NONINVASIVE ASSESSMENT OF RESPIRATORY, MENTAL and PHYSICAL STRESSORS ON CARDIOVASCULAR FUNCTION

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A thesis submitted to the

Graduate School – New Brunswick

Rutgers, The State University of New Jersey

in partial fulfillment of the requirements

for the degree of

Master of Science

Graduate Program in Biomedical Engineering

and

The Graduate School of Biomedical Sciences

University of Medicine and Dentistry of New Jersey

Written under the direction of

Prof. John K. Li

and approved by

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New Brunswick, NJ

October 2007

ABSTRACT OF THE THESIS

Noninvasive Assessment of Mental, Physical and Respiratory Stressors on Cardiovascular Function

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Stress is encountered in daily life from relationships with people, work obligations, and our environment. Often viewed from the realm of the social sciences, this field of study was perceived to be limited to descriptive measures which only captured information discretely. As medicine started to investigate the biological ramifications of the stress response, it elevated the importance of stress as a precursor to several chronic diseases, which manifest both physically and mentally. As a result, many sub-areas of research have emerged to examine the relationship of stress to various systems within the human body which include the endocrine, cardiovascular, respiratory systems. This thesis compared three types of stressors and sought to determine which cardiovascular parameters were most sensitive to stress. Young volunteers were subjected to mental, physical and respiratory stressors while their variability of heart rate, blood pressure and blood flow velocity were measured to examine the role of stress on the cardiovascular system.

From comparisons between the rest and different stressor interventions for each subject, statistics from paired t-tests show significant differences exist between the physical stressor and rest phase for the heart rate, described by beats per minute (BPM) and frequency. In addition, testing of the null hypothesis against T wave amplitude, QT

interval, R-P onset, frequency, beats per minute, change in blood pressure and change in blood flow velocity reveal the most sensitive cardiovascular parameters are beats per minute and frequency. These cardiovascular parameters can be used as indicators to study the course of coronary artery disease, myocardial infarction, atherosclerosis and hypertension.

The present study can be improved by redesigning a durable pressure transducer, and securing the position of the transducer and Doppler flat probe angle throughout the experiment. It would increase the fidelity of the data acquired. In addition, it would be beneficial to test more subjects and design stressor tasks to elicit a greater stress response. Finally, including measurement of glucose and stress hormones during stressor interventions would enhance our understanding of the stress response. This would aid in studying other stress induced diseases outside the realm of the cardiovascular system.

ACKNOWLEDGEMENTS

I would like to thank Dr. Li for giving me the opportunity to work in his lab and introducing me to Dr. Hongjun Zhang, who was invaluable in training on the equipment and answering my many questions. I have appreciated both of their advice and support during my time at Rutgers. I would also like to thank Dr. Shoane and Dr. Drzewiecki for their feedback throughout the writing of this thesis. Lastly, the pursuit of this degree would not be possible without the support of my mother and father, who have encouraged my educational endeavors throughout the years.

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CHAPTER 1: INTRODUCTION

1.1 Traditional vs. Clinical Definition of Stress

Stress is a term often used subjectively, making it difficult to pinpoint a definition. It can be attributed to the fact that stress is generally perceived as a physical, mental or emotional strain, where individuals respond uniquely to specific triggers. Some examples of triggers are noise in the environment, interaction with people with conflicting personalities, or a difficult task. Stress was mainly considered intangible response to difficulties in daily life. Its presence was felt but could not be seen or heard. It was a challenge to define the intangible. Even amongst researchers, a consensus of a well defined concept of stress could not be reached. Difficulties in identifying the essence of stress hindered growth in this field research, escaping the firm grasp of scientific inquiry.

Science has been working toward understanding stress and its link to health since the beginning of the last century. Perceived as a discipline proceeding in a linear, directional manner with an accepted knowledge base, many imagine researchers chipping away at falsehoods to unearth the "Great Truth". However, scientists have actually been moving their knowledge toward a "Great Truth" to counteract their field from moving too far away to support a different "Great Truth". In the last century of psychology, William James emphasized a "Great Truth" that the mind is the organ of philosophizing. However, a new great truth emerged mid-century, which stated the mind was an organ that produced measurable behavior, shaped by rates of positive and negative reinforcement, called behaviorism (McEwen and Lasley, 2002). In medicine, the "Great Truth" of Western thought has been reductionism, the theory that every complex

phenomenon can be explained by analyzing the simplest, most basic physical mechanisms that are in operation during the phenomenon. It has been responsible for discoveries of vaccines, the human genome and treatments for genetic disorders. However, it could not account for illnesses without a discernable cause, such as depression, where stress is thought to be at its center. As with many disciplines, new truths emerged to account for the inadequacies of earlier truths. For the field of medicine, the next "Great Truth" was that one cannot understand a disease outside the context of the person with the disease. Under this new truth, the holistic approach considered non-reductive risk factors of the health of a patient. Such factors were the socioeconomic status, environment (i.e. access to clean water), lifestyle, psychological make-up, social relations patterns and stress, which involves stressors and coping with them.

Researchers have wrestled with the best definition for stress. Although not considered a disease state, stress was defined in terms of its symptoms. This list was a compilation of mental and physical conditions. For example, impatience, dissatisfaction, resignation and anxiety were placed on the same scale as muscle tension, dizziness, sweating, tiredness, depression, heart palpitations and stomach problems (Brattburg, 2005). Stress tests have been developed and designed to address these symptoms and have its respondents rate its severity on a number scale, where the total score indicated the stress level. While this technique was useful to indicate behavior and lifestyles encouraging stress onset, it could not objectively rate the stress severity since each person perceives and reacts to different triggers uniquely. In addition, if subjects were to be tested repeatedly to observe stress symptoms over long periods of time, the stress tests were administered at carefully chosen timeframes to avoid false high reliability, which

could occur when the respondents remembered how they answered previously (Brattburg, 2005). By design, data that is collected is discrete in nature, allowing any change in parameters between stress tests to go unnoticed.

1.2 Allostasis in Animals: Past and Present

One of the earliest examples of guarding homeostasis in an organism is the use of heat shock proteins in bacteria. It is believed the hormonal response is linked to the heat shock response, used to respond to environmental stress. An extreme example of allostatic load can be seen in salmon, which have a similar set of fight or flight responses to humans. The brain, cardiovascular system, glands and immune system of the salmon all respond in relation to the hypothalamic-pituitary-adrenal (HPA) axis. When salmon make the long trek back up river to their birth waters, cortisol skyrockets and the fish stop feeding. Extreme elevations in cortisol exhaust the stores of energy and destroy the immune system, leading to massive infection. Interestingly, the salmon's sex glands mature and its sex hormones are elevated to levels that allow them to reproduce! Once they reproduce, they die from the effects of extreme cortisol levels. Another use of the HPA axis is the ability to avoid environments salmon fear. When salmon sense copper, their cortisol levels elevate above normal and swim away from the source. Triggering the HPA activates risk perception. However, their system is not optimal. Salmon will swim toward cadmium, known to kill the fish, but they show no stress signs such as elevated cortisol levels. Many animals rely on the "fight or flight" response for their safety and next meal (Fig. 1.1). In normal circumstances, the animal will return to its resting state once the stressor is absent. However, sudden changes in the environment such as weather

can cause the stress response to induce behavioral changes for adaptive purposes. For 500 million of years, animals have relied upon allostasis to make important decisions for survival. Animals have adapted to, died from or in some cases, developed abnormally from stress, when prolonged. How did humans convert the stress response, designed to protect, into stress related diseases? Sapolsky, a neuroendocrinologist, suggested that animals only used the stress response during the time of stress, not in times of peace. In his novel, "Why Zebras Don't Get Ulcers", he argues that zebras do not flood themselves with stress hormones wondering when the next predator will show up, only when there is a lion present (McEwen and Lasley, 2002).

When animals experience allostatic load from exposure to repeated or prolonged stress, they exhibit symptoms similar to stress related diseases seen in humans. In one of the earliest quantitative studies of stress from the 1930s, a Dr. Hans Selye conducted experiments on laboratory animals exposed to different noxious physical and emotional stimuli which included blaring light, deafening noise, heat or cold or frustration. From his numerous experiments, he found that all laboratory animals showed the same pathological changes of stomach ulcers, shrinking lymphoid tissue and enlarged adrenal glands. Later, he demonstrated persistent stress could cause the animals to develop various diseases such as heart attacks, stroke, kidney disease and rheumatoid arthritis, all seen in humans (American Institute of Stress, 1983). Dr. Selye's work radically shifted the paradigm of the underlying contributing factors of disease. Pathogens were no longer the only cause of ill health.

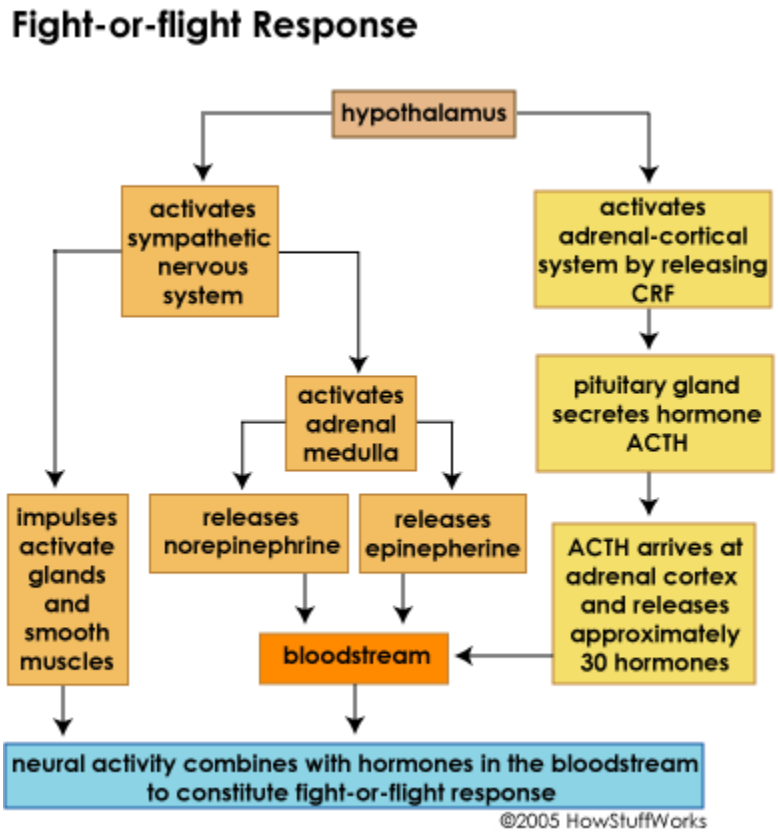


Figure 1.1 Block diagram illustrating the Fight or Flight Response (Layton, 2005).

The development of a formal definition stress was formed from the breakthroughs of Dr. Selye's work and the psychological viewpoint of stress as an intervening variable, the link between a stimulus or stressor and the behavioral response (Selye, 1985). Stress was defined as a condition, where a specific response by the body to a stressor or stimulus, such as fear or pain, disturbs or interferes with the normal physiological equilibrium of an organism. This discrepancy elicited a pattern of compensatory responses. During stress, when the body senses a disruption to homeostasis, the sensation is compared to a set point within the system (Goldstein, 1995). From this comparison, the compensatory response level was determined. An analogy to relate stress sensing is a thermostat. When the ambient temperature is below the set point, the furnace will be turned on to allow the temperature to reach the set point.

1.3 Types of Stress: Eustress and Distress (Acute and Chronic)

At its most basic level, Selye divided stress into two categories: eustress and distress (Fig. 1.2). Eustress is positive stress that is beneficial because it conditions the body to adapt for a similar future situation. For example, it may act as a motivator increase awareness and alertness during "fight or flight", when an animal senses danger and increases its adrenaline to escape or fight its way out of the situation. When stress becomes a negative or noxious, termed distress, it can take a toll on the physiological system, especially when a person cannot adapt to recurring negative stress. Eustress and distress cause similar physiological responses but it is the perceived effect that determines whether the stress causes "malfunction" in the body. For instance, a performer may have increased heart rate from excitement that will return to normal upon the start of

the performance whereas another may have increased heart rate due to stage fright that may the duration of the performance.

Firdaus Dhabhar, who believed stress was not meant to simply hinder the body's defenses, wanted to uncover evidence that stress could act in favor of the body. At the time, chronic stress studies dominated the discourse in the stress research field. Dr. Dhabhar wanted to challenge the notion that stress only suppressed the immune system. In his studies, he focused on the activity of WBCs during acute stress and determined that while WBC counts decreased in the bloodstream, they migrated to the lymph nodes and skin. Once the stress ceased, the WBCs returned to the blood stream. Dr. Dhabhar termed this activity "stress-induced trafficking", where stress enhanced WBC immune activity. From this study, he established the concept of protection in the short term versus wear and tear in the long term (McEwen and Lasley, 2002). Therefore, Selye's eustress corresponded to the current term acute stress and distress corresponded to chronic stress.

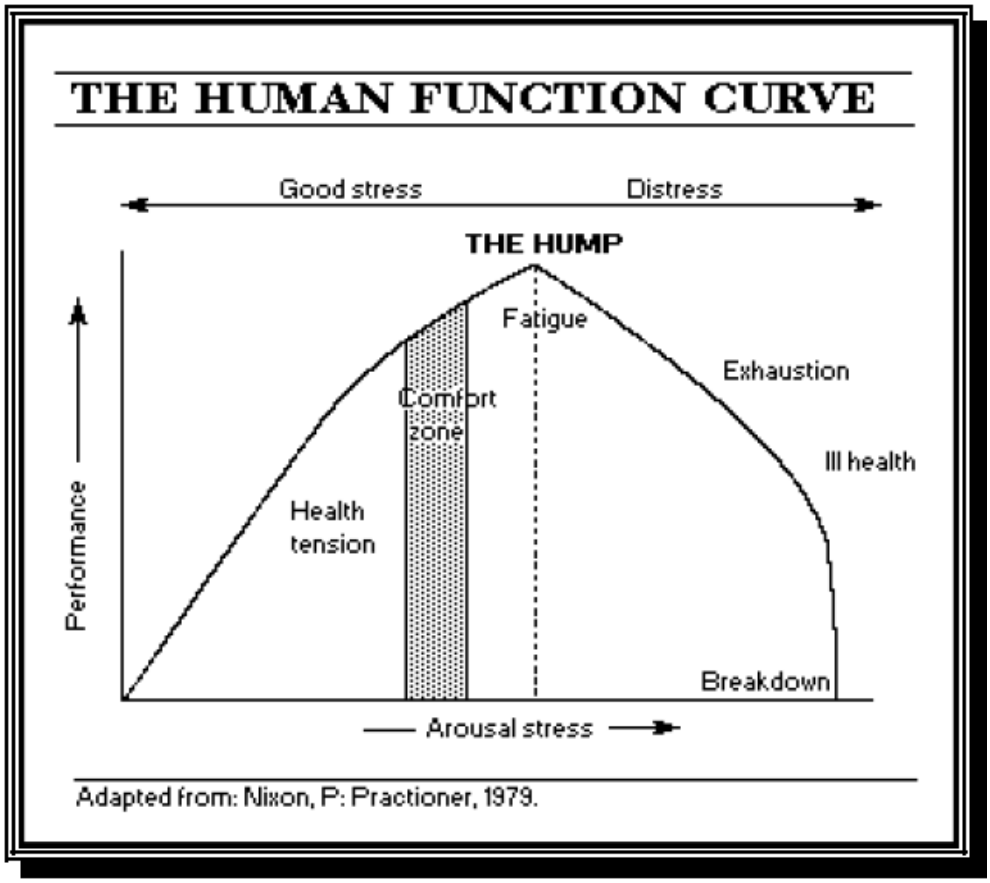


Figure 1.2 Illustration of Eustress (good stress) and Distress (American Institute of Stress).

1.4 How the body copes with stress

To explain how stress was dealt with, Dr. Selye introduced General Adaptation Syndrome (G.A.S.), the manifestation of stress in the whole body, as it develops over time. The syndrome consists of three stages: the alarm reaction, the stage of resistance, and the stage of exhaustion (Selye, 1985). Most of the physical or mental exertions, which act upon the body during a limited time, produce compensatory responses relating to the first and second stages: initially the stressors upset and alarm us, but the body copes with them through adaptation. The stage of exhaustion and death is reached quickly when the body is overwhelmed by the stressors. G.A.S. describes the role of adaptation in differentiating the positive and negative types and effects of stress. Figure 1.3 provides a visual representation of General Adaptation Syndrome. The ability for the body to adapt to stress is the "fine line" between eustress and distress. Selye proposed that the excessive, repeated, maladaptive distress responses would lead to "diseases of adaptation". These diseases are categorized as hyperfunctional, hypofunctional, and dysfunctional. Some of diseases falling into these umbrellas are cancer, arthritis, diabetes, allergies and hypertension (Goldstein, 1995).

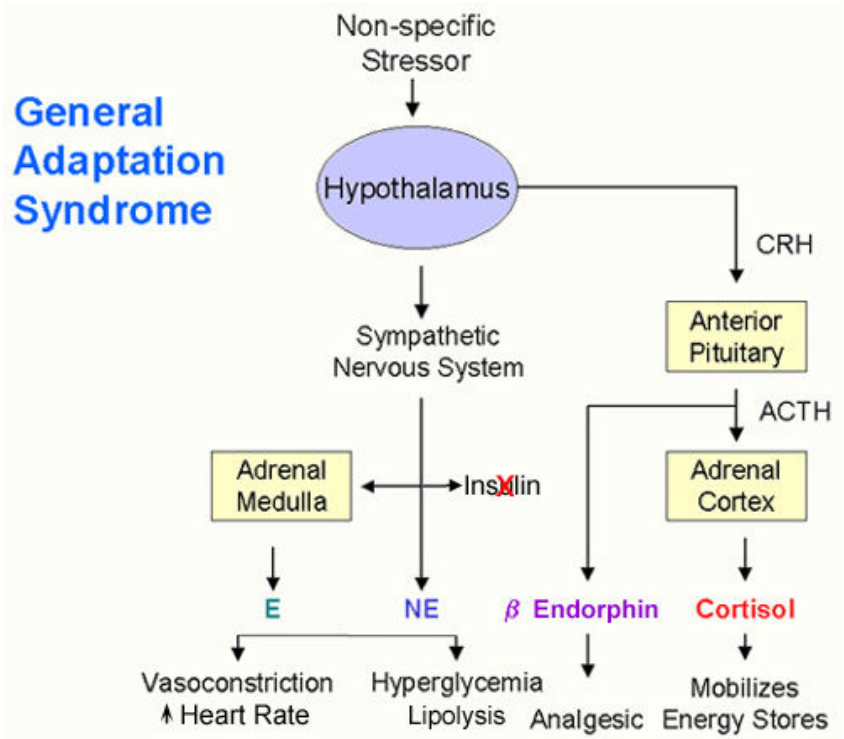


Figure 1.3 General Adaptation Syndrome (Lynch, 2007).

Missing from Dr. Selye's experiments was the influence of emotions. Because he considered emotions unscientific, the links between emotions, the stress response and the brain were missing. In Selye's day, the brain was not deemed the master coordinator of the stress response. However, they recognized the role of the endocrine system as the workhorse of the stress response, controlled by the pituitary (McEwen and Lasley, 2002). Walter Cannon, one of the first scientists to bring stress into the medical field's consciousness, encouraged the study of emotional stress and relief to benefit psychosomatic medicine in 1928 (McEwen and Lasley, 2002). He felt it would address the concerns of World War I army physicians treating embattled soldiers with symptoms of illness with no discernible cause. In the 1950s, Dr. Mason repeated part of Selye's experiment to separate psychological stress from nocuous stimuli to determine whether psychological could induce G.A.S. Choosing to study the stress of fasting, one group of monkeys were fed placebo pellets with no nutrients and the other group nutritional food pellets. Dr. Mason found that the placebo group did not show psychological stress, as evidenced from the stress hormone levels (McEwen and Lasley, 2002). In Mason's second experiment, in which monkeys try to avoid receiving shocks, showed that the anticipation of the shock was more of a stressor than the shock itself. Frustration increased the stress hormones in the monkeys. When monkeys received shocks randomly, they quickly stopped "fighting" or trying to avoiding the shocks once they learned they had no control to stop it. This is called "learned helplessness", where the stressor cannot be escaped. Loss of the ability to cope with a stressor has applications to clinical depression, where apathetic behavior and high levels of cortisol are common characteristics. These experiments established how psychological stimuli can affect the

output of stress hormones. One of the reasons for the lack of consensus for the role of the brain in the stress response was lack of evidence of a network of nerves from the brain to the pituitary had not been found. It was not until the discovery of the hypothalamus link to the pituitary, called the HPA axis, by Geoffrey Harris of Oxford University, that the brain was officially a major component of the stress response. In turn, it lead to the discovery of the corticotrophin releasing factor (CRF) hormone, which initiated the stress response in 1983 (McEwen and Lasley, 2002).

To aid the underrepresented field, additional terms were used to define the components of stress. Introduced in the 1980s, allostasis emphasized the ability of allostatic systems to help keep the body stable by allowing their parameters to change (McEwen and Lasley, 2002). Such parameters included heartbeat, breathing, the amount of glucose in the blood and the amount of stored energy. Allostasis is associated with the "fight or flight" response, where the body will prepare to do one of the two scenarios in extreme cases (Fig. 1.4). It links the brain, endocrine and immune systems together. The brain perceives the threat, initiating the endocrine system to mobilize the whole body and the immune system to ready its internal defenses. To fight or flee, oxygen flow must increase to the muscles, breathing will accelerate, heart rate speeds up to deliver oxygen through the bloodstream to the muscles, skin blood vessels constrict to reduce bleeding from injury. To fuel the "fight or flight" response, glands will exhaust stored carbohydrates into the blood sugar. In addition, infection fighting white blood cells (WBCs) attach to the blood vessel walls, ready to depart to sites of injury. If a stressful situation is prolonged, the immune response is dampened in favor of the heart and lungs, which need the energy the most. This scenario can lead to stress induced illnesses, which

will not subside until the stressor is removed. A term to describe this event is allostatic load, the cumulative wear and tear of life, in which the stress response turns against the body (McEwen and Lasley, 2002). When allostasis is activated to deal with a stressful situation, the stress response is ignited, often for situations where fighting or fleeing are not viable options. Examples of some scenarios are caring for a seriously ill family member or working for an overbearing boss. Since the stress response cannot help reach a resolution swiftly, the system designed to protect us will cause wear and tear, inviting illness to take over the body.

Allostatic load, also described as being "stressed out", will occur during three situations: (a) when it does not shut off at the right time, (b) when it does not adapt to a repeated stressor or (c) when it repeatedly or inappropriately activates the major body systems, causing them to turn on and eventually break down. An allostatic load scenario can take the form of unremitting stress, where chronic stress takes a toll immediately on the heart. Stress aggravates the clogging of blood vessels where damage in the coronary arteries can lead to atherosclerosis. Many sudden escalations in blood pressure can lead to myocardial infarction (MI) from clogged blood vessels. An example is the social disruption, where monkeys are constantly placed with a new group monkeys without having a chance to acclimate themselves to the other inhabitants and hostile environment. The trauma of being trapped in a hostile environment accelerated the process of atherosclerosis and risk of MI, allowing some monkeys to die prematurely. A parallel has been evidenced in the workplace. When massive reorganization occurs in a department of an organization, employees become wary from the uncertainty of their jobs. From the Whitehall studies, employees in this situation faced increased likelihood of stroke,

increased cholesterol, and body mass index. Therefore, instability can take a toll on one's life and health.

One question without a clear answer is why does allostasis convert into allostatic load in some people but not in others? To date, the top three factors are genetics, emotional reactions and lifestyle. For example, one's lifestyle can contribute to allostatic load, not just coping with change or an emergency. Sleep deprivation, common in students and professionals in demanding jobs, is often seen as a necessary to meet deadlines. In the long term, it will cause increased levels of glucose, cortisol, which will contribute to accumulation of abdominal fat and bone mineral loss. Eating a rich diet of fatty foods will increase metabolic load and fat storage, accelerating hardening of the arteries. Lack of exercise will disable the body's ability to counteract the increase in glucose and metabolic load from eating rich foods. Lastly, anticipation or anxiety, involving the perception and physiological response, can become strong enough to set off the flight or fight response, known as allostasis or stress response, by simply imagining a threat.

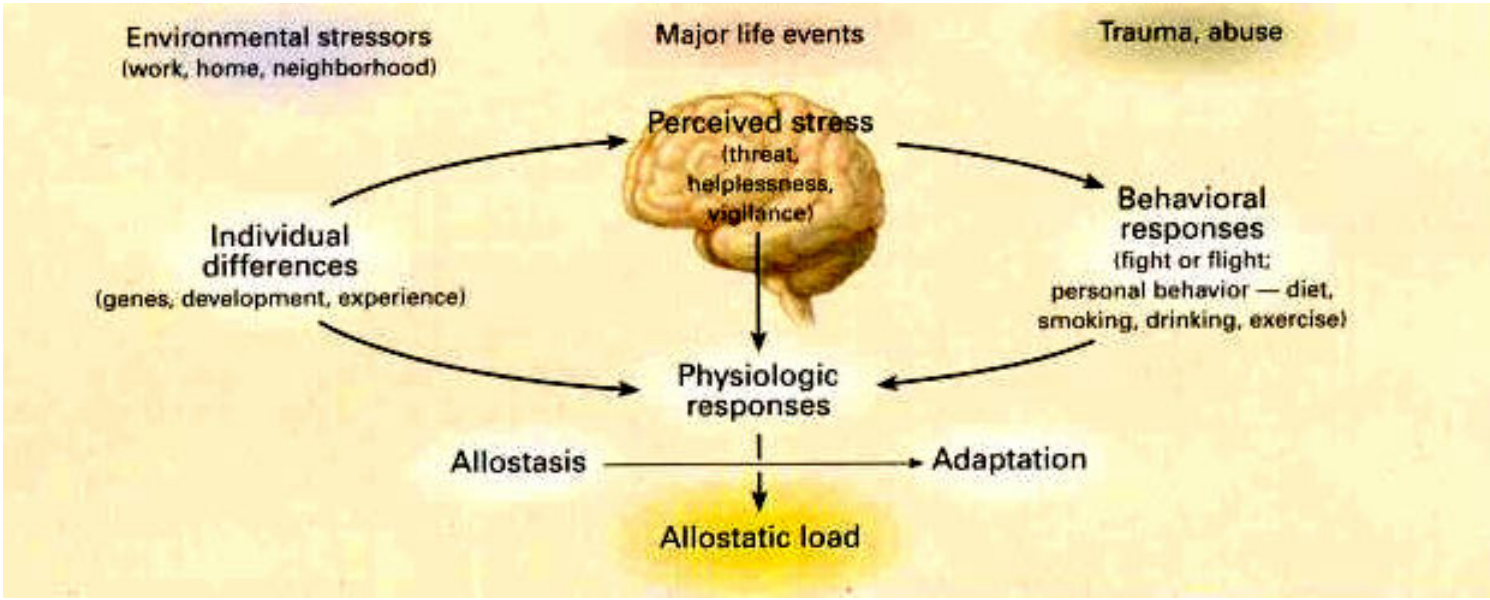


Figure 1.4 The Stress response and Development of Allostatic Load (Allostasis). Perceived stress from the environment, trauma or major life events can manifest into physiological responses, imposing allostatic load on the body. However, individual differences from a person's genes, development or prior experiences can alter the course of a physiological response to the stressor. In addition, behavior towards a perceived stress may also change the way the body responds to the perceived stress. Allostasis, the process where the body copes with stress, tries to alleviate the challenge presented by the stressor thought adaptation. Modification of behavior

1.5 Stress as a 21st century concern

From the discussion of allostasis and allostatic load, it appears that maintaining our physical and emotional health is of utmost importance. Lifestyles must be examined for the appropriate course of action to alleviate stress. If stress is not unique to the people of this century, it is certainly a concern. In the last century, people lived shorter lives because they died from diseases which had no cure in those times. They also lived harder lives, experiencing hardships. Some may argue that people died of stress although it may have been due to depression or post-traumatic stress disorder (PTSD). However, the life expectancies of American have increased by 50 percent (McEwen and Lasley, 2002). They no longer die of the same diseases which were responsible for many deaths in the last century, such as pneumonia and tuberculosis (Fig. 1.5). In addition, death by childbirth and fires has reduced with the help of medical treatment and safety protocols. Stress is not equivalent to hardship. Ironically, in the age of convenience, stressors are on the rise. People have unprecedented access to live streaming information through television, newspapers, the internet, which is much more than anyone in the last century had to process all at once. Technology has allowed people to become physically inactive, isolated from friends and loved ones. Advances in travel have allowed families to live farther apart, further weakening family face to face contact. Competition at work, school and sports has elevated in the pursuit of promotions, scholarships or fame and endorsements respectively. In today's society, stressors are increasing while the social and physical mechanisms to deal with them may not be able to keep up with the rapid changes.

