing and stressful situation. The study proposed to determine whether levels of cardiovascular reactivity are related to levels of heart rate variability during stress or paced breathing.

Another aspect of this study was the investigation of the influence of performing both a relaxing task and a stressful task on cardiovascular reactivity. When sequences of tasks are performed, it is possible that a previous task will influence response to the present task. This is especially important if both relaxing and stressful tasks are performed in the same session. The present study was designed to allow observation of whether the order of the tasks being performed affected the cardiovascular reactivity. Other factors such as personality and familial history of diseases were also investigated for their influences on cardiovascular reactivity.

2.1.1 Eligible Subjects

For this study, 10 subjects volunteered their time to participate. The original criteria for the choice of subjects were as follows: eligible subjects were male, have an age range of 18 to 35 years, in good health, have no history of psychological disorders or chronic illnesses, were not on any drugs or tobacco products, did not experience any recent negative life event (i.e., death in the family), had no class exam or any other activity that might induce additional stress, and were not math-phobic. These criteria were chosen to keep the subjects similar to each other and have differences attributable only to those factors being investigated, i.e., personality and familial history of disease. Eliminating gender and health differences provided for an easier analysis in this pilot study. The ability to perform mathematical activities was important because of the mathematical activities all the subjects had to complete. Each subject was also required to have a normal breathing rate of greater than 10 breaths-per-minute (bpm) so that it would be distinguishable from the 10-bpm paced breathing exercise, one of the activities the subjects had to perform in the study. In addition, it is important that the subjects have breathing rates greater than 10-bpm in order to keep the vagal tone for the breathing exercises in the parasympathetic, or HF, region. Any breathing rates lower than 10-bpm causes the vagal tone to appear in the LF region.

However, the subjects used in this study did not rigorously follow the subject criteria. Of the ten, two subjects had breathing rates at exactly 10-bpm. One subject had 3 - 4 cups of caffeine a day and smoked regularly, and another was taking some medication (an antibiotic and an allergy medicine). Due to the small number of subjects

that participated in this study, no subjects were dropped. According to Melissa DePrince, who conducted the study, a minimum of eight (8) subjects was needed to provide enough data to perform meaningful statistical analysis. Exclusion of any of the subjects who did not meet the criteria would reduce the number of subjects to less than eight. It was decided that all subjects would be used for the study. Also, due to unforeseen circumstances, it was not possible to recruit more subjects to continue the study.

2.1.2 Discussion of Test Activities

The study was performed on three separate days for each subject, none of which were required to be consecutive. All the activities were performed at approximately the same time each day, i.e., the session was conducted starting at approximately 10 AM for all three days. This was necessary to perform readings on the body in approximately the same condition on all three days. It would be erroneous to test one subject during the morning for one session and during the late afternoon for the other. The difference of going through a whole day's activities might affect the readings drastically, i.e., increased heart rate due to fatigue later in the day. Although the subject might not go through the exact same situations each day, the chance of the body being in a similar condition as the other days would be greater if the sessions were all performed at approximately the same time. Performing the sessions at the same time also takes into account that the body will be in the same period of its circadian rhythm, thereby increasing the likeliness that the body will be in a similar state for each session. The first day was primarily for baseline recordings, respiratory rate determination, and personality characterization. Determining personality characteristics was done through a number of questionnaires and "tests," which included the Marlowe-Crowne Scale, the Beck Depression Inventory, a hostility inventory list, a personal and health statistics questionnaire, a life changes index scale, and other questionnaires pertaining to the subjects' general well-being and current emotional status with emphasis on level of anger and stress. The author evaluated the questionnaires. Appendix B contains the questionnaires used for this study.

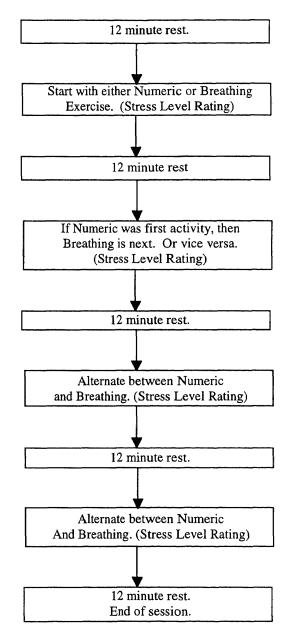


Figure 2.1 Flowchart of schedule for test sessions

The second and third days were the actual test sessions when the volunteers were subjected to the test activities. Figure 2.1 is a flowchart for the schedule of the test activities on these days. For this study, the second day was known as Session I and the third day was known as Session II. The schedule for these days called for a 4-minute mental arithmetic stress activity (the stressor) and a 4-minute non-stress activity (the stressor's control); a 4-minute paced-breathing activity and its 4-minute control (normal breathing); and five 12-minute rest periods, one before each activity and another one after the final activity. After each 4-minute activity, the subject was also asked to rate the activity on how stressful the activity was (from 1 which was not stressful to 10 which was very stressful) and answer some questions about the activity. The stressor used for this study was a mental arithmetic (MA) subtraction exercise. The exercise requires the subject to continuously subtract a set value starting from a given initial number until instructed to stop. According to [17], the most dramatic changes in heart rate and blood pressure occurred during the mental arithmetic task. Therefore, this was chosen as the stressor to be applied to the each subject. A number of other studies have also used a mathematical stressor [1] [11] [16]. This was also the easiest task to set-up when compared to other stressors used in previous studies such as the handgrip stressor (where the subject squeezes a pressure-measuring device) or the Stroop Color-Word Interference (where the subject chooses the correct color name corresponding to a given colored word) [1]. No additional equipment is needed to set up the arithmetic exercise and every healthy, normal person who has had up to 4th grade level education should be able to perform basic subtraction. Additionally, stress level can be easily increased through addition of distractions or introducing a psychological variable such as the mention of payment dependent on how well the subject performs in the mental arithmetic task. The control activity to mental arithmetic is the repetition of numbers. Relative to the MA, the repetition should not be perceived as stressful by any of the subjects as little mental ability is required to repeat numbers.

A 10-bpm paced breathing activity is part of the experiment to observe cardiovascular activity during periods of relaxation. At 10-bpm, the body is in a more relaxed state when compared to a normal breathing rate greater than 10-bpm. The control-counterpart would be breathing at the subject's normal rate, which was determined on the first day of the study. To better control the breathing rate, the subject follows a light metronome that is set to the breathing rate being performed. After each activity, the subject is asked to rate the activity on how stressful the activity was.

The length of times chosen for each activity is significant. At four minutes, the body should have enough time to "adjust" to the given activity. At shorter time lengths, the readings and the resulting data can be tainted with the effects of the body's "adjustment period" as it goes from a stress to a non-stress situation, or vice-versa. During these transitionary periods, the heart rate readings may be higher or lower than the actual value; giving the body time to "adjust" to a desired response level should achieve vagal tone readings that are close to the actual values. The twelve-minute rest periods should give adequate time for the body to "erase" the effects of any previous activity. The whole experimental session lasted an average of 90 minutes and did not exceed 2 hours.

2.2 Data Acquisition

Beat to beat arterial finger blood pressure was measured by an Ohmeda 2300 Finapres blood pressure monitor and respiratory rate was determined by impedance pneumography. Impedance pneumography is based on the relationship between respired volume and changes in transthoracic impedance during breathing [12]. The frequency of the impedance change was recorded and used in respiratory rate determination. EKG was collected using a Quinton stress test monitor. The Q4000 stress test monitor acquires EKG signals of various lead configurations and generates a pulse train, which was synchronized with the detection of the QRS complexes. Respiration rate, EKG, synchronous pulse train and blood pressure signals were connected via an interface box to a Keithley-Metrabyte DAS-16 board, which is a 16-chanel A/D converter. EKG, respiration, blood pressure, and the synchronous pulse were acquired continuously during the experiment and were stored as ASCII files in a computer. Each signal was sampled at 200 Hz. Therefore, for a signal that is 20 seconds long, there were 4000 measurements recorded.

Computer analysis was done with *MatLab* and *JMP In!*. *MatLab* is an interactive program that aids with numeric computation and data visualization. Fundamentally, *MatLab* is built upon a foundation of sophisticated matrix software for analyzing systems of equations. *MatLab* is especially designed for working with number matrices. Since the measurements are stored in a simple matrix format, using *MatLab* was very advantageous in this study. Programs can also be written in *MatLab* that can perform specific functions that are not included in the program, such as the programs written to

extract the HRV power spectrum. *JMP In!* is a statistical software package. All statistical analysis was done with *JMP In!*.

2.3 Data Processing

The EKG measurements were converted into the heart rate variability spectrum using *MatLab*. Figure 2.1 shows a flowchart of how the HRV spectrum is produced. As was mentioned before, the heart rate variability spectrum can be used as a noninvasive index of parasympathetic activity, or vagal tone, by measuring the area under the high frequency region that is part of the spectrum. Using *MatLab*, the synchronous pulse train signal was converted into the HRV spectrum. Refer to Appendix A for the *MatLab* programs with explanatory comments used in this study. The programs also calculated the area under the HF and LF regions. The synch pulse train records the time of occurrence of the R waves. Figure 2.2 shows an example of a recorded synchronous pulse signal recorded from one of the subjects and displayed using *MatLab*. Figure 2.3 shows an example of a recorded EKG signal from the same subject. Notice how in Figure 2.2

the signal only records the occurrences of the R waves and produces a spike of a set height while in Figure 2.3 the signal records the entire EKG signal which has R waves of varying heights. These varying heights causes the *MatLab* program to miss R waves that are not "tall enough." The synch pulse train was used because it produced results that were more accurate than using the EKG signal. To obtain the HRV for the desired activity (MA, Repetition, 10-bpm, or Normal breathing), the appropriate time frame for the activity must be isolated. Since the measurements were continuously recorded 200

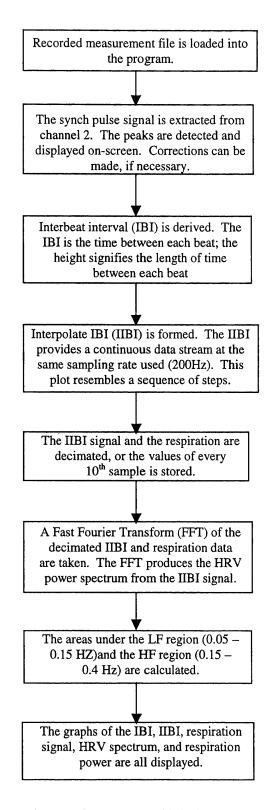


Figure 2.2 Flowchart of *MatLab* program which derived the HRV power spectrum

times per second for the duration of the session (and each session averaged 90 minutes or 5400 seconds), there is an average of 1,080,000 measurements per synch pulse signal per subject per session. By calculating the time frame of each of the four activities, it is possible to isolate the measurements pertaining to any of the activities. This task was made easier since the time when each activity began and ended were all recorded. From these time frames, each signal was further reduced to the middle two minutes of each activity. The middle two minutes was used because the first minute will most likely be tainted with the effects from the previous activity. Also, choosing the middle provides a "safety margin" in case there is miscalculation as to the time frame of the activity. From this resulting signal, the HRV spectrum was extracted and the area under the HF region calculated.

The resulting spectrum yields one value for the overall parasympathetic activity. This allows for the comparison of the overall vagal tone for each task, or the comparison of the overall vagal tone for the same tasks between different subjects. Figure 2.4 shows an example of an HRV spectrum with the areas under the LF and HF region calculated. This HRV spectrum is from the MA exercise of d420 or the MA exercise for the second session of subject "d." The HRV spectrum shows sympathetic and parasympathetic activities. Each subject had four HRV spectrums (one for each test activity) per session. Refer to Appendix C for all the HRV spectra.

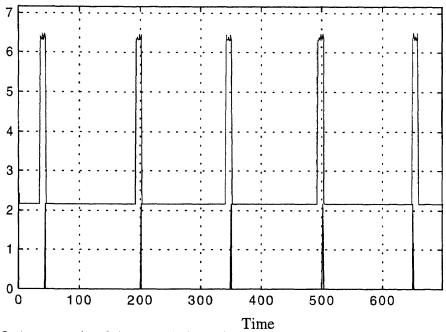


Figure 2.3 An example of the recorded synchronous pulse train as displayed by MatLab

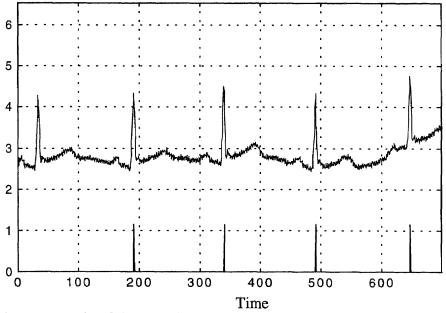


Figure 2.4 An example of the recorded EKG as displayed by *MatLab*. This is the same EKG used to derive the synch pulse of Figure 2.3. The lower spikes correspond to R waves and are used to derive the HRV. Notice the first spike is missing. Missing spikes cause inaccurate HRV's. The pulse train is more accurate.

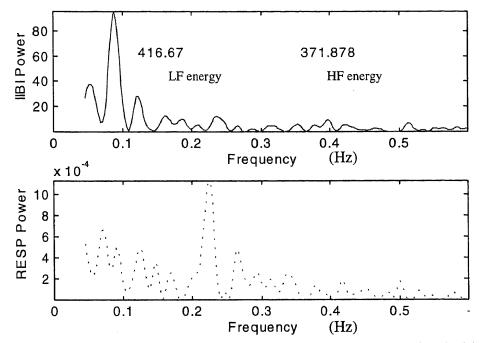


Figure 2.5 HRV spectrum with areas in the LF and HF regions calculated. The bottom graph shows the respiration power spectrum with the tallest spike at the frequency of respiration. The respiration power is not used in this study.

CHAPTER 3

ANALYSIS

3.1 *MatLab* Analysis

As was described in Chapter 2, raw measurements were converted into numerical data with the use of *MatLab*. Taking the synchronous pulse train measurement that was recorded from each subject, the HRV spectrum was extracted. From this spectrum, the areas under the LF region and the HF region were calculated for each of the four activities and recorded in Table 3.1. The rows correspond to one session per subject; each subject performed one session in one day, for a total of 2 test sessions and 20 rows in the table. In the first column, the letters correspond to the name of the subject while number corresponds to the date of the session in month-day format. Since only the HF areas were needed, the LF areas were taken out, as can be seen in Table 3.2. The area under the HF region provides an index of parasympathetic activity and is therefore important for this analysis.

3.2 Statistical Analysis

Statistical analysis is used to correlate the numerical data obtained from the cardiovascular readings from the subjects with information present about each subject and about the variables being investigated. The results are discussed in Chapter 4.

