ABSTRACT

USING HEART RATE VARIABILITY TO MEASURE THE EFFECTS OF MANUAL MEDICINE ON AUTONOMIC ACTIVITY

by
Michael Zullow

A pilot study was performed to test the theory that manual medicine techniques known as the CV4 (compression of fourth cerebral ventricle) maneuver and a sacral hold/iliac bridge hold combination increase parasympathetic activity. Manual medicine techniques are widely practiced by Doctors of Osteopathy and Chiropractors to treat physical and mental health problems. Those who have received manual therapy have reported to experience less pain, better mobility, and a greater feeling of relaxation. To help us learn about the effects of manual therapy, autonomic activity was measured scientifically rather than subjectively in this study by monitoring respiration rate and heart rate, and computing heart rate variability. In order to establish a baseline for comparison we measured activity during intervals without any contact. These were then compared to intervals where interventions took place. In addition to the CV4 and sacral/iliac bridge holds a sham technique called the shoulder squeeze was used to test for placebo effects that the patient-physician contact may have had upon the results. Results indicated an increase in parasympathetic influence as a result of the CV4 maneuver and sacral holds.

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

Biomedical Engineering Committee

October 1997

APPROVAL PAGE

THE USE OF HEART RATE VARIABILITY TO MEASURE THE EFFECTS OF MANUAL MEDICINE ON AUTONOMIC ACTIVITY

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ACKNOWLEDGEMENT

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CHAPTER 1

INTRODUCTION

1.1 The Skull

It is often assumed that the skull is a rigid and fixed structure. This is because we often tend to think of bones as rigid bodies that do not bend. However, bones when alive do in fact have a small degree of elasticity and bend on a millimetric scale when stresses are applied to them. In addition, the skull can be mistakenly perceived as a continuous bony structure[1,8]. However, the skull is made up of several sections including the cranial and facial bones that are interconnected via stiff soft tissue joints known as sutures. (Figure 1.1) Presently it is known that some movement can occur about the soft tissue sutures between plates of the skull. Recent three dimensional motion detection techniques have enabled changes in shape and volume of the cranium to be measured. For about eighty years Doctors of Osteopathy have acknowledged what they call the cranial rhythmic impulse. This is the circulation of the cerebrospinal fluid that they feel causes movement of the parietal bones about the sagittal suture of the skull (Figure 1.2) at regular cycles[1,9]. Recent studies indicate that external pressures can affect compliance of the skull sutures and can even affect fluid flow of cerebrospinal fluid.

Bregma

Pterion

Lambda

Glabella

Posterior pole

Nasion

Nasal bone

Lacrimal bone

Inion or external
occipital protuberance

Anterior nasal aperture

Anterior nasal spine

Asterion

External acoustic meatus

Tympanic part

Mastoid process

Styloid process

Posterior border of ramus

Mental
protuberance

A. *Lateral view*

Angle of mandible

Inferior border of mandible

Parietal bone

Mandible

Figure 1.1   Picture of the human skull illustrating its plates which are connected by
sutures or rigid joints. (From Agur, A., Lee, M., Grant's Atlas of
Anatomy, Baltimore, Md., Williams & Wilkins 1991)

Sagittal suture

Parietal foramen

Parietal bone

Lambda

Lambdoid suture

Occipital bone

External occipital
protuberance and crest

Supreme (highest) nuchal line

Superior nuchal line

Inferior nuchal line

Mastoid process

Occipitomastoid
Suture

Mandible
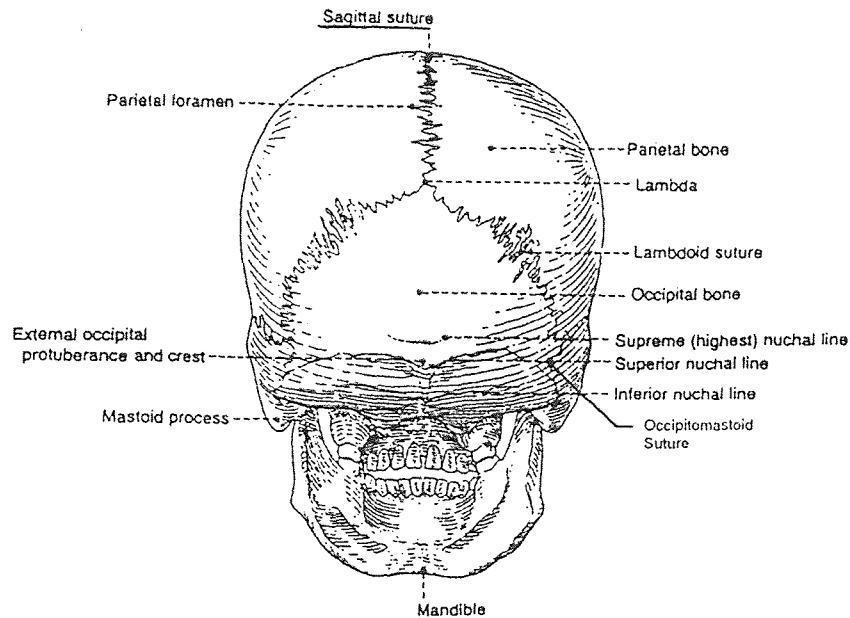
Figure 1.2   Posterior view of the skull illustrating the sagittal suture, a joint in the
back of the skull. (From Chung, K.W., Gross Anatomy, Baltimore,Md.,
Williams & Wilkins 1991.)

1.2 Craniosacral Manipulation

Craniosacral manipulation techniques were discovered in the early twentieth century by a

Doctor of Osteopathy named William Garner Sutherland. During craniosacral

manipulation the cranium, sacrum and other regions are manipulated to affect the cranial

rhythmic impulse. The cranial rhythmic impulse refers to the oscillations involved in

movement of the cerebrospinal fluid and the bones and membranes associated with it.