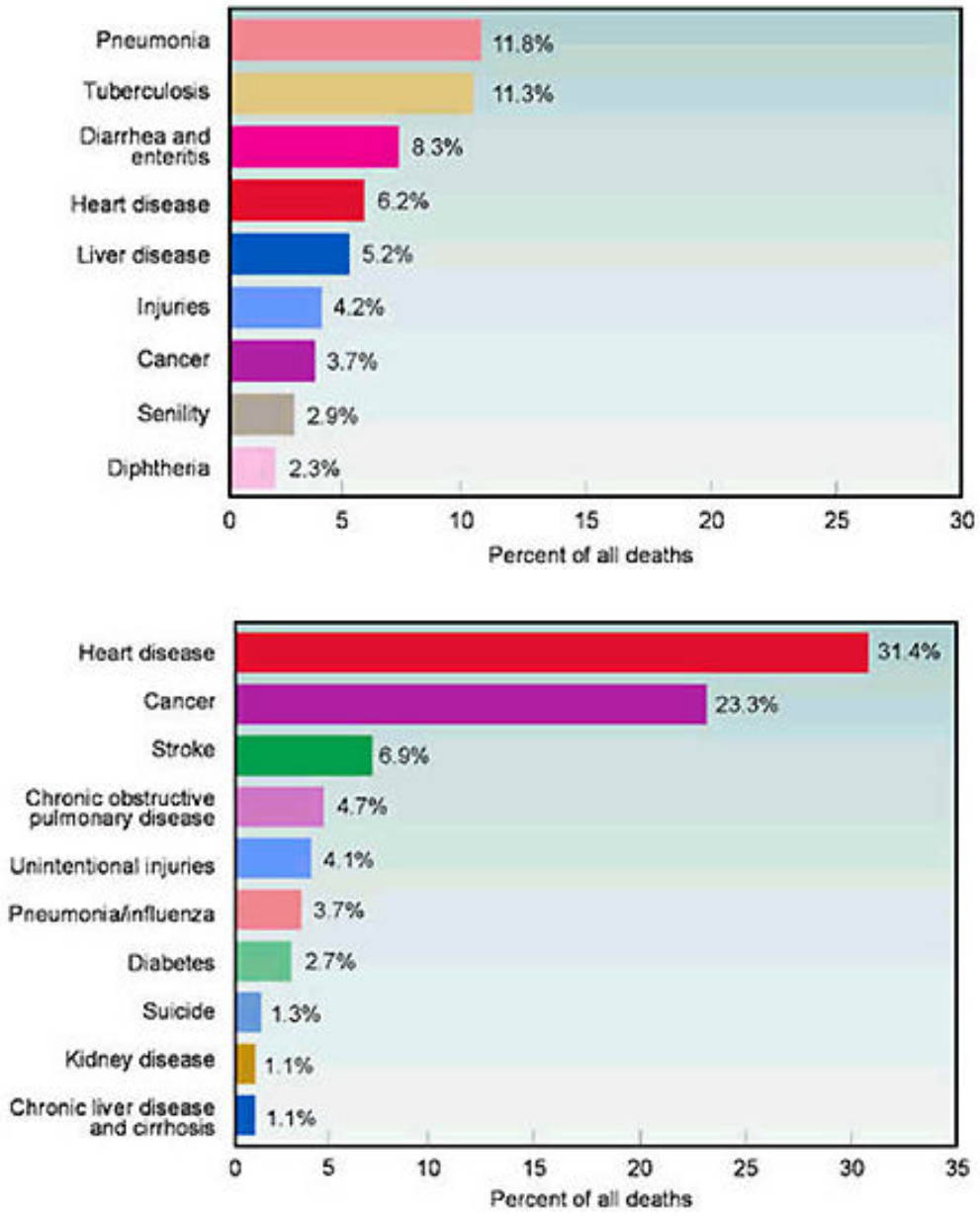


Figure 1.5 Leading Cause of Death 1900 (*top*) and in 1997 (*bottom*) (CDC, 1997).

1.6 Stress response circuit

In the first wave of defense, allostasis begins in the brain, which releases endorphins. Oxygen intake into the lungs and brain is increased. Next, the hypothalamus signals the adrenal glands directly via sympathetic stimulation. In response, the adrenal glands release adrenaline (epinephrine) which constricts the blood vessels, and increases heart rate and respiration. Glucose levels in the bloodstream are increased from the release of glucose from fat stores in the body. Adrenaline also produces more fibrinogen to prevent clotting in case of a blood vessel rupture.

In the second wave of defense, the hypothalamic-pituitary-adrenal (HPA) axis unites the nervous system, glands and immune system to maintain the balance between the systems. The HPA axis adjusts the phase of the allostatic response. The hypothalamus releases CRF to signal the pituitary gland, which releases ACTH to signal the adrenal glands, which release cortisol and adrenaline. Cortisol moderates immune system activity to aid in the response to an infection or injury. In excess, cortisol suppresses the immune system. However, when the body is deficient in cortisol, allergies and inflammation develop. Adrenaline temporarily increases the heart rate to send extra blood to muscles and organs and constricts the blood vessels supplying the skin to reduce potential bleeding (McEwen and Lasley, 2002).

1.7 Stress and the Cardiovascular System

The cardiovascular system is a good area to observe the "double-edged" sword of allostasis in action. It is sensitive to the demands of allostasis and the strain of allostatic load. Parameters include elevated levels of oxygen, glucose, heart rate and blood pressure. When allostatic load is reached from a combination of the above mentioned parameters in the cardiovascular system, several health conditions can develop. These conditions may arise from lifestyle choices, the environment where one works or social interactions. The clinician will rely on the following cardiovascular measurements: blood pressure, echocardiography and blood flow.

Blood pressure measures the force blood exerts in the blood vessels. Since blood pressure drops as the blood flows from arteries to capillaries and veins, clinicians measure it from the arteries, which take blood away from the heart. It can be measured invasively via cannulation or non-invasively via auscultatory or oscillometric methods. Clinicians can measure the systolic and diastolic pressures to describe the peak and resting phases of the cardiac cycle respectively. Such measurements can provide the mean arterial pressure, pulse pressure and indicate whether a patient has abnormally high blood pressure, called hypertension or pressure that is too low, called hypotension.

The electrocardiogram (ECG) gives an overall picture of electrical activity in the heart (Fig. 1.7). An electrical impulse or action potential precedes each heartbeat or mechanical contraction. Composed of five phases (0, 1, 2, 3, 4), the action potential correlates with the ECG waveform, which records the summation of the action potentials in the muscle cells in the atria and ventricles (Fig. 1.6). In Phase 4, the action potential has returned to its resting potential. Potassium slowly leaks out of the cell, allowing the

inside of it to become negative relative to its environment. In Phase 0, when the muscle cell is stimulated, the rapid influx of sodium and slow efflux of calcium cells cause depolarization. This correlates with the P wave in the atria and the QRS complex in the ventricles (Lipman and Cascio, 1994). The muscle cell returns to its resting state through repolarization, where the inside of the cell is returned to its negatively charged state and its environment positively charged. Repolarization is represented by Phases 1, 2, and 3. In Phase 1, fast sodium channels close while the slow calcium channels remain open. In Phase 2, known as the plateau phase, calcium influx is balanced by potassium flow out of the cell. This phase correlates with the ST segment of the ECG waveform. For Phase 3, the calcium channels eventually close while potassium continues to leak out of the cell. On the ECG, the T wave correlates with Phase 3.

The simplest ECG electrode configuration consists of three leads which are placed at the left arm, right arm and lower right or left leg. This creates an Einthoven's triangle. The clinician can determine the heart rate, size and position of the chambers, the presence of damage to the heart, and view the effects of drug or medical device treatments used to regulate the heart. The waveform provides this information through the examination of distinct segments composing the cardiac cycle. The P wave, the start of the heart beat where atrial depolarization occurs, begins in the sinoatrial (SA) node towards the atrioventricular (AV) node, spreading across the atria from right to left. The Q wave signifies depolarization of the interventricular septum via the Bundle of His originating form the AV node. Proportional to the muscle mass, the R wave occurs as the electrical impulse travels from the right and left branches of the Bundle of His to their corresponding Purkinje fibers in the ventricles (Fig. 1.8). Afterwards, the S wave,

representing the terminal forces of ventricular depolarization, appears as the electrical impulse spreads backwards towards the posterior surface of the heart (Ritota, 1975). Together, the Q, R and S waves comprise the QRS complex, which describes the electrocardiographic pattern of ventricular depolarization prior to contraction (Ritota, 1975). The T wave, representing the end of repolarization of the ventricles or diastole, occurs immediately after the QRS complex. It has been the topic of stress literature due to its role as a marker of repolarization abnormalities indicative of ventricular arrhythmias (Ikeda *et al.*, 2000).

Blood flow is measured using Doppler ultrasound, where reflected sound waves evaluate blood flow as it passes through major arteries and veins of the arms, legs, and neck. It can detect blocked or reduced blood flow through narrowing in the major arteries of the neck that could cause a stroke or embolism. During the test, Doppler ultrasound meters continuously transmit high frequency sound (8 MHz for shallow vessels or 4 MHz for deep vessels) which travels through the vessel wall and into the flowing liquid. An ultrasound gel is applied to the transducer and passed lightly over the skin above a blood vessel to enhance the signal. The transducer sends and receives sound waves that are amplified through a microphone. When sound waves bounce off solids, including blood cells, the movement of blood cells cause a change in pitch of the reflected sound waves, known as the Doppler Effect. If there is no blood flow, the pitch will not change. Information from the reflected sound waves can be processed by a computer to provide graphs or pictures that represent the flow of blood through the blood vessels (Nissl, 2005).

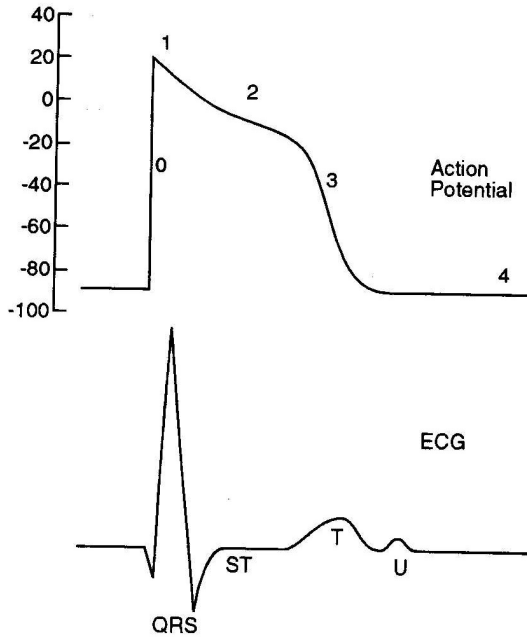


Figure 1.6 The Action Potential correlates to the ECG waveform. (Lipman and Cascio, 1994)

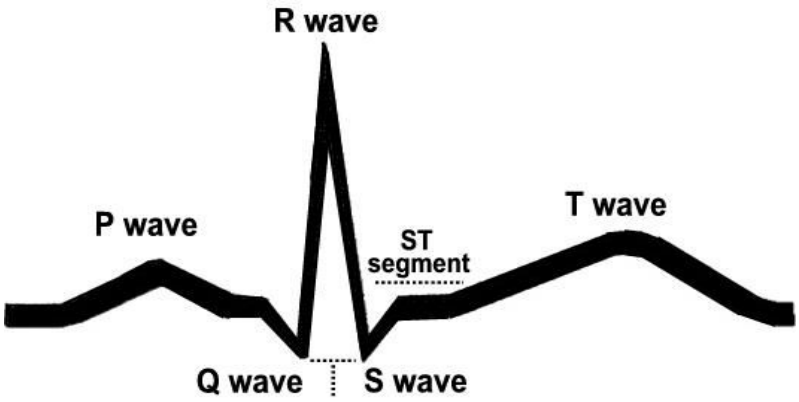


Figure 1.7 ECG waveform (Atrial Fibrillation, 2007).

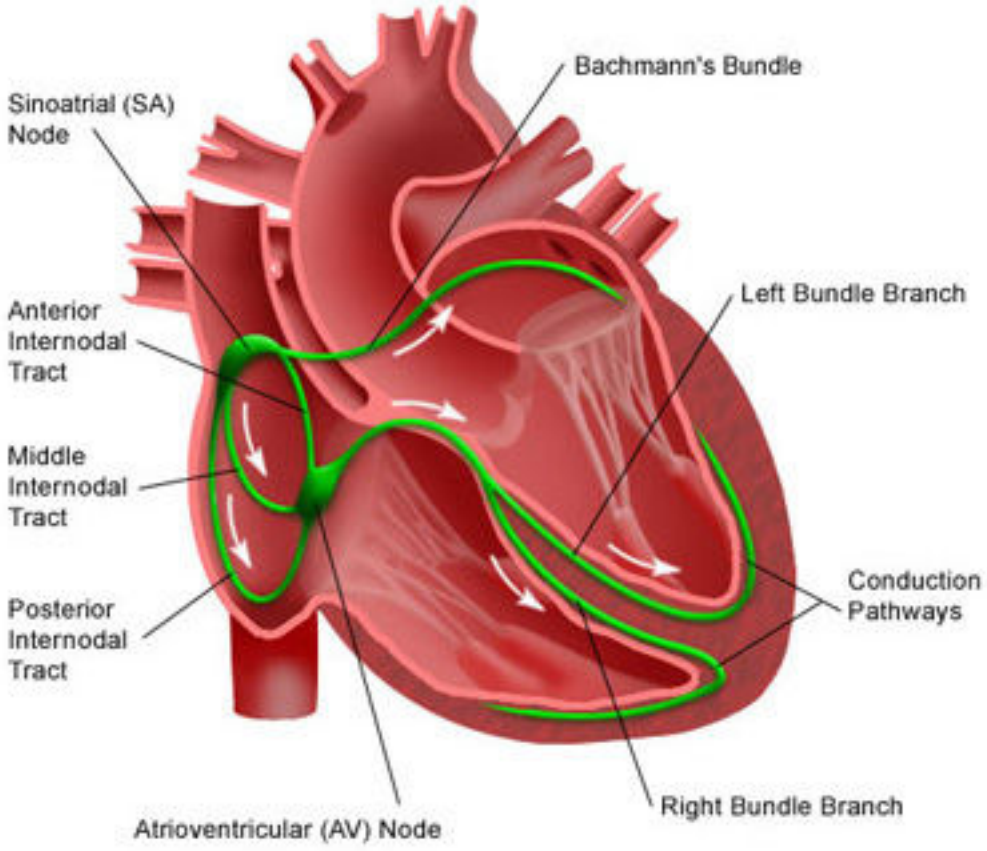


Figure 1.8 Electrical System of the Heart (Ministry Health Care, 2006).

The autonomic nervous system (ANS), which connects the brainstem to the rest of the body, controls the reflexes, respiration and heartbeat (Fig. 1.9). The system is divided into the sympathetic and parasympathetic nervous systems with the visceral afferent system acting as a network of nerves running from the organs back to the brain to relay messages from the body (McEwen and Lasley, 2002). The sympathetic nervous system mobilizes the body by increasing the heart rate and blood pressure, whereas the parasympathetic nervous system restores the body to a resting state via a decrease in blood pressure and heart rate. The vagus nerve, considered the "brake" of the parasympathetic nervous system, acts as a built in protector against heart disease. A healthy small beat to beat variation due to synchrony with respiration, known as respiratory sinus arrhythmia (RSA), is a sign of a properly functioning vagal brake. However, poor use of the vagal brake, where the heart rate and blood pressure cannot be controlled, is seen in people who exhibit hostility, depression and anxiety. For example, hostile individuals, prone to surges in blood pressure, were found to have a cardiac output lower than calm people (McEwen and Lasley, 2002). Therefore, the hearts of the hostile people were beating harder, moving less blood, ushering an elevation in their blood pressure. The maladaptive response did not have a corresponding counteractive measure to deal with the challenge or stressor. Without the vagal brake working properly, the body is unable to cope with the stresses from a new or hostile environment, leading to heart disease development such as hypertension.

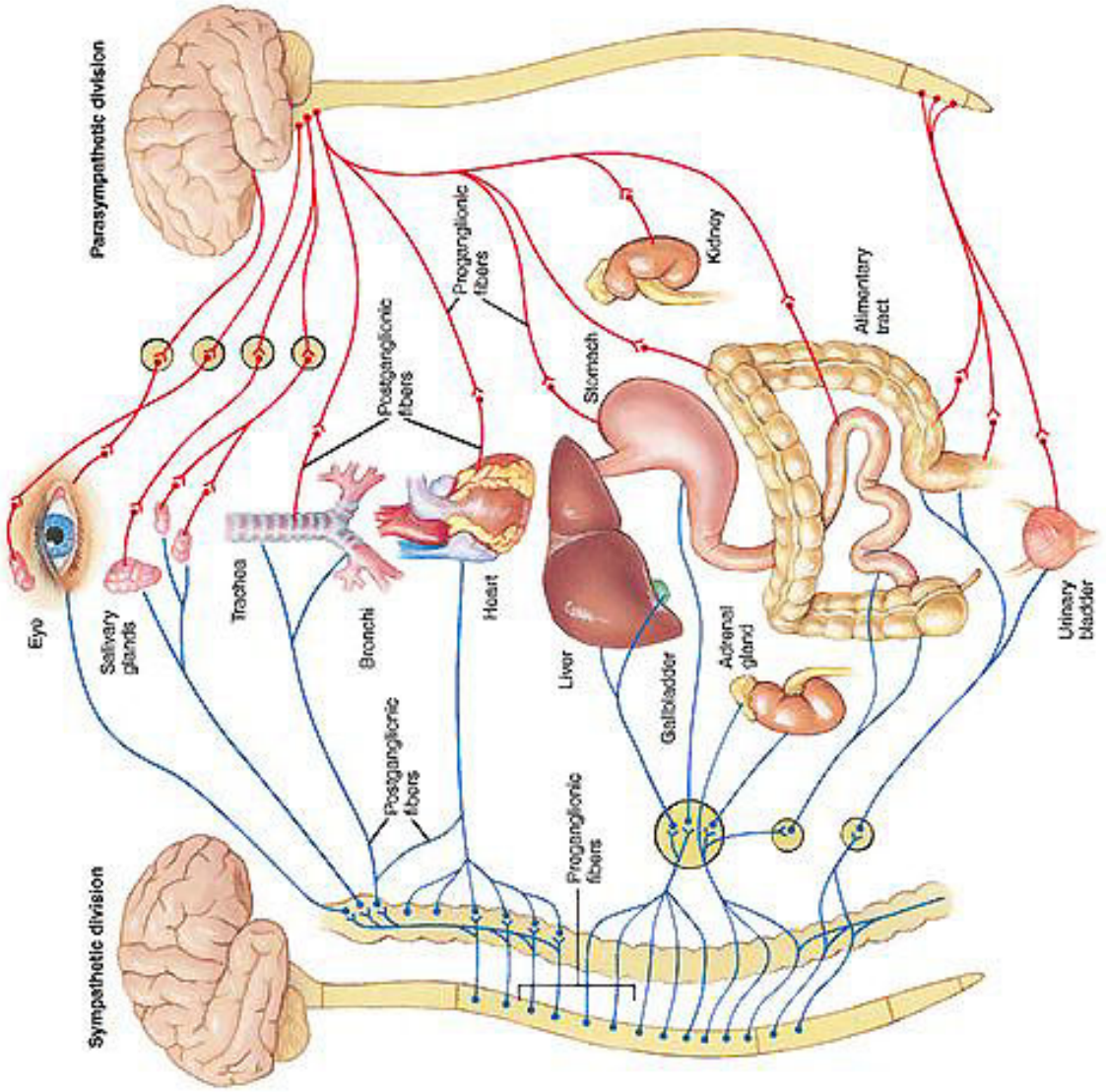


Figure 1.9 Sympathetic and Parasympathetic components of the autonomic nervous system. (PTSD Forum, 2006)

1.8 Stress as an Indicator of Disease

Overall, stress studies have sought different paths to elucidate the inner workings of stress. There has been wide use of psychological stress tests which take the form of questionnaires that grade the level of stress experienced with a scale. This has been popular in psychology based experiments, which seek to identify the source of stress through behavior and lifestyles. In an attempt to understand the physical manifestations of the stress response, specific parameters of organ systems have been selected to describe the state of the system during stress. Of recent interest is the use of biomarkers in epidemiological studies of stress. One notable example is plasma fibrinogen, which has an increasing trend of usage in the study of stress related disorders (Thorell, 2003). Their levels have been shown to be elevated in prolonged stress exposure at work. More established biological measures involve catecholemines, stress hormones of the endocrine system that are sensitive to stressors. One such hormone is adrenaline, which is activated during the "fight or flight" response to increase readiness in danger. Another hormone widely studied is cortisol. During stress, cortisol is secreted into the bloodstream to regulate blood pressure and cardiovascular function, in addition to the metabolism and distribution of proteins, carbohydrates and fats. Long term elevated levels have been linked to various heart diseases. The latest trend in stress research has focused on measurement of biological system specific parameters. Throughout the study of cardiovascular disease, stress has been noted to play a larger role in its onset and progression. In the cardiovascular system, heart rate, diastolic and systolic blood pressures are the most common parameters in stress research. Changes in these parameters define many of the cardiovascular disease symptoms. For example, chronic

elevated blood pressure or hypertension is linked to increased risk for stroke, myocardial infarction, and heart failure (Rabin, 2005).

CHAPTER 2: AIMS AND SIGNIFICANCE OF THESIS

The stress response is a unique system in which the whole body is temporarily mobilized to respond to a challenge or stressor. Based on the assessment of the situation, the brain prepares the body to fight or flee from the challenge. In normal circumstances, the stress response dissipates once the stressor disappears. However, in humans, the response can be triggered before the stressor appears or last after the stressor has been dismissed. Often, the stress response is triggered in situations where humans imagine fear or entrapments in daily situations in which the response cannot help them resolve their current "danger".

The objective of this thesis is to explore which types of stressors exert the most influence on the cardiovascular system. From prior stress studies, it is known that stressors can influence the health of an individual when they are repeated or prolonged and cannot be resolved. However, there have not been studies on what types of stresses people are most susceptible to in daily life. In the university setting, stresses often come from sports, class work and work obligations. Since life as a graduate student requires one to balance various activities inside and outside the classroom, university graduate students were selected as our group of subjects.

Young volunteers were subjected to mental, physical and respiratory stressors while their variability of heart rate, blood pressure and blood flow velocity were measured to examine the role of stress on the cardiovascular system. Respiratory stress consisted of holding breath for forty seconds, while the mental stress was induced from playing a video game with levels of increasing difficulty for five minutes and the physical stress was imposed by holding a five pound weight with the right arm. Each stressor was

preceded by a two minute rest period. The subject's complete data was divided into the segments of rest, respiratory, mental and physical. Furthermore, each segment narrowed down to the last forty seconds to observe the maximum change in stress. From these segments, five beats, consecutive or individual, were selected to take the fast Fourier Transform (FFT) of the corresponding blood pressure (p) and blood flow (v) waveforms to measure the ratio of the FFT(p) and FFT (v) to obtain the impedance. For the individual beat analysis, the ratio of the change in blood pressure and the change in blood flow velocity were determined in addition to the QT interval, T-wave amplitude and the R-wave to start of the blood pressure waveform, identified as the R-P onset, were measured from the 5 beats selected per segment. Each subject's rest recording served as the control to be compared to the interventions.

The present study offers insight into quantitative measurement of different types of stressors from the cardiovascular point of view. As noted by other stress physiologists, the cardiovascular system is a prime example of observing the effects of stress during the fight or flight response to a challenge or stressor. It is also ideal due to the non-invasive nature of acquiring cardiovascular measurements during stress testing. From this thesis, the outcome can further our knowledge of stress physiology and its impact on cardiovascular health from the evaluation of select cardiovascular parameters.

CHAPTER 3: METHODS

3.1 Experimental Subjects

Ten graduate students ranging in age from 22-35 years of age were selected from the Biomedical Engineering department to participate in an experiment to study the cardiovascular responses to different types of stress in college students. Participants were warned not to consume food or caffeine, exercise or drink alcohol for one hour prior to the experiment because they could affect their cardiovascular measurements. Subjects were asked to disclose any history of heart problems for evaluation of whether they were still eligible to participate. Appointments were made on an individual basis to accommodate for varying class schedules. Subjects came into the lab during late mornings, mid afternoons or early evenings. The protocol was explained to the subjects as they rested prior to taking baseline blood pressure measurements using the cuff method.

3.2 Experimental Setup

3.2.1 Electrocardiography

A three lead electrocardiogram circuit (Fig. 3.2) was designed with differential operational amplifiers and filters to acquire ECG signals with maximal signal to noise ratio. Coupled to a computer with AcqKnowledge 3.1 software (BIOPAC Systems Inc.), the ECG signals of each patient were recorded from the circuit for the duration of the experiment. A DC power supply voltage of +/- 12 volts was used in the ECG circuit. The three wires from the circuit were attached to an Electrode Lead Assembly, connecting the electrodes on the subject to the circuit. All three ECG leads were clipped onto the gel

electrodes placed on the upper right and left chest immediately underneath the clavicles and inner left ankle. Male subjects were asked to shave away hair just underneath the clavicle area to ensure good contact between the gel electrodes and the skin. The chest area was chosen over the arms to minimize the motion artifacts that would occur when executing the physical task and mental task. All recordings were band pass filtered to 0-50 Hz and digitized with a sampling frequency of 200 Hz.

3.2.2 Blood Pressure

A blood pressure circuit (Fig. 3.1) was also built with differential operational amplifiers and filters to acquire blood pressure waveforms when coupled to AcqKnowledge 1.3 (BIOPAC Systems Inc.). A DC power supply voltage of +/- 5 volts was used in the blood pressure circuit. There are 4 leads originating from the circuit. All leads were consolidated into a wire casing while its ends were soldered to a pressure transducer. Lastly, the transducer is connected to a Velcro cuff made to fasten around the wrist for steady acquisition of radial artery blood pressure measurements. All recordings were band pass filtered to 0-25 Hz and digitized with a sampling frequency of 200 Hz. To calibrate the blood pressure measurements from volts to mmHg, it was assumed the cuff diastolic pressure was equivalent to the resting diastolic pressure measured from the transducer. The resting pulse pressure was divided by the difference of the average systolic and average diastolic pressures per intervention (1-1) to obtain the pulse pressure multiplying factor in mmHg/volt. To obtain the pulse pressure in mmHg, the pulse pressure factor was multiplied by the raw data difference between the systolic and

diastolic pressures in volts. Finally, systolic pressures were calculated as the sum of the pulse and diastolic pressures in mmHg.

(Ps - Pd) / (avgMaxP - avgMinP) = [mmHg/volt] (1-1)

Diastolic pressure (Pd) = resting diastolic pulse pressure (mmHg)

Pulse pressure = mmHg/volt * (systolic pressure (volts) - diastolic pressure (volts))

Systolic pressure (Ps) = Pulse pressure (mmHg) + Diastolic pressure (mmHg)

3.2.3 Doppler Blood Velocity Measurement

A Dual Frequency Directional Doppler (Model 909) by Parks Medical Inc. was used to capture the blood flow velocity in the radial artery from an adult flat flow probe prepared with ultrasound gel to enhance signal acquisition. The probe was attached to a homemade Velcro cuff to be wrapped around the wrist a few inches away from the blood pressure transducer. The flat probe was adjusted to increase its angle to the skin surface to 45 degrees, the recommended angle. Without the angle increase, the sensor was elevated 15 degrees above the skin surface. It was also convenient because the multiplier used to calculate the velocity was one. The Doppler instrument is capable of sensing blood flow occurring in opposite directions when the ultrasonic beam from the probe intersects a vessel. The parameters used during the experiments were the emitted frequency (Fe = 8.2MHz), angle between the acoustical axis and blood velocity (a = 45 degrees), received frequency (Fr, as recorded from AcqKnowledge 1.3), velocity of sound (c = 1.56x10^6 cm/sec), the mean frequency shown in meters on the Doppler (also known as the area under the flow curve to the zero flow line) and the velocity of the scattering medium or blood cells. Calibration measurements for the Doppler flow meter

were as follows: 0 Hz→ 3mV (circuit output) and Calibration A (0.73 kHz as read from the flow meter → 450 mV). Once the Doppler measurements were in Hz, they were divided by the baseline radial artery velocity of 19.1 cm/s (Chong et al., 2006). To obtain the unit of cm/s, the values were divided by their respective beat durations, measured in seconds. Together, each component was captured on separate channels simultaneously throughout the duration of the experiment per subject.

3.3 Protocol

Subjects may not exercise or consume caffeine 1.0 hours before test.

Subjects may not talk during experiment.

The experiment is non invasive and will take 20-30 minutes to complete.

1. Take Diastolic and Systolic pressure with cuff (for conversion from volts to mmHg).

2. Take pulse at radial artery of left arm and attach blood pressure transducer, prepare Doppler flat probe position on radial artery and attach three ECG leads

- Upper left chest (underneath clavicle area) = WHITE lead
- Upper right chest (underneath clavicle area) = RED lead
- Inner right ankle = BLACK lead (above ankle bone)

3. Set up AcqKnowledge 3.1 to read three channels and acquire for 30 minutes at 200Hz.

EXPERIMENT BEGINS

4. 2 minutes (120 sec) of rest (baseline)

5. Hold breath for 40 seconds and release for 2 minutes (120 seconds) of rest.

6. 5 minutes (300 sec) of a mental task (ROAD BLOCK video puzzle game)

Reward for highest level achieved.

7. 2 minutes (120 sec) of rest

8. 5 minutes (300 sec) physical task (lift 5 lb. weight with left arm keeping rest of body as still as possible)

9. Save raw data to file.

3.4 Data Analysis

Each circuit contained components to filter the signal as it was acquired. In addition, AcqKnowledge 3.1 software allowed for additional data filtering from noise or movement artifacts as well as data analysis (i.e. FFT). From the ECG waveforms, the following parameters were calculated from measurements with Acqknowledge 1.3:

- **R-R interval**: distance between consecutive R- waves of the ECG waveform; used as marker of duration of one beat or beat to beat cardiac period. Select beats were highlighted to measure their duration in seconds.
- **R-P onset:** R-wave peak to start of the blood pressure waveform. Duration measured in seconds.
- **Beats per Minute (BPM)**: Number of heart beats per 60 seconds.
- **Frequency (Hz)**: Rate of heart beats per second
- **QT interval**: Time elapsed between the onset of ventricular excitation to the end of ventricular depolarization. This segment serves as in indicator of the efficiency of ventricular excitation, mechanical response and electrophysiological recovery (Ritota, 1975). For select beats, the initial negative deflection to the end of the second positive deflection was highlighted from to measure their duration in seconds.
- **T wave amplitude**: Representative of ventricular repolarization duration. Changes in serum potassium levels have been known to change the amplitude of the T wave (Lipman & Cascio, 1994).
- **Change in blood pressure (Δ p)**: Difference between systolic and diastolic pressures. Select beats were highlighted for FFT analysis.

- **Change in blood flow (Δ v)**: Difference between maximum and minimum blood flow velocity corresponding to the systolic and diastolic blood pressures. Select beats were highlighted for FFT analysis.

Fast Fourier transforms of blood pressure and velocity were obtained to determine the impedance or ratio of FFT (Δp)/ FFT (Δv) per intervention from all subjects. These plots were analyzed to observe changes occurring during each portion of the experiments.