The vagal tone data obtained from the HRV spectrum was statistically analyzed using *JMP In!*, a statistical analysis software package. The analysis methods used on the data were distribution, paired-t test, and stepwise regression analysis. The vagal tone data for the main stressor, the relaxer, and their control-counterparts, were used in the

19

Subject	10-bpm LF	10-bpm HF	Normal LF	Normal HF	Mental LF	Mental HF	Repetition LF	Repetition HF
d412	177.293	1684.91	14820.7	38497.6	398.105	378.223	157.777	989.026
d420	606.236	1901.41	440.78	1739.44	416.67	371.878	210.132	491.339
h625	190.907	415.222	114.102	359.71	350.043	147.023	360.367	331.447
h628	207.182	710.474	59.417	422.35	918.785	402.567	395.858	252.514
j625	489.63	702.66	207.921	645.148	441.791	170.339	120.601	201.671
j627	1172.63	1080.24	153.405	661.351	609.326	215.498	299.085	237.462
m508	420.05	476.156	301.434	218.786	1345.13	419.947	520.206	420.315
m604	1392.88	1130.69	331.422	648.037	1535.07	312.601	1799.93	362.919
r707	602.939	681.785	166	169.422	321.333	320.921	300.484	186.561
r709	386.387	268.394	65.616	39.868	283.993	174.772	106.605	37.106
s425	780.802	2076.95	1171.58	1762.87	1131.32	1058.52	731.859	798.055
s509	3072.95	2190.45	1964.5	2656.98	1434.71	771.931	934.692	1310.3
s705	2352.64	4662.99	136.963	617.161	724.496	312.332	143.662	608.223
s706	2154.16	2087.41	277.291	1092.41	1373.97	394.129	214.574	419.134
v630	510.938	585.923	79.117	540.023	178.829	113.376	42.000	124.343
v703	246.466	478.453	27.098	118.571	52.565	107.009	22.835	113.67
z425	43.152	250.144	21.379	43.963	457.407	132.849	575.804	239.209
z602	131.426	136.957	21.371	36.833	382.676	152.361	320.854	72.448
z704	1141.28	711.849	532.906	227.348	661.529	410.672	841.474	250.067
z705	615.51	527.8	67.278	376.048	942.845	187.54	543.075	317.823

Table 3.1 Table of LF and HF region areas as calculated using MatLab. The areas have no units.

Subject	10-bpm HF	Normal HF	Mental HF	Repetition HF
d412	1684.91	38497.6	378.223	989.026
d420	1901.41	1739.44	371.878	491.339
h625	415.222	359.71	147.023	331.447
h628	710.474	422.35	402.567	252.514
j625	702.66	645.148	170.339	201.671
j627	1080.24	661.351	215.498	237.462
m508	476.156	218.786	419.947	420.315
m604	1130.69	648.037	312.601	362.919
r707	681.785	169.422	320.921	186.561
r709	268.394	39.868	174.772	37.106
s425	2076.95	1762.87	1058.52	798.055
s509	2190.45	2656.98	771.931	1310.3
s705	4662.99	617.161	312.332	608.223
s706	2087.41	1092.41	394.129	419.134
v630	585.923	540.023	113.376	124.343
v703	478.453	118.571	107.009	113.67
z425	250.144	43.963	132.849	239.209
z602	136.957	36.833	152.361	72.448
z704	711.849	227.348	410.672	250.067
z705	527.8	376.048	187.54	317.823

Table 3.2 Table of HF region areas. The areas have no units.

statistical analysis. Information such as aggression and depression scores was also included in the JMP analysis. The table of the data as it appears in *JMP In!* is shown in Table 3.3. This table contains the data that was used in the various statistical analyses in *Jmp In!*. The table contains the following information: HF areas for each activity; the stress-level ratings given by each subject for each of the activities performed; the depression and aggression scores of each subject taken from the Beck Depression Inventory and the aggression questionnaire (refer to Appendix B) respectively that each subject filled out on the first day (the higher the score the more depressed/aggressive a subject is); and numerical labels for the test session (1 for Session II and 2 for Session III) and order of activities (1 for breathing exercise performed first and 2 for numeric exercise performed first).

First, the data was analyzed for distribution information. This is to assess the range of values of the data. Information such as minimum, maximum and median values, and standard deviations resulted including a graphical representation of the values. This provided a better picture of the range of values for each of the activities from each subject's sessions. Table 4.1 lists the results of the distribution analysis.

Stepwise regression is an approach to selecting a subset of effects for a regression model. This *JMP In!* feature computes estimates that are the same as those of other least square platforms, but facilitates searching and selecting among many models [14]. Stepwise regression tests different combinations of predictors which provide the best fit for a regression model. For this test, the responses were the vagal tone readings of each subject for each activity. The following predictors were used to determine what model would best suit the responses: Aggression, Depression, either Session or Order, and the

interaction of Aggression/Depression and Order/Session. Session and Order were used separately because the effects of Order on the regression model would cancel the effects of Session. Session as a predictor was used to see if performing multiple tasks on different days would affect vagal tone readings. The major assumption with using Session as a predictor was that the days were identical to each other. When it is taken into account that both days were different from each other because each day had a different order of activities, then the effects of Session become moot since there is no repeatability in the days. The effects of Order already assume that each of the two days are different: each day had a different order of activities. However, if there were more than two sessions, i.e. 4, then the effects of Session would be more significant since there would be two days where the same Order was repeated.

As a result of performing the stepwise regression analysis, a two-way analysis of variance (ANOVA) is performed on the best combination of factors and/or interactions between factors that affect the respone. Interaction means that the response is not only the sum of a separate function for each term; rather each term affects the response differently depending on the level of the other term in the model being studied. An example of a situation where interaction exists as used in [14] is the cooking of popcorn. The variables of type of popcorn, whether plain or gourmet, and size of the batch of unpopped kernels control the yield of the popcorn. By varying the type of popcorn, or the size of the batch, different yield amounts of popcorn result. However, the varying sizes of the batch have different influences on the yield of the plain popcorn than on the gourmet popcorn. The variables not only influence the outcome but influence how the

Table 3.3 Data entered into *JMP In!* Statistical Analysis Software. The table contains the aggression and depression scores of each subject, the vagal tone readings for each activity, the stress level ratings for each activity as rated by the subjects, and the numerical labels for the session and order of the activities. The areas have no units.

Subject	Aggression	Depression	10	Stress	Normal	Stress	Mental	Stress	Repetition	Stress	Session	Order
			bpm	Level		Level	Arith.	Level		Level		j
				10		Norm		MA		Rept.		
d412	53	0	1684.91	8	38497.6	8	378.223	7	989.026	4	1	2
d420	53	0	1901.41	3	1739.44	3	371.878	7	491.339	5	2	1
h625	69	7	415.222	1	359.71	5	147.023	6	331.447	1	1	1
h628	69	7	710.474	1	422.35	1	402.567	8	252.514	1	2	2
j625	100	9	702.66	2	645.148	1	170.339	4	201.671	1	1	1
j627	100	9	1080.24	1	661.351	2	215.498	4	237.462	1	2	2
m508	50	1	476.156	1	218.786	2	419.947	9	420.315	4	1	1
m604	50	1	1130.69	1	648.037	1	312.601	8	362.919	4	2	2
r707	65	5	681.785	3	169.422	8	320.921	9	186.561	2	1	1
r709	65	5	268.394	1	39.868	8	174.772	9	37.106	2	2	2
s425	65	4	2076.95	3	1762.87	3	1058.52	8	798.055	7	1	1
s509	65	4	2190.45	5	2656.98	5	771.931	8	1310.3	6	2	2
s705	80	12	4662.99	7	617.161	9	312.332	7	608.223	1	1	2
s706	80	12	2087.41	3	1092.41	6	394.129	8	419.134	1	2	1
v630	75	7	585.923	1	540.023	2	113.376	7	124.343	3	1	2
v703	75	7	478.453	1	118.571	3	107.009	9	113.67	2	2	1
z425	63	8	250.144	4	43.963	6	132.849	9	239.209	2	1	2
z602	63	8	136.957	4	36.833	7	152.361	5	72.448	2	2	1
z704	80	10	711.849	5	227.348	5	410.672	6	250.067	5	1	1
z705	80	10	527.8	6	376.048	3	187.54	6	317.823	4	2	2

other variable influences the outcome; this is interaction. Interaction is important in this study in that it shows which factors affect the vagal tone readings and how they are affected. The most important part of the interaction analysis was the lack-of-fit test, which tested whether any factor or variable left out of the model is significant. The lack of fit test produced a p-value or alpha level as its result. The significance of the alpha level was the key element in determining whether a factor or interaction was neglected or whether the included factors did not affect the yield variable: a significant alpha level (0.05 or lower) denotes missing factors or interactions, while a non-significant alpha level (greater than 0.05) denotes that the factors or interactions being tested has effects on the output.

The HF region areas for stressor and relaxer activities and their counterpart were analyzed with a paired t-test analysis. The paired t-test was used because of its value in determining the correlation, if any, of a pair of measurements coming from the same experimental subject. The paired t-test tests the means of two groups of measurements over matched pairs. Responses that are positively correlated to each other will have the same means, while those that are negatively correlated will not. A typical situation that involves a pair of measurements in which a t-test is useful is a before-and-after reading from a single subject. In effect, the vagal tone readings for each pair of activities can be considered a before-and-after situation and so the paired t-test can be applied.

Other factors in the subjects' background were also taken into consideration as to their effects on the vagal tone readings. As was mentioned before, each subject's level of aggression and depression of the study was used to correlate to the readings. These two variables affect the way a person reacts to stress situations and can lead to a better understanding of reactivity. Familial history of diseases, particularly heart disease and hypertension, was also reviewed. This will hopefully show what effects these diseases have on the reactivity of a person. The effects of exercise and caffeine intake were also taken into account. All of these factors were used to correlate, if possible, any differences in the vagal tone readings between the different subjects.

CHAPTER 4

RESULTS and DISCUSSION

4.1 Distribution Results

Figures 4.1 - 4.4 show a graphical representation of the distribution of the vagal tone readings from each subject for each activity as produced by *JMP In!*. The bars represent the number of data points present in a certain value range: the taller the bar the more points in that value range. The curve that is overlaid through the bars is the normal curve, or the bell-shaped curve. The apex of the curve shows where the median of the values is. Each point in the upper box or the normal quantile box plot is a vagal tone

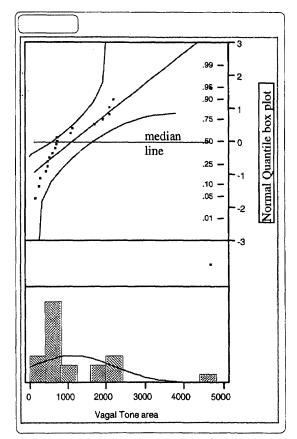


Figure 4.1 Distribution graph for 10 bpm HF vagal tone area from *Jmp In*!

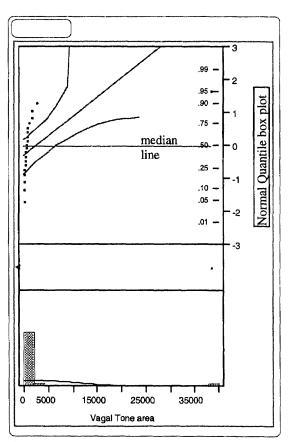
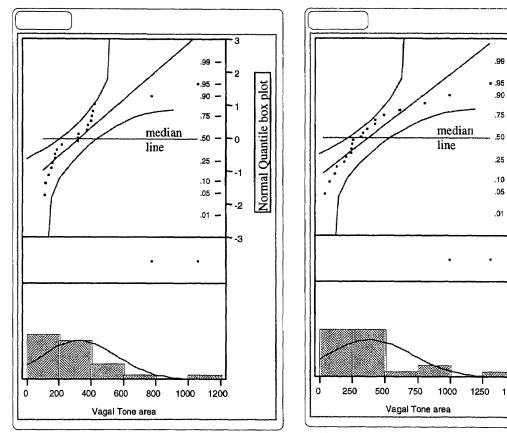


Figure 4.2 Distribution graph for Normal HF vagal tone area from *Jmp In!*

reading and the line through the .50 or 0 point on the vertical axis represents the median point of the readings. The points are vertically positioned according to how far they are from the median and are horizontally positioned by their actual values. The diagonal line shows where the points would lie if the data were normal; the curves on each side of the diagonal line are confidence limits. The slope of the diagonal line is the standard deviation of the data. Table 4.1 lists the results of the distribution analysis.

As can be seen from Table 4.1, the vagal tone readings for all the activities have high standard deviation values, with the breathing exercises surpassing the thousands range. Such high standard deviation values show the subjects' different cardiovascular



Distribution graph for

Figure 4.3

Mental HF vagal tone area Jmp In!

Figure 4.4 Distribution graph for Repetition HF vagal tone area Jmp In!

.99

.90 .75 -

.50

.25 -

.10 -.05 -

.01

1500

Normal Quantile box plot

0

-2

-3

responses to the activities; each subject has different reactivities to the stress/non-stress situations they encountered. Were the standard deviation values low, it would mean that all the subjects had similar responses to the identical activity. For the Normal activity the unusually high reading in d412, as can be seen in Table 3.3, caused the extremely high standard deviation of 8,490.20. Figures 4.1 - 4.4 show that the vagal tone readings for each activity produced non-uniform distribution curves. A possible explanation for these results is that there is an insufficient amount of data points present to obtain a uniform distribution curve.

An unusual reading occurred in the Normal activity of d412 as listed in Table 3.3. The vagal tone area reading is the highest, reaching 38,000. No information is available to explain such a high vagal tone reading (the second highest reading is 4662.99) or a high difference from the other readings. Subject "d," according to these readings, was extremely relaxed during the Normal breathing activity of Session I.

 Table 4.1 Results of Distribution Analysis for Vagal Tone readings for each activity

 from all subjects

Activity	Minimum	Maximum	Median	Mean	Std. Deviation
10 bpm	137.0	4663	706.6	1130.04	1065.80
Normal breathing	37.0	38496.0	481	2543.7	8490.20
Mental Arithmetic	107.0	1058.5	312.5	327.72	233.76
Repetition	37.1	1310.3	285.2	388.18	322.40

Surprisingly, not all the subjects found the activities easier to perform during Session II as can be seen from the decreased vagal tone readings and the increase or same stress level ratings in Session II, as listed in Table 3.3. It was expected that since the subjects already knew what the activities were and were familiar with the activities, the subjects would be more relaxed. The 10-bpm activity showed the largest number of

increased vagal tone readings from Session I to Session II (6 subjects) followed by Normal (5), MA (4), and Repetition (3). Subject "j" is the only subject that had increased vagal tone readings for all activities in Session II, meaning he was more relaxed in Session II. Eight subjects showed increased vagal tone readings from Session I to Session II in at least one activity. Of those 8 subjects, 5 subjects had an order of activities of 1 - 2 for Sessions I and II, meaning these subjects performed breathing exercises first in Session I and started with the numerical exercises in Session II. Four of those five subjects showed an increase in vagal tone readings in the breathing activities. This suggests that the 4 subjects found the breathing exercises less stressful compared to the numeric exercise they performed first, suggesting Order affects the resulting vagal tone readings.

For the breathing activities, the vagal tone readings were higher during the 10bpm compared to the Normal, as listed in Table 3.3. This trend follows the expectation of the results: since the 10-bpm activity is more relaxing than the Normal breathing activity, the vagal tone readings for the 10-bpm would be higher than for the Normal. There is one discrepancy with d412, as the 10-bpm reading is lower compared to the Normal. This occurrence can not be explained with presently available information. Fourteen (14) readings for the numerical activities were lower for the Repetition activity than the MA, which is expected since the Repetition exercise is more relaxing than the MA. Of the five subjects who did not display this trend, only subject "r" had smaller Repetition vagal tone readings occurring on both days, as seen in Table 3.3 on lines r707 and r709. These subjects were more stressed during the Repetition exercise than the MA. No definite information can be linked to these discrepancies.