Such movement causes an alternation between outward flexion and inward extension of

the plates in the skull (Figure 1.3) along with its related membranes and bodily regions.
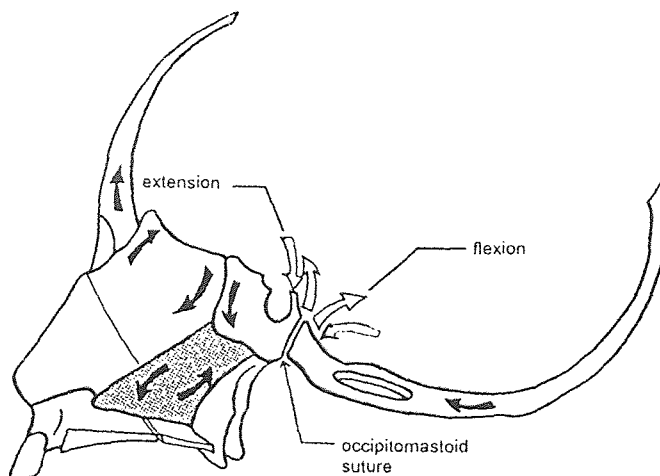
Figure 1.3   Movement of skull plates about the cranial sutures or joints. Flexion is the expansion of the skull while extension is the inward movement of plates within the skull. (From Gehin, A., Atlas of Manipulative Techniques for the Cranium & Face, Seattle, Washington, Eastland Press 1985.)

It was found by Sutherland that manipulation about certain sutures or joints within the skull can reduce the movements of the cranial rhythmic impulse to a state called the still point during which movements temporarily stop[1,2,3]. Following the still point a person may undergo physiological changes including changes in oscillation rate of cranial rhythmic impulses, heart rate, respiration rate, dermal temperatures and autonomic activity[1,2,3,4].

In the following experiments described by this paper three manual techniques were studied over the course of each trial; the sacral/iliac hold, the CV4 hold and the "shoulder squeeze", a sham intervention that acted as an object for comparison and a placebo. At the beginning and end of the sacral/iliac hold, the CV4 technique and shoulder squeeze was a more mild form of each intervention: the sacral hold for the sacral/iliac hold, the cranial vault hold for the CV4 technique, and a light touch on the shoulders for the

shoulder squeeze. These acted as buffer zones between the rest periods and intervention

periods to reduce the rate of change and allow the subject's autonomic nervous system to

stabilize during transitions.

The cranial vault hold, a mildly intrusive technique, was performed for one minute

before and after the CV4 hold which lasted for three or four minutes depending on the

protocol. This involved taking outstretched hands and placing them on either side of the

skull (Figure 1.4) allowing the cranial rhythmic impulse to be monitored and to create a

buffer zone between rest and the more intrusive CV4 hold.

The CV4 hold involved cupping the back of one hand in the other while laying the

thumbs flat against the palm. With the hands in this position the experimenter placed her

hands underneath the back of a subject's skull while he/she lay flat on an examination table.

Figure 1.4   Application of the cranial vault hold. Note the position of the hands and
fingers upon the subject's cranium. (From Gehin, A., Atlas of
Manipulative Techniques for the Cranium and Face, Seattle,
Washington, Eastland Press1985.)

This was done so that the bottom of both thumbs made contact (Figure 1.5) about the

occipitomastoid suture, a skull bone joint in the back of the head[3]. (Figure 1.3,1.2) The

CV4 hold had been chosen for this study because it is often considered to be central to

craniosacral manipulation, has been associated with vagal regulation, and is used widely by

osteopathic physicians. The CV4 maneuver is one that evokes a still point by restricting

flexion but allowing extension.(Figure 1.3) Once the still point is met, restriction upon

cranial movements are removed and the cycles involved in craniosacral movement are

monitored.
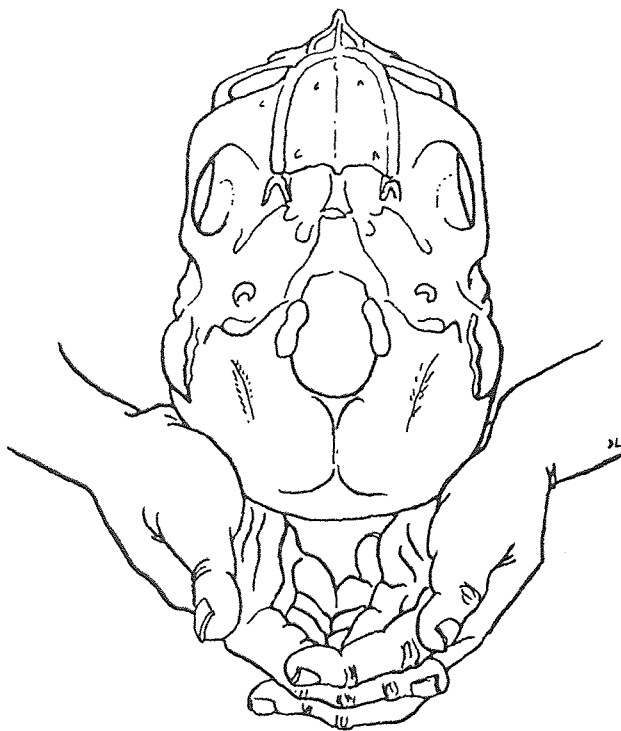
Figure 1.5   Illustration of the CV4 maneuver. Note the positioning of the lower
thumbs upon the cranium of the subject. (From Gehin, A., Atlas of
Manipulative Techniques for the Cranium & Face, Seattle Washington,
Eastland Press 1985.)

Sutherland believed that compression of the human skull could affect the circulation of cerebrospinal fluid within the fourth ventricle of the brain. Within the fourth ventricle, he stated, were vital vasomotor control centers that acted as regulators of heart rate. These control centers would then accelerate the heart via the sympathetic nervous system and slow the heart via the parasympathetic nervous system or vagus nerve. Sutherland claimed that compression of the fourth ventricle for a period of minutes or even seconds would lead to deepened breathing or sighing, changes in heartrate, more balanced cranial movements and a better state of equilibrium for all systems of the body. This could include outcomes such as fever reduction and headache relief[1,2,3,4]. As the name "craniosacral manipulation" implies it involves more than just regions about the head; cerebrospinal fluid also flows through membranes surrounding the spinal cord and compression of the sacrum can affect cerebrospinal fluid flow to evoke a still point[3].