3.5 Statistical Analysis

Paired Student's t-tests were applied to TWA, R P onset, Frequency, BPM, QT interval, delta blood pressure and delta blood flow velocity when compared to rest over all subjects. All were tested with the null hypothesis of no difference between rest and the intervention, H0: $\mu 1 - \mu 2 = 0$, whereas the alternative hypothesis for all comparisons was Ha: $\mu 1 - \mu 2 \neq 0$ ($\mu 1$ = mean of Rest and $\mu 2$ = mean of Intervention). Mean and standard deviations were obtained from T wave amplitude (TWA) data per subject. In addition, percent changes were calculated from the TWA measurements between rest and stress interventions of respiratory, mental and physical stressors.

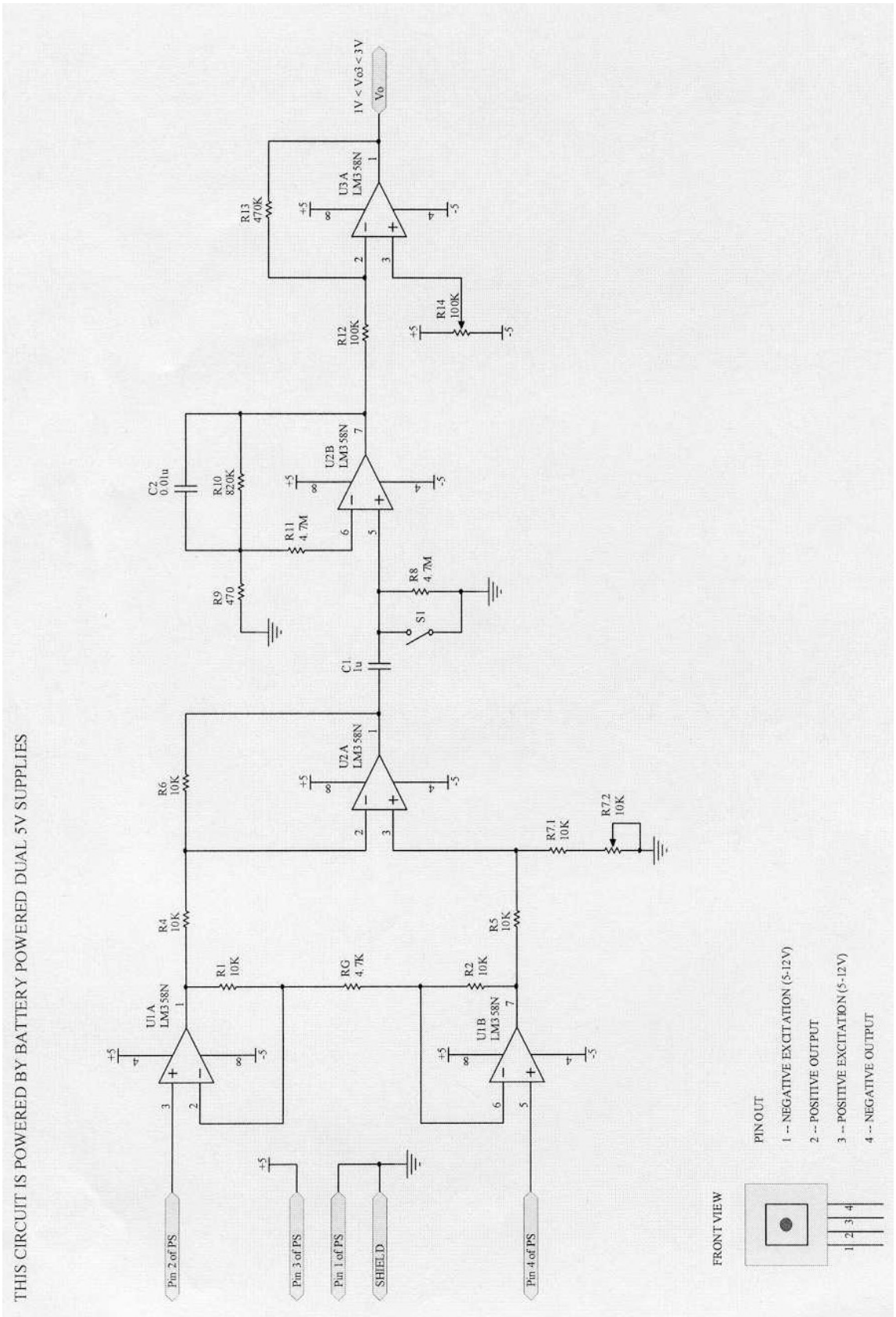


Figure 3.1 Blood Pressure Amplifier schemati

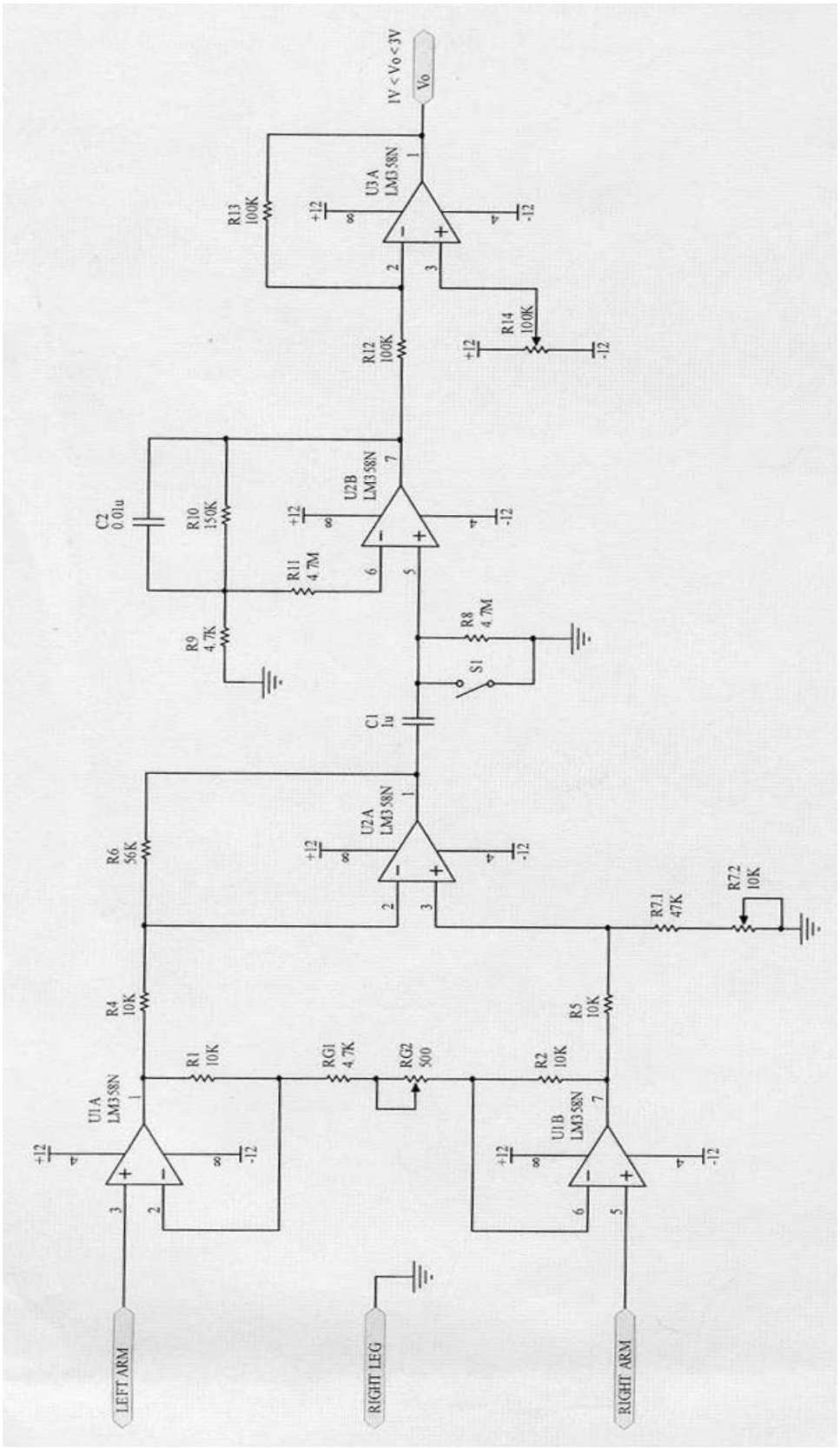


Figure 3.2 ECG Amplifier schematic.

CHAPTER 4: RESULTS

After collecting data from ten subjects, three subject data recordings could not be used. Problems with the data were attributed to poor Doppler readings and blood pressure transducer malfunction during the experiment. The raw data was streamlined into an analysis of 5 beats per rest and intervention for a total of 140 beats. Beats were chosen from the last 20-40 seconds of each stressor task to observe the greatest change induced by it.

Subject	Gender	Age	Resting Systolic	Resting Diastolic	Time of Day	Doppler probe angle (degrees)
1	Male	35	106	68	Afternoon	45
2	Male	24	117	80	Evening	45
3	Male	24	109	63	Morning	45
4	Female	26	90	60	Afternoon	45
5	Female	31	104	74	Evening	45
6	Male	24	108	68	Afternoon	45
7	Male	24	122	76	Morning	45

Table 4.1a. Subject age, gender and respective baseline conditions.

Subject	Intervention (Stressor)	Average R-R interval Duration (sec)	Average Frequency (Hz)	Average BPM	# Beats
1	**REST**	4.43	1.12865	67.7201	5
	RESPIRATORY	4.56	1.0965	65.789	5
	MENTAL	4.21	1.1875	71.259	5
	PHYSICAL	4.235	1.17925	70.7547	5
2	**REST**	5.575	0.89685	53.81165	5
	RESPIRATORY	4.795	1.04275	62.56515	5
	MENTAL	5.505	0.90825	54.4959	5
	PHYSICAL	1.7	1.18344	71.0058	2
		1.725	1.15942	69.56522	2

		0.88	1.11111	66.6667	1
3	**REST**	4.72	1.0593	63.5593	5
	RESPIRATORY	4.69	1.0695	64.1711	5
	MENTAL	4.195	1.18625	71.1744	5
	PHYSICAL	3.88	1.287	77.2201	5
4	**REST**	3.94	1.26905	76.14215	5
	RESPIRATORY	3.65	1.36985	82.1918	5
	MENTAL	4.285	1.17235	70.34	5
	PHYSICAL	4.08	1.2255	73.525	5
5	**REST**	4.425	1.12865	67.7201	5
	RESPIRATORY	4.285	1.16685	70.01165	5
	MENTAL	4.325	1.15475	69.28405	5
	PHYSICAL	3.835	1.3038	78.22685	5
6	**REST**	5.15	0.97085	58.25245	5
	RESPIRATORY	5.56	0.89605	53.76345	5
	MENTAL	4.945	1.01215	60.72875	5
	PHYSICAL	4.485	1.1136	66.81515	5
7	**REST**	4.01	1.2469	74.81295	5
	RESPIRATORY	5.24	0.956	57.3614	5
	MENTAL	4.095	1.221	73.26005	5
	PHYSICAL	3.86	1.292	77.5194	5

Table 4.1b. Experiment data for all subjects during Rest, Respiratory, Mental and Physical stressors.

4.1 Impedance from Fast Fourier Transform of blood pressure and blood flow velocity

Using AcqKnowledge software, blood pressure and blood flow velocity raw data was transformed via Fast Fourier Transform (FFT). The impedance, or ratio of the FFT (blood pressure) and FFT (blood flow velocity), was plotted in Figures 4.1-4.7. In the context of this experiment, impedance is the opposition to blood flow, characterized by blood pressure and blood flow velocity. It is expected that subjects will show increased impedance in the form of higher blood pressures and lower flow velocities as they experience strain from each stressor. Descriptions of the locations of the impedance peaks or valleys will be denoted as (velocity, Pulse Pressure), with units in Hz. The results from each subject are discussed below:

SUBJECT 1 (Fig. 4.1)

During rest, Subject 1's impedance exhibited a decreasing trend. This was interpreted to show the resistance to blood flow had dropped, decreasing the blood pressure and increasing the flow. Initial decrease in impedance at (0.6, 3) followed by an increasing opposition to flow at (1.4, 33) during respiratory stress. During mental stress, impedance gradually increases to its secondary maximum at (1, 15) before continuing its ascent to its maximum at (1.6Hz, 35Hz). Maximum impedance is thought to occur at the end due to increasing game level difficulty. For physical stress, the highest impedance occurs at (0.6, 20) followed by a secondary peak at (1.2, 14). The subject felt physical strain intermittently as evidenced by a trend of maximum followed by minimum impedances.

SUBJECT 2 (Fig. 4.2a and Fig 4.2b):

At rest, a high initial impedance (0.3, 1.25) gradually decreased to (0.85, 0.6) before gradually increasing to (0.95, 1.05). Pulse pressure was decreasing, allowing more blood flow at rest. During respiratory stress, the largest impedance was seen at (0.8, 2) followed by a secondary peak at (1.6, 1.5). Holding breath for extended periods of time temporarily increases pulse pressure as in respiratory sinus arrhythmia (RSA).
Mental stress was characterized by a low impedance at (0.3, 0.75) followed by a maximum impedance of (0.65, 1.3). Physical stress was analyzed from single or 2 consecutive beats. During the 2 beats at 945 seconds, large consecutive impedances were seen at (3.1. 2) and (5, 1.27) before lowering to (6, 1). Pulse pressure remained elevated from 3-5Hz (velocity), indicating strain from physical stress was causing higher pulse pressure. For the 2 beats at 965 seconds, impedance fluctuated, generally decreasing. Its maximum impedance occurred at (2, 1.29) while the lowest impedance was at (3, 0.9). This indicated that the blood flow was increasing as the pulse pressure dropped. For the single beat at 980 seconds, the impedance dropped sharply with a small increase in impedance at (2.5, 1.4). This beat occurred near the end of the physical task. The plot indicates a large drop in pulse pressure and increased flow to accommodate the prolonged exercise.

SUBJECT 3 (Fig. 4.3):

At rest, the subject's impedance increased and remained elevated. This may indicate that the subject was not relaxed. During Respiratory stress, there were two peaks of high impedance before it leveled at (0.8, 1.2). Elevated pulse pressures contributing to the high impedances were most likely due to deep inspirations. For mental stress,

maximum impedance occurred at (0.8, 1.38) and dropped sharply before it began its ascent. At the maximum impedance, a mental challenge from the game caused strain on the subject's cardiovascular system, dramatically increasing the pulse pressure. During physical stress, an initial peak at (0.4, 1) was followed by the maximum impedance at the end of the plot at (1.6, 1.4). This indicated a larger physical challenge was felt by this subject towards the end of the physical task. This may be attributed to fatigue felt at the end of the five minute exercise.

SUBJECT 4 (Fig. 4.4):

At rest, large impedances became smaller but were still present in lower magnitudes. During respiratory stress, the maximum impedance occurred at the beginning at (0.2, 5), followed by subsequent decreasing impedances. This may be due to more stress felt upon inspiration rather than holding one's breath. For mental stress, the maximum impedance occurred at the start and fell sharply before gradually rising back near its maximum impedance. This may be due to nervousness of playing a new game and being given a finite time. The impedance rose during the game, as expected, since the level of difficulty rises as one advances. During Physical stress, there are two large impedance peaks at (0.6, 11) and (1.6, 10). The larger impedance occurred first but the second impedance was not much smaller than the previous one.

SUBJECT 5 (Fig. 4.5):

At rest, the impedance was very low and maximum impedance was seen at (1, 25). It was unexpectedly high. At rest, impedances are low or decreasing. For respiratory stress, a small increase in impedance upon inspiration is followed by a maximum impedance at (1.4, 80) before drastically dropping in pulse pressure. This was

the recovery phase after holding ones breath. During mental stress, there was a gradual increase in impedance to (1.4, 230) before the pulse pressure quickly dropped at (1.6, 50). Stress was felt temporarily during the puzzle game. In physical stress, a small increase in impedance is followed by its maximum impedance towards the end at (1.4, 65). Maximum stress was felt at the end of the exercise, noted by increasing pulse pressure.

SUBJECT 6 (Fig. 4.6):

At rest, high initial impedance (0.2, 16) sharply decreased to (0.5, 1) before gradually increasing to (0.85, 8). Pulse pressure was decreasing, allowing more blood flow at rest. For respiratory stress, very low impedance was followed by secondary and maximal (0.8, 70) impedances towards the end. This subject was able to hold his breath for longer periods of time without strain compared to other subjects. During mental stress, there were secondary and maximal impedances with a sizable drop in impedance in between the two. During game play, the subject experienced greater mental strains twice (near the beginning, and towards the end). The peaks could be due to stress from a game level that took longer than expected to surpass. In physical stress, the largest impedance (0.6, 14.5) occurred near the beginning and gradually decreased to its lowest impedance. This may be attributed to the subject getting used to lifting the weight as time passed.

SUBJECT 7 (Fig. 4.7):

At rest, high initial impedance (0.2, 40) sharply decreases to (0.6, 1). Pulse pressure is decreasing, allowing more blood flow at rest. This subject was relaxed. During respiratory stress, the largest impedance occurred at the start of inspiration. The impedance gradually decreases to its lowest level towards the end of the breath holding

exercise. During mental stress, there is a single maximum impedance reached at (1, 5) before sharply decreasing. Pulse pressure is very low prior to and following the maximum impedance. This subject experienced mental strain in the form of higher pulse pressures but quickly calms (resolves stress) as evidenced by the immediate drop in impedance (increased blood flow and lower pulse pressure). During physical stress, peak impedance was reached near the beginning before sharply dropping. Later, the impedance gradually returned near its maximum impedance while exercising. This shows that this subject felt physical strain, recovered temporarily and began feeling another burst of strain.

4.2 T Wave Amplitude

T wave amplitudes (TWAs) of selected beats were averaged from the rest, respiratory, mental and physical interventions. In Table 4.2, the average mental stress T wave amplitude percent change from rest was compared to the highest mental game level achieved. It is interesting to note that subjects who reached the lowest level in the mental game exhibit an increase in T wave amplitude, while subjects who scored higher show a decrease in their TWAs. For comparison of T wave amplitudes between interventions, the mean TWAs for rest, respiratory, mental and physical stressors were plotted together for each subject in Figures 4.15a-4.21a. Additionally, the percent change from rest was plotted with standard deviations in Figures 4.15b-4.21b. To observe the trend over all subjects, mean TWAs and stressor TWA percent changes from rest was plotted in Figure 4.22. TWAs generally decreased from rest during respiratory and mental stressors. The percent change decrease ranged from a low of 13% to as high as 64%. However, Subject

7 was an exception to this trend. He showed a large increase in T wave amplitude for both respiratory (129%) and mental (119%) stressors. While physical stressors produced both positive and negative changes from rest, all but Subject 2 and Subject 6 produced a decrease in TWA from rest.

4.3 Individual Beat Analysis

4.31 Changes in Blood Pressure

During the respiratory stressor task, subjects were instructed to engage in breath holding for 40 seconds. Subjects 1 and 6 exhibited a steady descent in pulse pressure in Figures 4.8b and 4.13b respectively. The pulse pressure trends of the other subjects were varied. For Subject 7, there is an overall decrease with intermittent increases in pressure. For Subjects, 2 and 3, their pulse pressures dip before rising steadily. However, the pulse pressure of Subject 3 drops from its peak at 80 mmHg to 47 mmHg while Subject 2's pulse pressure continued to increase from 24-28 mmHg. Lastly, Subjects 4 and 5 both show a small increase before embarking on a moderate descent.

During the mental stressor, minimal changes in pulse pressure were exhibited in Subjects 1, 5 and 6. Their blood pressures fluctuated within a range of 4-6 mmHg. Subject 5 showed a stable trend at 25 mmHg. Subject 6 had a trend that showed slight intermittent increases in blood pressure as it decreased overall. However, the trend of Subject 1 had an initial increase in pressure followed by a descent over 2 beats before returning back to its initial pulse pressure at 29 mmHg. Subject 6 won the mental puzzle challenge by reaching the highest level within the allotted time of 5 minutes of play. Although the Subject 6 mentioned he was feeling frustrated, his pulse pressure did not

show any physical strain from the increasing difficulty in the game. Larger changes in blood pressure were seen all other subjects. Pulse pressure differences over five beats ranged from 11-27 mmHg. Subject 4 had a pulse pressure trend similar to Subject 1 in which the pressure increased temporarily before decreasing sharply and returning back to its initial pulse pressure. Subjects 2 and 3 are characterized by a steady decrease in pulse pressure whereas Subject 7 showed an initial increase in pulse pressure followed by a decrease and leveling at 75 mmHg.

The continuous lifting of a five pound weight exerted the greatest physical stress on Subjects 2 and 6. Both plots have one large peak following the first beat followed by a return to their lower initial values. However, the pulse pressure peak from Subject 2 reached a high of 170 mmHg from 50 mmHg before leveling off at 46mmHg whereas in Subject 6, the pulse pressure peak was gradually reached at 18mmHg before descending steadily back to 7 mmHg. The least strain from physical stress was seen in Subjects 1 and 5. They shared similar pulse pressure trends in which a small dip in pressure followed by in increase in pulse pressure. Subject 1's pulse pressure increased to 30 mmHg following the dip in pressure, just below it initial pulse pressure whereas Subject 5's pulse pressure increases slightly above its initial pressure, leveling at 23 mmHg. Lastly, Subject 3 had a pulse pressure temporarily leveling at 38 mmHg from 44 mmHg before increasing to 54 mmHg. Due to unfilterable data, the physical stressor data for Subject 4 could not be analyzed.

4.32 Changes in Blood Flow Velocity

Overall, changes in blood flow velocity were small. However, there were radial artery (RA) blood flow changes during the interventions compared to the resting state. In Subject 2, RA flow velocity increased from 17cm/s to 22 cm/s during the respiratory and physical stressors. During the mental stressor, the RA flow velocities resembled those from rest. In Subject 3, RA flow velocity increased slightly to a range of 21-24 cm/s during the mental and physical stressors. However, this subject's rest and respiratory RA flow velocities are similar. For Subject 4, RA flow velocities decreased to 21 cm/s from a resting range of 23-25 cm/s and increased to 26 cm/s during the respiratory stressor. In Subject 5, all interventions showed an increase in RA flow velocity from rest. Respiratory and mental stressors increased to 22 cm/s and 23 cm/s respectively from a resting range of 20-21 cm/s, whereas the physical stressor elevated the blood flow velocity to a range of 24-25 cm/s. For Subject 6, the greatest changes in blood flow velocity occurred during the mental intervention (22-24 cm/s), followed by the physical intervention in the range of 20-22cm/s. During the respiratory intervention, this subject's flow velocity fell between 15-18 cm/s from a resting range of 17-19 cm/s. Finally, Subject 7 showed an increase in flow velocities from rest during the mental and physical interventions and respiratory stressors decreased the flow velocity to 17 cm/s from a resting range of 20-26 cm/s.

4.4 Statistics

To find significant differences before and after the stressors were encountered, paired t-tests were selected for statistical analysis of the data. It was used to compare means of a particular parameter during rest for all subjects to the same parameters during the respiratory, mental, or physical stressor. The results of the paired t-tests were separated into means, variances and P values in Table 4.3. P values for each test were all compared to the significance level ($P<0.05$). This allowed all null hypotheses to be rejected in favor of the alternative hypothesis that a significant difference exists between rest and the stressor over all subjects. Significant P values were found during physical stress for the beats per minute and frequency parameters.

$H0: \mu1 - \mu2 = 0$ $\quad\quad$ $H1: \mu1 - \mu2 \neq 0$
where $\mu1$ = Mean of Rest and $\mu2$ = mean of Intervention

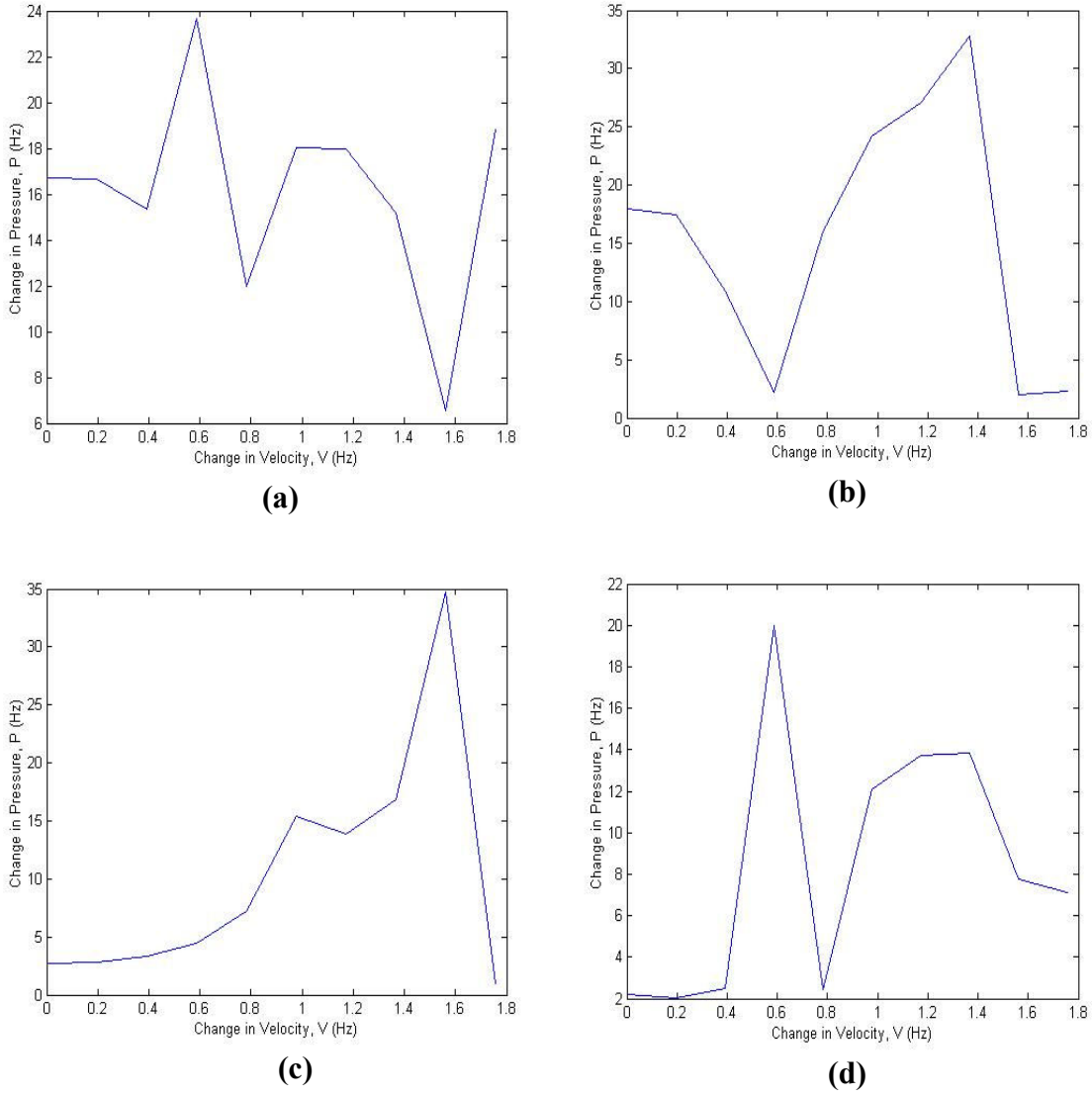


Figure 4.1 Subject 1 FFT Spectrum of Impedance (to 10 Hz) for a) Rest, b) Respiratory, c) Mental and d) Physical.