4.2 Stepwise Regression Results

The stepwise regression analysis was used to determine which combination of predictors, or variables, would best fit the regression model using each of the vagal tone readings of the activities as the response. The result from a stepwise regression analysis is a list of predictors for the response used which is put through an ANOVA analysis. The results of

the stepwise regression analysis can be see in Tables 4.2a and 4.2b.

The ANOVA test was used to determine how much the predictors affect the response and to show how well the predictors fit the model. The main points to look at in the ANOVA analysis are the significance of the alpha levels (or p-values) of the whole model and the lack-of fit test, and the adjusted R^2 value. The whole-model alpha level shows the probability of the model being incorrect; it is converted to a percent value by multiplying the alpha level by 100%. A whole model alpha level of 0.056 can be read as having a 5.6% probability of being incorrect. The alpha level has to have a value of 0.05 or less to be considered significant; the lower the alpha value, the higher the probability the model is correct. The lack-of-fit test tests whether any other predictor or interaction between predictors is missing from the model. For the lack-of-fit test, it is important that its alpha value be considered non-significant, or have a value greater than 0.05. A nonsignificant alpha level denotes that no other predictor or interaction is needed for the model. The adjusted R^2 value, when multiplied by 100%, gives a percentage of how much of the variations in the model is explained by the predictors used in the model. An adjusted R^2 value of 0.651 means that 65.1% of the variations in the model is explained by the predictors. The adjusted R^2 value range from zero (0) to one (1), where 1 means 100% of the variations in the model is explained by the predictors.

As can be seen from Tables 4.2a and 4.2b, the predictors that best fit the regression for the responses are identical for the two separate analyses. The Depression state of a subject affects the vagal tone readings for all the activities except the 10-bpm activity. Stepwise regression found no predictor or interaction that would best fit the 10-bpm response in a model. For the Normal activity, Order/Session also affects the vagal tone readings, along with its interaction with Depression. The results of the analyses also show that lack-of-fit test for the Normal activity is significant when Order is used, suggesting that a missing predictor and/or interaction is missing. When Session was used the lack-of-fit test had an alpha level that was barely non-significant, which denotes that no other interaction or predictor is needed. The whole model has a significant alpha level, though it is very close to 0.05, which means that the probability that this model is

Table 4.2a Stepwise Regression analysis results using Order as a predictor. The "*" between Depression and Order denotes interaction between the two.

Activity	Predictors	ANOVA Analysis results					
		Whole Model alpha level	Lack-of-Fit alpha level	Adjusted R ²			
10-bpm	None	N/A	N/A	N/A			
Normal	Depression, Order, Depression*Order	0.0459	0.0003	0.26959			
Mental Arith.	Depression	0.1815	0.0011	0.046775			
Repetition	Depression	0.1121	0.0035	0.086152			

Table 4.2b Stepwise Regression analysis results using Session as a predictor. The "*" between Depression and Session denotes interaction between the two.

Activity	Predictors	ANO	VA Analysis results		
		Whole Model alpha level	Lack-of-Fit alpha level	Adjusted R ²	
10-bpm	None	N/A	N/A	N/A	
Normal	Depression, Session, Depression*Session	0.0564	0.0005	0.248982	
Mental Arith.	Depression	0.1815	0.0011	0.046775	
Repetition	Depression	0.1121	0.0035	0.086152	

incorrect is only 4.59% for Order, 5.64% for Session. However, the adjusted R^2 value is low; only about 27% of the variations in the model was explained by the predictors used in the regression model when Order was used and 25% when Session was used. Results show that previous activities affect vagal tone readings when measuring during a normal breathing activity. This can be seen from the fact that people do not recover from stressful activities, i.e., running for 20 minutes, in the same amount of time: some people need more time to rest while others need less.

For both of the numeric activities, the whole model alpha value is high, denoting non-significance and giving a high probability that the model is incorrect (greater than 10%). The lack-of-fit test results also show that both numeric activities have significant alpha levels, denoting that other predictors or interactions are missing. These predictors or interactions are not present in the study since this is the best fit for the model as analyzed by stepwise regression with predictors mentioned in Chapter 3. Another sign that the numeric activities models are weak is that the adjusted R^2 value is very small, denoting that only a small percentage of the variations in these models can be explained by the predictors and interactions used. The results of the analyses are a bit surprising. It was expected that Aggression would have some effect on the vagal tone readings, especially with the Mental Arithmetic activity. The expected result is that aggressive people would be better suited to dealing with stress and thereby have different readings than people who are less aggressive. However, according to these analyses, Aggression is not even a factor in affecting vagal tone readings. Order was also expected to have affected more than just one activity. On the other hand, it is not as surprising that there are no factors that affect vagal tone readings for the 10-bpm activity. According to the

analyses, the relaxing effects of being engaged in such a low-breathing rate activity effectively erase the effects of previous stressors and other activities.

4.3 Paired t-test Results

A paired t-test analysis was performed to determine if the vagal tone readings for each stressor/relaxer activity and its control-counterpart were positively correlated with each other. To make the paired t-test results useful, the vagal tone readings were put through a grouped, or independent means, t-test. The independent means t-test is used when analyzing responses from two groups that are unrelated and statistically different. The independent means t-test tests whether the difference between the means of the two data sets is significantly different from zero. If the independent means t-test has a small (<0.05) alpha level, or p-value, there is evidence that the difference between the means are significantly different from zero and that the two groups are different and unrelated. However, if the t-test has a p-value greater than 0.05, then it can be concluded that the means are not significantly different from zero and the two data sets are associated with each other. The paired t-test is more for analyzing two responses that form a pair of measurements coming from the same or related experimental subject sets. The paired ttest tests whether the mean of the difference between the two sets of data is significantly different from the hypothesized value of zero. A significant paired t-test result (an alpha level or p-value < 0.05) means the two responses are correlated and come from the same group of subjects. If the stressor/relaxer activity and its control-counterpart vagal tone readings "are positively correlated, the paired t-test gives a more significant alpha value. than the independent means t-test. If [the readings] are negatively correlated, the paired t-test will be less significant than the independent means t-test" [12]. Table 4.3 lists the results

Performing both the grouped t-test and the paired t-test, all the activities produced a non-significant alpha level. The non-significant alpha level of the independent means the t-test shows that both pairs of exercises are positively correlated with each other. In addition, the paired t-test results for both activity pairs produced a more significant alpha level than the independent grouped t-tests. This supports the positive correlation of both pairs of readings. However, although the independent means t-test results show nonsignificant alpha levels and due to the high values of non-significance in the alpha levels for the paired t-test, the readings may not have a strong correlation between each activity pair. Due to the small number of data, there might not be enough evidence to show that the readings sets have a strong positive correlation within each pair.

Activity	Alpha level (p-value) for:			
	Independent means t-test	Paired t-test		
10-bpm	0.4671	0.4624		
Normal Breathing				
Mental Arithmetic	0.5013	0.2338		
Repetition				

Table 4.3 Paired t-test analysis results

4.4 Other Factors

Other factors were also investigated in this study as to their effects on vagal tone readings. These factors were obtained from the information provided by each subject from the personal and health statistics questionnaire (refer to Appendix C for the questionnaire). Exercise, for one, did not have any effect on the readings. Subject "j" walked for 30 - 40 minutes a day at least three days a week, while subject "s" had never

exercise before. No significant differences between their vagal tone readings were apparent. It was expected that the subject who had a regular exercise routine would have a higher vagal tone reading during the MA activity than the subject who never exercised since the subject who exercised would be accustomed to stress situations. The subject with the highest aggression score (subject "j") rated the MA activity the lowest in stress level; other subjects with high aggression scores (as seen in Table 3.3) rated the MA activity similarly to the other subjects who didn't. Again, no significant differences between their vagal tone readings can be found. It was expected that those subjects with high aggression scores would rate the MA activity as not too stressful and have higher vagal tone readings for the MA activity as they would be used to stressful situations; subjects who were not as aggressive were expected to have the opposite effect. Other factors such as regular caffeine and alcohol intake and smoking and familial history of diseases also did not produce any significant results in the vagal tone readings. Table 4.4 lists information from each subject that was thought to be most pertinent to this study. The information here was not used in any statistical analysis since it was not known how to quantify the information to fit any analysis. However, the available information was examined as a possible explanation to any discrepancies found within the vagal tone readings for each activity. None were found.

Additional analysis was performed while excluding the subjects who did not meet the subject critera as stated in Section 2.1.1 and the subject who had a very high vagal tone reading during the Normal breathing activity. No improvement was seen in the stepwise regression analysis results. The results were either comparable or degraded to the results using all the subjects.

Subject (Sessions)	Exercise	Caffeine Intake	Alcohol Intake	Smoking	Familial History of Disease
d (d412/d420)	1-2 times /week	None	None	None	asthma in parents; sibling
h (h625/h628)	1 – 2 times /week	None	None	None	history of cancer in mother; grandparents; subject takes medication for allergy_
j (j625/j627)	30 – 40 min/day walking	None	None	None	maternal grandfather had heart attack; father had hyperthyroid
m (m508/m604)	1 – 2 times /week	3 – 4 cups / day	None	None	None
r (r707/r709)	Never exercised	None	None	None	maternal grandfather; mother had heart disease
s1 (s425/s509)	1 – 2 times /week	None	1 – 2 cups within past 24 hours	None	maternal grandfather; father had heart disease; sibling has asthma
s2 (s705/s706)	1 – 2 times /week	3 – 4 cups / day	None	more than 4X / day	mom has high blood pressure; asthma in family
v (v630/v703)	1 – 2 times /week	None	None	None	father has high blood pressure
z1 (z425/z602)	1 – 2 times /week	1 – 2 cups within past 24 hours	None	None	None
z2 (z704/z705)	1 – 2 times /week	3-4 cups within past 24 hours	None	None	grandfathers had heart disease; mom has high blood pressure

Table 4.4 Information about each subject from personal and health statistics questionnaire

CHAPTER 5

CONCLUSION

The objective of this study was to investigate what variables affect the reactivity of a person to the stresses introduced to the person's environment. Results from this pilot study show that only the subject's depression state had any effects on the vagal tone readings for three of the activities: Normal breathing, Repetition, and Mental Arithmetic. No factors affected the vagal tone readings for the 10-bpm activity. The Normal breathing activity was also affected by the Order of the activities, performing the activities on different Sessions, and the interaction of each with Depression. Taking into account the depression state of a subject would lead to more accurate data when performing studies that involve vagal tone readings, i.e., HRV studies. Also, when taking vagal tone measurements during normal breathing, i.e., for baseline recording, since Order and Session were factors that affect the vagal tone measurement, it is recommended that the measurement be taken before any other activity is performed.

The main deterrent to formulating any concrete conclusion is the small number of subjects used for this study. No concentrations of values in specific ranges were present whose differences between other values were attributable to at least one of the factors that was being investigated. Because of the small number of subjects, there was too much variety and range in the values and not enough similarities, as was seen in the distribution results. Even if there were similarities present, those similarities can not be explained by any information that was gathered about the subject and appear only as random similarities.

To determine a suitable number of subjects for future studies, a power analysis is performed on the existing statistical data. Power analysis was performed using a program designed specifically for power analysis, G^*Power [3]. For the ANOVA analysis to have an alpha level, or p-value, of at least .05 for significance, the number of subjects performing the breathing exercises (10-bpm and Normal breathing) have to be at least 54 while the number of subjects needed to perform the numeric exercises (MA and Repetition) is 76. These results are based on the assumptions that the values of the mean for each group of data from each activity (Refer to Table 4.1) stays the same, the standard deviations (Refer to Table 4.1) for each group is approximately 2000 for the 10bpm/Normal pair and 100 for MA/Repetition pair, and that each subject performs the activities in two sessions.

A number of issues from this study can be further investigated in future studies. An investigation on what other factors that affect any of the activities should be performed. More detailed information about a subject's personality and background, i.e., job stress or personal stress, could be used as a source for additional factors. The question as to the exact nature of how Depression affects vagal tone readings needs to be answered. That answer is not clear in this study since the differences in vagal tone readings cannot be linked to Depression scores. The biggest change that must take place in such an investigation, which could also answer some of the questions from this study, is an increase in the number of subjects. Other studies have had subject groups that are greater than 20 [1] [10] [14]. The information garnered from a more complete and thorough investigation will impact similar studies and how they are conducted to optimize data acquisition in heart rate variability studies.

APPENDIX A

MatLab PROGRAM USED IN THIS ANALYSIS WITH EXPLANATORY

COMMENTS.

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% this program. This program takes recorded data of EKG, synch pulse, and

% respiration and displays it in a graph format.

clear clc	% Clears all values from program memory.% Clear command window. CLC clears the% command window and homes the cursor.
global signal peaks RESPs	 % Defines the global variables: signal, peak % and RESPs. These variables will be % available for use to other functions.
<pre>bpm=input('Is this 10 BPM file? ','s'); if bpm == 'y' LF = 1; else LF = 0; end</pre>	 % User input as to the nature of the data. % Due to the nature of the 10-bpm data, % its HF region is calculated differently % from the other activities.

[ECGs,RESPs] = getdata; % Calls the function getdata. [signal,peaks] = getpeaks(ECGs,FirstStep); % Calls the function getpeaks.

clear ECGs ucontrol ; display ;

FirstStep=250;

- % Clears the value from the variable ECGs.
- % Calls the function ucontrol.
- % Calls the function display.

% This function gets the data that is stored as a computer file in the computer. The % measurements are stored in the computer as a file with six columns of numbers, each % column representing a different channel. The measurements are taken by measuring % the signals at a sample rate of 200 Hz. This means the computer recorded the value of % the signal being measured 200 times in one second and stored it in its proper column. % The file is then saved as a simple ASCII text file. This function returns the two % signals in ECGsig and RESPsig to the main program and stored in the variables ECGs

% and RESPs.

```
function [ECGsig,RESPsig] = getdata
```

```
[fname,pname]=uigetfile('*.txt','Open Data File "ASCII" ');
% Command to get the data file
```

if isstr(fname) == 0 disp(' Cannot find file') dbquit end	 % Tests to see if filename exists % in the present directory. If not % the program quits with an error % message.
Filename = [pname fname];	% An existing file is being used.% pname is the path name.
<pre>load(Filename) ; fname = strtok(fname,'.'); k=eval(fname);</pre>	 % The file is loaded into memory. % The .extension is dropped % A MatLab command that % evaluates the validity of the file, % i.e., file is in the correct format.
ECGsig=k(:,2);	% ECG signal is located on channel,% or column two.
RESPsig=k(:,1);	% Respiration signal is stored in

clear pname Filename k

% channel 1.

% This function will calculate where the peaks are in the EKG signal. These peaks

% correspond to the R wave. The distance between these R waves helps create the HRV

% spectrum.

function [signal,peaks] = getpeaks(signal,FirstStep)

signal=signal(:); V = mean(signal(2:length(signal))); % Calculates the mean of values of the EKG. sig=rot90(signal); % Rotates the matrix by 90°. sig(1) = V; % Modify first point.