Sacral mobilization was performed in two forms, a form of lighter touch known as the sacral hold, followed by a more restrictive maneuver that included both the sacral and iliac bridge holds. The sacral hold was performed by placing a hand with fingers together and palm upward, under the sacrum of the subjects as they lay flat on their backs. The sacral/iliac bridge hold combination was performed by applying light pressure upon the front side of the iliac or hip bone using fingers while simultaneously administering the sacral hold. This provided a greater amount of restriction and leverage since the cranial rhythmic impulses will move the sacrum about the iliac.

1.3 Nervous System Physiology

1.3.1 Neurons

The basic unit of the nervous system is the nerve cell or neuron. The nerve cells generate electrical signals to carry information from one end of the cell to the other and then release chemical signals to communicate with adjacent nerve cells. Most neurons contain four regions: the cell body, the dendrites, the axon and the axon terminals. (Figure 1.6)
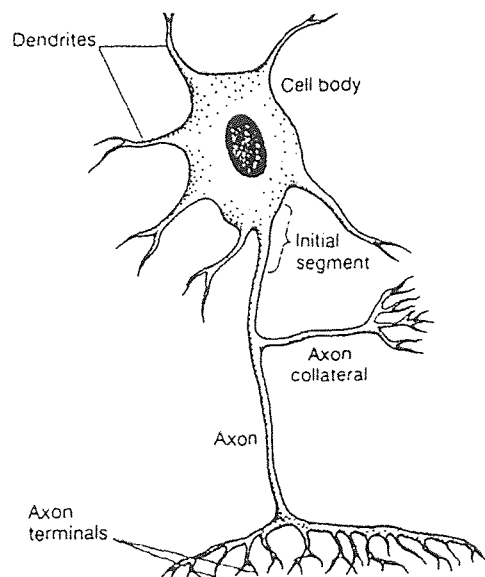
Figure 1.6   Illustration of a nerve cell and its regions: cell body, axon, dendrites, and axon terminals. (From Vander, A.J., Sherman, J.H. and Luciano, D.S., Human Physiology, New York, Applicon & Lange 1993.)

The cell body contains genetic information and the machinery for protein synthesis. The dendrites branch out from the cell body to collect signals from other neurons. The axon, also known as the nerve fiber, extends away from the nerve cell and generates propagating

electrical signals to branches that release chemical signals to surrounding cells. The axons of some neurons are covered by a myelin sheath that helps to insulate and accelerate electrical signals. Neurons can be classified as afferent, efferent or interneurons. Afferent neurons transmit signals from organs and regions of the body to the central nervous system; efferent neurons carry information from the central nervous system to muscle or gland cells; the interneurons connect the afferent and efferent neurons to one another within the central nervous system[5].

1.3.2 The Central Nervous System

The brain and spinal cord make up the central nervous system. The spinal cord lies within the vertebral column while the brain is contained within the skull. Between the soft neural tissue and bone containing it are three membranous layers or menanges: the dura mater next to the bone, the arachnoid in the middle and pia matter on the outside next to the neural tissue. (Figure 1.7) A space between the arachnoid space and pia called the subarachnoid space contains the cerebrospinal fluid. The spinal cord is a long, thin cylinder of soft tissue that carries information to and from other organs or regions within the body via the peripheral nerves. The brain is made up of six subdivisions: cerebrum, diencephalon, midbrain, pons, medulla oblongata and cerebellum.

Spinal cord

Spinal nerve

Pia mater

Dura mater

Dorsal primary ramus

Ventral primary ramus

Posterior intercostal artery

Intervertebral foramen

Rami communicantes

Transverse process

Vein
Intercostal   Artery
Nerve

Anterolateral view

Sympathetic trunk

Ventral rootlets

Arachnoid mater

Vertebral venous plexus

Extradural (epidural) fat

Hemizygous vein

Anterior longitudinal ligament

Aorta

Thoracic duct

Azygos vein

prevertebral structures

Figure 1.7   The spinal cord and its surrounding soft tissue and bone. Between the
spinal cord and vertebrae are three membranous layers: the dura mater
next to the bone, aracnoid in the middle and pia mater on the outside
next to the spinal cord. (From Agur, A., Lee, M., Grant's Atlas of
Anatomy, Baltimore, Ms., Williams & wilkins 1991.)

The diencephalon and cerebrum together make up the forebrain while the pons, medulla,

and midbrain constitute the brainstem[5]. (Figure 1.8)

Forebrain { Cerebrum
Diencephalon

Brainstem { Midbrain
Pons
Medulla
oblongata

Cerebellum
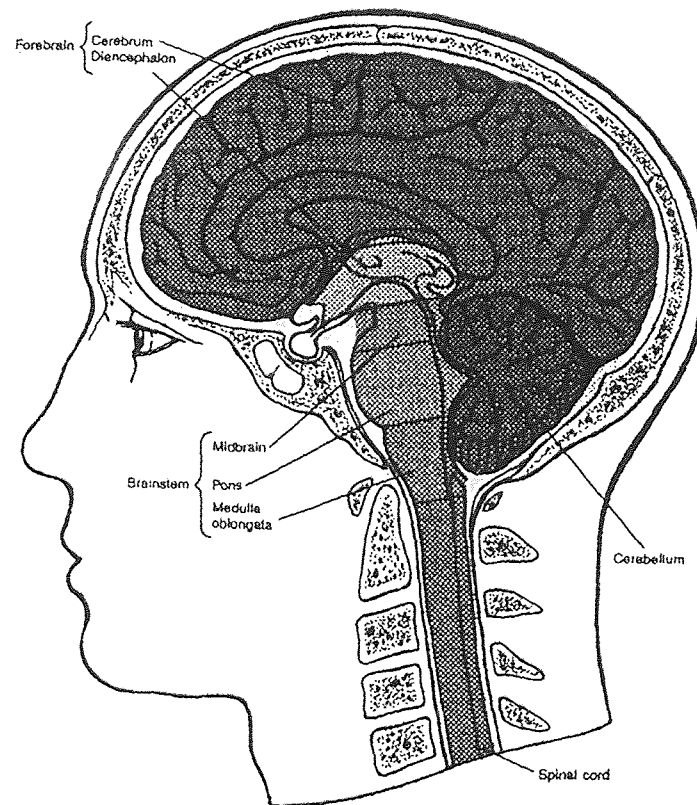
Spinal cord

Figure 1.8   Regions of the brain and spinal cord. The brain is made up of six regions: cerebrum, diencephalon, midbrain, pons, medulla oblongata and cerebellum. (From Vander, A.J., Sherman, J.H. and Luciano, D.S. Human Physiology, New York, Applicon & Lange 1993.)