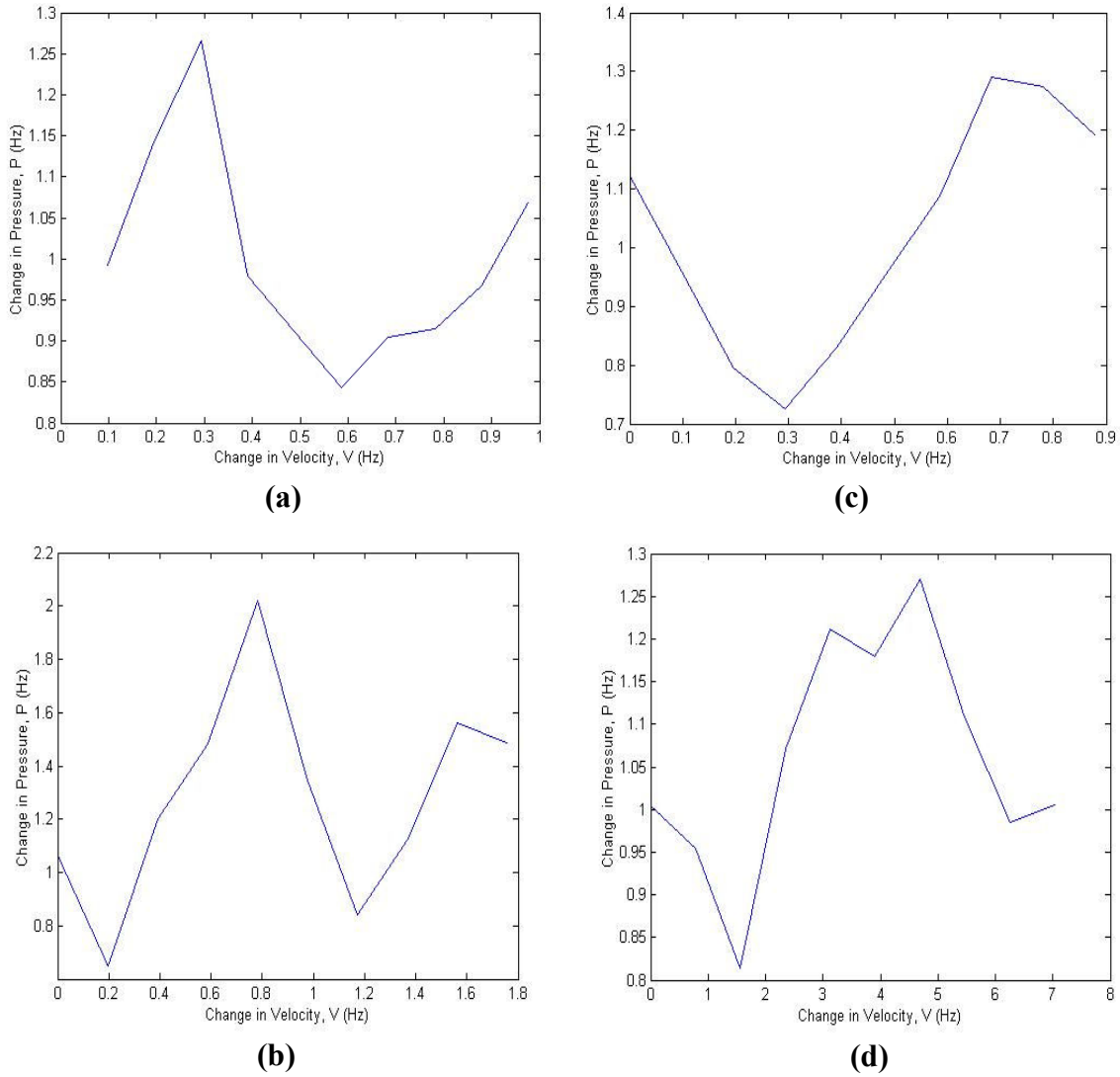


Figure 4.2a Subject 2 FFT Spectrum of Impedance (to 10 Hz) for a) Rest (5 beats), b) Respiratory (5 beats), c) Mental (5 beats at 520 seconds) and d) Physical (2 beats at 945 seconds)

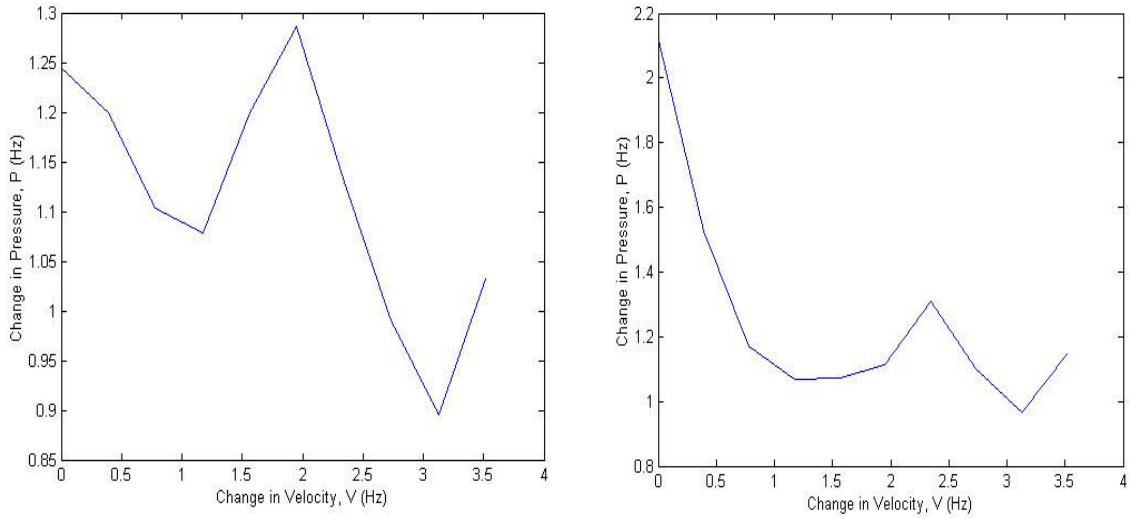


Figure 4.2b Subject 2 FFT Spectrum of Impedance (to 10 Hz) for e) Physical (2 beats at 965 seconds) and f) Physical (1 beat at 980 seconds)

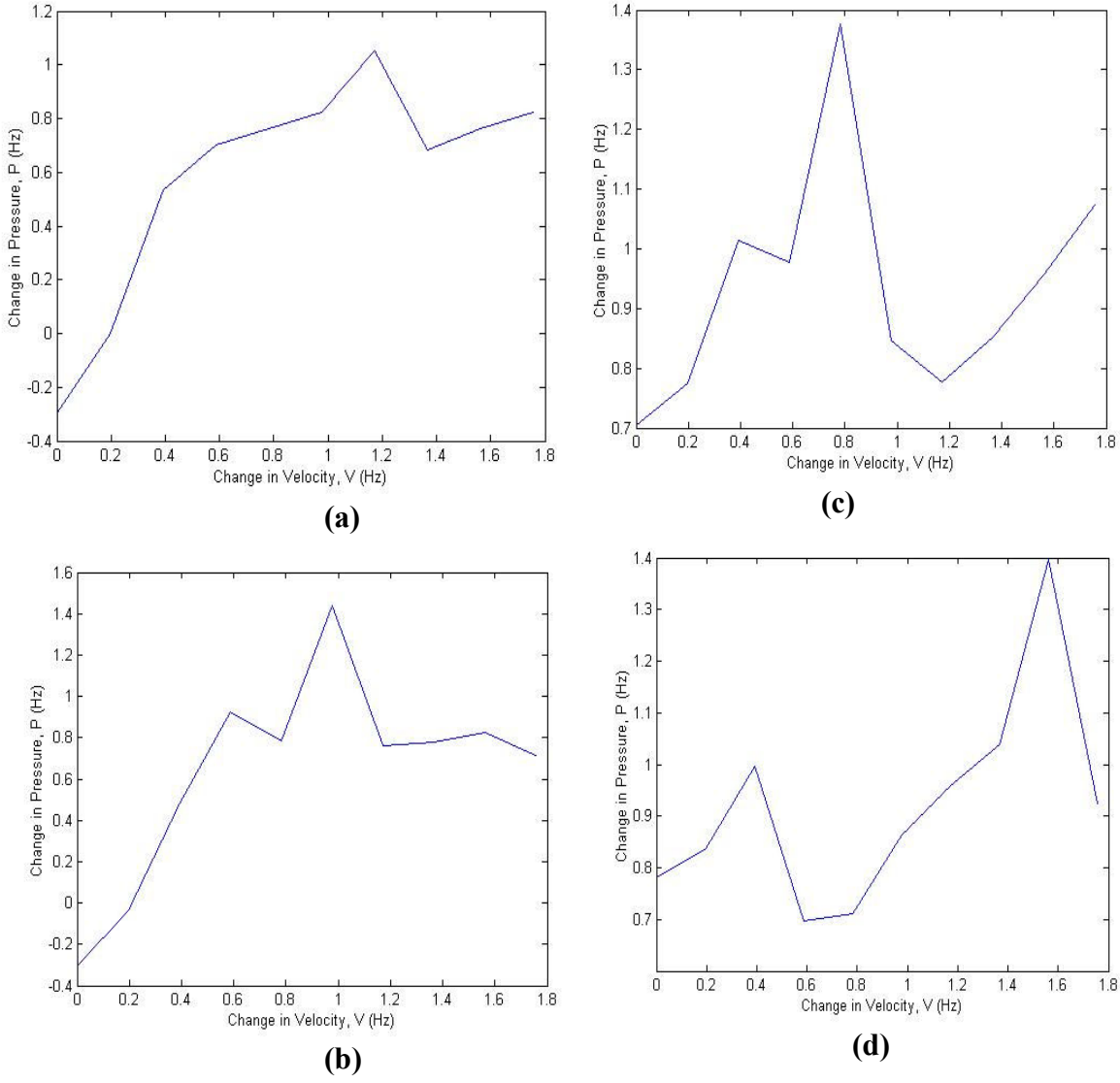


Figure 4.3 Subject 3 FFT Spectrum of Impedance (to 10 Hz) for a) Rest (5 beats), b) Respiratory (5 beats), c) Mental (5 beats) (d) Physical (5 beats)

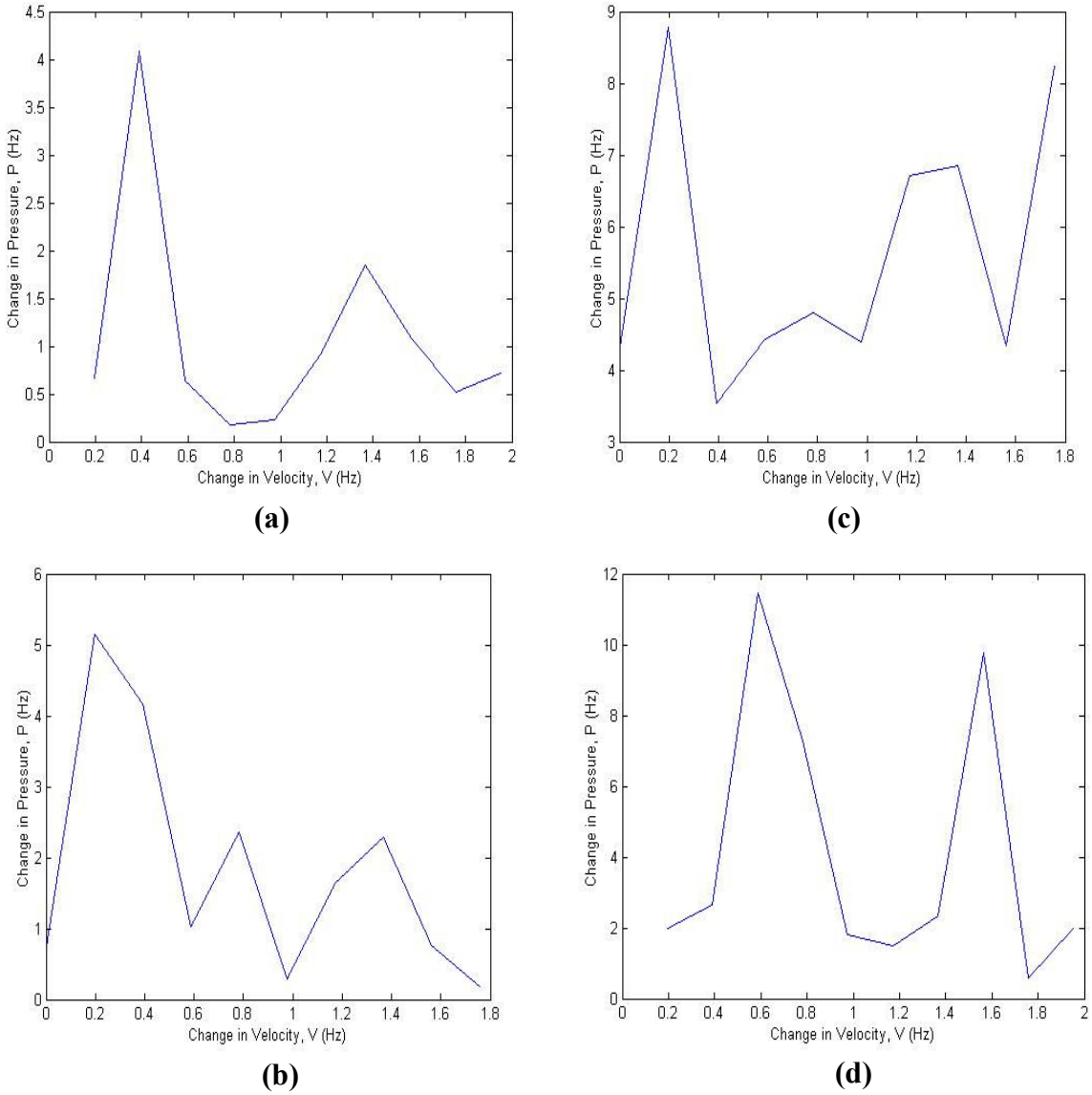


Figure 4.4 Subject 4 FFT Spectrum of Impedance (to 10 Hz) for a) Rest (5 beats), b) Respiratory (5 beats), c) Mental (5 beats) (d) Physical (5 beats)

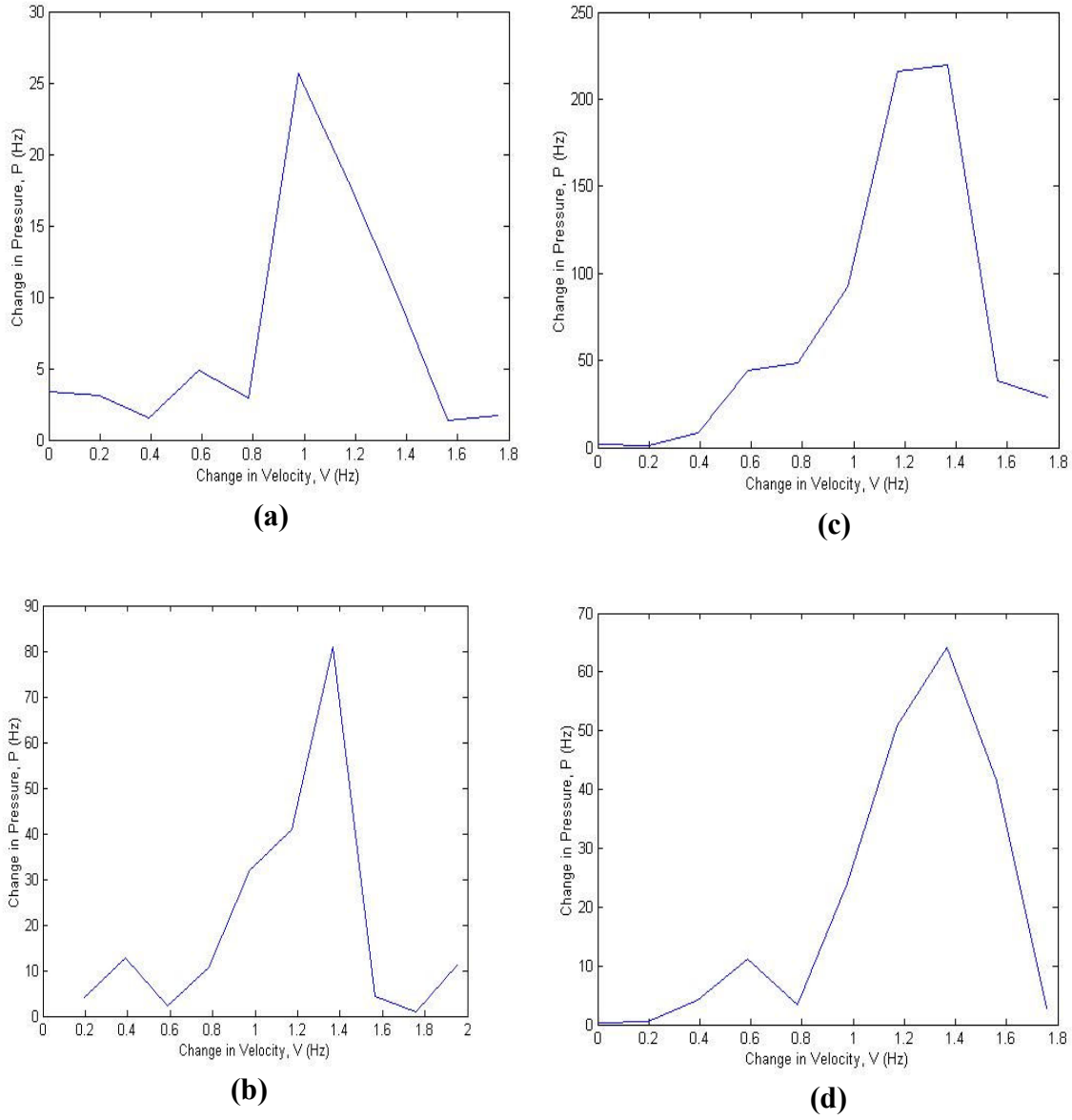


Figure 4.5 Subject 5 FFT Spectrum of Impedance (to 10 Hz) for a) Rest (5 beats), b) Respiratory (5 beats), c) Mental (5 beats) (d) Physical (5 beats)

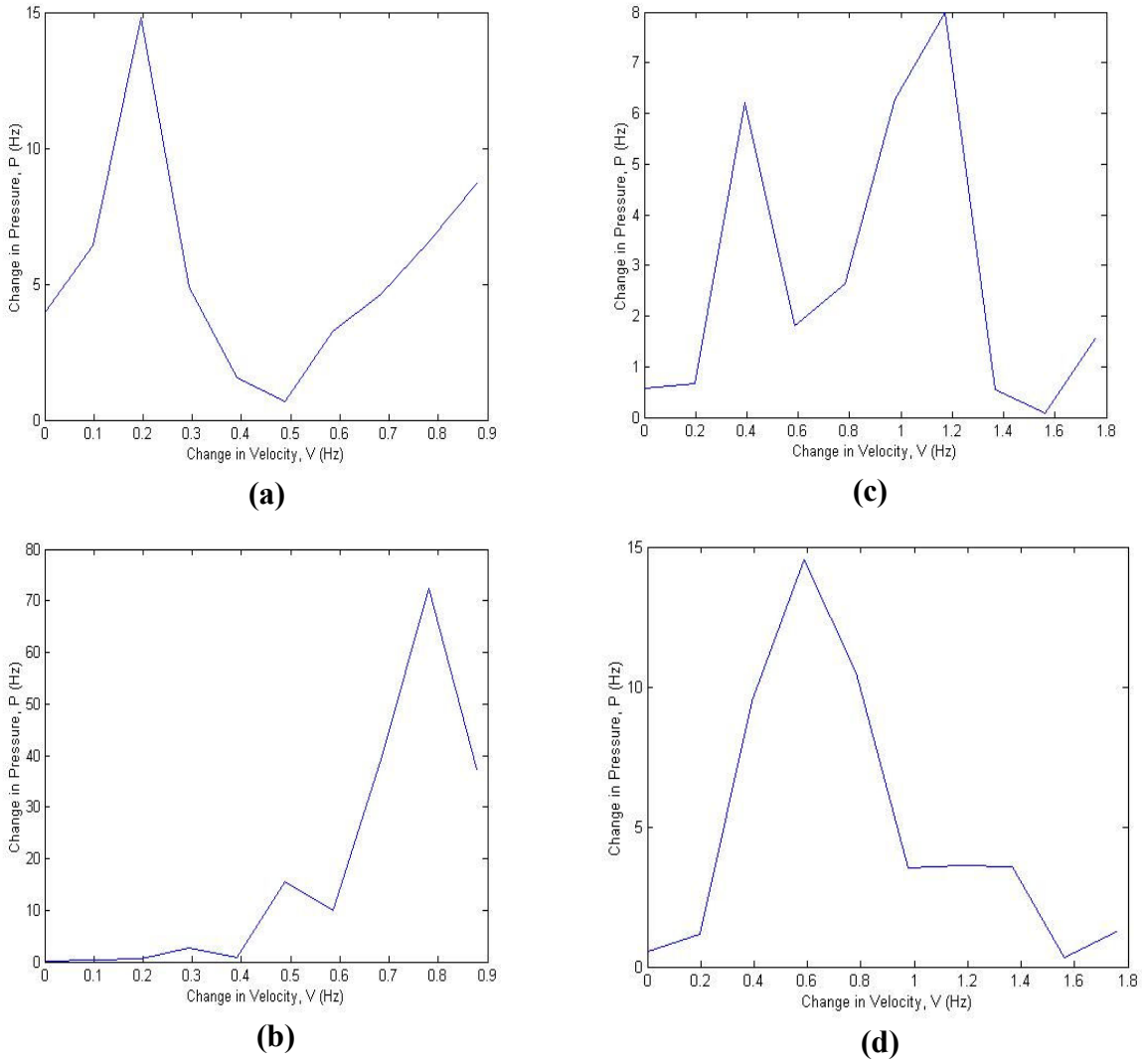


Figure 4.6 Subject 6 FFT Spectrum of Impedance (to 10 Hz) for a) Rest (5 beats), b) Respiratory (5 beats), c) Mental (5 beats) (d) Physical (5 beats)

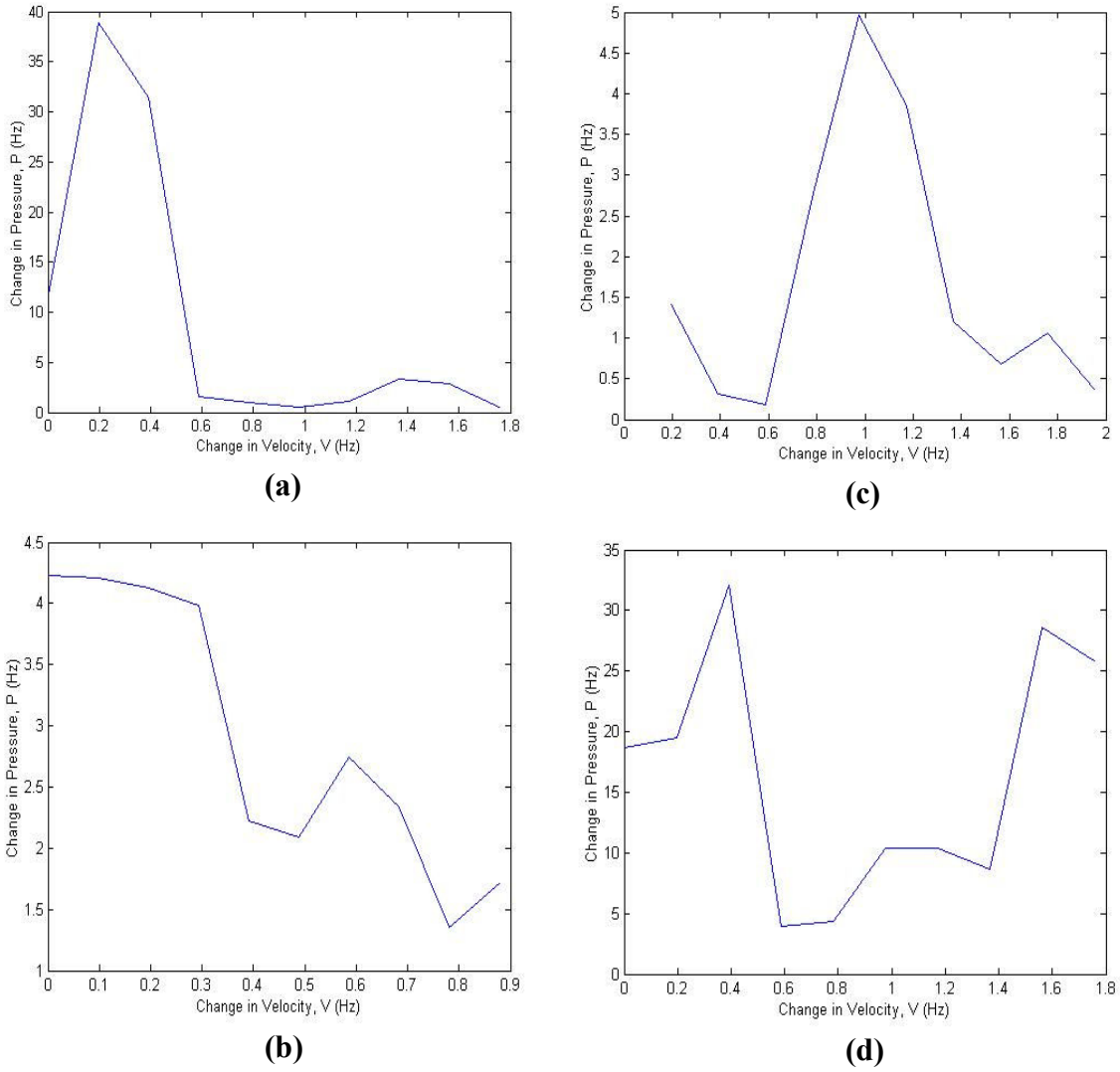
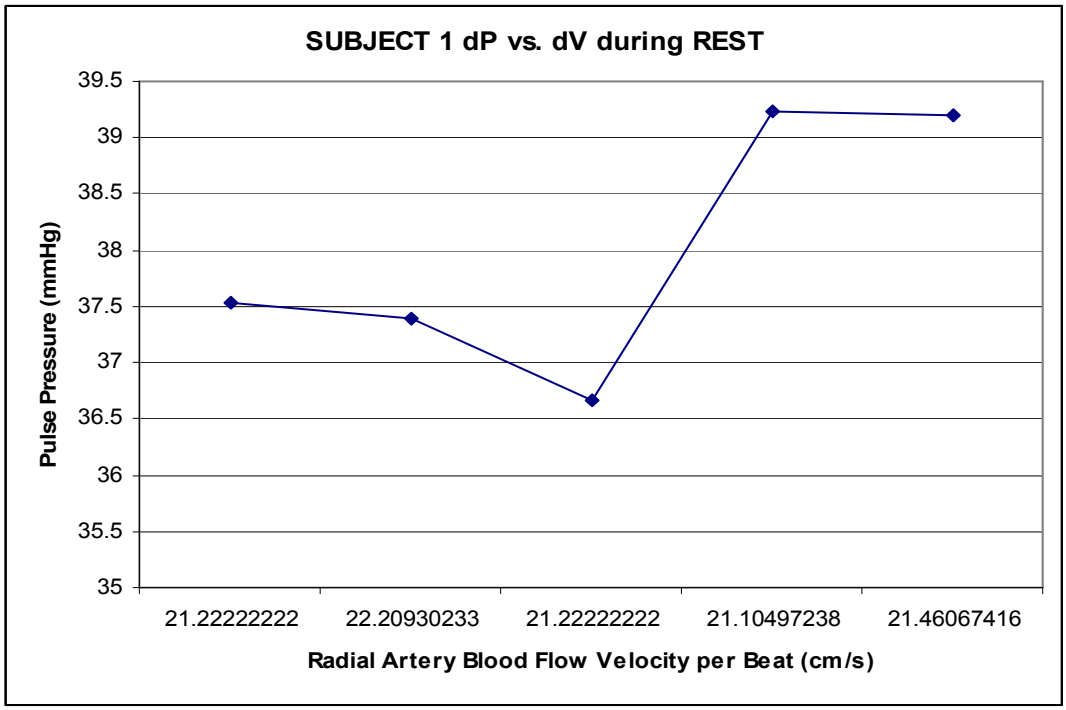
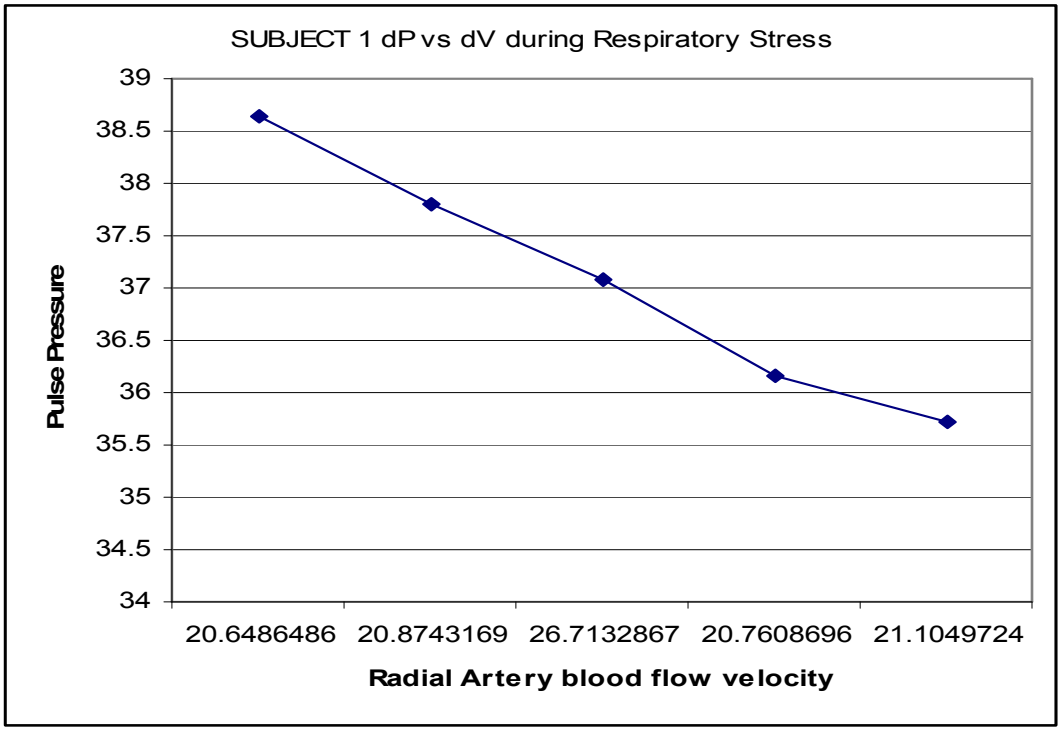


Figure 4.7 Subject 7 FFT Spectrum of Impedance (to 10 Hz) for a) Rest (5 beats), b) Respiratory (5 beats), c) Mental (5 beats) (d) Physical (5 beats)

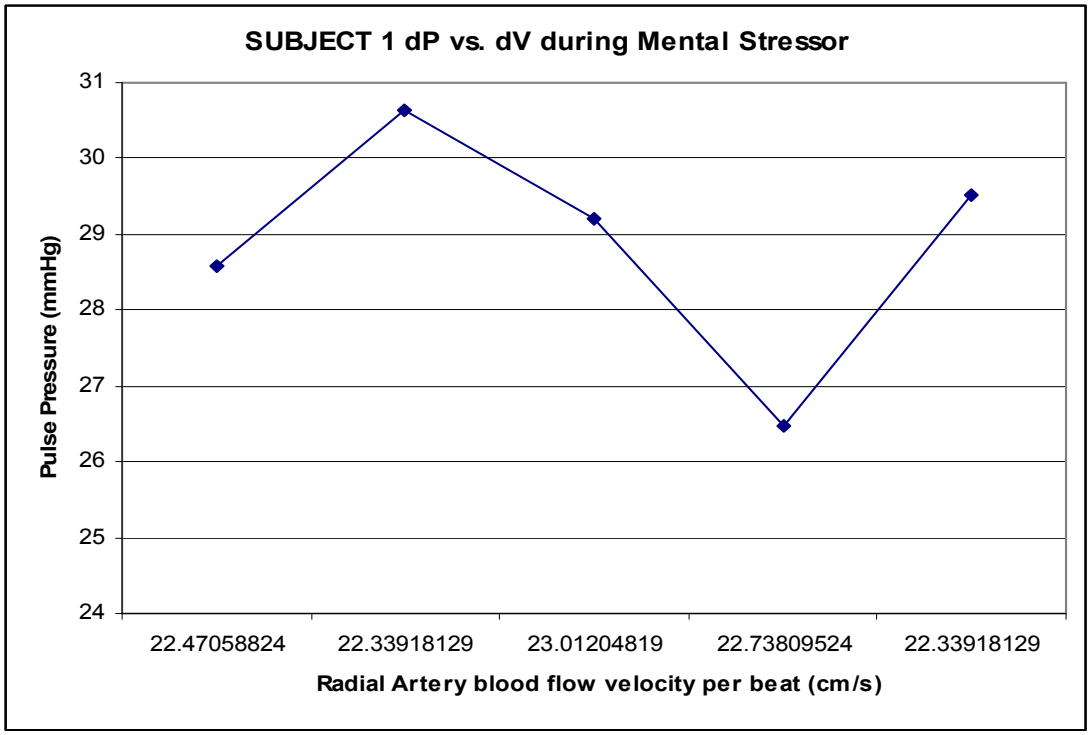


(a)