2
l 4 points

clear A B C V

V = mean(m); A = [V m V]; B = [V V m]; C = [m V V]; s = A + B + C; s=s(2:length(s) - 1)/4; clear A B C V

LENGTH=length(s); TOP=max(s); [MAX,k]=max(s(1:FirstStep)); peaks=zeros(1,LENGTH); peaks(k)=TOP;

%%%%%%%	testing for the peaks	%%%%%%%%
N1 = 200 ;		% This variable is to be used for interval% threshold.
n=fix(0.2*(k + N1) i=k+n;));	% The initial count of points per cycle.
THERMOMETER	t = bar(0, Detecting the H)	Peaks '):
		% The 'Thermometer' is a real-time
		% graphical meter displaying the amount of
		% task completed.
while i < LENGT	н	
	% increment count	
n=n+1;		
	.25*MAX + 0.2*TOP	% Check for threshold
[M.	AX,T] = max(s(i:min(i+1)))	0,LENGTH)));
N=	n+T-1;	% update current interval
pea	ks(i+T-1)=TOP;	% a peak
n=f	ix(0.2 *(N + N1));	
	+T+n-1;	% Jump forward
	= N;	% previous interval
bar	(i/LENGTH)	% update THERMOMETER

end

end close(THERMOMETER);

signal=sig - min(sig)+TOP;

clear m s MAX TOP k n N LENGTH

% This function displays the signal taken by the getpeaks function and displays it. % Along with the signal, another graph of spikes is displayed. Each spike correspond to % a peak In the signal.

function ucontrol

global SLIDER PATCH signal peaks

LENGTH=length(signal);

PATCH = 700;

h=figure;

whitebg

% Background of the graph is default white

% This list of commands creates a user interface control with the MatLab command

% uicontrol. Three buttons appear on top of the graph: correct, done, and background.

% The first two call other functions. The background button changes the background of % the graph.

SLIDER=uicontrol('Style', 'Slider', 'Position', [0 0 600 16],...

'Max',[LENGTH],'Min',[PATCH+1],'Value',PATCH+1,'Callback','display');

PUSHBUTTON1=uicontrol('Style', 'Pushbutton', 'Position', [430 352 60 20],...

'String', 'Correct', 'BackGroundColor', 'w', 'ForeGroundColor', 'r', 'Callback', 'correct');

PUSHBUTTON2=uicontrol('Style','Pushbutton','Position',[340 352 60 20],...

'String', 'Done', 'BackGroundColor', 'w', 'ForeGroundColor', 'r', 'Callback', 'upslwsu');

PUSHBUTTON3=uicontrol('Style', 'Pushbutton', 'Position', [2 352 84 22],...

'String', 'Background', 'BackGroundColor', 'w', 'ForeGroundColor', 'r', 'Callback',

'whitebg');

MENU=uimenu('Label','Work Space','Position',5);

uimenu(MENU,'Label','Whole signal','Position',1,'Callback','allsig'); uimenu(MENU,'Label','Doublicate','Position',2,'Callback','gdouble'); uimenu(MENU,'Label','One Half','Position',3,'Callback','ghalf');

clear LENGTH PATCH Signal Peaks

- % This function processes the data by calling several functions and terminates by
- % plotting 3 plots required for the study. The variables available for use and for further
- % processing are IBI, IIBI, respiration, and frequency and spectrum of both IIBI and % respiration.
- % pi and fi are the power and the frequency of IIBI respectively.
- % pr and fr are the power and the frequency of respiration respectively.

global peaks RESPs clear g ibi rsp rpd iibi % Closes current figure close(gcf) tit le = ';peaks=peaks(:) ; % Rotates the matrix 90°. peaks=rot90(peaks); [g,ibi,rpd]=grep(peaks,RESPs); % Calls the function grep. clear peaks x=iibi(g,ibi); % Calls the function iibi. clear g [iibi,rsp]=seq(x,rpd); % Calls the function seq. clear x rpd % Calls the function ugraph. [pi,pr,fi,fr] =ugraph(iibi,rsp,ibi,tit_le,LF); stdgraff(pi,fi,pr,fr,tit_le); % Calls the function stdgraff % Prints the elapsed time. toc

% This function detects Interbeat interval(IBI) and extracts the respiration signal (rpd).

% The IBI is the given time between each beat. The height of the spike corresponds to % the length of time between beats.

function [g,ibi,rpd]= grep(file1,file2) % The EKG or synch pulse is stored jks=file1; in the % variable jks. g=diff(jks); % Calculates the column of matrix % differences. % For-end loop to detect IBI. j=0; for i = 1:1:length(g)if(g(i) > 0)j=j+1; ibi(j)=i; end end % The IBI signal is stored in the ibi=diff(ibi); % variable ibi. rpd = file2;% The respiration signal is stored in % the variable rpd. clear jks j i

% This function generates the Interpolated Interbeat Interval, or the IIBI. The spectrum % of the IIBI is also known as the HRV spectrum.

```
function x = iibi(g,ibi)

j=0;

s=length(g)-max(size(ibi))-2;

x(s)=0;

h=bar(0,'finding iibi....');

for i=1:1:length(ibi)

n=round(ibi(i));

for k =1:1:n

j=j+1;

x(j)=ibi(i);

end

bar(i/length(ibi));

end

close(h);

clear j t s n
```

% This function generates decimated Interpolated IBI and decimated respiration signal.

```
function[iibi,rsp]=seq(x,rpd)

q=1;

for(p=1:10:length(x))

iibi(q) = x(p);

q=q+1;

end

w=1;

for(r=1:10:length(rpd))

rsp(w)=rpd(r);

w=w+1; % For Respiration

end

clear q p w r
```

-

 $\%\,$ This function plots the Interbeat interval and Respiration plots. Here the function

% spectrak is called to get the power spectrum values. The parameter 0 passed is

% no overlap (number of overlapping section), the 20 is the decimated sampling

% frequency, 8192 is the best number point FFT.

function[p_ii,p_r,f_ii,f_r] = ugraph(iibi,rsp,ibi,tit_le,LF)

[p_ii,f_ii]=rspect(iibi,8192,0,hanning(length(iibi)),20);

[p_r,f_r]=rspect(rsp,8192,0,hanning(length(rsp)),20);

```
figure('Name','IBI : IIBI : Respiration')
```

z=1:length(iibi);

subplot(311)

```
plot(ibi/200,'.b');
xlabel('Beat Number'), ylabel('IBI'),
title('Inter beat interval');
```

```
subplot(312)
```

plot(z,iibi/200,'b'); xlabel('Time'), ylabel('IIBI'), title('IIBI');

subplot(313)

plot(rsp,'b'); xlabel('Time'), ylabel('Resp'), title('Resp');

figure('Name',' Power spectrum Vs Frequency')

subplot(211)

```
f_iic=f_ii(20:500,:);
p_iic=p_ii(20:500,:);
areain=p_iic/200;
ymax=max(p_iic/200);
ymin=min(p_iic/200);
plot(f_iic,p_iic/200,'r');
```

```
axis([0 0.6 ymin ymax]);
```

xlabel('Frequency')
ylabel('IIBI Power');
title(tit_le);

% This is where the HF and LF areas are calculated. Also, here is where the input about

% the 10-bpm file applies. If the file is a 10-bpm file, then LF = 1. This is to more

% accurately reflect the affects of the 10-bpm activity, which borders on the HF and LF % regions.

if LF == 1

a1=area(0.05,0.12,areain); a2=area(0.12,0.4,areain);

elseif LF == 0

a1=area(0.05,0.15,areain); a2=area(0.15,0.4,areain);

end

gtext(sprintf('%g',a1))
gtext(sprintf('%g',a2))

- % Enables the user to place the HF and LF
- % area values on the graphs with the
- % mouse.

subplot(212)

f_rc=f_r(20:500,:); p_rc=p_r(20:500,:); ymin=min(p_rc/200); ymax=max(p_rc/200); plot(f_rc,p_rc/200,'g:'); axis([0 0.6 ymin ymax]); xlabel('Frequency') ylabel('RESP Power');

% Power spectrum of Respiration.

% This function plots the spectrum plot of IIBI and Respiration in single plot. The scale % of only IIBI has been made visible. The spectrum plot of the IIBI is also known as the % HRV spectrum.

function stdgraff(pi,fi,pr,fr,tit_le)

clear h1 figure('Name','Power Spectrum : IIBI and Respiration') rect=[.1,.1,.8,.8]; h1=axes('position',rect); plot(fi,pi/200,'r'); %ymin=min(pi/200); %ymax=max(pi/200); %axis([0 0.5 ymin ymax])

axes('position',rect);
plot(fr,pr/200,'g:');

set(gca,'visible','off'); title(tit_le) ylabel('Power') xlabel('Frequency')

gca=h1;

set(gca,'fontsize',10)
set(gca,'fontweight','bold')

 $\%\,$ This function corrects the spikes that correspond to the signal peaks. It gives the user

% the ability to add spikes missed or remove extra spikes.

```
global PATCH peaks signal SLIDER
```

% By clicking on the screen on the left and right side of where a spike is or should be, % the user can add or remove a spike.

```
[Xcord,y] = ginput(2); % Graphical input from the mouse.
current = round(get(SLIDER,'Value'));
LEFT = current - PATCH + fix( min(Xcord(1),Xcord(2)));
RIGHT = current - PATCH + ceil(max(Xcord(1),Xcord(2)));
```

[MAXPEAK,LOCAT] = max(peaks(LEFT:RIGHT));

```
if MAXPEAK > 0
peaks(LEFT + LOCAT -1) = 0;
```

else

```
[T,LOCAT]= max(signal(LEFT:RIGHT));
peaks(LEFT + LOCAT - 1) = max(peaks);
```

end

```
clear current LEFT RIGHT LOCAT
```

display

% Display the corrected signal.

APPENDIX B

3

QUESTIONNAIRES USED IN PRESENT STUDY

B.1 Persoanl and Health Statistics Questionnaire Personal and Health Statistics

1. What is your date of birth?

2. How much do you weigh?

3. Have you exercised in the past 24 hours?[] yes []no

4. How often have you exercised in the past week? [] never []1-2 times/week []3-4 times/week []5-7 times per week

5. How often have you exercised in the past year? [] never []1-2 times/week []3-4 times/week []5-7 times per week

6. How many caffeinated beverages do you drink per day? [] none [] one-two [] three-four [] more than four

7. How many caffeinated beverages have you drank in the past 24 hours? [] none [] one-two [] three-four [] more than four

8. How many cigarettes or other nicotine products do you use per day? [] none [] one-two [] three-four [] more than four

9. How many cigarettes or other nicotine products have you used in the past 24 hours? [] none [] one-two [] three-four [] more than four

10. How many times/week do you drink alcoholic beverages? [] never []1-2 times/week []3-4 times/week []5-7 times per week

11. How many alcoholic beverages have you had in the past 24 hours? [] none [] one-two [] three-four [] more than four

12. Do you have high blood pressure?

[] I am certain that I have high blood pressure

[] I am certain that I don't have high blood pressure

[] I think that I do have high blood pressure

[] I think that I do no have high blood pressure.

[] I don't know

13. Does your mother have high blood pressure?

[] I am certain that she has high blood pressure

[] I am certain that she doesn't have high blood pressure

[] I think that she does have high blood pressure

[] I think that she doesn't have high blood pressure.

[] I don't know

14. Does your father have high blood pressure?

[] I am certain that he has high blood pressure

[] I am certain that he doesn't have high blood pressure

[] I think that he does have high blood pressure

[] I think that he doesn't have high blood pressure.

[] 1 don't know

15. Do any of your siblings have high blood pressure

[] I am certain that at least one of my siblings has high blood pressure

[] I am certain that none of my siblings have high blood pressure

[] I think that at least one of my siblings have high blood pressure

[] I think that none of my siblings have high blood pressure.

[] I don't know

[] I have no siblings

heart disease	definitely yes	definitely not	l think so	I think not	I don't know	comments:
auto- immune disorder	definitely yes	definitely not	I think so	I think not	I don't know	comments:
asthma	definitely yes	definitely not	I think so	I think not	l don't know	comments:
hyper- thyroid	definitely yes	definitely not	I think so	I think not	I don't know	comments:
hypo- thyroid	definitely yes	definitely not	I think so	I think not	I don't know	comments:
Raynaud's Disease/ Syndrome	definitely yes	definitely not	I think so	I think not	l don't know	comments:
diabetes	definitely yes	definitely	I think so	I think not	I don't know	comments:
respiratory illness	definitely yes	definitely not	I think so	I think not	I don't know	comments:
other serious illness (please specify)	definitely yes	definitely not	I think so	I think not	I don't know	comments:
Cancer	+	1	1		1	1

16. Do you have any history of the following (check appropriate box for each condition listed)

17. Specify whether any of your grandparents have/had any of the following conditions? In comments please specify whether referring to maternal grandmother, maternal grandfather, paternal grandmother, or paternal grandfather.

heart	definitely	definitely	I think	I think not	I don't know	comments:
disease	yes	not	SO		KIIOW	
auto- immune disorder	definitely yes	definitely not	I think so	1 think not	I don't know	comments:
asthma	definitely yes	definitely not	I think so	I think not	1 don't know	comments:
hyper- thyroid	definitely yes	definitely not	I think so	I think not	I don't know	comments:
hypo- thyroid	definitely yes	definitely not	I think so	I think not	I don't know	comments;
Raynaud's Disease/ Syndrome	definitely yes	definitely not	I think so	I think not	I don't know	comments:
diabetes	definitely yes	definitely not	I think	I think not	I don't know	comments
respiratory illness	definitely yes	definitely not	I think so	I think not	I don't know	comments:
other serious illness (please specify)	definitely yes	definitely not	I think so	I think not	I don't know	comments
lancer			-1			

18. Specify whether your mother has/had any of the following:

heart	definitely	definitely	I think	I think not	I don't	comments:
disease	yes	not	SO		know	
auto- immune disorder	definitely yes	definitely not	I think so	I think not	I don't know	comments:
asthma	definitely yes	definitely not	I think so	1 think not	I don't know	comments:
hyper- thyroid	definitely yes	definitely not	I think so	I think not	I don't know	comments:
hypo- thyroid	definitely yes	definitely not	I think so	I think not	I don't know	comments:
Raynaud's Disease/ Syndrome	definitely yes	definitely not	I think so	I think not	I don't know	comments:
diabetes	definitely yes	definitely not	I think	I think not	I don't know	comments:
respiratory illness	definitely yes	definitely not	I think so	1 think not	I don't know	comments:
other serious illness (please specify)	definitely yes	definitely not	I think so	I think not	I don't know	comments:
Cancer			1			

19. Specify whether your father has/had any of the following:

heart	definitely	definitely not	I think	I think not	I don't know	comments:
disease	yes	not	SO		KIIOW	
auto- immune disorder	definitely yes	definitely not	I think so	I think not	I don't know	comments:
asthma	definitely yes	definitely not	I think so	I think not	I don't know	comments:
hyper- thyroid	definitely yes	definitely not	I think so	I think not	I don't know	comments:
hypo- thyroid	definitely yes	definitely not	I think so	I think not	I don't know	comments;
Raynaud's Disease/ Syndrome	definitely yes	definitely not	I think so	I think not	I don't know	comments:
diabetes	definitely yes	definitely not-	I think so	I think not	I don't know	comments
respiratory illness		definitely not	I think so	I think not	I don't know	comments
other serious illness (please specify)	definitely yes	definitely not	I think so	I think not	I don't know	comments
Concer	1					

20. Specify whether siblings have/had any of the following

heart	definitely	definitely	I think	I think not	I don't	comments:
disease	yes	not	SO		know	
auto- immune disorder	definitely yes	definitely not	I think so	I think not	I don't * know	comments:
asthma	definitely yes	definitely not	I think so	I think not	I don't know	comments:
hyper- thyroid	definitely yes	definitely not	I think so	I think not	I don't know	comments:
hypo- thyroid	definitely yes	definitely not	I think so	I think not	I don't know	comments:
Raynaud's Disease/ Syndrome	definitely yes	definitely not	I think so	I think not	I don't know	comments:
diabetes	definitely yes	definitely not	I think so	I think not	I don't know	comments:
respiratory illness	definitely yes	definitely not	I think so	I think not	I don't know	comments:
other serious illness (please specify)	definitely yes	definitely not	I think so	I think not	I don't know	comments:

,

21. Do you regularly take prescription medication for a medical condition? [] yes []no

22. If yes, specify the nature of the medical condition and the medication that you take.

23. Are you temporarily on prescription medication for an infection or virus or other short term illness or allergy (If yes, please write down the name of the medication)
 (1) yes []no

24. Have you been on prescription medication in the past month for an infection, virus or some other short term illness or allergy? (If yes, please write down the name of the medication)

[] yes []no

25. Have you taken any non-prescription medication in the past 24 hours? (If yes, please write down the name of the medication)

[] yes {] no

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Aggression Questionnaire

For the following items please rate how characteristic each is of you. Using the following rating scale record your answer in the space to the left of each item.