The brain also has four contiguous cavities containing cerebrospinal fluid called the cerebral ventricles. (Figure 1.9) The cerebrospinal fluid surrounds and bathes the brain and spinal cord, as mentioned above, providing a cushion from movements or blows. The fluid is secreted into the ventricles by the choroid plexuses (Figure 1.9)which makes up part of the lining of the four ventricles. The cerebrospinal fluid flows into the brainstem where it passes to the brain and spinal cord through small openings[5,6].

Corpus callosum

Septum pellucidum

Fornix

Anterior commissure

Hypothalamus

Interventricular foramen
Massa intermedia

Thalamus
Choroid plexus

Parieto-occipital
sulcus (fissure)

Pineal body (gland)

Calcarine sulcus
(visual area)

Cerebral aqueduct

Cerebellum

4th ventricle

Median aperture

Brain stem:

Midbrain

Pons

Medulla oblongata
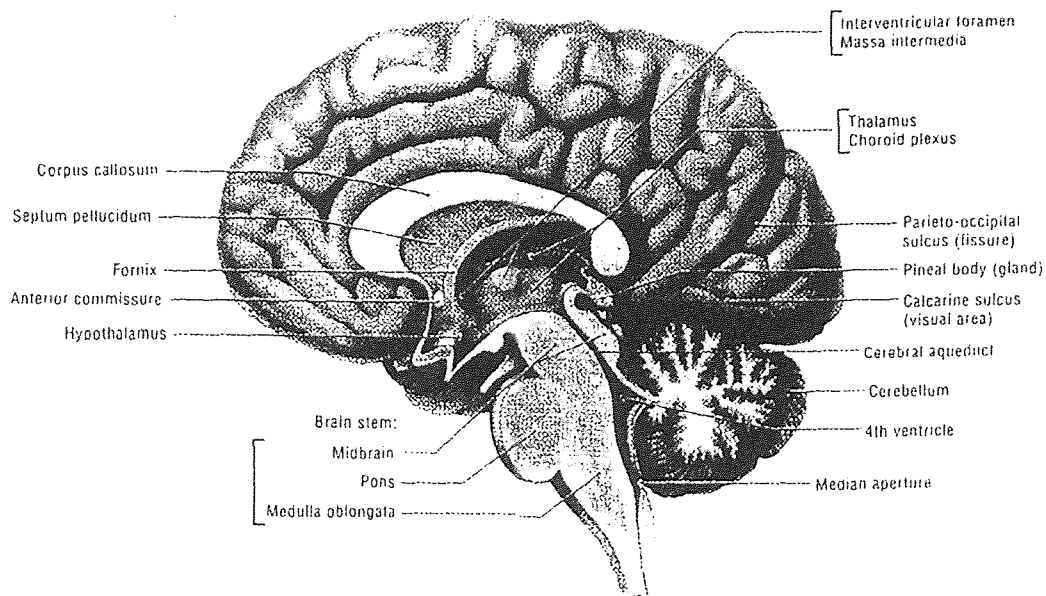
Figure 1.9   Crossection of the brain illustrating areas including the choroid plexus and fourth ventricle. The choroid plexus secretes cerebrospinal fluid, depositing it into the ventricles of the brain. (From Agur, A., Lee, M., Grant's Atlas of Anatomy, Baltimore, Md., Williams & Wilkins 1991.)

Its circulation is aided by pressure changes within the circulatory and respiratory system and those created by postural changes. After circulating throughout the central nervous system the fluid eventually arrives at the top of the surface of the brain where most of it is reabsorbed into the blood stream[5,6].

1.3.3 The Peripheral Nervous System

Peripheral nerve fibers transmit information between the central nervous system and all other parts of the body. The afferent nerves transmit information from receptors within or about organs in the body to the central nervous system while the efferent nervous system