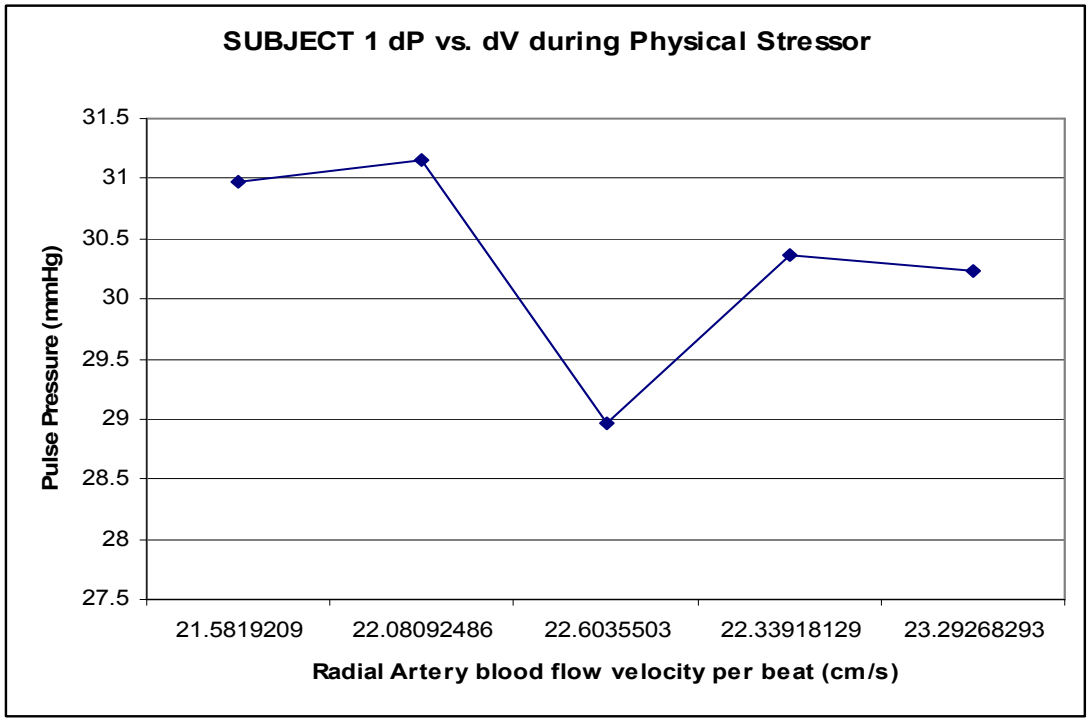


(b)

Figure 4.8a. Subject 1 plots of Delta Pressure vs. Delta blood flow velocity during a) Rest (5 beats) and b) Respiratory Stressor (5 beats)

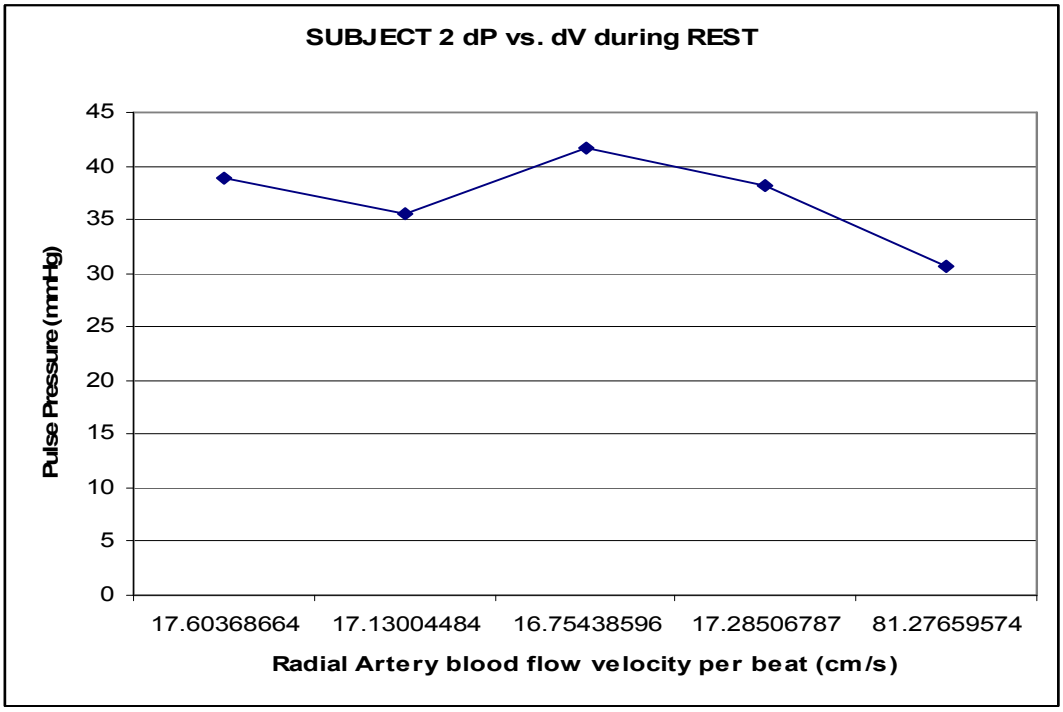


(c)

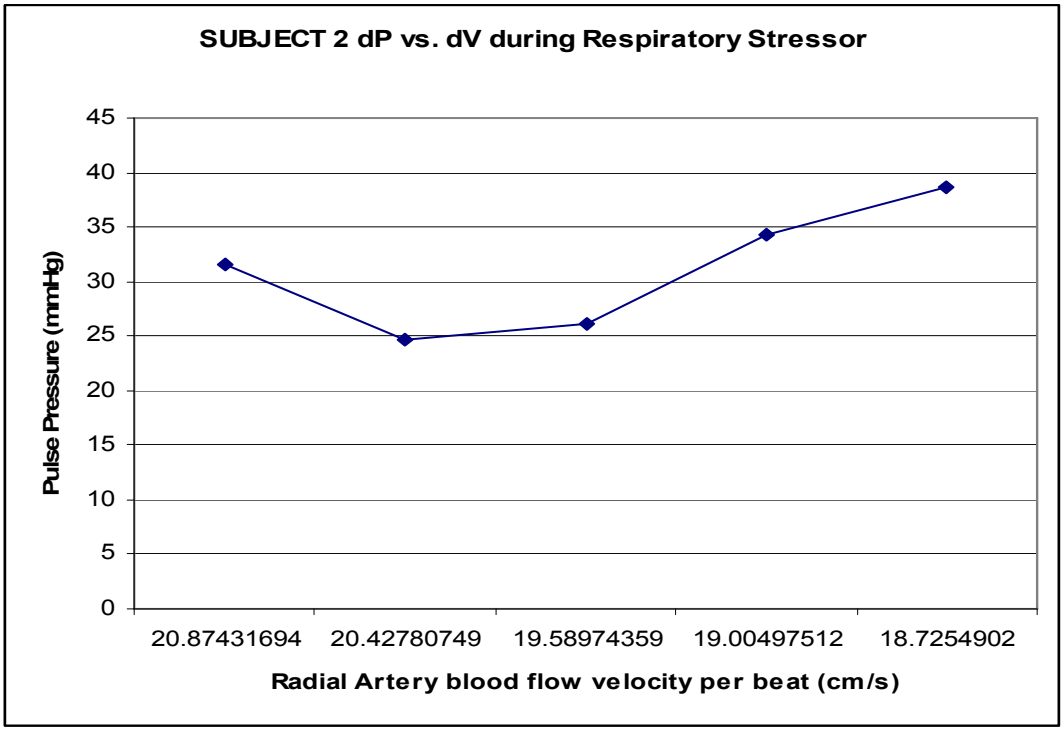


(d)

Figure 4.8b. Subject 1 plots of Delta Pressure vs. Delta blood flow velocity during c) Mental (5 beats) and (d) Physical Stressors (5 beats)

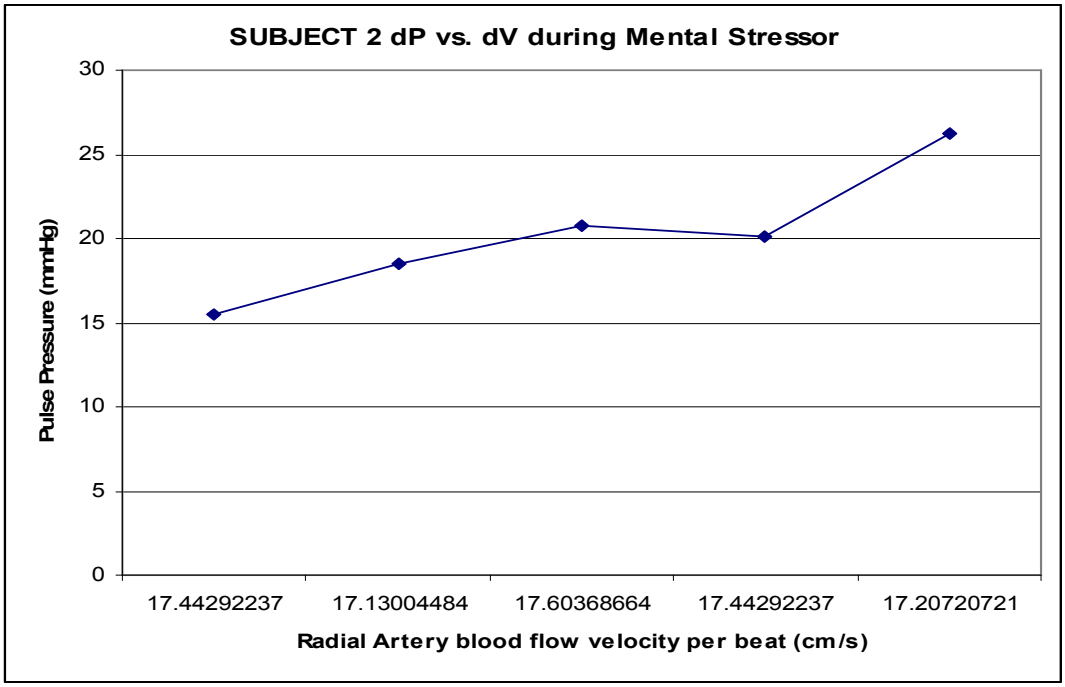


(a)

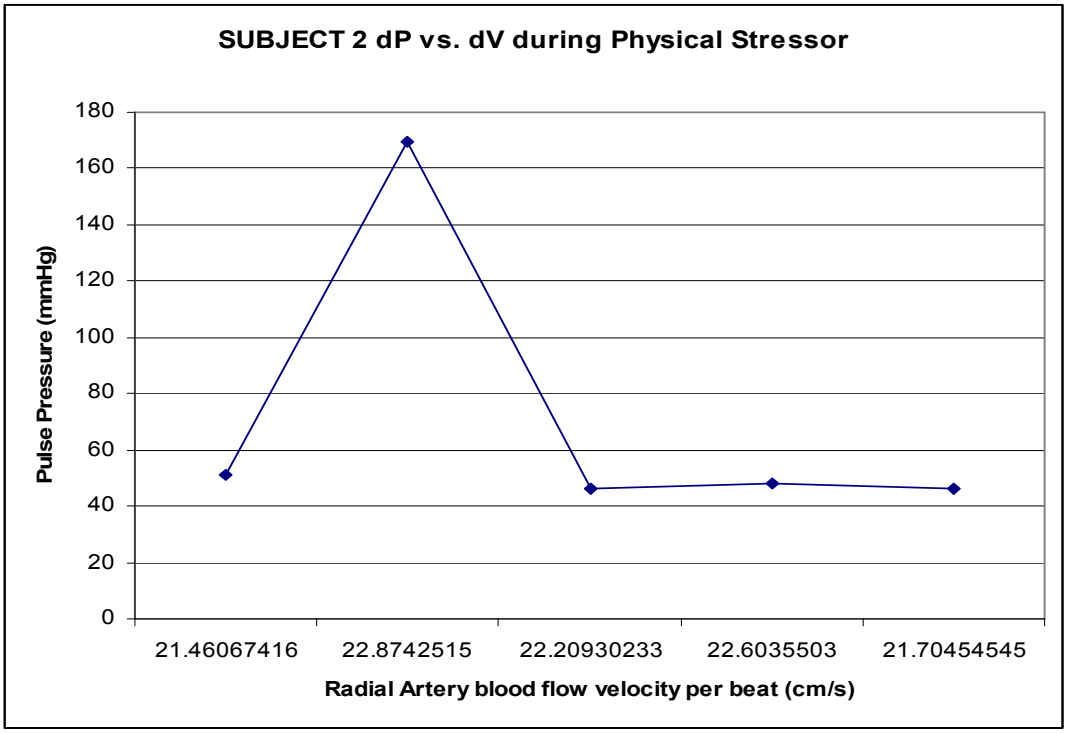


(b)

Figure 4.9a. Subject 2 plots of Delta Pressure vs. Delta blood flow velocity during a) Rest (5 beats) and b) Respiratory Stressor (5 beats)

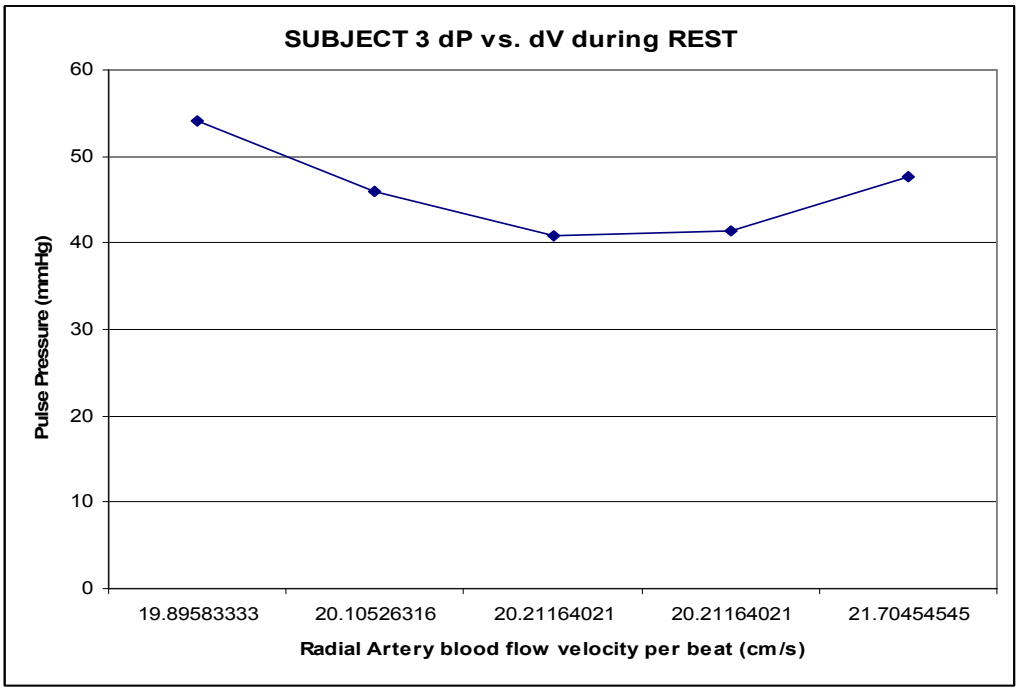


(c)

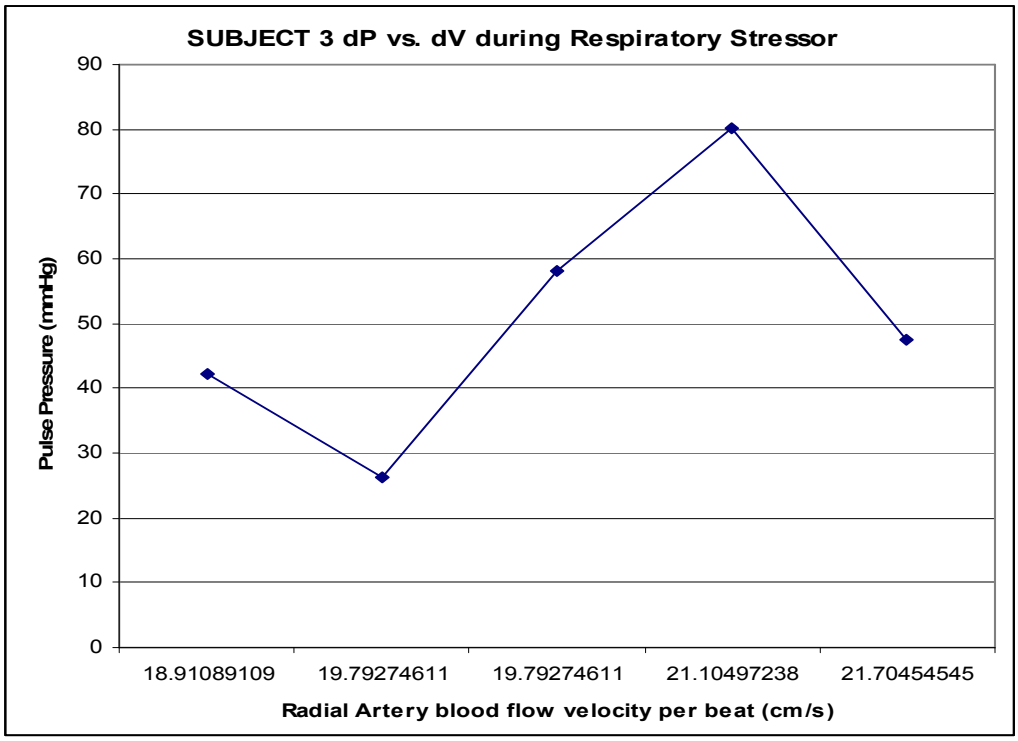


(d)

Figure 4.9b. Subject 2 plots of Delta Pressure vs. Delta blood flow velocity during c) Mental (5 beats) and (d) Physical Stressors (5 beats)

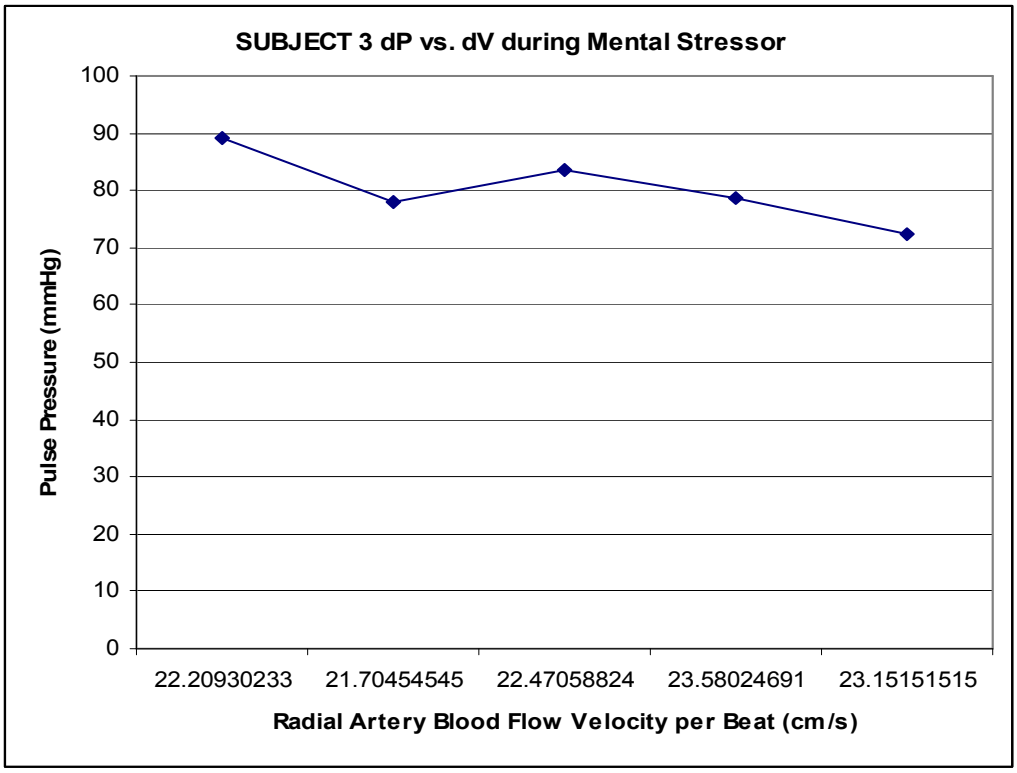


(a)

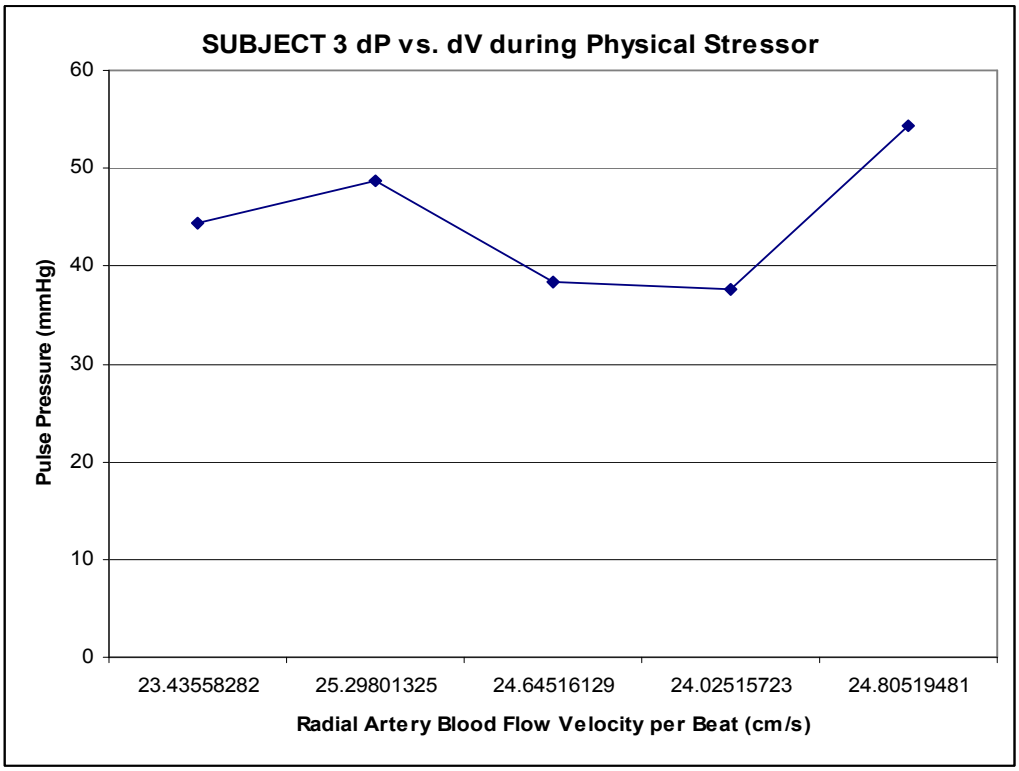


(b)

Figure 4.10a. Subject 3 plots of Delta Pressure vs. Delta blood flow velocity during a) Rest (5 beats) and b) Respiratory Stressor (5 beats)

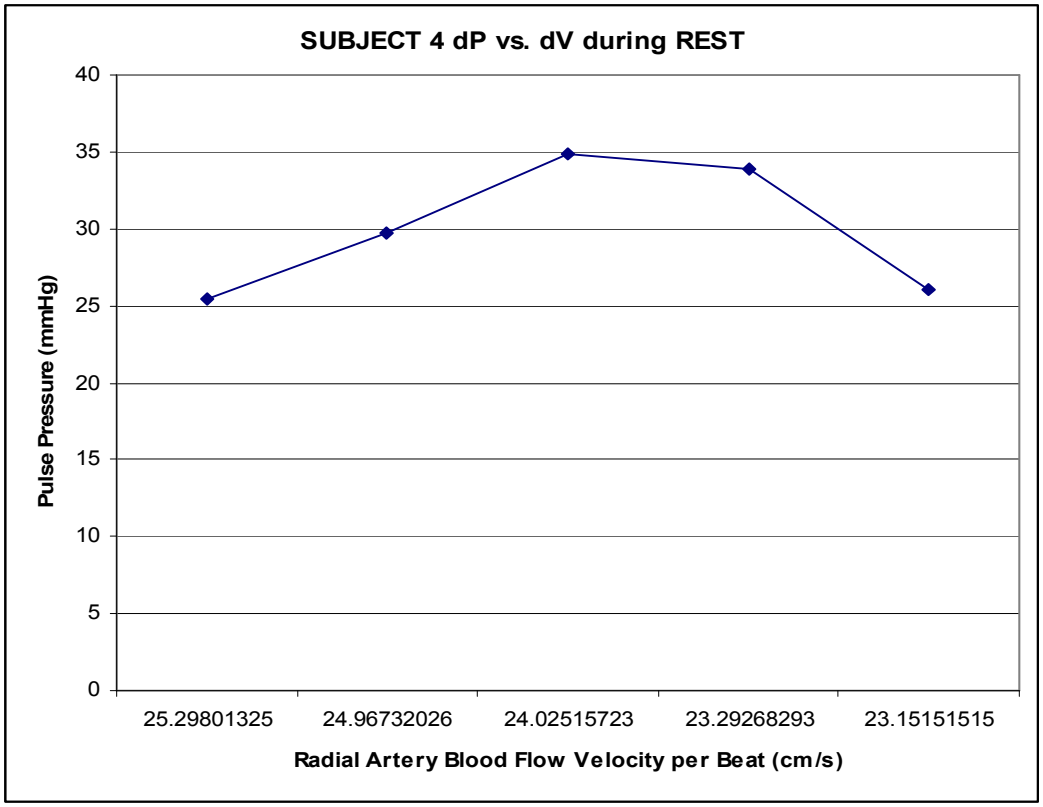


(c)

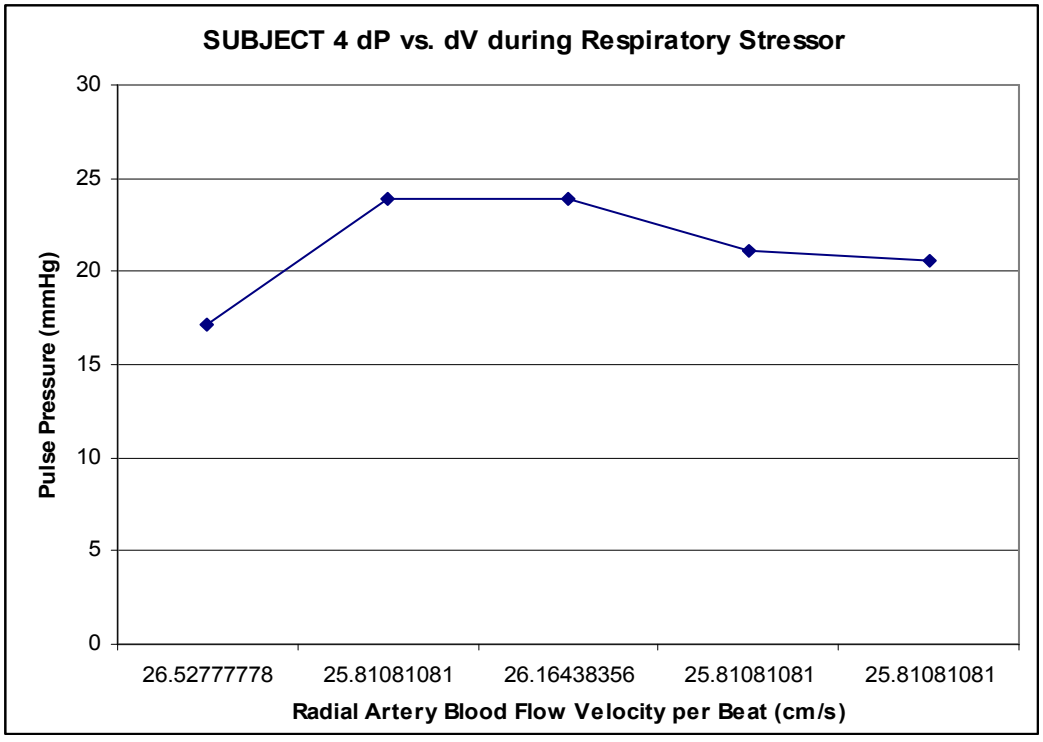


(d)

Figure 4.10b. Subject 3 plots of Delta Pressure vs. Delta blood flow velocity during c) Mental (5 beats) and (d) Physical Stressors (5 beats)