- 1 = Extremely uncharacteristic of me
- 2 = Somewhat uncharacteristic of me
- 3 = Only slightly characteristic of me
- 4 = Somewhat characteristic of me
- 5 = Extremely characteristic of me
- 1. Once in a while I can't control the urge to strike another person.
- 2. I tell my friends openly when I disagree with them.
- _____ 3. I flare up quickly but get over it quickly.
- _____ 4. I am sometimes eaten up with jealousy.
- 5. Given enough provocation, I may hit another person.
 6. I often find myself disagreeing with people.
 7. When frustrated, I let my irritation show.

- At times I feel I have gotten a raw deal out of life.
- 9. If somebody hits me, I hit back.
- 10. When people annoy me, I may tell them what I think of them.
- _____ 11. I sometimes feel like a powder keg ready to explode.
- _____ 12. Other people always seem to get the breaks.
- _____ 13. I get into fights a little more than the average person.
- I can't help getting into arguments when people disagree with me. _____ 14.
- _____ 15. Some of my friends think I'm a hothead.
- I wonder why sometimes I feel so bitter about things.
- If I have to resort to violence to protect my rights, I will.
- _____ 18. My friends say that I'm somewhat argumentative.
- Sometimes I fly off the handle for no good reason.
- I know that "friends" talk about me behind my back. _____ 20.
- There are people who pushed me so far that we came to blows.
- _____ 22. I have trouble controlling my temper.
- _____ 23. I am suspicious of overly friendly strangers.
- I can think of no good reason for ever hitting a person. _____ 24.
- I sometimes feel that people are laughing at me behind my back. _____ 25.
- _____ 26. I have threatened people I know.
- ____ 27. When people are especially nice, I wonder what they want.
- 28. I have become so mad that I have broken things.
- _____ 29. I am an even-tempered person.

B.4 Subject's Defensiveness State Questionnaire Marlowe-Crowne Scale

Listed below are a number of statements concerning personal attitudes and traits. Read each item and decide whether the statement is true or false as it pertains to you.

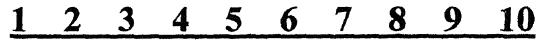
	-	a more that the state of the state of the
T	F	 Before voting I thoroughly investigate the qualifications of all the candidates.
т	F	I never hesitate to go out of my way to help someone in trouble.
т	F	 It is sometimes hard for me to go on with my work if I am not encouraged.
T	F	4. I have never intensely disliked anyone.
T	F	5. On occasion I have had doubts about my ability to succeed in life.
T	F	6. I sometimes feel resentful when I don't get my way.
T	F	7. 1 am always careful about my manner of dress.
T	F	 My table manners at home are as good as when I eat out in a restaurant.
T	F	If I could get into a movie without paying and be sure I was not seen, I would probably do it.
T	F	 On a few occasions, I have given up doing something be- cause I thought too little of my ability.
Ŧ	F	11. 1 like to gossip at times.
۲	F	12. There have been times when I felt like rebelling against people in authority even though I knew they were right.
Т	,F	13. No matter who I'm talking to, I'm always a good listener.
T	F	14. 1 can remember "playing sick" to get out of something.
.T	F	 There have been occasions when I took advantage of someone.
Т	F	16. I'm always willing to admit it when I make a mistake.
T	F	17. Lalways Iry to practice what Lpreach.
T	F	 I don't find it particularly difficult to get along with loud- mouthed, obnoxious people.
T	F	19. I sometimes try to get even, rather than forgive and forget.
T	F	20. When I don't know something I don't at all mind admitting it.
T	F	 21. 1 am always courteous, even to people who are disagree- able.
T	F	22. At times I have really insisted on having things my own way.
T	F.	23. There have been occasions when I felt like smashing things.
T	F	 I would never think of letting someone else be punished for my wrongdoings.
7	F	25. I never resent being asked to return a favor.
T	F	 1 have never been irked when people expressed ideas very different from my own.
Ţ	F	 1 never make a long trip without checking the safety of my car.
Ť	F	 There have been times when I was quite jealous of the good fortune of others.
T.	F	29. I have almost never felt the urge to tell someone off.
r	F	30. I am sometimes irritated by people who ask favors of me.
т	F	31. I have never felt that I was punished without cause.
т	F	32. I sometimes think when people have a misfortune they only got what they deserved.
T	F	33. Thave never deliberately said something that hurt someone's feelings.

Activity Stress Level Rating

Indicate on a scale from 1 - 10: How stressful was the task you just completed ? 1= relaxing 5= not relaxing and not stressful 10 = very stressful $1 \ 2 \ 3 \ 4 \ 5 \ 6 \ 7 \ 8 \ 9 \ 10$

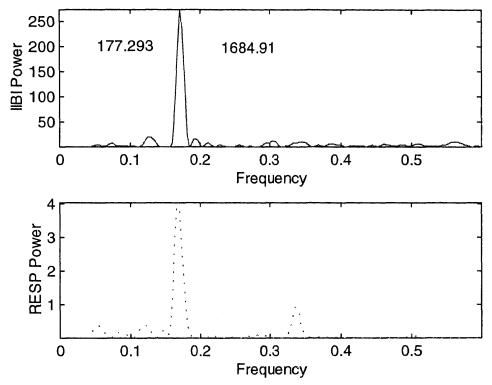
Indicate on a scale from 1-10: How difficult was the task you just completed? 1=easy