carry signals from the central nervous system to organs of the body. The efferent division of the peripheral nervous system is subdivided into two portions, the somatic nervous system and the autonomic nervous system.. The somatic division innervates skeletal muscle while the autonomic system innervates smooth and cardiac muscle, glands and neurons within the gastrointesinal tract. The heart, glands and smooth muscle are innervated by two subdivisions of the autonomic nervous system known as the parasympathetic and sympathetic components. The parasympathetic division prepares the activities of organs within the body for less stressful situations while the sympathetic nervous system prepares the body for more stressful situations. Sympathetic activity increases under conditions of stress and its effects have been referred to as the fight or flight response. This describes a situation where an animal would be forced to fight or run when attacked. Parasympathetic activity evokes a reduction in the bodily activity and is usually prevalent during maintenance functions like digestion. The parasympathetic and sympathetic nervous systems work together to regulate the activities of organs throughout the body. (Figure 1.10) The two are usually activated so one increases as the other decreases.
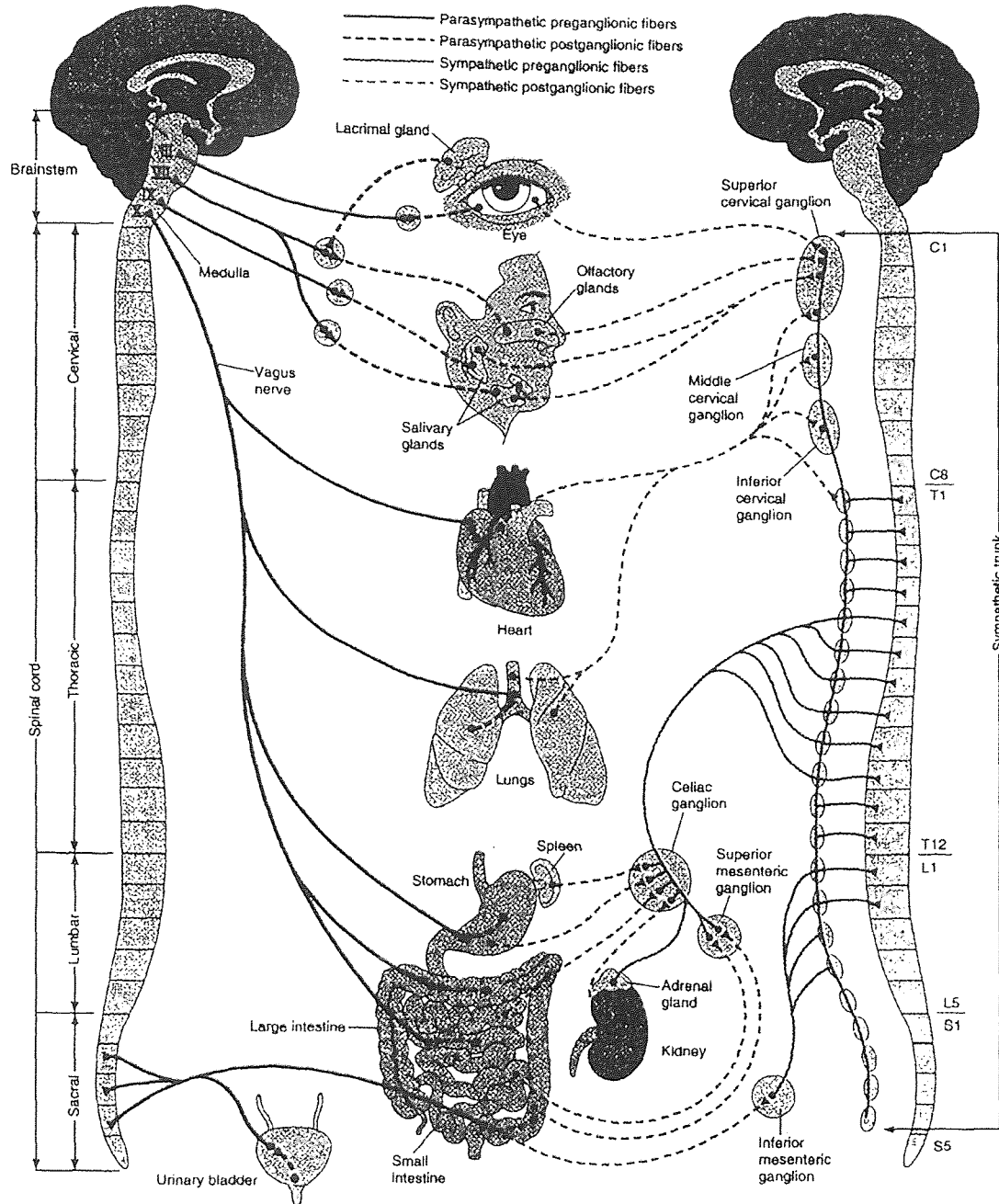
Figure 1.10   The parasympathetic and sympathetic nervous systems and the way they innervate the different systems throughout the body. (From Vander, A.J., Sherman, J.H. and Luciano, D.S., Human Physiology, New York, Applicon & Lange 1993.)

By working together they can regulate the systems they innervate with better control. For

example, if the parasympathetic division reduces activity, while the sympathetic division

increases activity it will cause the activities to increase much faster than the sympathetic nervous system alone. Autonomic functions are usually not controlled consciously, and the autonomic nervous system has also been referred to as the involuntary nervous system[5]. However, this is not a safe assumption since it has been shown that it can be controlled through learned behaviors such as meditation and possibly manual medicine techniques as shown here.

1.3.4 Regulation of Heart Rate

The activity levels of the heart are regulated by hormonal and chemical signals, temperature, and most importantly, autonomic nervous system activity. A balance between the simultaneous activities of the parasympathetic and sympathetic nervous systems plays an important role in regulating heart rate. The sympathetic nervous system works to increase heart rate while the parasympathetic nervous system works to decrease activity. Parasympathetic nerve fibers known as the vagus nerves and sympathetic nerve fibers end on the sino-atrial node. (Figure 1.11) The sino-atrial node, otherwise known as the "pacemaker" of the heart, is a region of the heart that sends signals to other regions of the heart to regulate heart-rate. Without any stimulation from nerves or hormones the sino-atrial node will cause the heart to beat at about one-hundred beats per minute[5,7].

Diagram labels: Superior vena cava, Atrioventricular node, Bundle of His, Sinoatrial node, Right bundle branch, Purkinje fibers, Left bundle branch
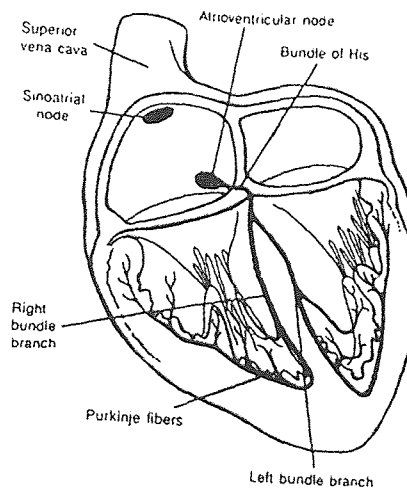
Figure 1.11   Conducting system of the heart illustrating the location of the sinoatrial node. It is the sinoatrial node that receives electrical signals from the autonomic nervous system to regulate heart rate. (From Vander, A.J., Sherman, J.H., and Luciano, D.S. Human Physiology, New York, Applicon & Lange, 1993.)

Depending upon the combined activities of the large number of parasympathetic and sympathetic nerve fibers that innervate the sino-atrial node, the heart rate will increase or decrease about this rate.