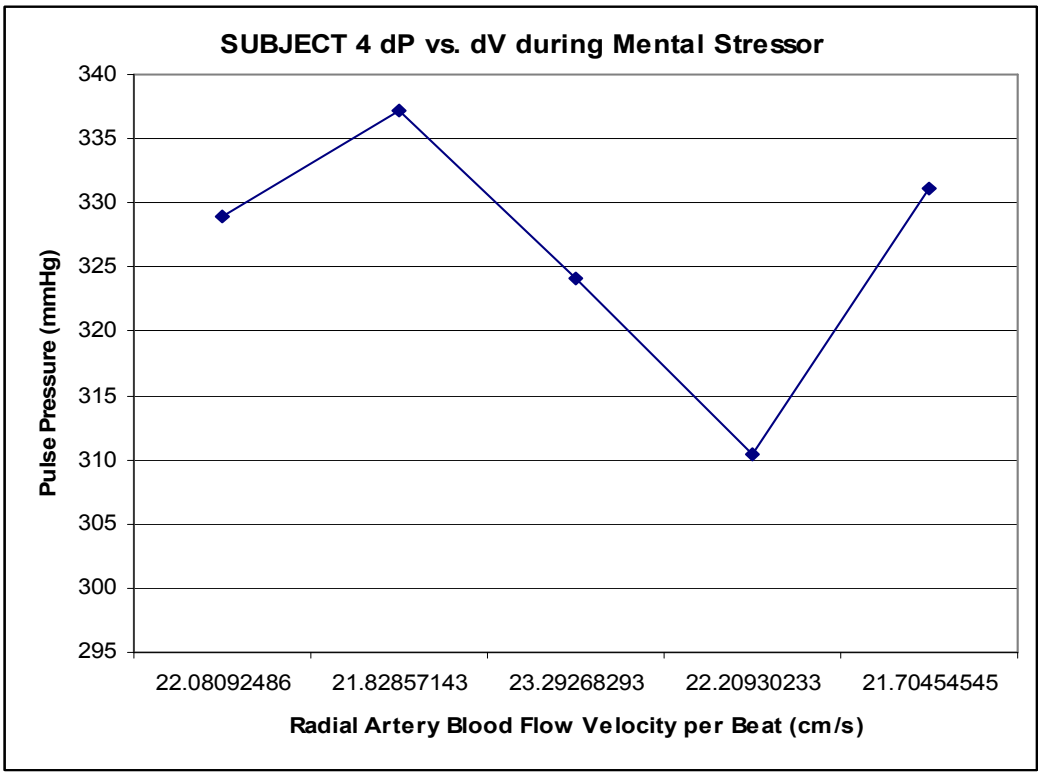


(a)



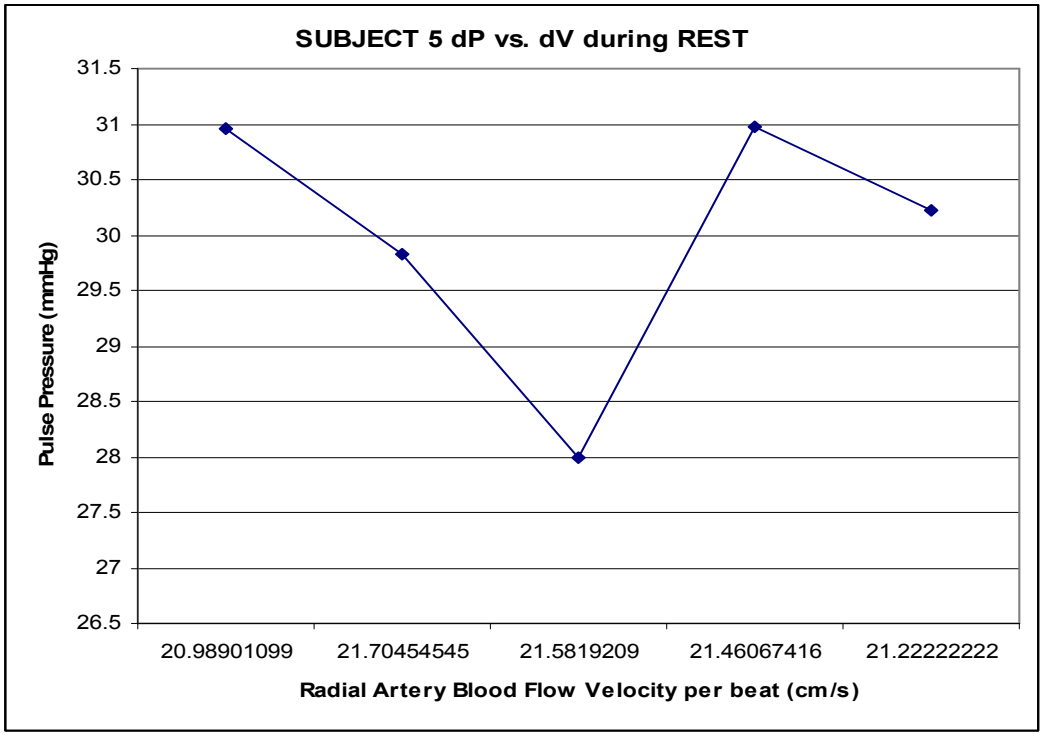
(b)

Figure 4.11a. Subject 4 plots of Delta Pressure vs. Delta blood flow velocity during a) Rest (5 beats) and b) Respiratory Stressor (5 beats)

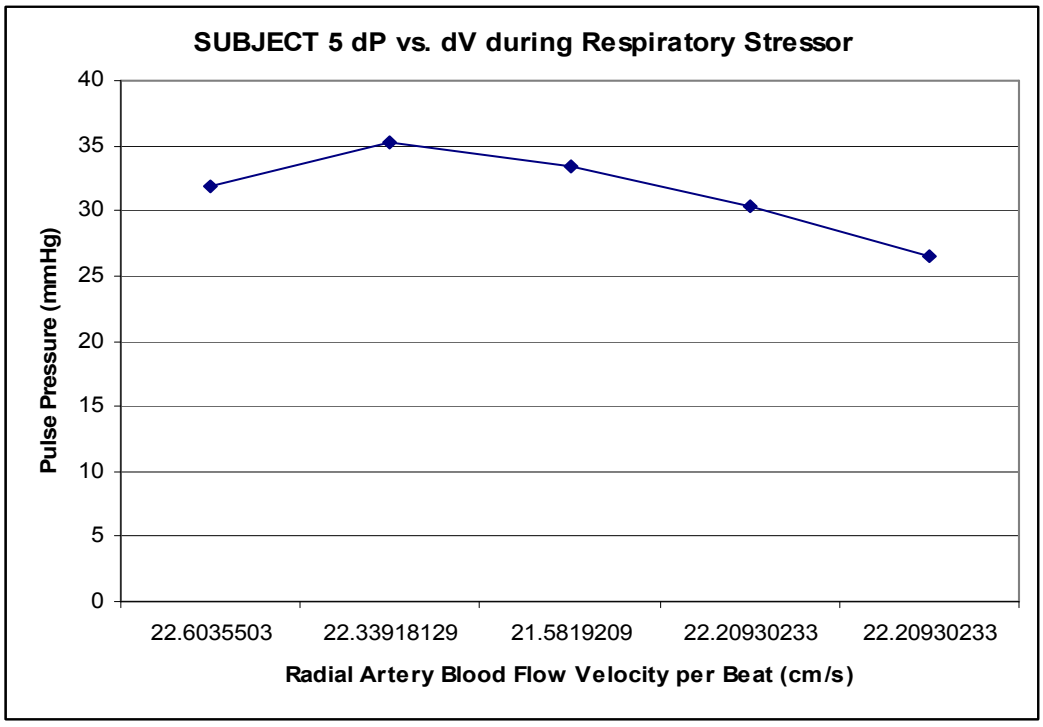


(c)

Figure 4.11b. Subject 4 plots of Delta Pressure vs. Delta blood flow velocity during c) Mental Stressor (5 beats) ** No Physical Stressor plot due to bad data

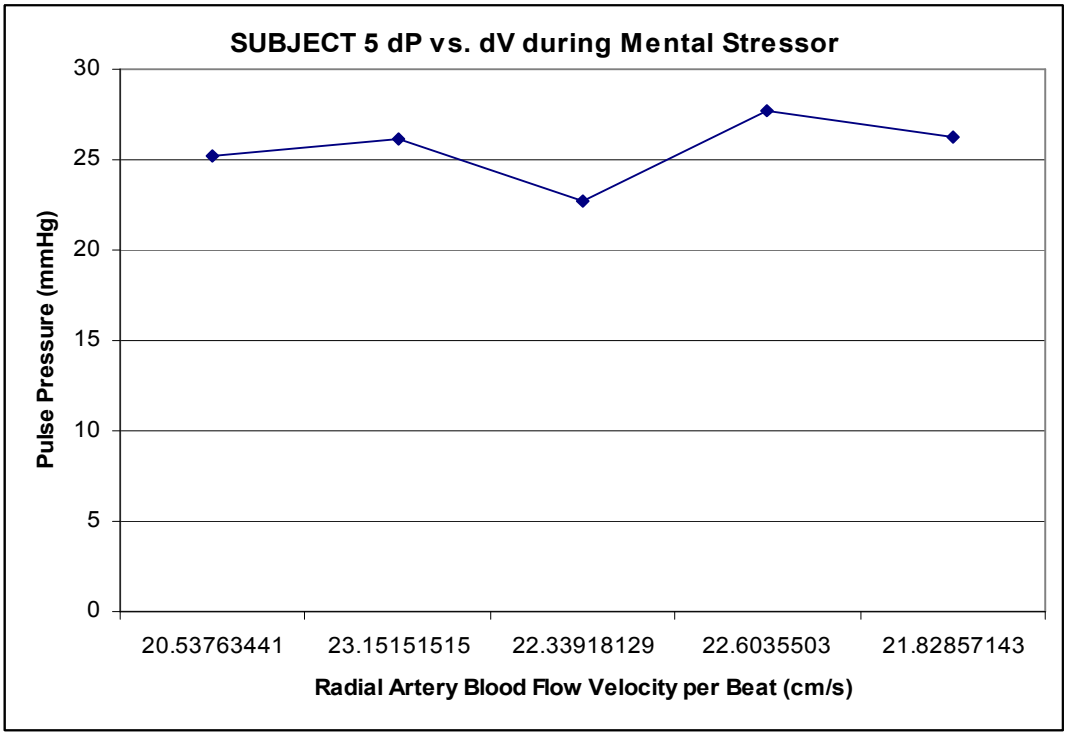


(a)

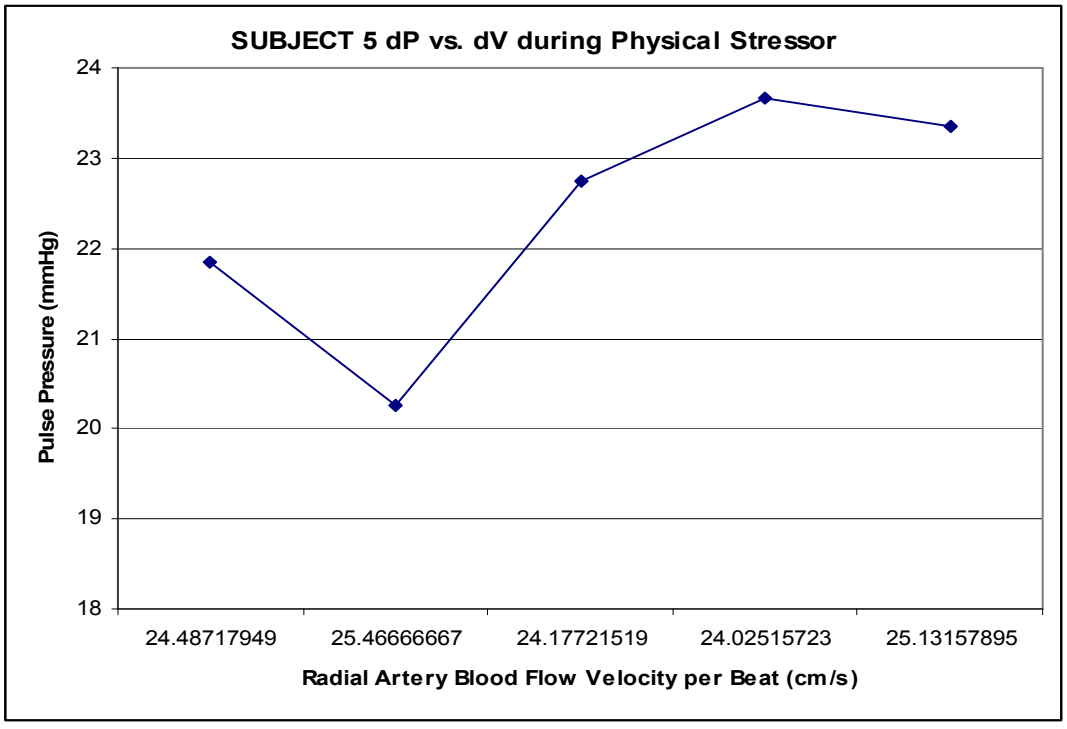


(b)

Figure 4.12a. Subject 5 plots of Delta Pressure vs. Delta blood flow velocity during a) Rest (5 beats) and b) Respiratory Stressor (5 beats)

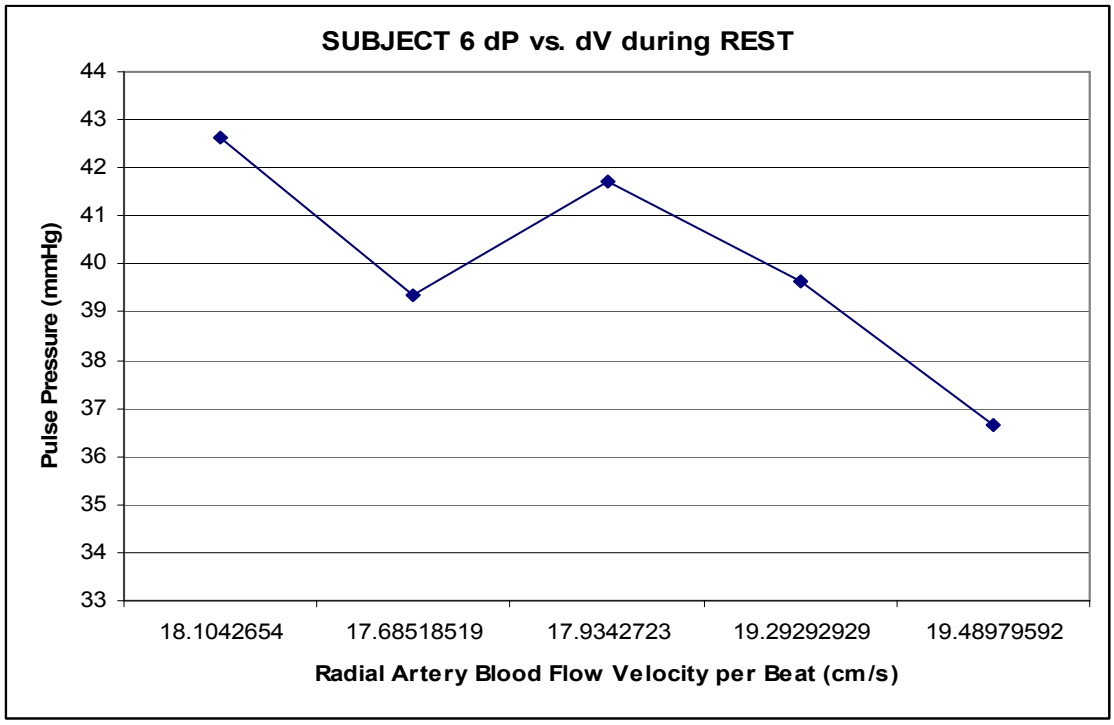


(c)

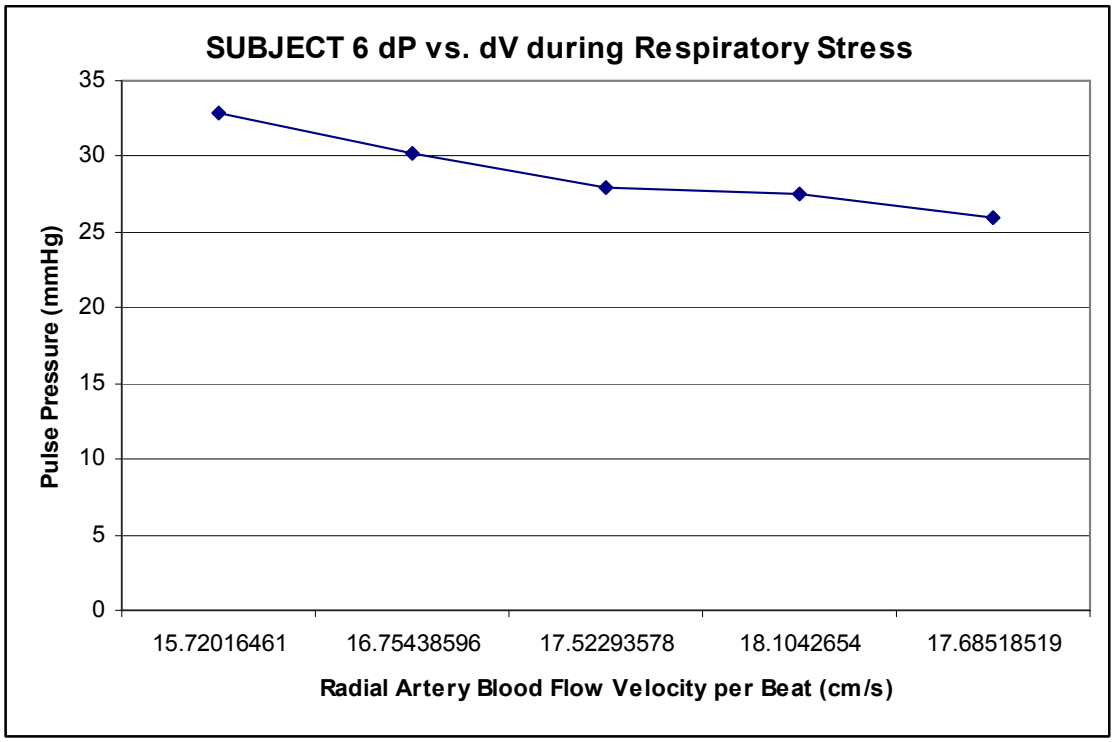


(d)

Figure 4.12b. Subject 5 plots of Delta Pressure vs. Delta blood flow velocity during c) Mental (5 beats) and (d) Physical Stressors (5 beats)

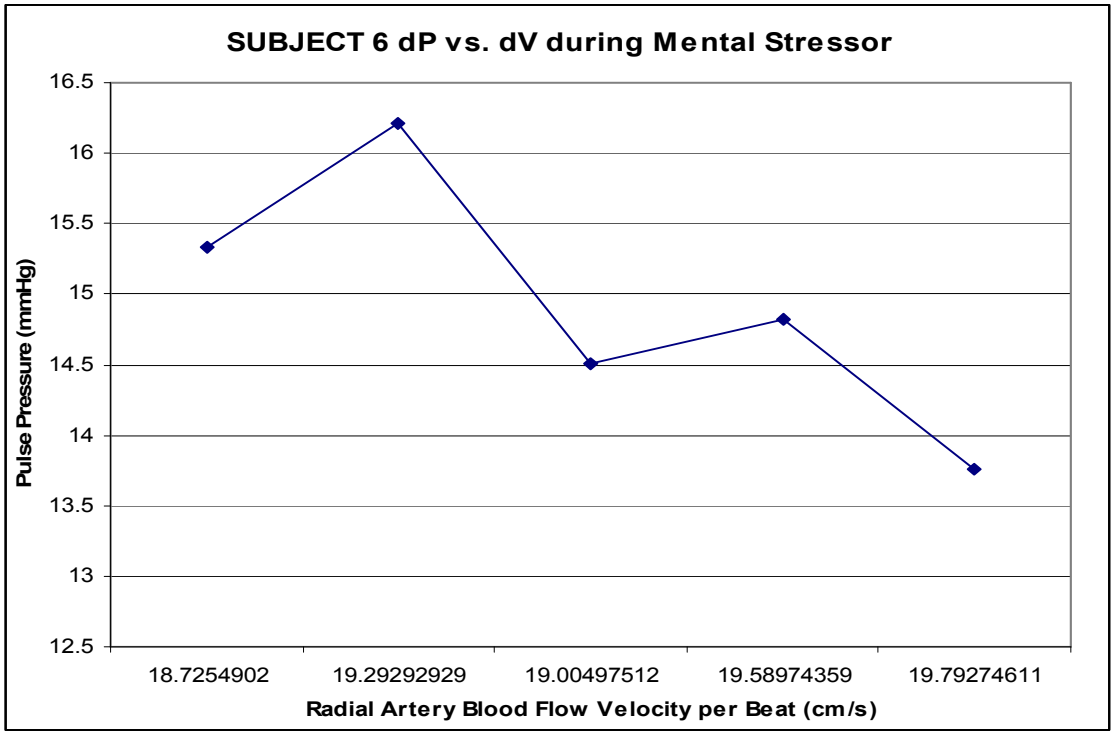


(a)



(b)

Figure 4.13a. Subject 6 plots of Delta Pressure vs. Delta blood flow velocity during a) Rest (5 beats) and b) Respiratory Stressor (5 beats)



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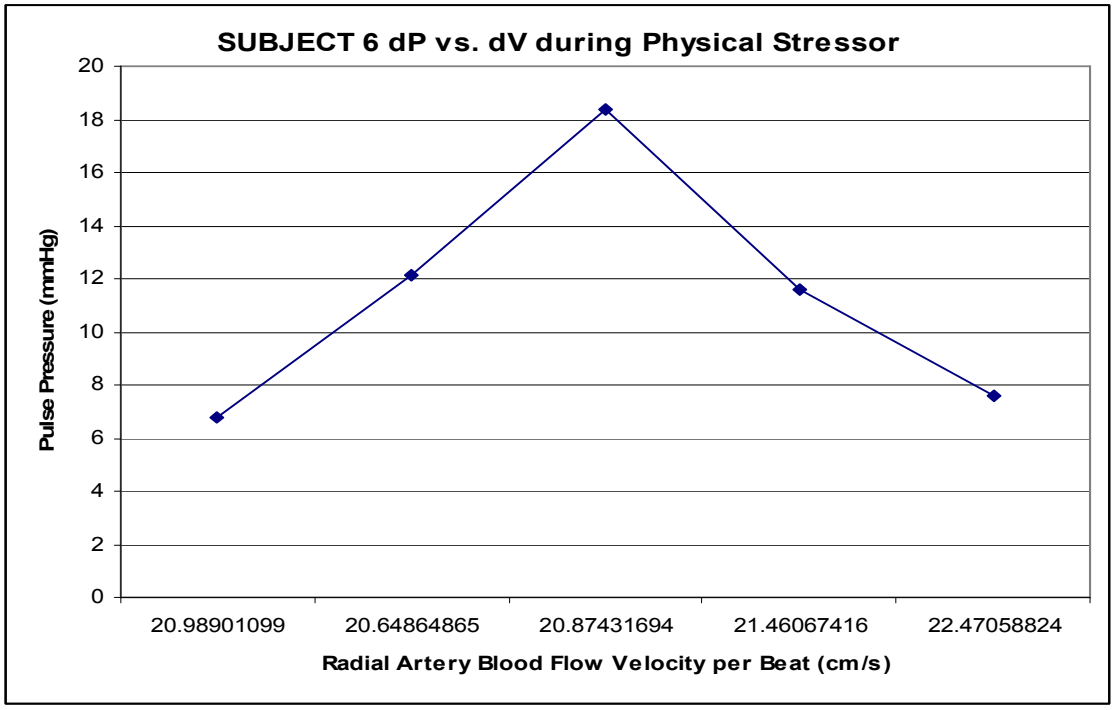
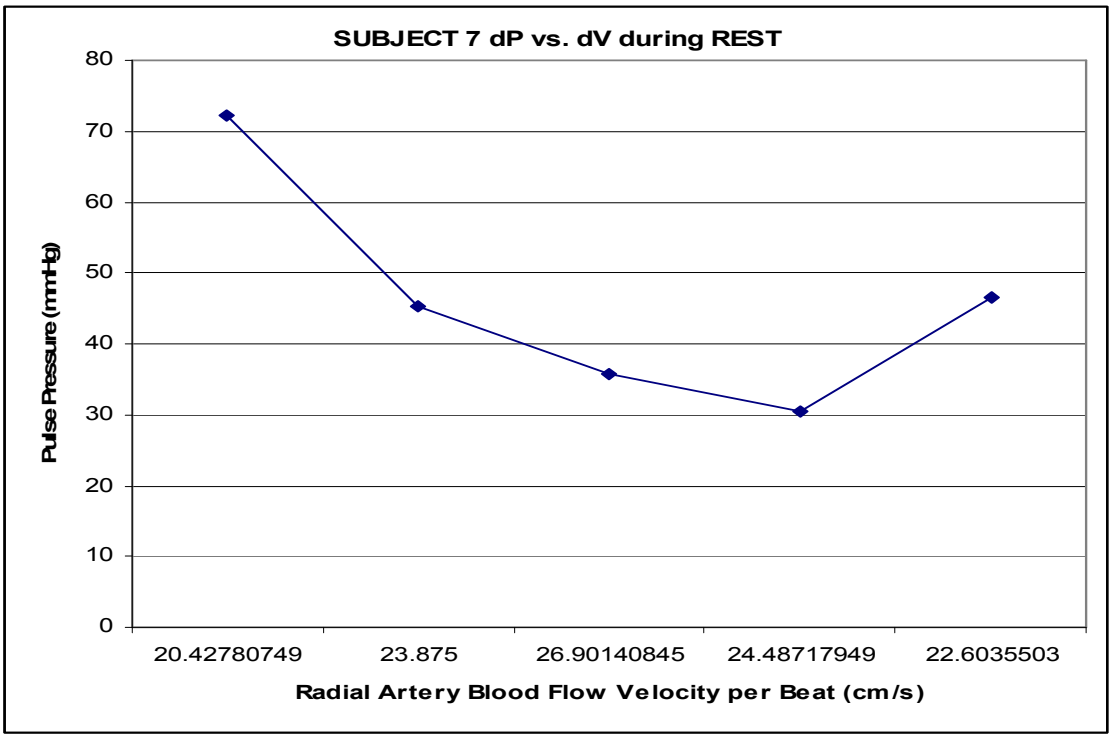
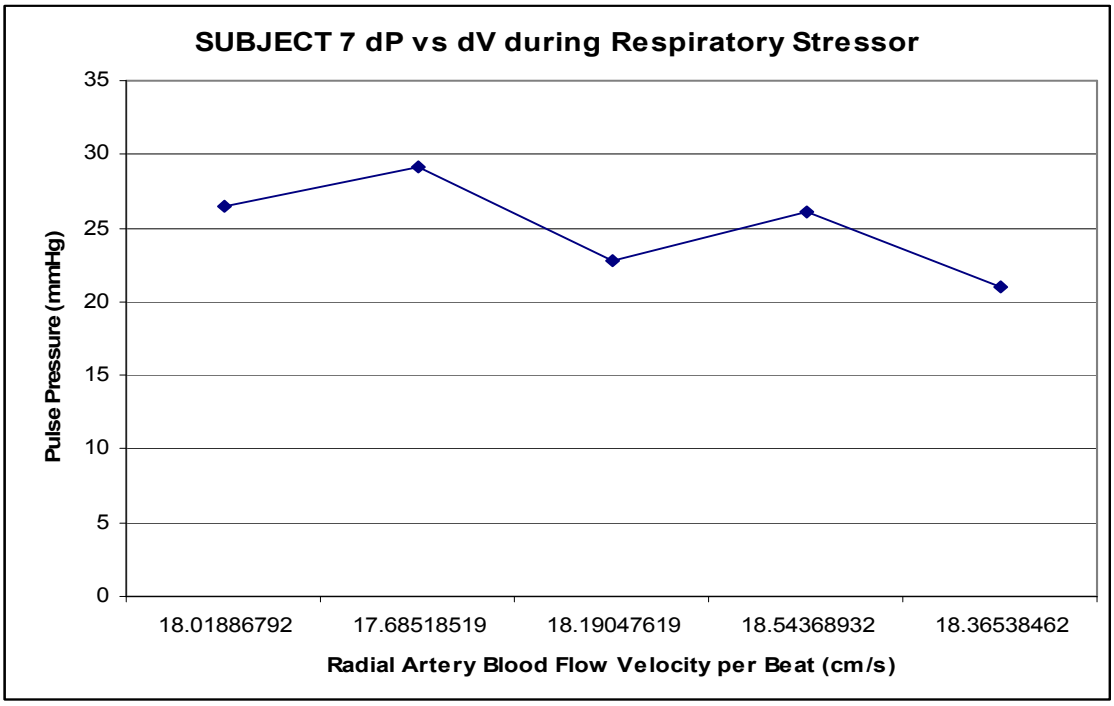


(d)

Figure 4.13b. Subject 6 plots of Delta Pressure vs. Delta blood flow velocity during c) Mental (5 beats) and (d) Physical Stressors (5 beats)

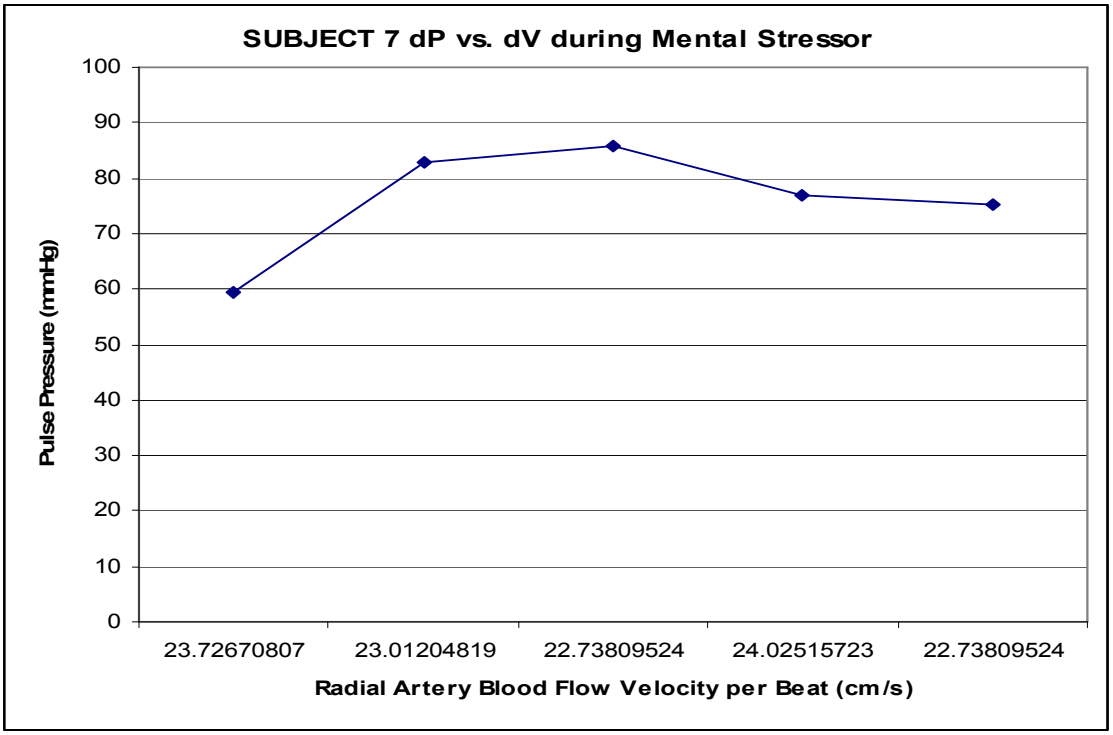


(a)