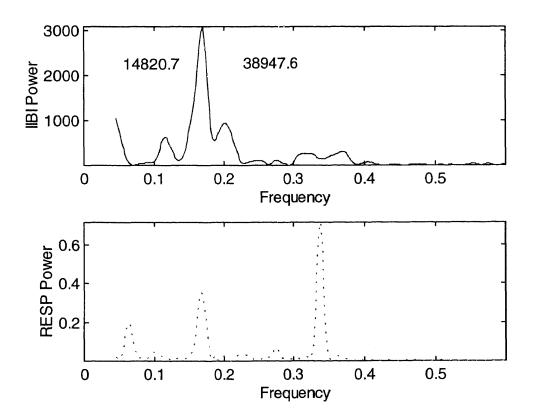
10= very difficult

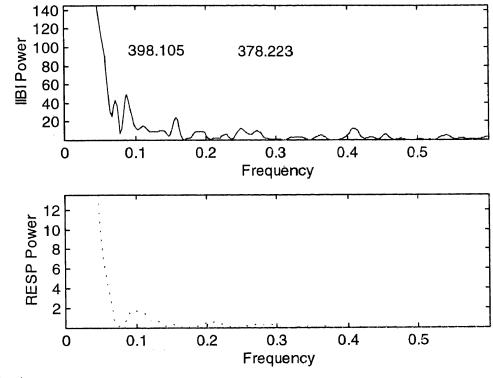


APPENDIX C

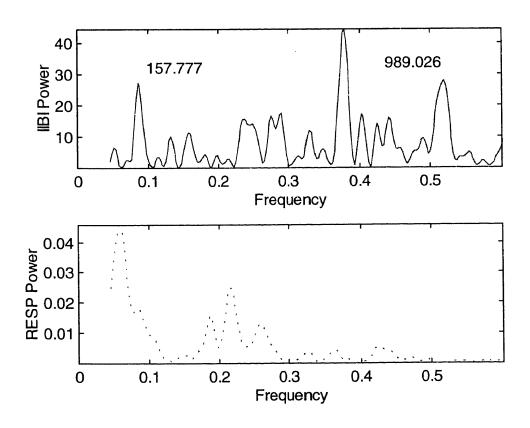
HRV POWER SPECTRUM GRAPHS FOR ALL SUBJECTS

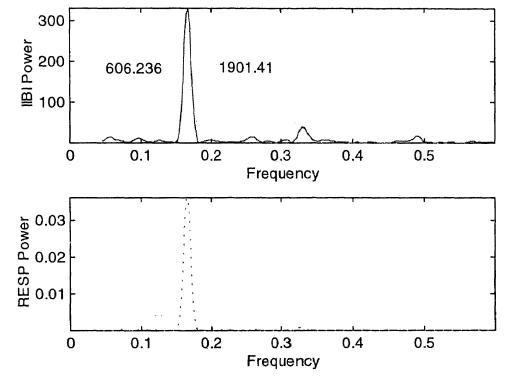


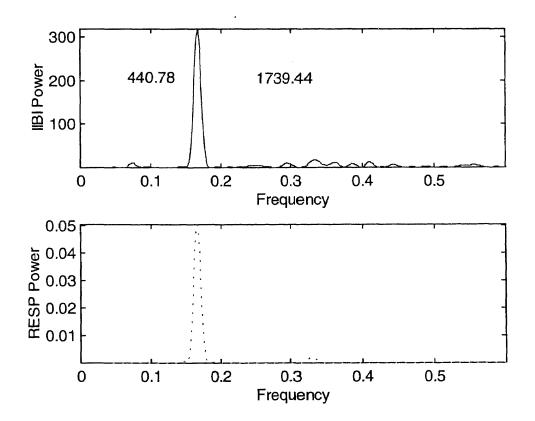


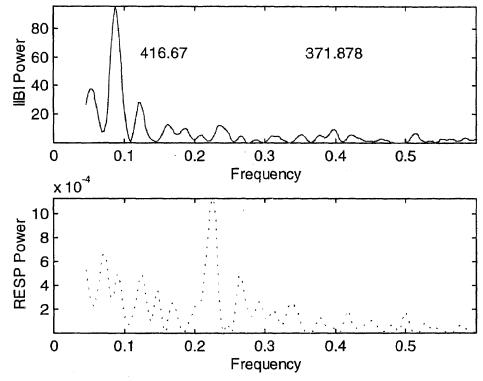




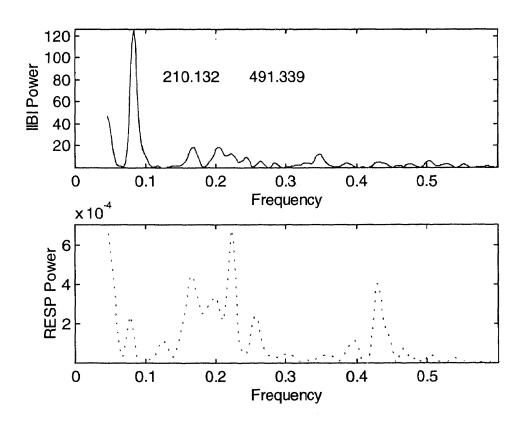


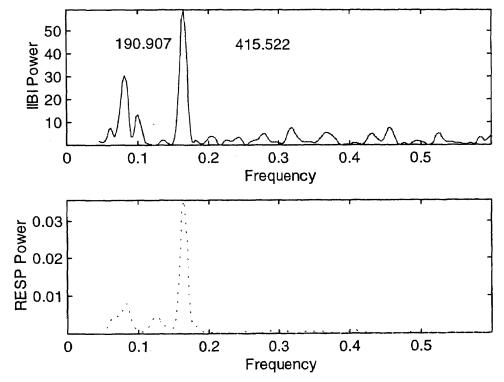


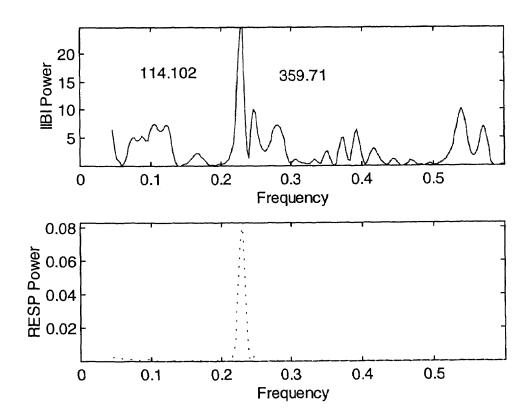


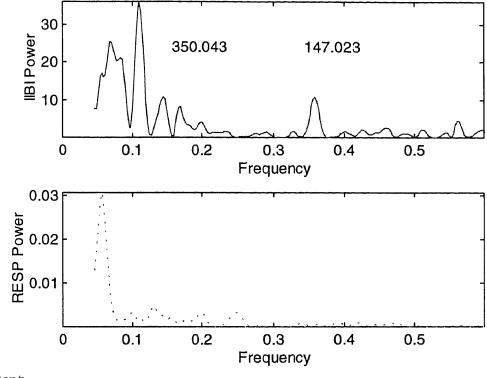


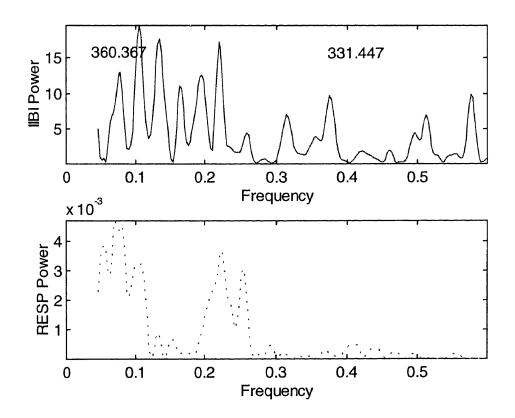


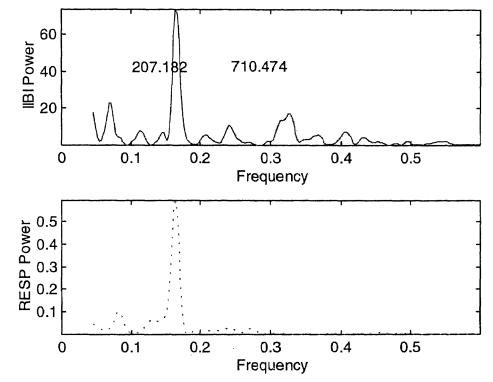


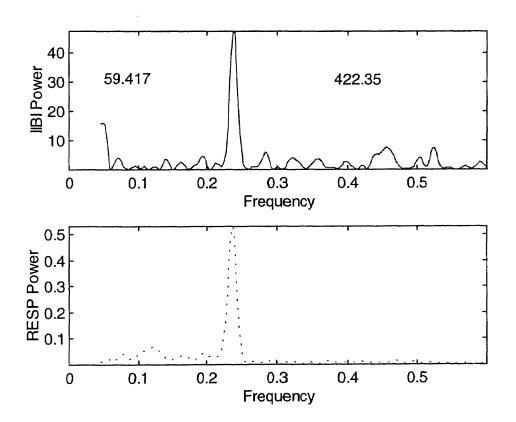


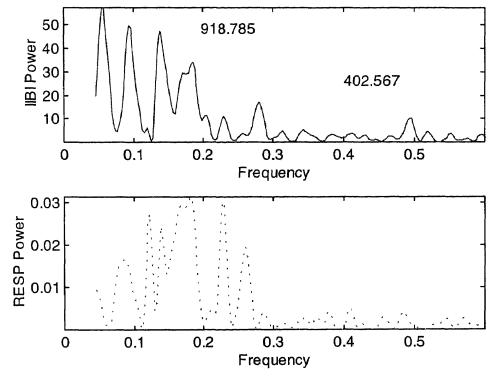


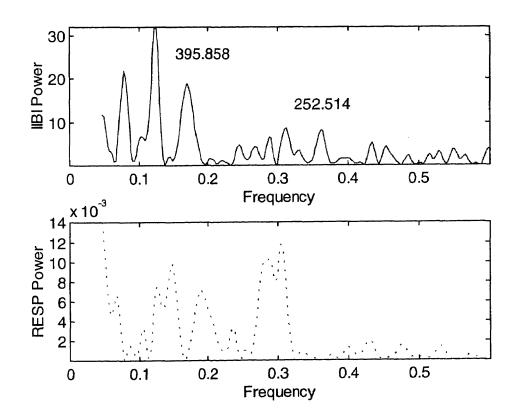


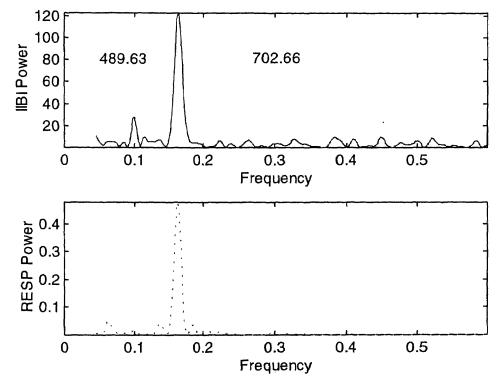


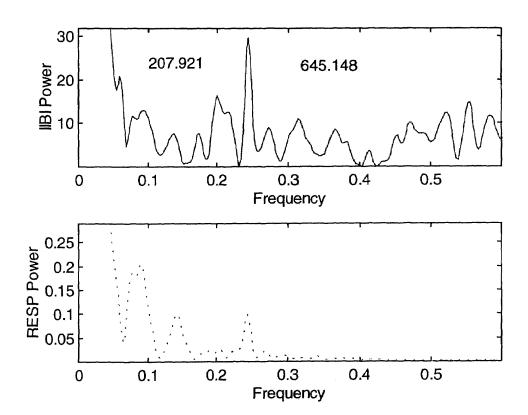


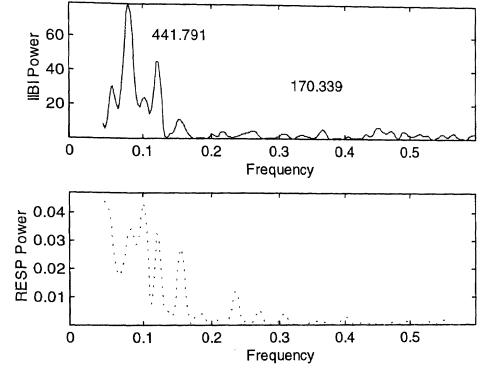


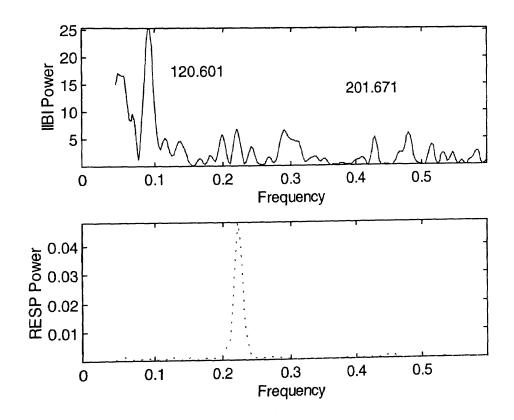


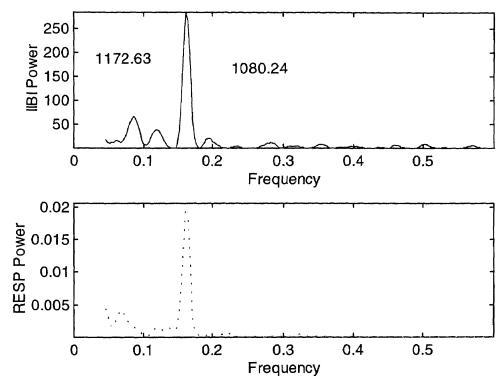


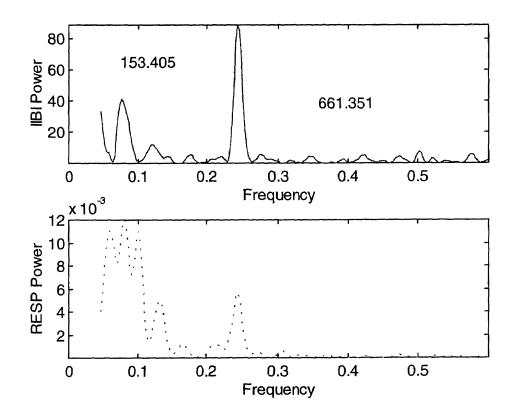


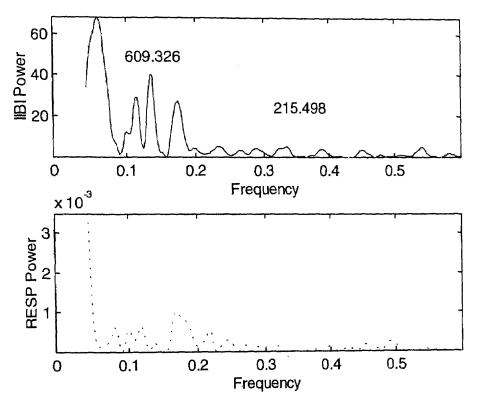


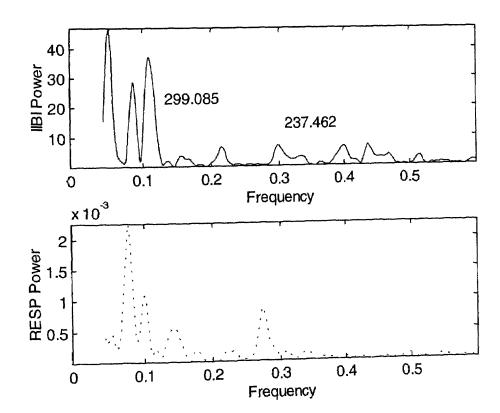


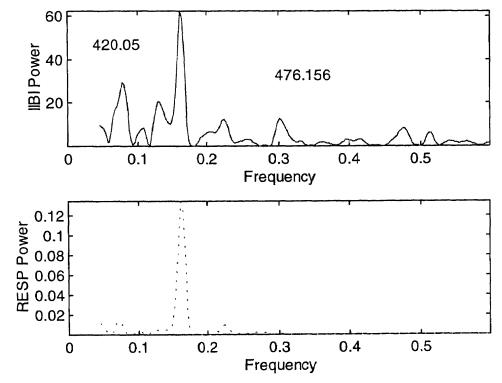


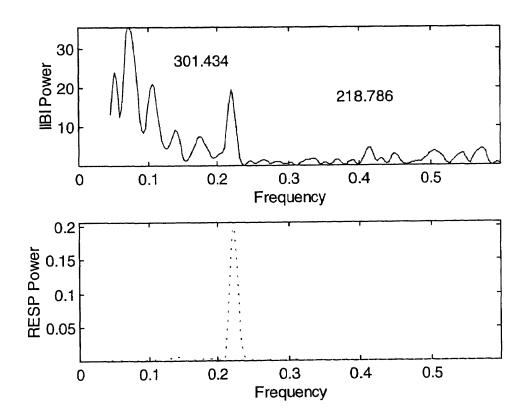


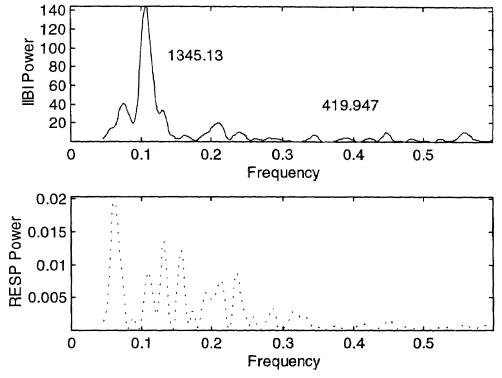