1.4 Heart Rate Variability

Heart rate variability is becoming an increasingly important tool for the medical field. It is especially useful in the field of cardiology and has been said to be the best means of predicting risk of mortality following myocardial infarction. When we exercise our heart rate will usually increase as we exert ourselves. This is done to supply more oxygen-containing blood to areas that must meet the higher energy demands during exercise. At rest the heart seems to beat at a steady rate[10]. But with sensitive instruments it can be

seen that the heart rate can vary, even during sleep. Heart rate is regulated by a number of

physiological mechanisms including the autonomic nervous system. The parasympathetic

nervous system slows the heart rate through the vagus nerve and nerve fibers connected to

the sino-atrial node. At the same time the sympathetic nervous system sends signals to the

sino-atrial node speeding up the heart.

As a result of the roles that the sympathetic and parasympathetic nervous systems

play in regulating heart-rate, we were able to indirectly monitor their activities using an

electrocardiogram. Information was obtained from the electrocardiogram by measuring

the time between ventricular beats using the synch pulse from a Q4000 Monitor (Quinton

Instrument Co., Seattle, WA). The synch pulse accompanies an electrocardiogram (Figure

1.12) signal giving a pulse wave each time an R wave within the ECG signal is present.

(Figure 1.12) The time intervals between these pulse waves were measured and then

placed consecutively in a time series to generate an inter-beat interval (IBI) signal. (Figure

1.13) This signal was then interpolated using a backward step function to produce an

interpolated inter-beat interval signal (IIBI). (Figure 1.13)
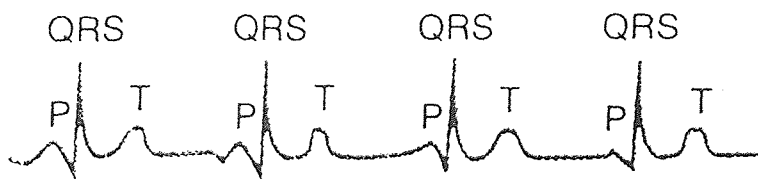
Figure 1.12   Electrocardiogram signal produced by the electrical activities that drive
the contractions of the heart muscle. The P wave represents an atrial
contraction, the QRS (or R) wave (with R at its peak) represents a
ventricular contraction and the T wave represents a repolarization of the
ventricular conducting system. (From Vander, A.J., Sherman, J.H., and
Luciano, D.S., Human Physiology, NY, Applicon & Lange, 1993.)

Interbeat Intervals

Beat Number

(a)

- IIBI

Time

(b)
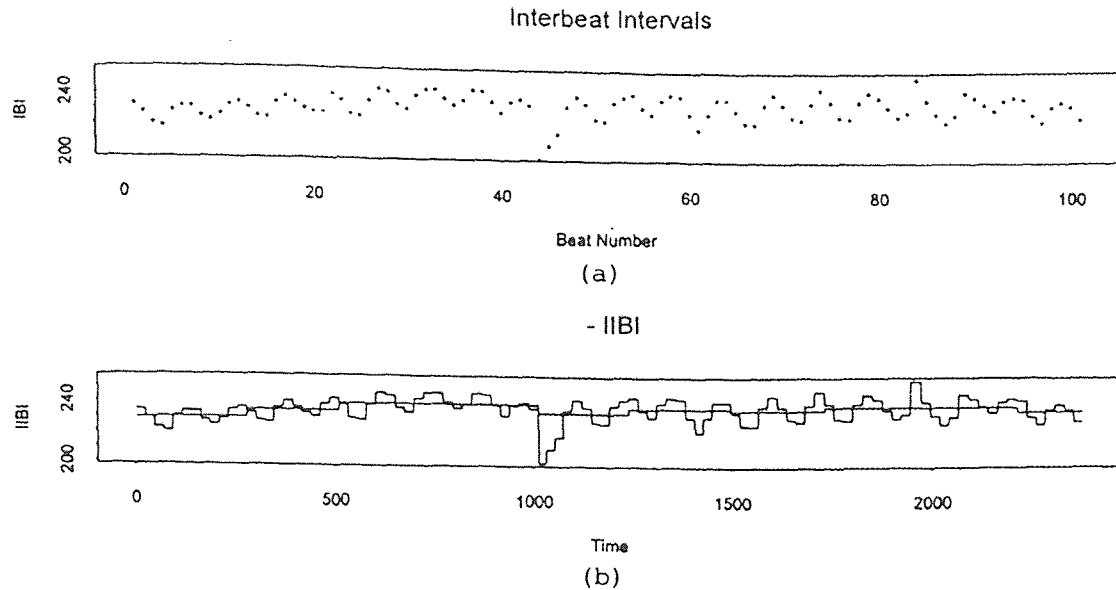
Figure 1.13   In Figure 1.13a is an interbeat interval plot. In figure 1.13b is the interbeat interval plot with an interpolation line drawn through it. (200 = 1 second) It is this signal that the Fourier transform is used upon to generate the frequency results representative of physiological activities.

1.4.1 Power Spectrum Analysis

After the IIBI signal was produced, the Fourier Transform was acquired to represent the frequency components within the interpolated inter-beat interval signal. The Fourier Transform is a signal processing technique that is used to represent a function in the time domain as a function within the frequency domain. It does this by representing the components of a non-periodic signal as a sum of complex exponentials or sinusoids. The equation for the Fourier Transform is as follows:

$$X(\omega) = \int_{-\infty}^{\infty} x(t)e^{-j\omega t}dt \qquad (1.1)$$

$X(\omega)$ represents the time function, $x(t)$ of a signal within the frequency domain and $e^{j\omega t}$ represents the complex exponentials or sinusoids that make up the signal[20]. Once the frequency domain signal is obtained using the Fourier transform we can analyze the frequency content of the signal. This can be achieved by plotting the Fourier spectrum (Figure 1.14) which indicates the relative proportion of different frequencies within a signal. These frequency components are measured by comparing magnitudes, a measurement proportional to amplitude and frequency of occurrence.
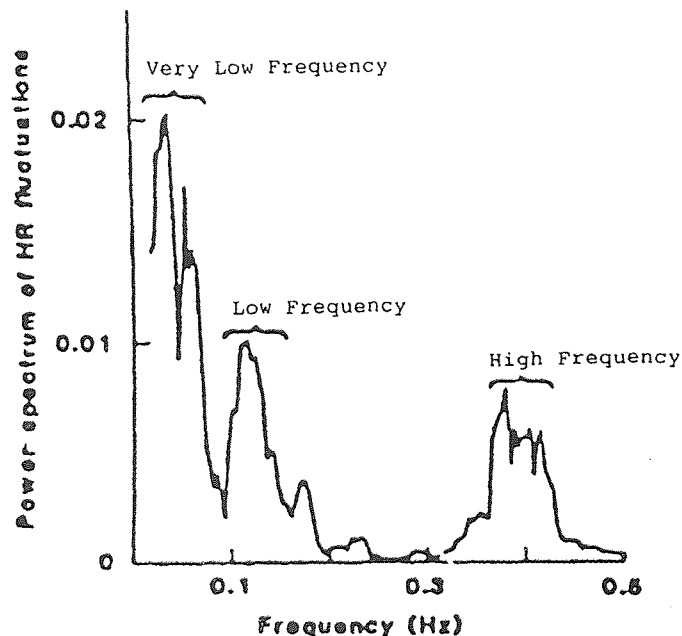
Figure 1.14   The peaks representative of the different frequency ranges within a power spectrum graph. The portion below 0.05 Hz represents the very low frequency range, the range from 0.05 Hz to 0.15 Hz represents the low frequency range, and the interval from 0.15 Hz to 0.4 Hz represents the high frequency range. (From Tang, X., "Heart Rate Variability Studies on Ambulatory Subjects Using EKG Derived Respiration", Master's Thesis, New Jersey Institute of Technology, 1995)

With the frequency spectrum we can measure the contribution of sympathetic or parasympathetic activity to heart rate variability. The frequency spectrum often contains peaks within three different frequency ranges that are associated with different systems which control heart rate.

The low frequency band in the range of 0.05Hz to 0.15Hz is strongly correlated with baroreceptor-mediated control, high frequency range of 0.15Hz to 0.4 Hz is associated with respiratory sinus arrhythmia and the very low frequency range, less than 0.05 Hz, represents the renin-angiotensin system and vasomotor and/or temperature control[11]. (Figure 1.14) Experiments using parasympathetic blockades have yielded frequency spectra with peaks in the high frequency band eliminated and with peaks in the low frequency range reduced. Tests using sympathetic blockades have yielded reduction in the low frequency ranges. These studies indicate that frequencies in the range of 0.05Hz to 0.15 Hz represent a mixture of sympathetic and parasympathetic activity and those in the range of 0.15 Hz to 0.4 Hz represent parasympathetic activity[11,12]. These frequency ranges are valid under the condition that a subject breathes at the rate of at least 10 breaths per minute. This is important because of the interaction between the circulatory and respiratory system rates. The high frequency range can be attributed to respiratory sinus arrhythmia, which can be described as variation in heart rate that accompanies respiratory activity. If respiration rate is less than 0.15 Hz, then the respiratory sinus arrhythmia would be affected by both sympathetic and parasympathetic activity[7,13,14].

Measurements of high and low frequency activities are measured by taking the area under the frequency spectrum between the high and low frequency limits mentioned above. This area is in units of power thus giving this form of measurement the name power spectrum analysis. A larger amount of power for the high and/or low frequency ranges is representative of a greater amount of autonomic activity taking place while a smaller amount of power is representative of less activity.

1.4.2 Time-Frequency Analysis

Currently, the use of frequency spectrum analysis for the measurement of autonomic activity has become commonplace. However, taking such measurements can put the experimenter at a disadvantage because a frequency spectrum is obtained that allows only one measurement over the course of a time interval, two minutes or longer. The spectral analysis of a signal indicates which frequencies are present over a period of time but not when those frequencies occur during the course of the time interval. For example, the frequency spectrum on the bottom of figure 1.15 indicates which frequencies occurred over the time period of 1.4 seconds, but it does not show precisely when each of the frequency components were present as does a time-frequency plot. In the time-frequency picture at the top of figure 1.15 we can see that during the first 0.6 seconds we have frequencies in the range of 175Hz to 260Hz and from 0.6 to 1.4 seconds we have frequencies in the range of 260 Hz to 335Hz. Thus it is known when certain frequency ranges were present and for what durations.

When performing time-frequency analysis the IIBI signal that is used for power spectrum analysis is used. When analyzing the IIBI for time-frequency analysis the signal is analyzed in portions known as window periods along the length of the signal
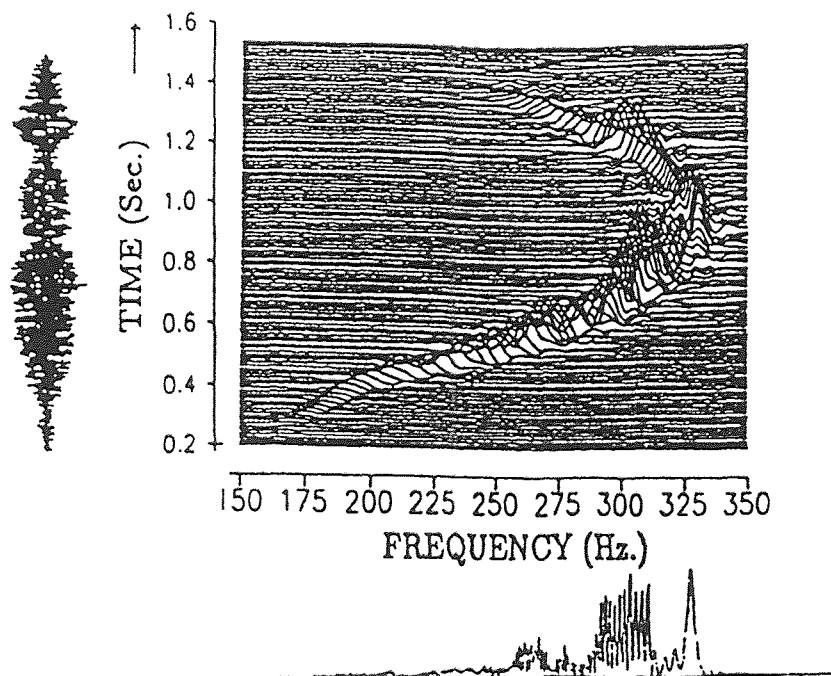
Figure 1.15   The above shows a signal (on the left) whose frequency composition is plotted over time using time-frequency analysis (top). Below the time-frequency graph is the frequency spectrum which tells us the sum total of each frequency present within the signal. (From Amin, M. et al. "Methods and Applications for Time-Frequency Analysis", Conference Notes, University of Michigan, 1993)