(b)

Figure 4.14a. Subject 7 plots of Delta Pressure vs. Delta blood flow velocity during a) Rest (5 beats) and b) Respiratory Stressor (5 beats)



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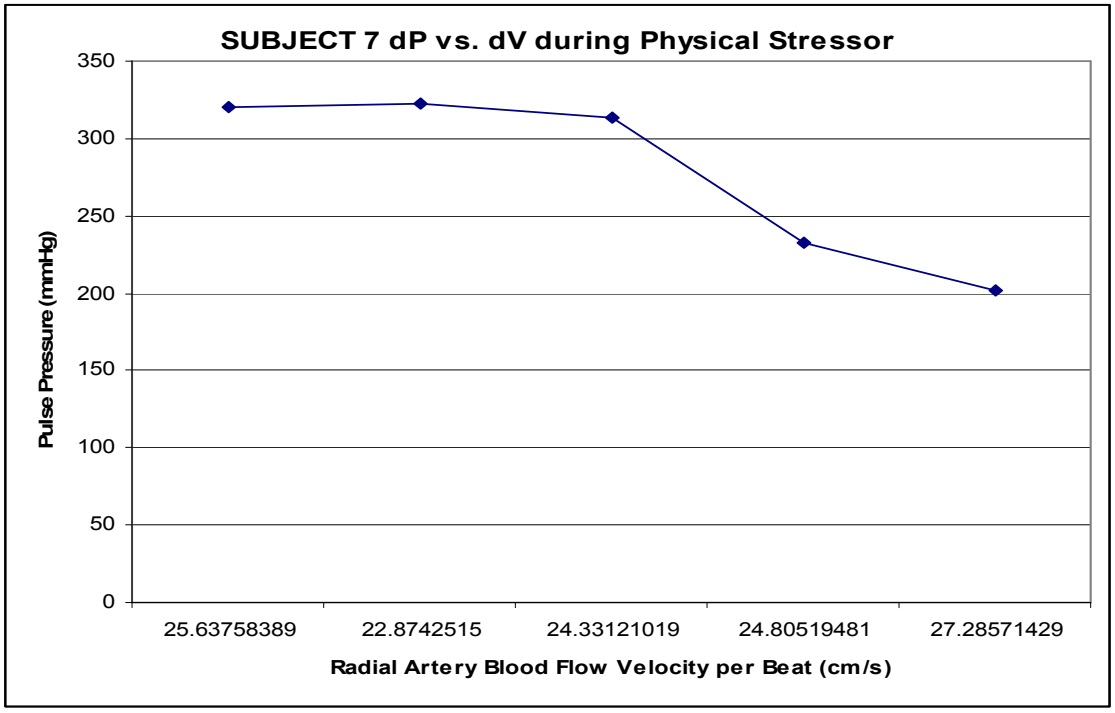
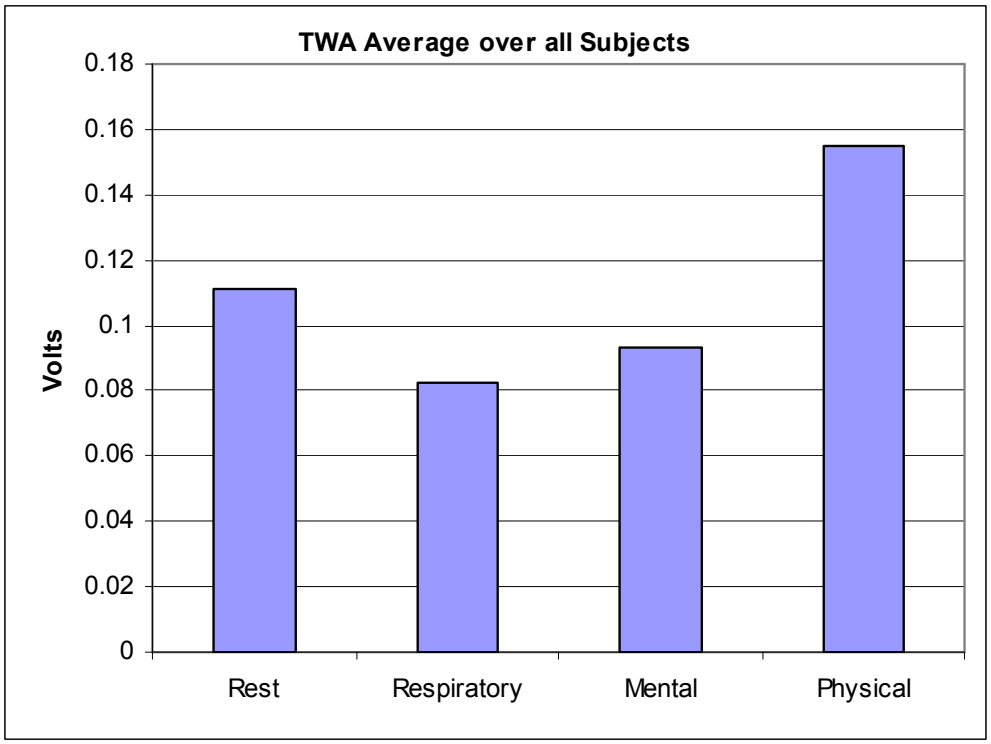


(d)

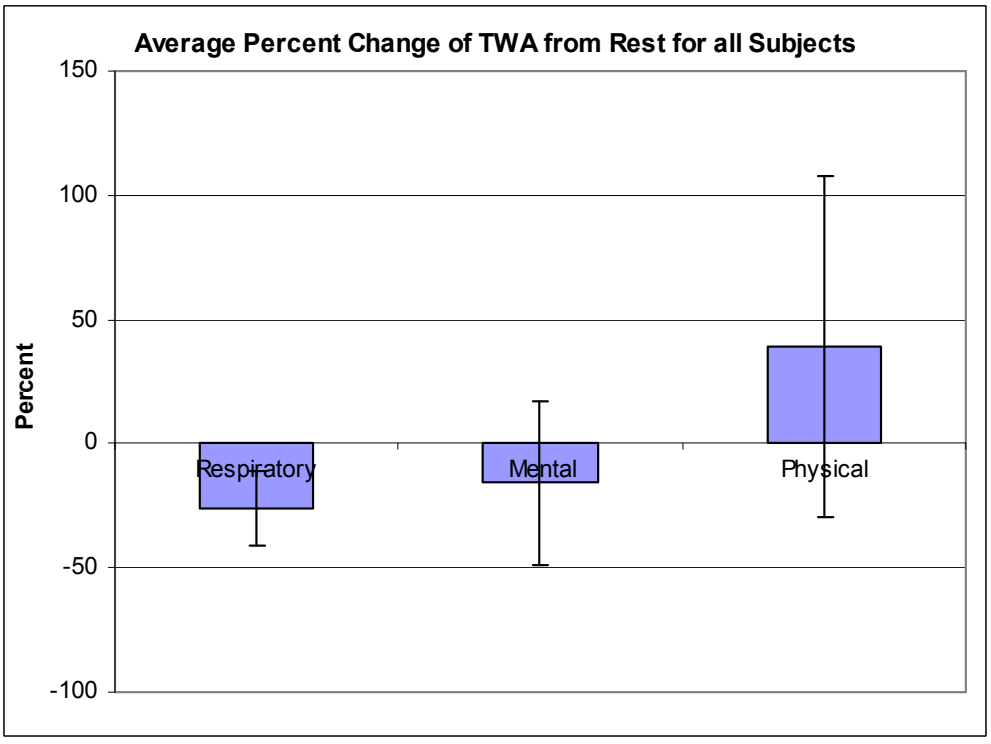
Figure 4.14b. Subject 7 plots of Delta Pressure vs. Delta blood flow velocity during c) Mental (5 beats) and (d) Physical Stressors (5 beats)

Subject	**Average Mental stress T wave Amplitude % change from Rest**	**Highest Mental Game Level Achieved**
1	-28.0290255	8
2	-75.59621655	10
3	-3.775821807	8
4	**9.993619397**	**7**
5	**16.97351927**	**7**
6	-10.28563998	12
7	**119.1473318**	**7**

Table 4.2 Percent Change in T wave Amplitude and Mental Game Level for each subject.

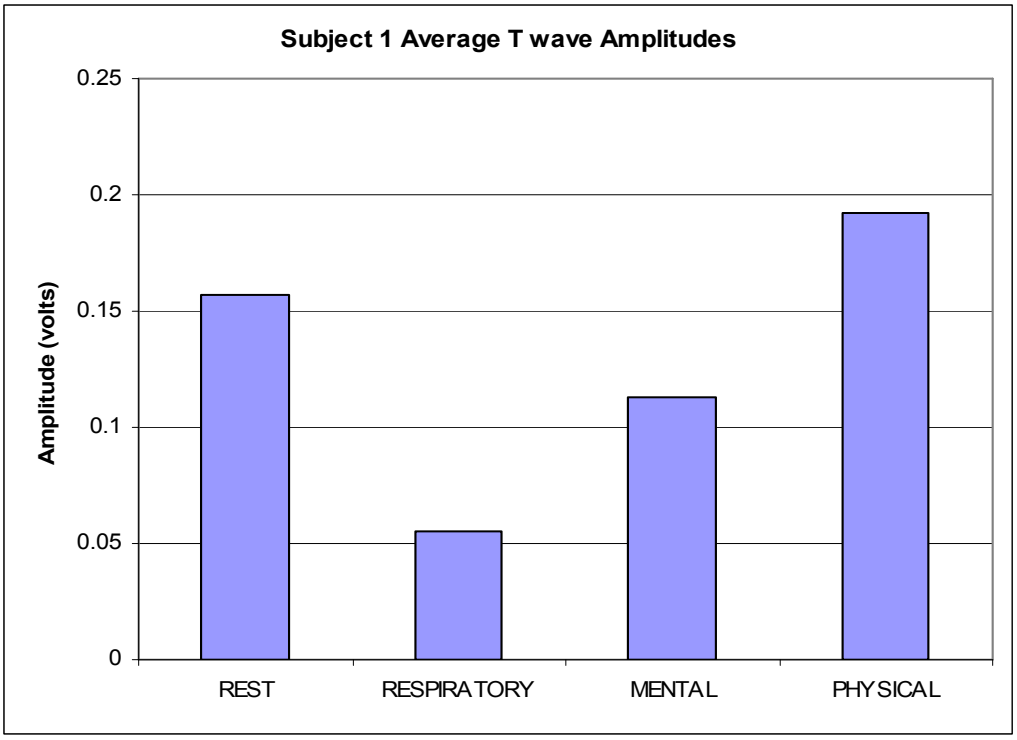


(a)

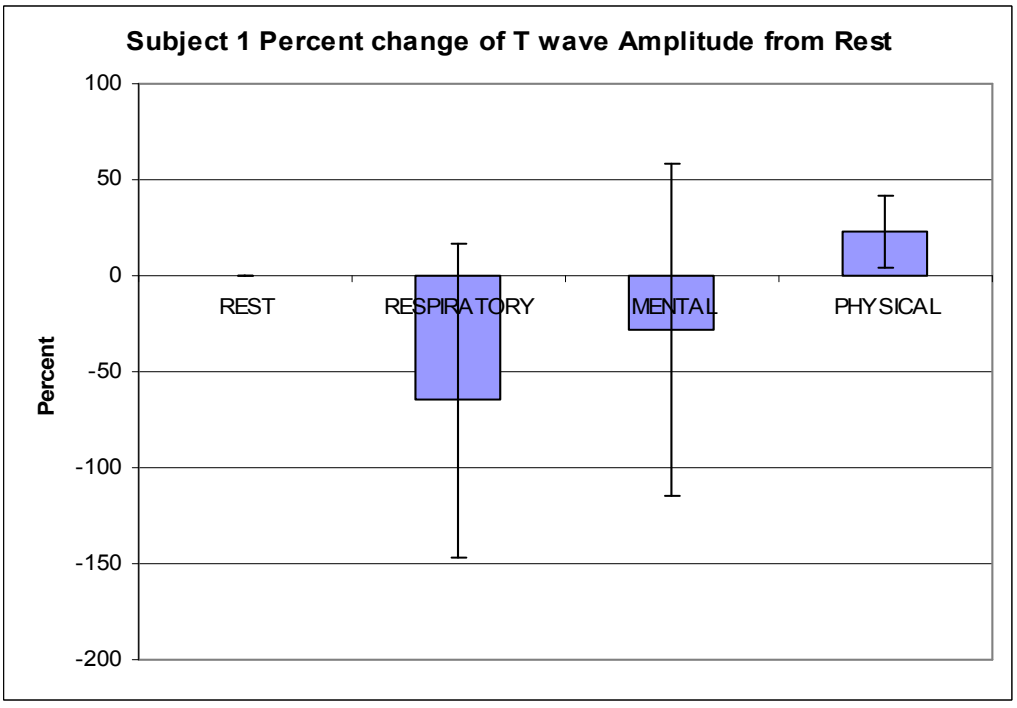


(b)

Figure 4.15 T wave amplitudes per intervention (a) and the percent change of T wave amplitude from rest (b) for all subjects.

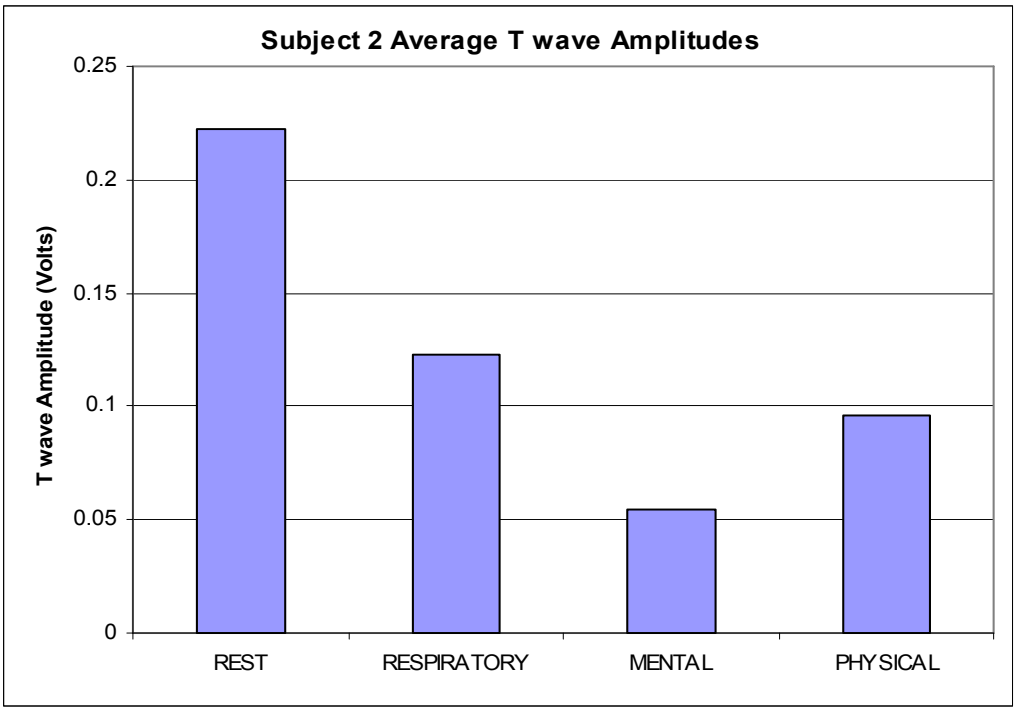


(a)

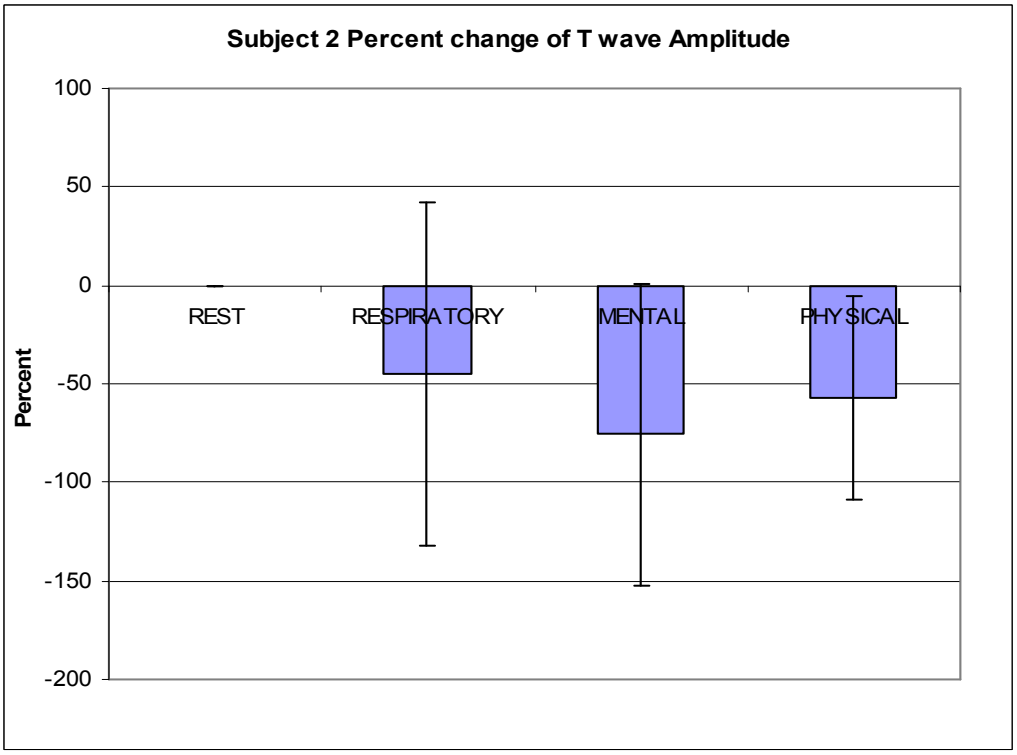


(b)

Figure 4.16 Subject 1 (a) T wave amplitudes per intervention and (b) the percent change of T wave amplitude from rest.

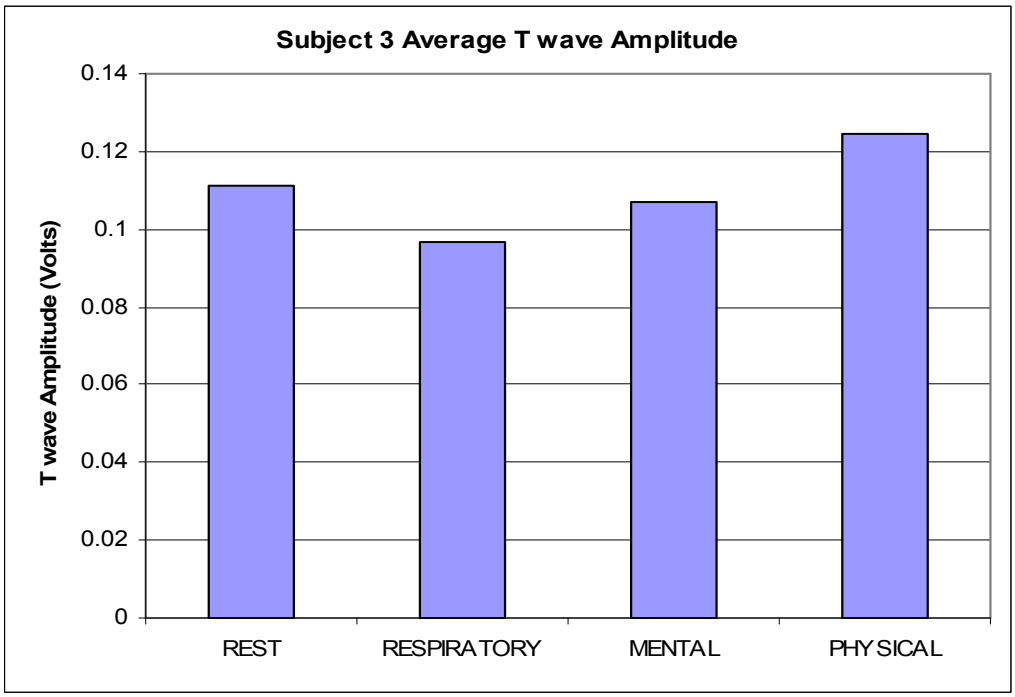


(a)

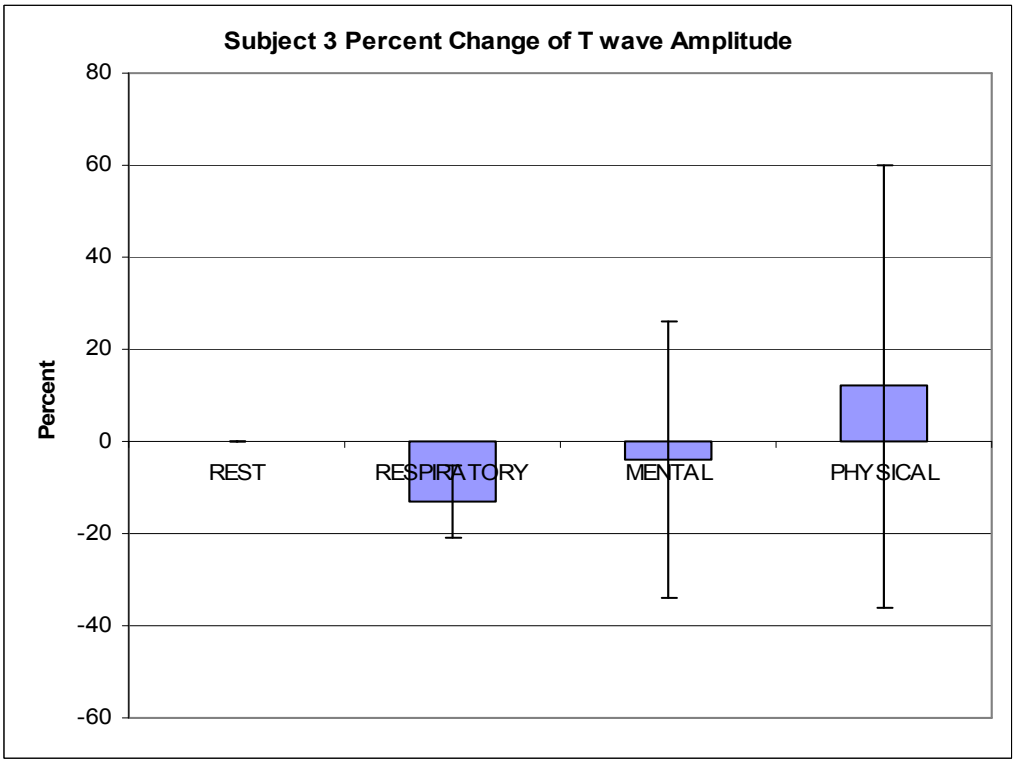


(b)

Figure 4.17 Subject 2 (a) T wave amplitudes per intervention and (b) the percent change of T wave amplitude from rest.

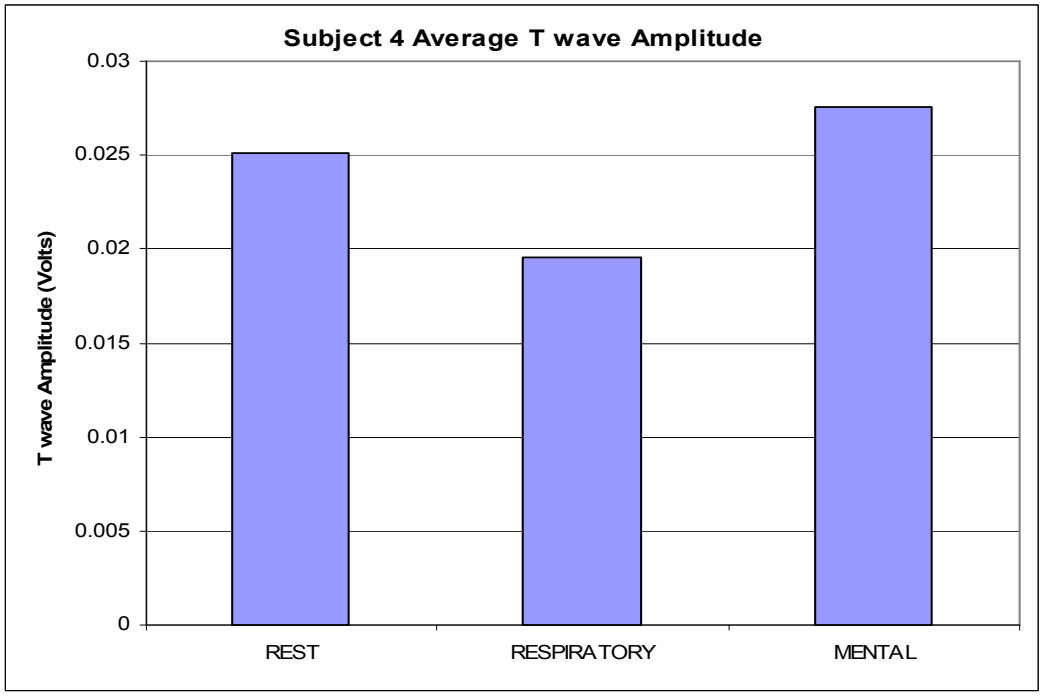


(a)

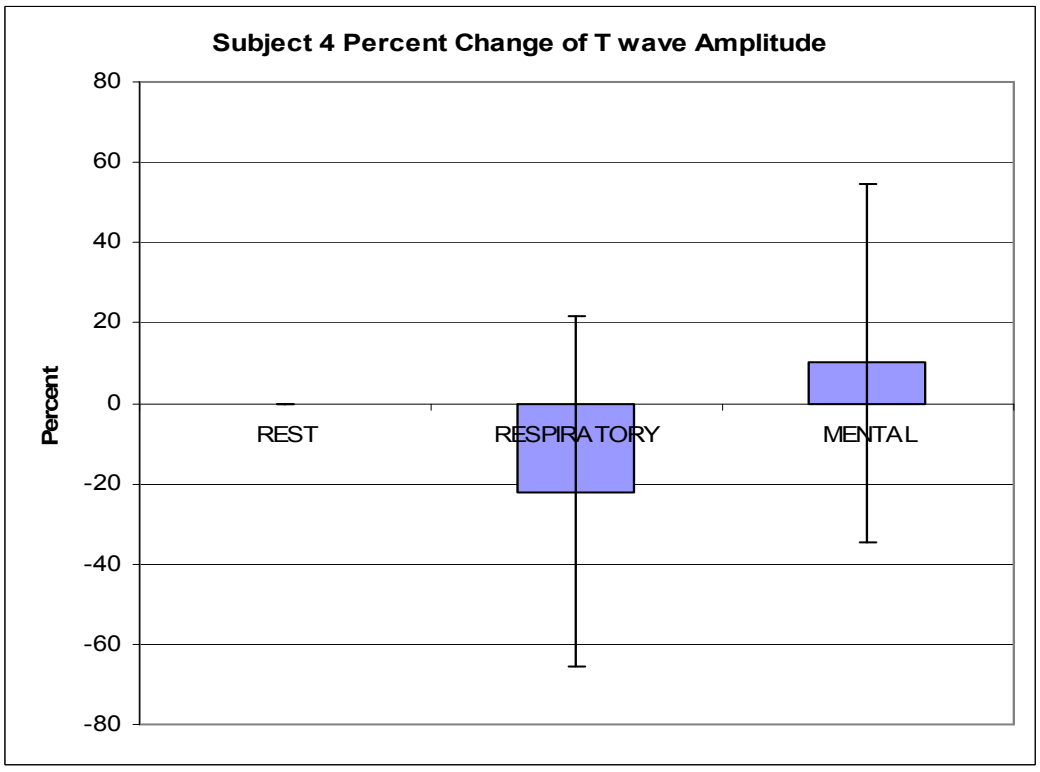


(b)

Figure 4.18 Subject 3 (a) T wave amplitudes per intervention and (b) the percent change of T wave amplitude from rest.