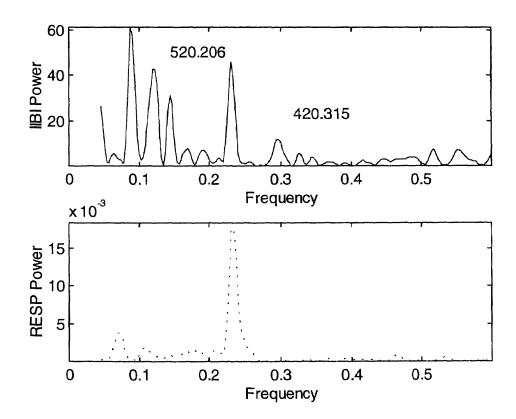


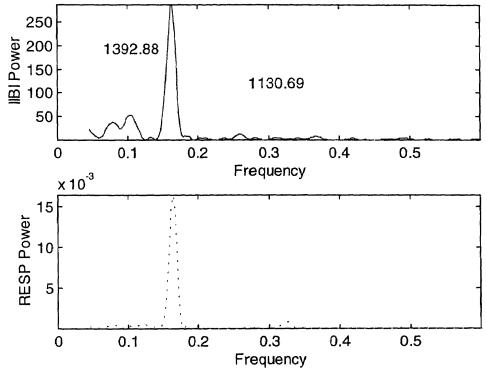


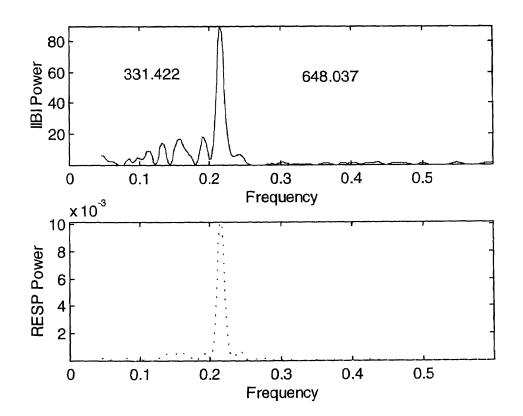


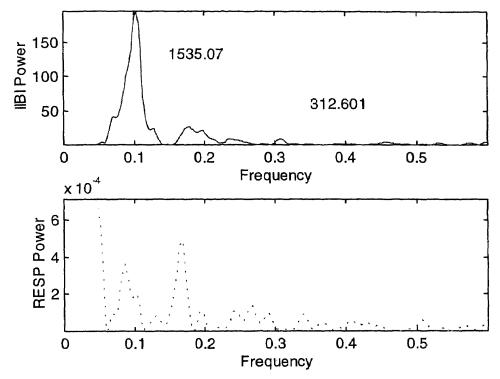


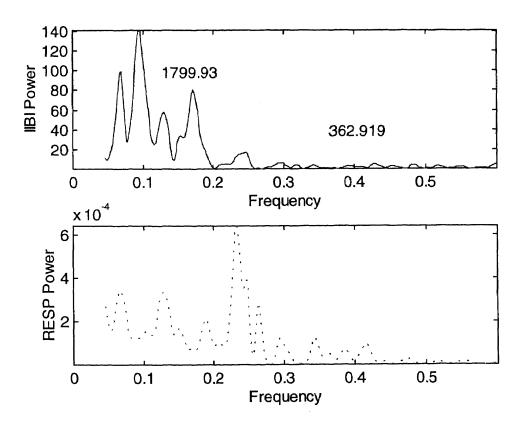


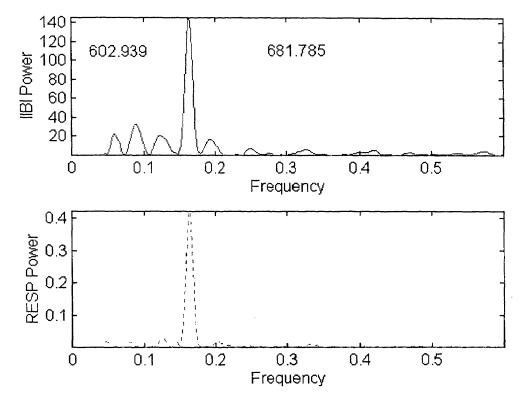


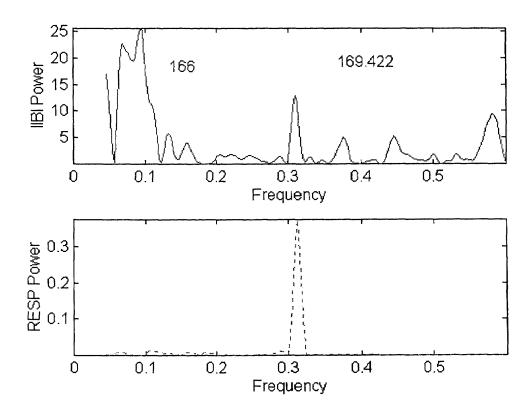


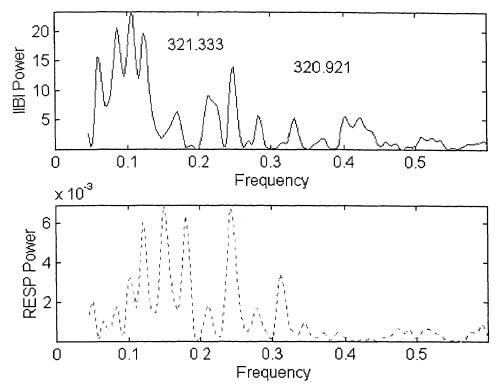


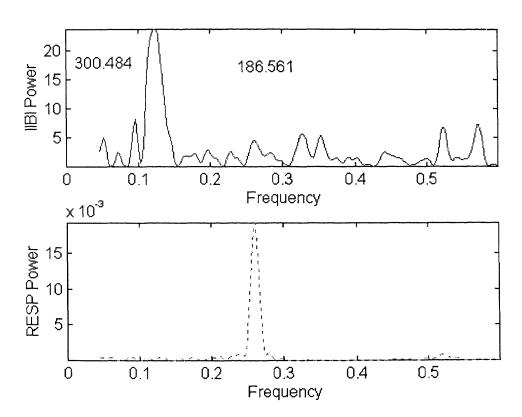


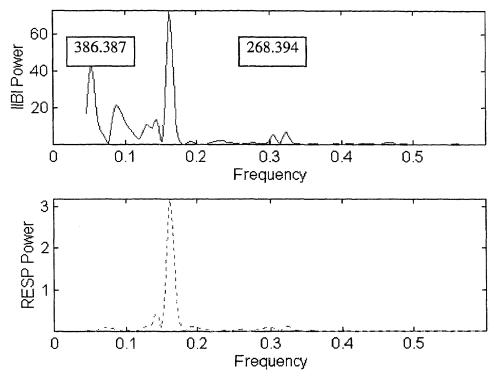


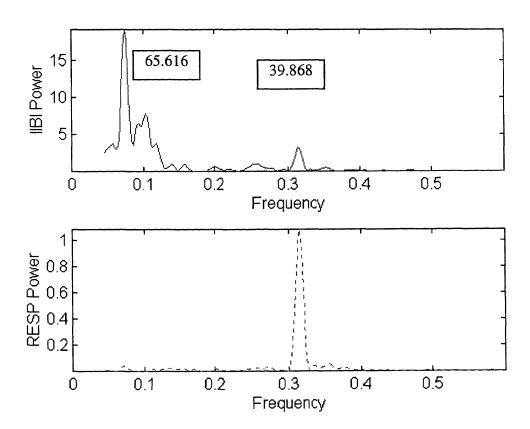


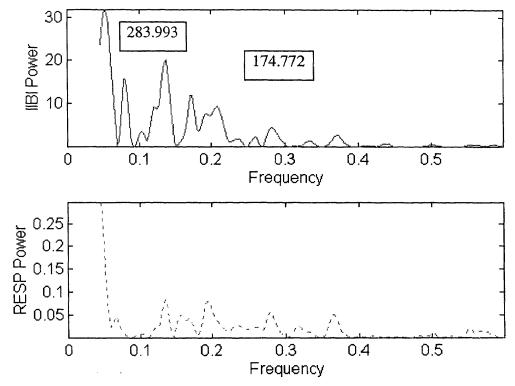


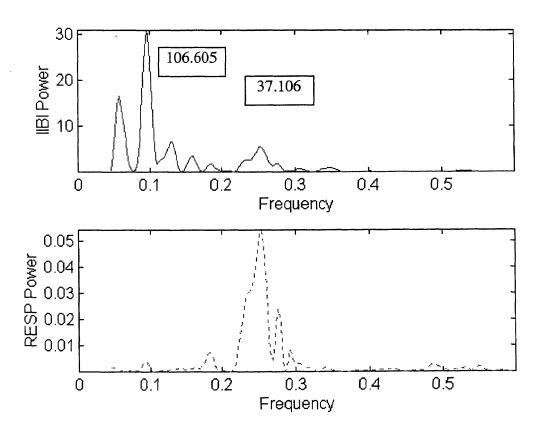


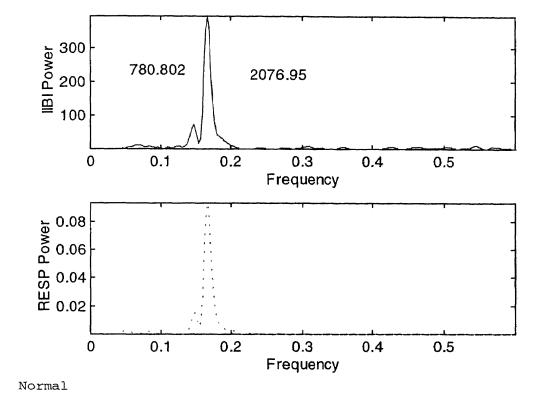


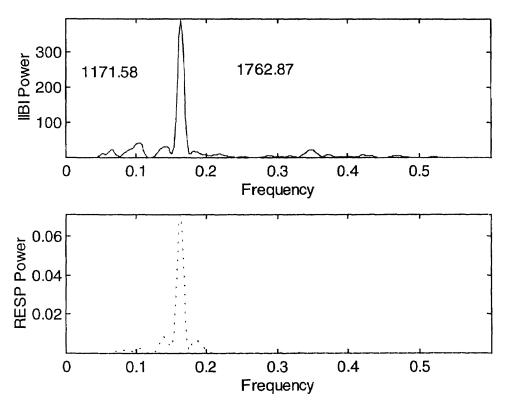




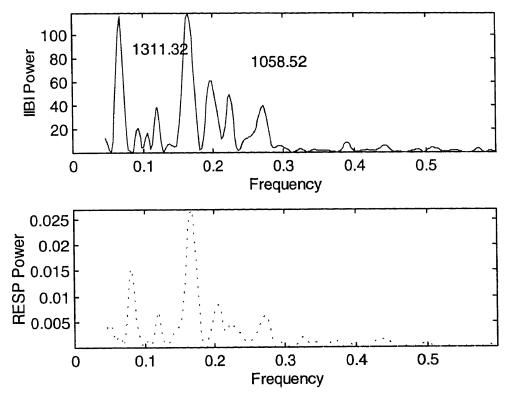


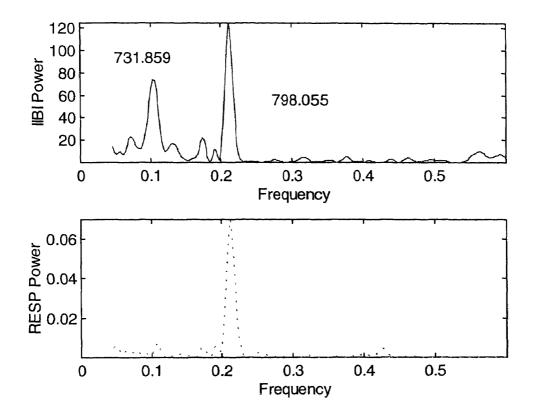


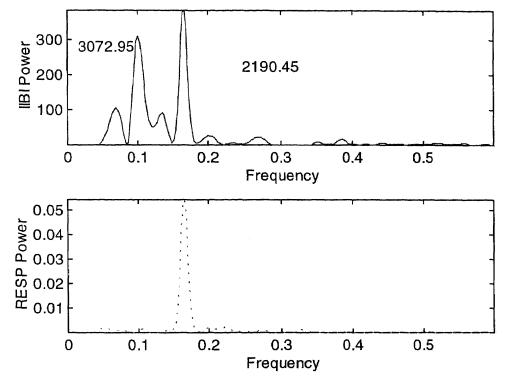


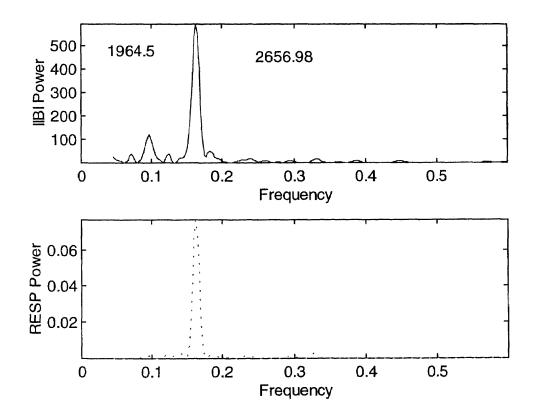


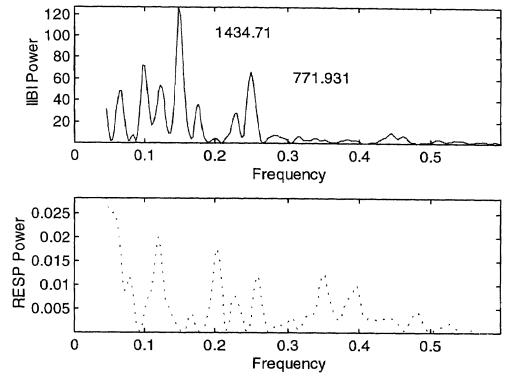


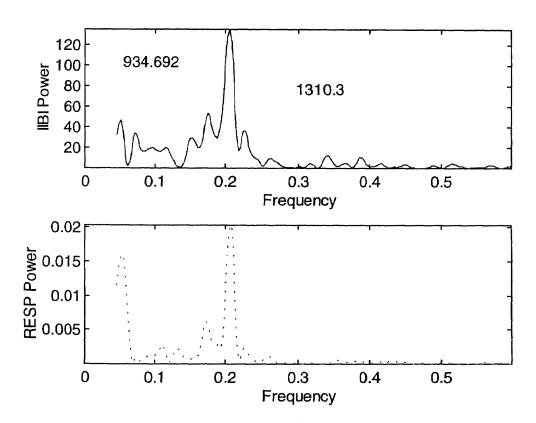


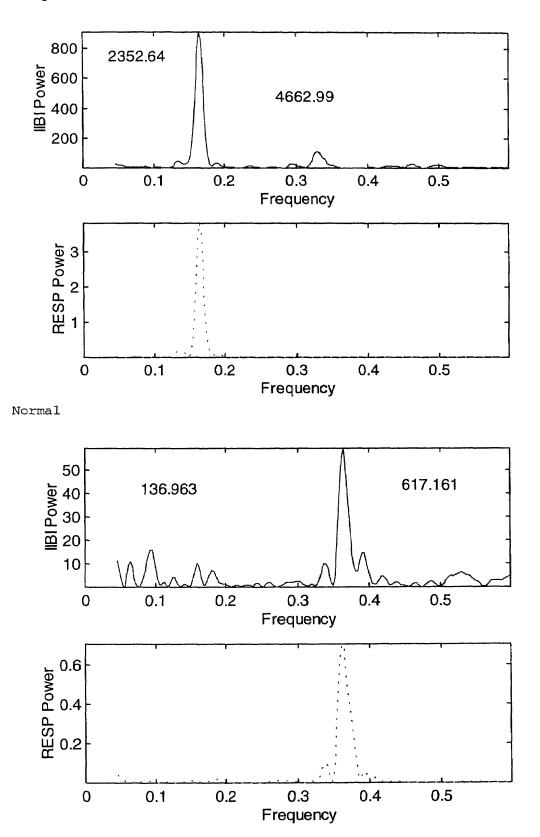


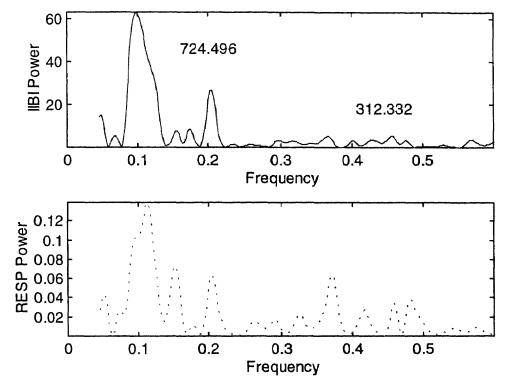


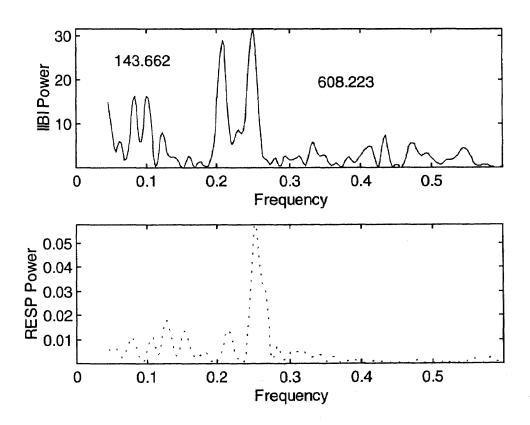


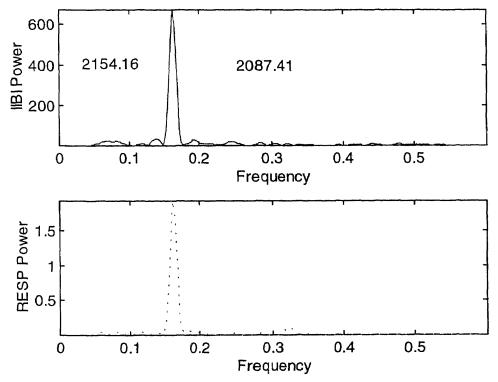


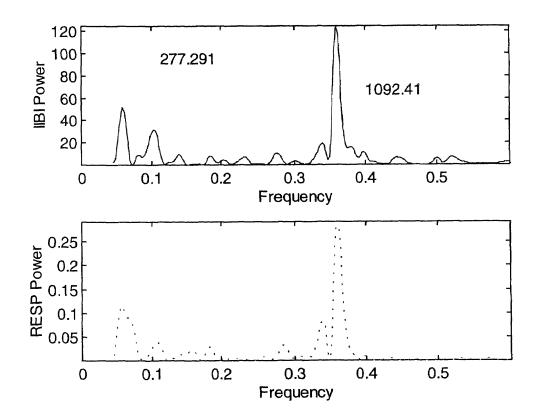


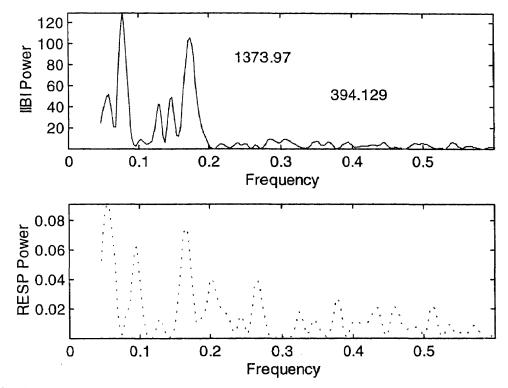