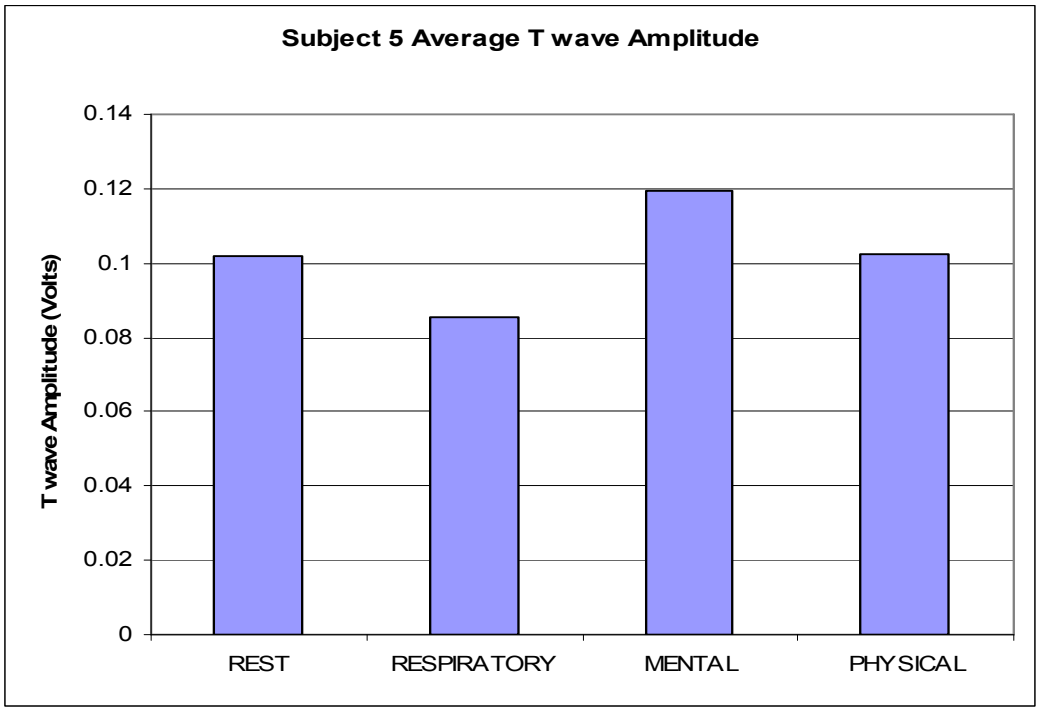


(a)

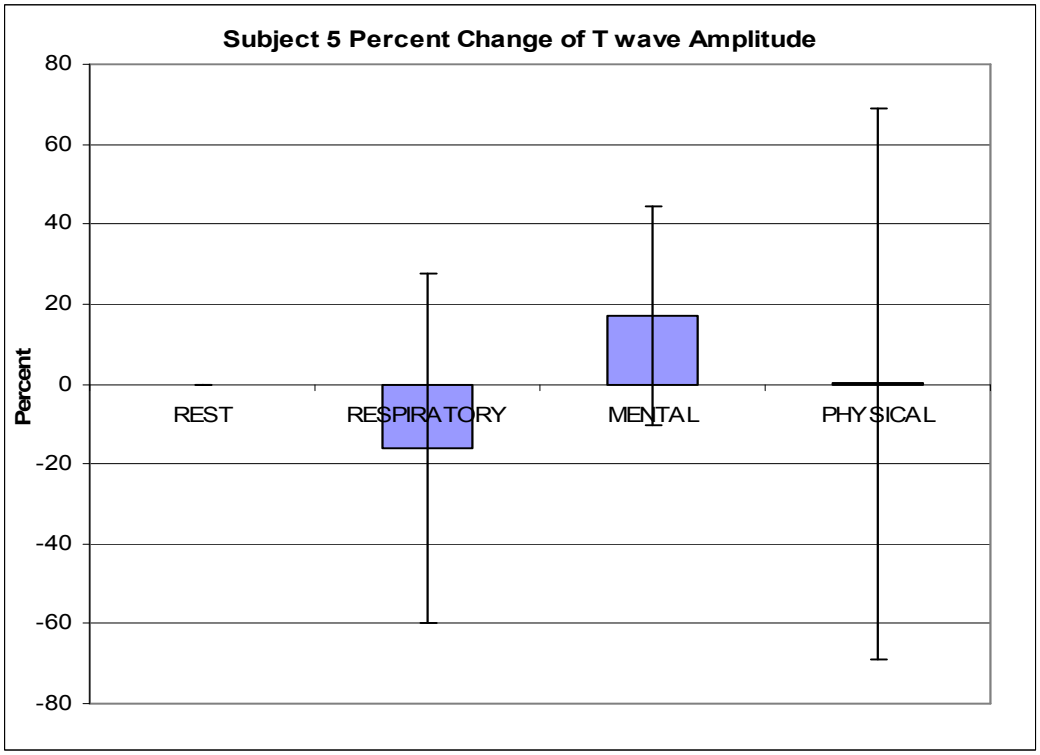


(b)

Figure 4.19 Subject 4 (a) T wave amplitudes per intervention and (b) the percent change of T wave amplitude from rest.

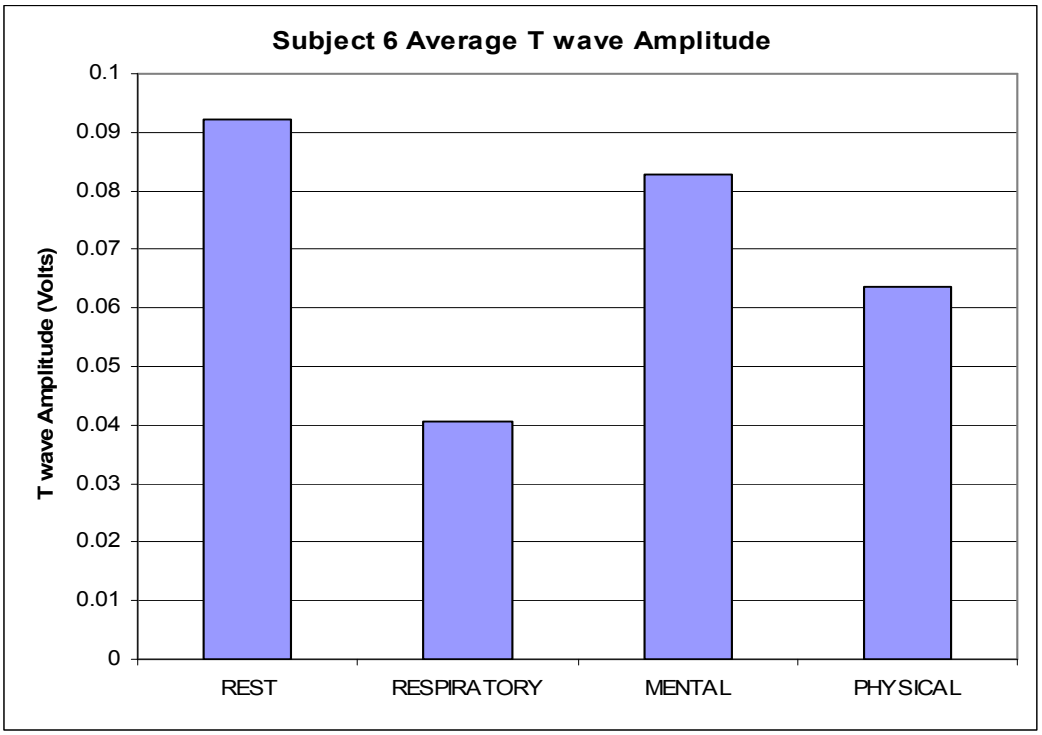


(a)

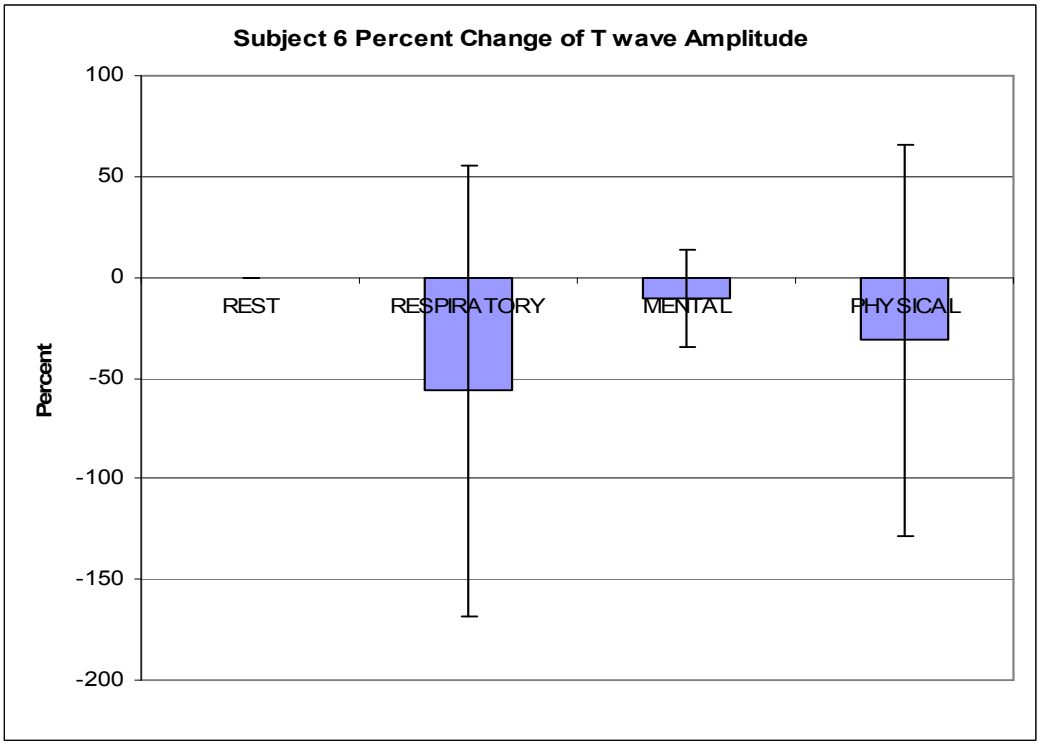


(b)

Figure 4.20 Subject 5 (a) T wave amplitudes per intervention and (b) the percent change of T wave amplitude from rest.

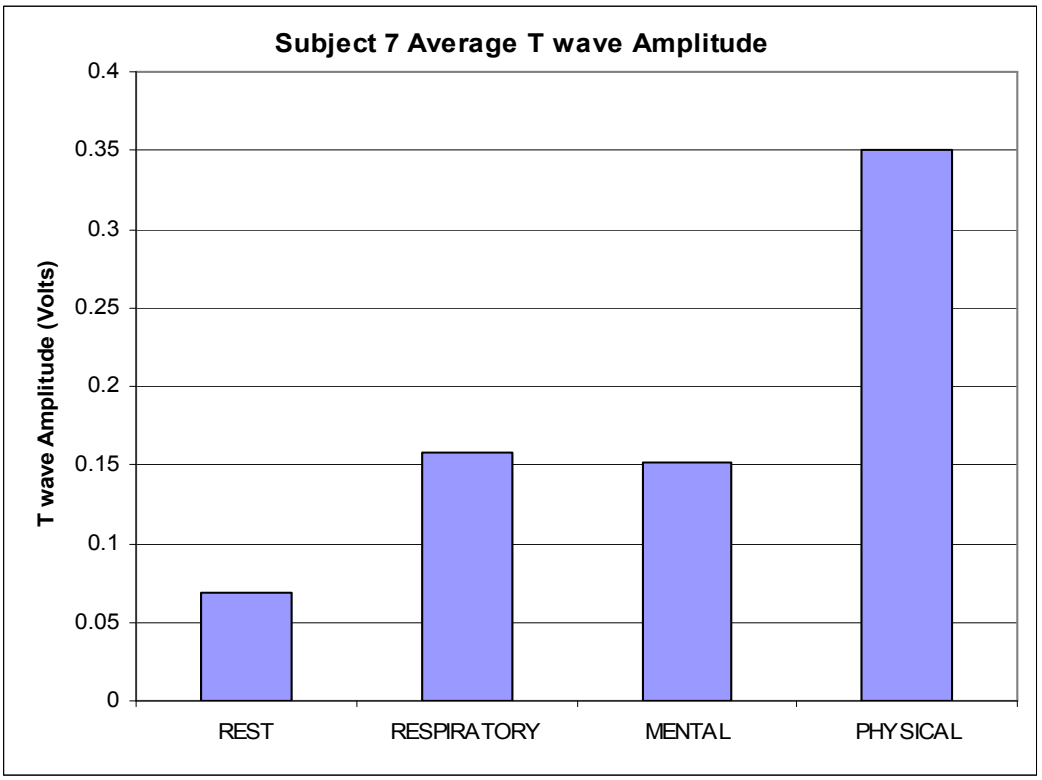


(a)

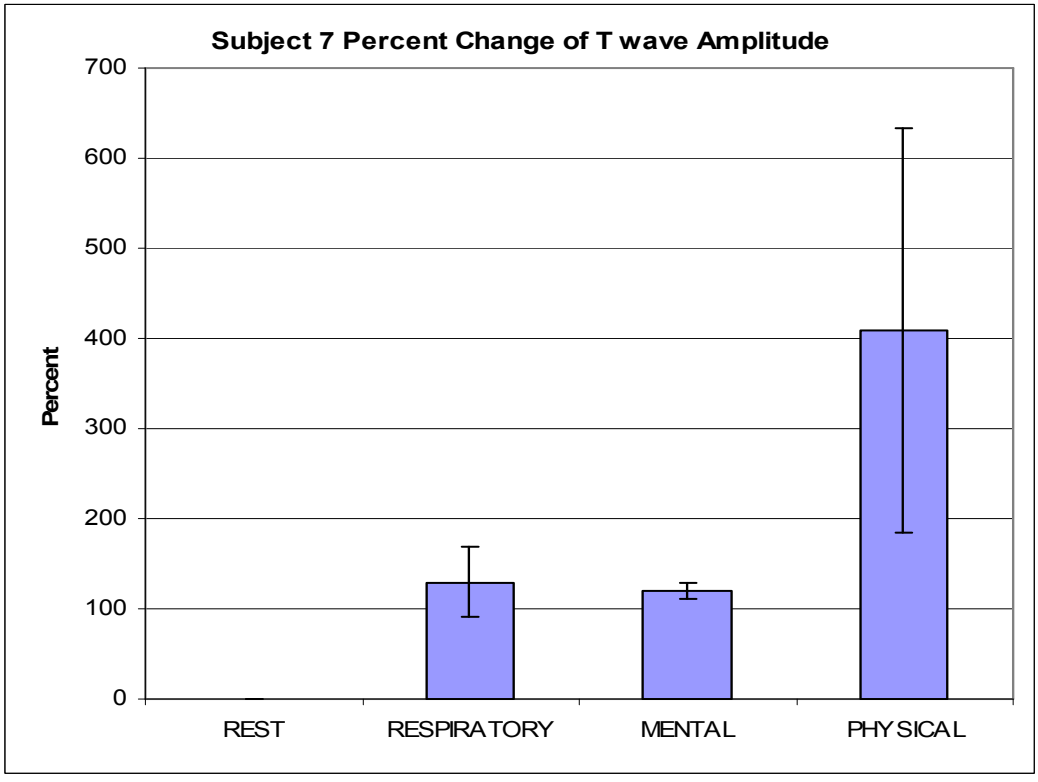


(b)

Figure 4.21 Subject 6 (a) T wave amplitudes per intervention and (b) the percent change of T wave amplitude from rest.

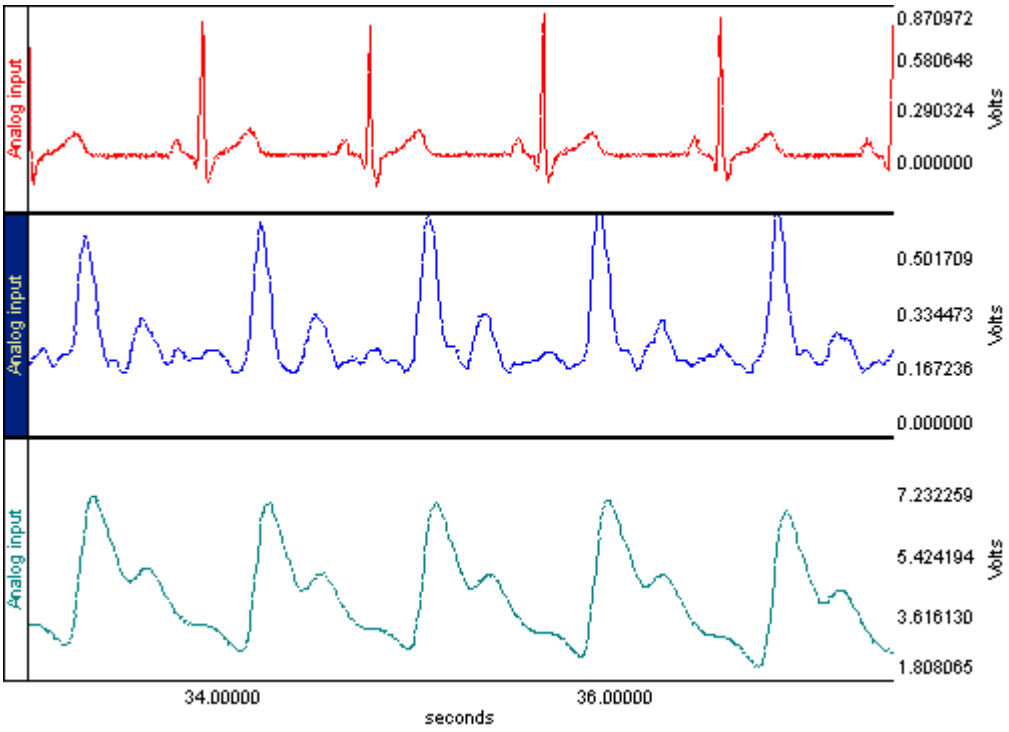


(a)

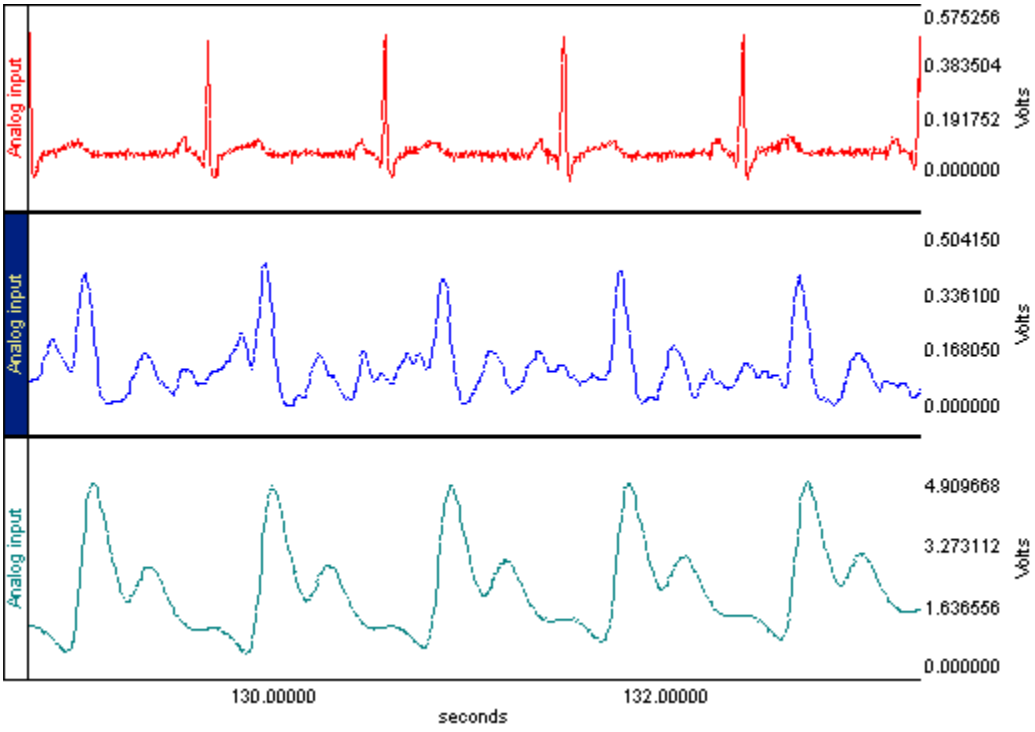


(b)

Figure 4.22 Subject 7 (a) T wave amplitudes per intervention and (b) the percent change of T wave amplitude from rest.

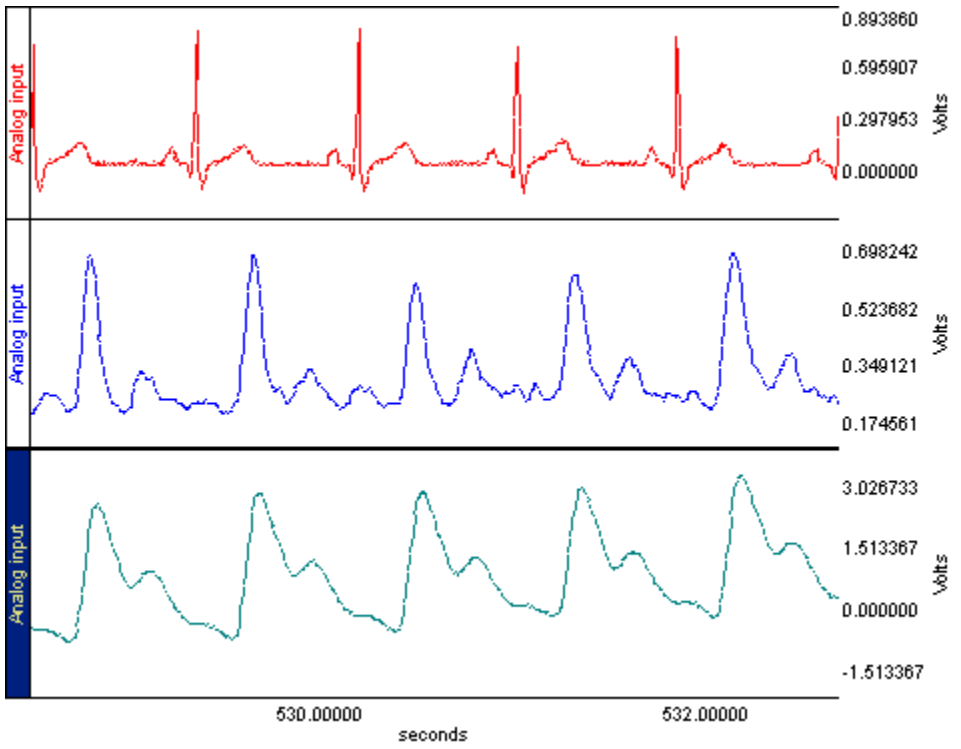


(a)

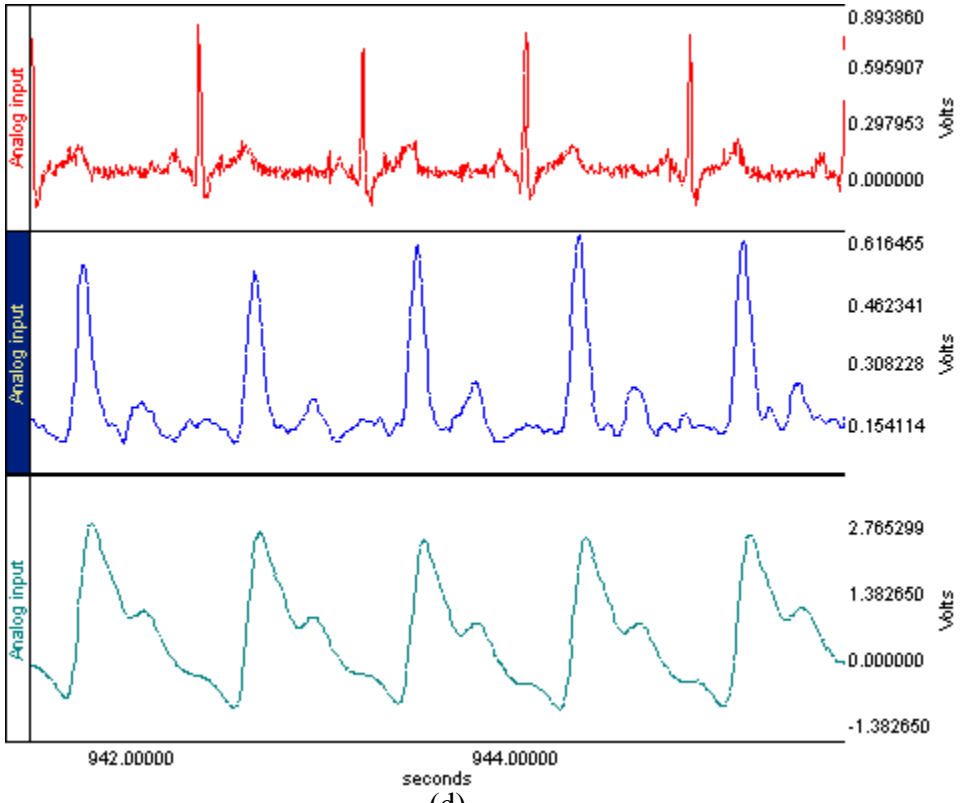


(b)

Figure 4.23a. Several beats of each intervention from Subject 1 during (a) Rest, and (b) Respiratory interventions.



(c)



(d)

Figure 4.23b. Several beats of each intervention from Subject 1 during (c) Mental and (d) Physical interventions.

Parameter	Statistics	REST	RESPIRATORY	MENTAL	PHYSICAL
T wave Amplitude	Mean	0.111282571	0.082663857	0.093583714	0.154937667
	Variance	0.004020123	0.002348346	0.001756789	0.011045756
	P value		**0.291177**	**0.563546**	**0.620515**
BPM	Mean	66.00085857	64.67355429	67.047664	73.12386933
	Variance	66.83090745	82.396693	46.88662106	23.10051762
	P value		**0.696425021**	**0.527859751**	**0.009641**
Frequency	Mean	1.098649429	1.077892571	1.117460286	1.218730333
	Variance	0.018093336	0.022887779	0.013024196	0.006416676
	P value		**0.7081415**	**0.490433183**	**0.008387**
R-P onset	Mean	0.245142857	0.230285714	0.227285714	0.2295
	Variance	0.003055476	0.002044571	0.003056905	0.0076411
	P value		0.169746443	**0.332893**	**0.471576**
Blood flow velocity	Mean	22.79693009	20.79110628	21.34470125	23.32082282
	Variance	13.74562695	8.733086348	4.70107957	2.431563012
	P value		**0.26947557**	**0.479933**	**0.691793**
Pulse Pressure	Mean	38.14285714	32.24901535	81.75346221	76.4871449
	Variance	43.47619048	91.915131	12344.7656	10184.59617
	P value		0.123127219	**0.349471**	**0.396549**
QT interval	Mean	0.423746286	0.404857143	0.424142857	0.390683333
	Variance	0.004567229	0.001959476	0.005114143	0.001533322
	P value		**0.2003288**	**0.964153**	**0.342156**

Table 4.3 Statistics from two tailed paired t-tests on each cardiovascular parameter (T wave amplitude (volts), Beats per Minute (BPM), Frequency (Hz), R-P onset (sec), blood flow velocity (cm/s), pulse pressure (mmHg), and QT interval (sec)) between rest and each intervention over all subjects ($P < 0.05$).

CHAPTER 5: DISCUSSION AND SUGGESTIONS FOR FUTURE RESEARCH AND IMPROVEMENT

Prior research in cardiovascular reactivity and stress has used questionnaires, select biological parameters from the cardiovascular or endocrine system or a combination of questionnaires and biological parameters. As the need for quantitative data became increasingly relevant to understand the onset of stress and its relation to disease, stress research became the subject of study in science and medicine. The use of quantitative data can reduce the lack of agreement amongst studies testing the same parameters. Following an extensive review of stress literature, studies which have included cardiovascular parameters limited themselves to heart rate and blood pressure. Additional variables for consideration include cardiac output, stroke volume, and blood flow velocity. They would be useful in giving a more detailed assessment of how the heart is functioning mechanically. While diastolic blood pressure is widely studied in the literature, few studies have mentioned pulse pressure as a measurement variable. Pulse pressure is a surrogate measure of arterial stiffness, which occurs at 50+ years. Diastolic pressure is a measure of peripheral resistance. In studies using young adults, it is suitable to use diastolic pressure. However, studies using older patients continued to use mean or diastolic pressure when pulse pressure has been proven a better indicator of heart disease. Pulse pressure rather than diastolic pressure is the best predictor of coronary heart disease risk in older subjects (Blacher, *et al.* 2000).

In the present study, several cardiovascular parameters were examined during respiratory, mental and physical stressors. Pulse pressure and blood flow velocity, not included in many stress studies, by obtaining the Fast Fourier Transform for each parameter and plotting their impedances. This data enabled the observation of the

relationship between pressure and radial artery flow velocity during rest, respiratory, mental and physical interventions. Rest was characterized by falling impedance or low pressure and increased blood flow. Once deep inspiration began, some subjects showed higher strain or impedance at the beginning of the task, followed by low impedance. However, subjects that were able to hold their breath for long periods of time, such as Subject 6, showed very low impedance at the onset of inspiration, with a large impedance peak at the end of the breath exercise. During mental stressor task, which involved playing a puzzle game, several subjects showed trends of increasing impedance with recovery between the highest levels of impedance. This was an indication of subjects experiencing mental strain from the game at certain levels. Impedance dropped sharply once the strain or difficult levels were surpassed. Lastly, physical stress showed two trends: high impedance followed by low impedance, suggestive of acclimation to the physical task, and low impedance followed by high impedance, which points to fatigue experienced by those subjects. In the plots of pulse pressure (delta blood pressure) and change in blood flow velocity in the radial artery (delta blood flow velocity), the results of these plots coincide with the impedance plots. They would be more useful to a clinician because the change in blood flow and pressure are in cm/s and mmHg respectively.

T wave amplitude has been the topic of many stress studies. However there is argument over its reliability due to its changeable nature. For most subjects, the T wave amplitudes (TWA) of the mental and respiratory stressors decreased from rest while the amplitudes increased during the physical stressor. However, there were a few subjects who exhibited decreased T wave amplitudes during physical stress or an increase from

mental stress (Subjects 4, 5 and 7). I expected these to be variable and based on the level of stress felt during each intervention. The results coincide with the results of the highest game level achieved. Subjects 4, 5 and 7 scored the lowest level during mental stress. Increases in T wave amplitudes during mental stress indicate that subjects were reacting to this stressor physiologically by having their ventricles pump more blood to supply to their brains to assist in their decision making process. In addition, subjects with increased T wave amplitudes during physical stress are more physically fit compared to the subjects with those with decreased amplitudes.

From statistics generated from the paired t-tests between the rest and each intervention over all subjects, the only parameter with a significant change from rest is the heart rate or BPM during physical stress ($P<0.05$). While BPM and Frequency were expected to be sensitive to stress, it was surprising that it was the only sensitive parameter. This may be attributed to data acquisition techniques, insufficient number of subjects or that the respiratory and mental stressors did not elicit a significantly different stress response compared to the resting state.

Cardiovascular reactivity, a term from cardiovascular behavioral medicine, describes physiological changes from a resting state to a psychological or physical stressor. It is a way to measure how stress related tasks can induce the onset of cardiovascular diseases (e.g. hypertension, coronary heart disease). While research continues, the general public and health care professionals should be aware and prepared to identify stress before it becomes a chronic condition. Further research is needed to clarify the relationship between stress related diseases and the cardiovascular system to highlight better intervention and management techniques.

The present study can be improved by redesigning a durable pressure transducer, and securing the position of the transducer and Doppler flat probe angle throughout the experiment. It would increase the fidelity of the data acquired. In addition, it would be beneficial to test more subjects and design stressor tasks to elicit a greater stress response. Finally, including measurements of oxygen, glucose and the stress hormones, cortisol and adrenaline, during stressor interventions would enhance our understanding of the stress response. This would aid in studying other stress induced diseases outside the realm of the cardiovascular system.

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