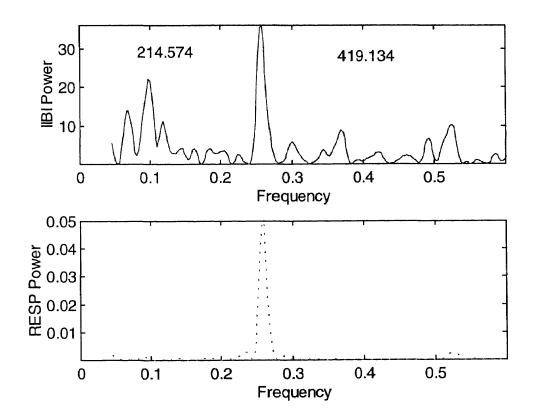


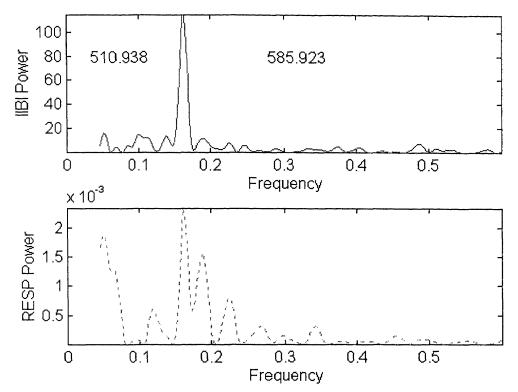


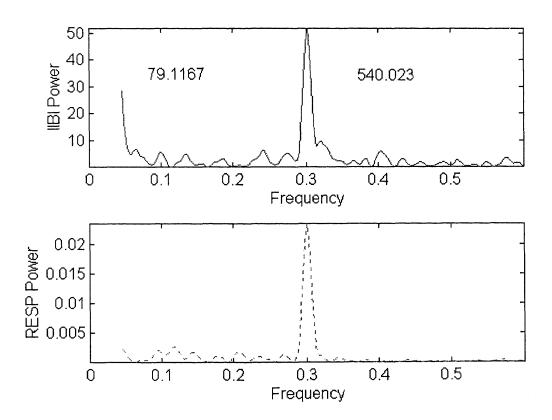


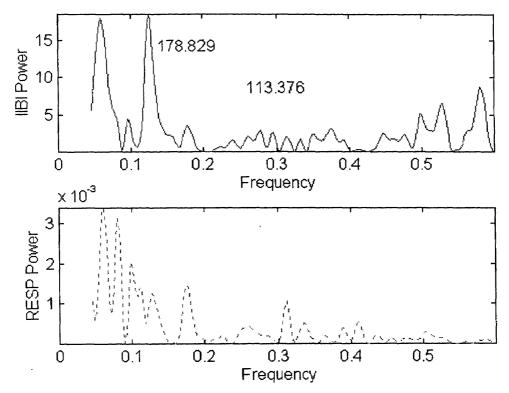


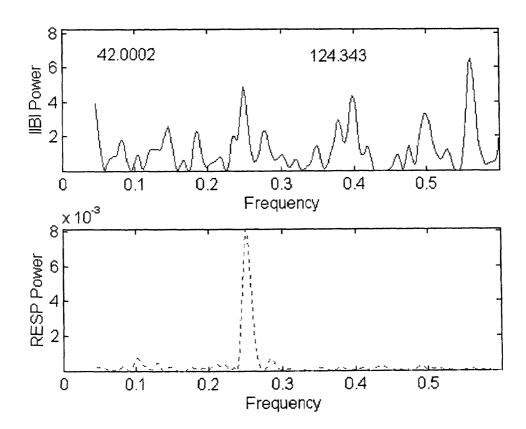


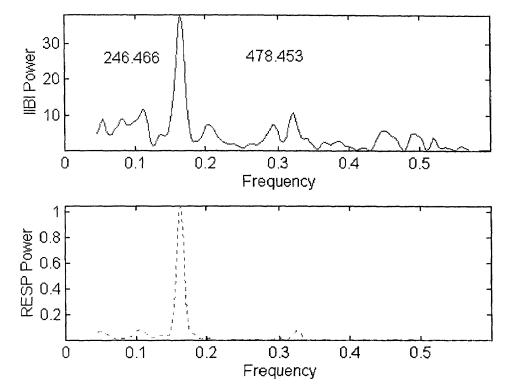


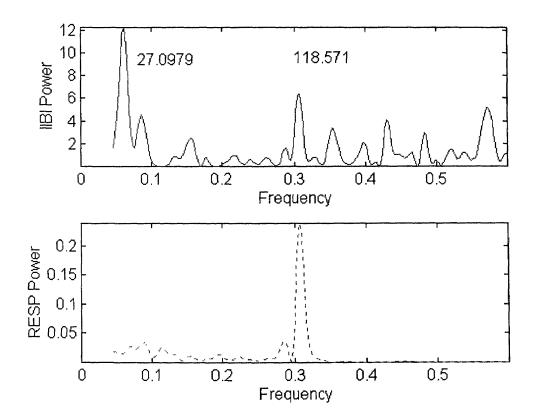


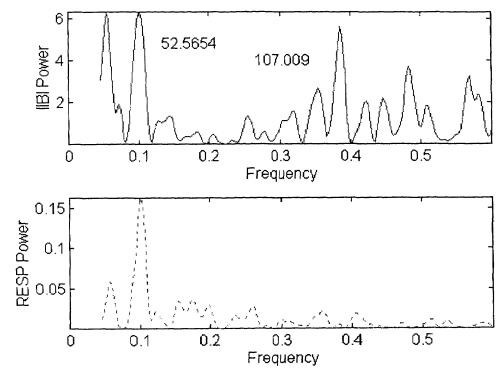


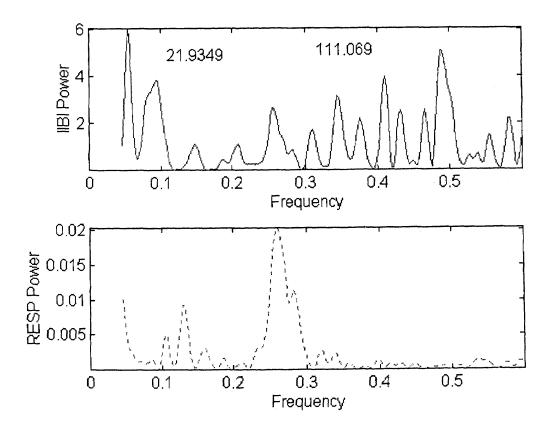


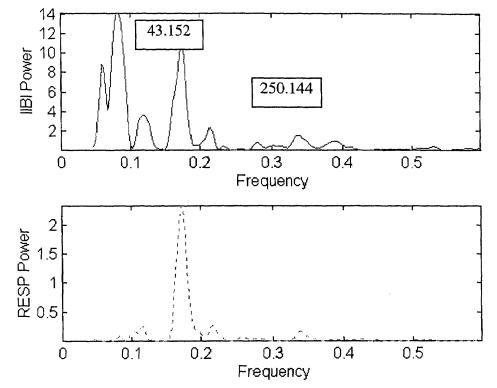


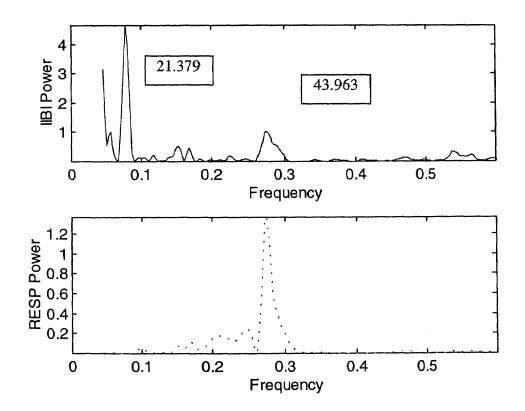


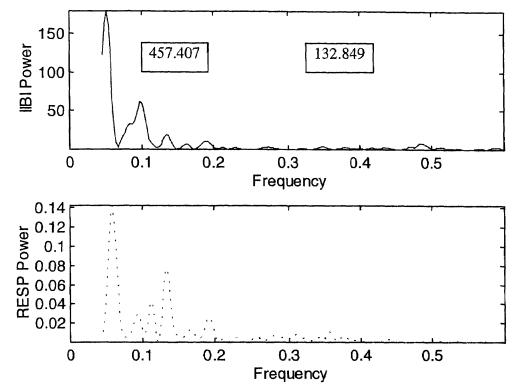


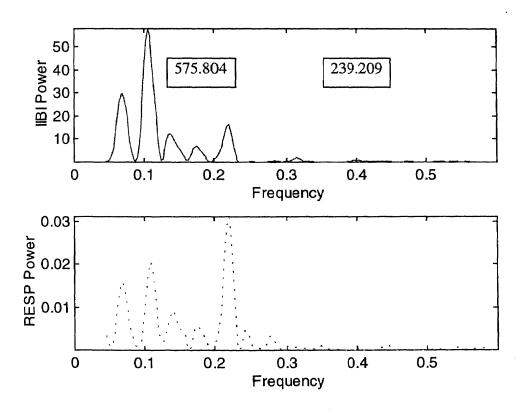


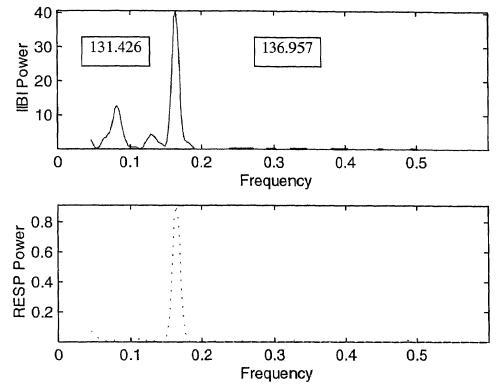


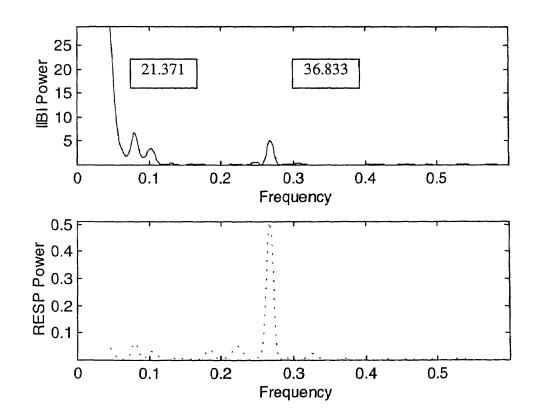


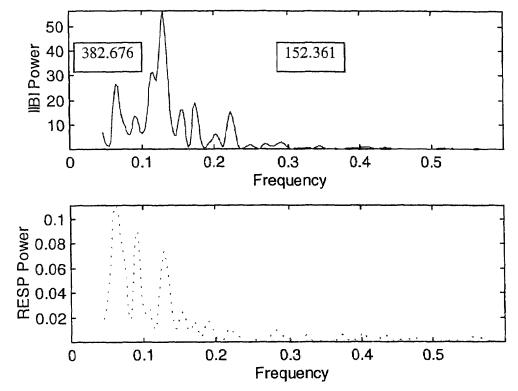




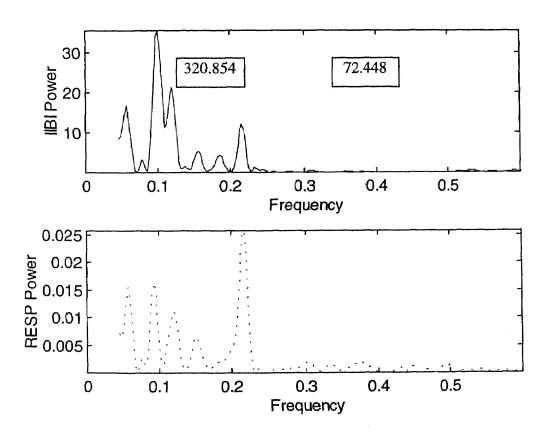


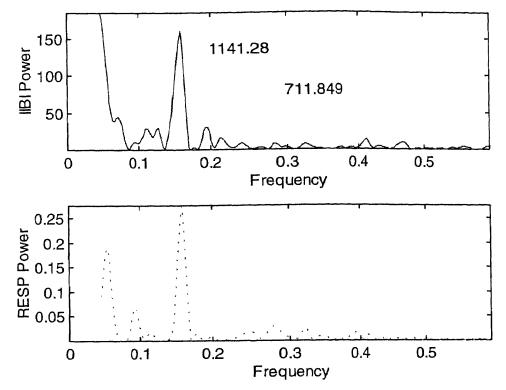


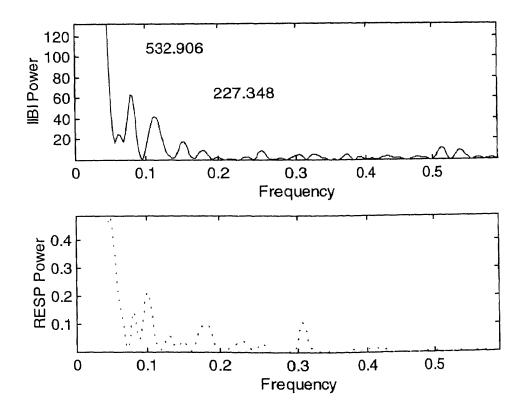


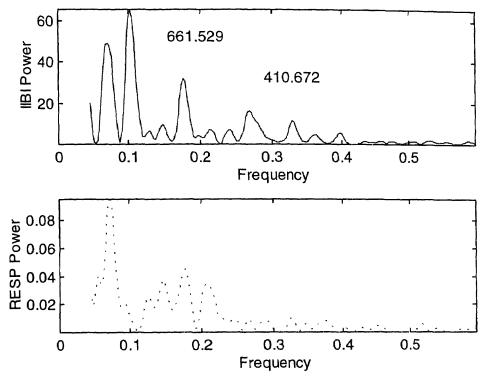


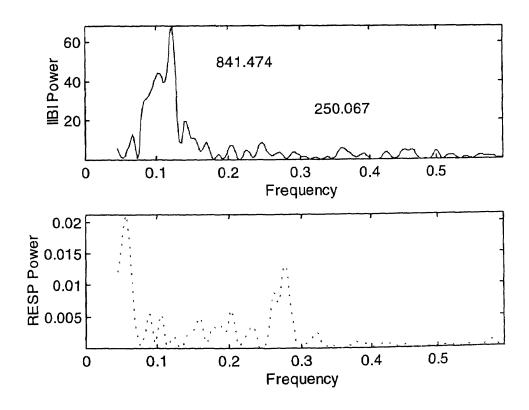
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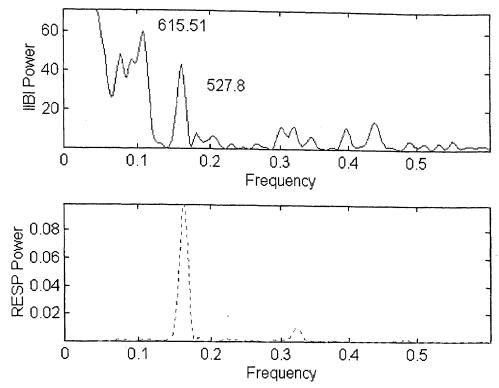


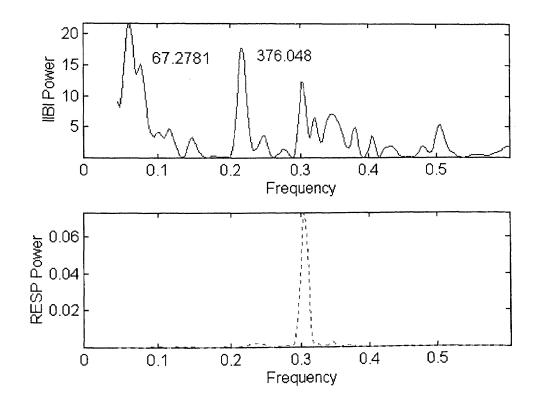


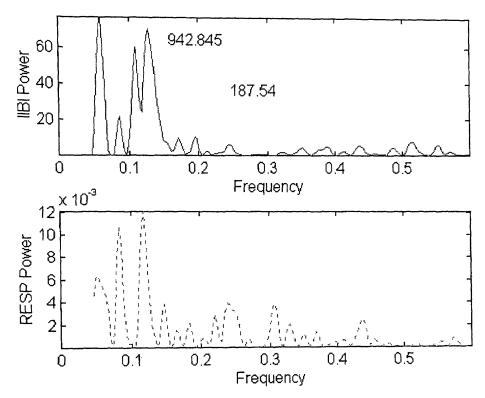


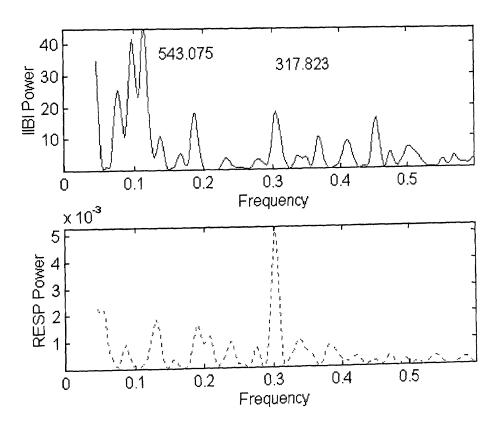












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