These window periods are portions of the signal centered at time t with their ends at t-Δ and t+Δ so that their durations are equivalent to a fixed time-length 2Δ. (Figure 1.16) [13,14]. Window periods are taken at fixed intervals of time (1.25 seconds in this study) so that a result is obtained every 1.25 seconds corresponding to the information within each window period.
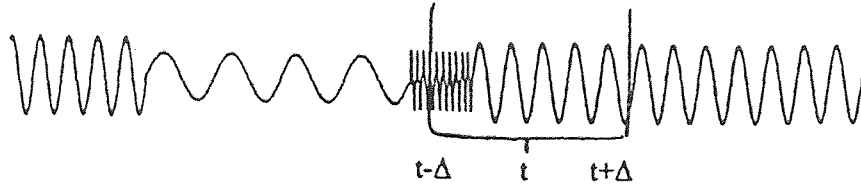
Figure 1.16   Illustration of window period taken about a point in time, t. The short-time Fourier transform is taken for each window period when using the Fourier transform for time-frequency analysis. (From Amin, M. et al. "Methods and Applications for Time-Frequency Analysis", Conference Notes, University of Michigan, 1993)

When using the Fourier transform to separate the contents of the window periods into its frequency components the short-time Fourier transform is used. The equation for the short-time Fourier transform is as follows:

$$X_{st}(\omega) = \frac{1}{\sqrt{2\Pi}} \int_{-\infty}^{\infty} e^{-j\omega\tau} x_w(\tau) d\tau \qquad (1.2)$$

$X_{st}(\omega)$ represents the short-time Fourier transform [13,14] as a function of frequency and $e^{-j\omega\tau}$ represents a set of complex exponential or sinusoidal functions. The function $x_w(\tau)$ represents $x(\tau)$ multiplied by the window function, $h(\tau)$ to give a weighted signal with τ centered about time t. [13,14]. The equation for $x_w(\tau)$ is as follows:

$$x_w(\tau) = x(\tau)h(\tau) \qquad (1.3)$$

When the short-time Fourier transform is used frequency resolution is sacrificed for the sake of time resolution. This is because the window periods used are narrow about the point of interest giving a wide spectrum as described by the uncertainty principle. [13,14,20]. This difficulty was overcome in this paper by using a Matlab program called sympar written by Sanjay Fernando which used the Wigner distribution. This produced results with superior time-frequency resolution. The Wigner distribution was introduced by Wigner in 1932 and later used for signal analysis by Ville in 1948. [13,14]. The distribution is expressed according to the following equation:

$$D_w(t,f) = \int_{-\infty}^{\infty} e^{-j2\Pi f t} x^*(t - \frac{\tau}{2}) x(t + \frac{\tau}{2}) d\tau \qquad (1.4)$$

$D_w(t,f)$ represents the Wigner distribution, and $e^{-j2\Pi f t}$ represents a set of sinusoidal or complex exponential functions. The original function of time $x(t)$ is written as $A(t)e^{j\Phi(t)}$. $A(t)$ is the envelope or amplitude and $\Phi(t)$ signifies the phase. In the above equation the signal is shifted to the right by $\frac{\tau}{2}$ and then multiplied by the signal when shifted to the left by $\frac{\tau}{2}$. This is done for each time t to yield $x^*(t - \frac{\tau}{2}) x(t + \frac{\tau}{2})$ as seen above. The Fourier transform is then taken of the signal $x^*(t - \frac{\tau}{2}) x(t + \frac{\tau}{2})$ (note that $\int_{-\infty}^{\infty} e^{-j2\Pi f t} d\tau$ represents the Fourier transform) to give us a result for the Wigner Distribution. [13,15]. After the Wigner Distribution is performed for each period along the

IBI signal the result is partitioned into its high and low frequency components to give us a high and low frequency point at each time t. This generates two time series of points representing the sum of all signals over time within the designated high and low frequency ranges.

1.5 Scope of Thesis

The purpose of this study was to test the theory that the sacral/iliac hold and CV4 hold evoke an increase in parasympathetic activity. It was important to determine whether or not changes in parasympathetic activity actually occurred. To accomplish this task both time-frequency analysis and power spectrum analysis were used. Not only was it important to determine whether or not changes were taking place, but it was crucial to measure when the activities occurred and the dynamics involved. The use of time-frequency analysis allowed us to collect data within the time domain, whereas employing the power spectrum analysis revealed the frequency distribution. One objective of these experiments was to establish a means for quantifying the results of the time-frequency analysis. This would provide us with concrete measurable information rather than less definitive data based on pattern recognition open to human interpretation. Once we derived numbers, we then used paired t-tests in making comparisons and drawing conclusions from the data.

A means of determining what effects may have taken place as a result of the manual medicine techniques was established by taking measurements for intervals when no treatments or mild treatments occurred and comparing them to those intervals when

manual techniques were administered. Three distinctly different forms of manual treatment were employed in this study. Two techniques were genuine manual medicine techniques, (the sacral/iliac and CV4 holds). The third, (the shoulder squeeze,) devised as a bogus form of treatment, played an important role in establishing whether or not significant changes or effects were truly taking place as a result of the genuine manual medicine techniques. It allowed us to test for placebo effects that could have resulted from the researcher's physical contact with the subjects.

CHAPTER 2

METHODS

2.1 Data Acquisition Equipment

Respiration rate and heart rate were monitored to gather the necessary information for heart rate variability computations. Respiration rate was monitored by using a Resp I (UFI, Morrow Bay CA) impedance pneumatograph while heart rate was monitored using a Q4000 Monitor(Quinton Instrument Co., Seattle, WA) ECG machine. ECG adhesive silver/silver chloride surface electrodes (Medtronic, Haverhill MA), transferred electropotential information from the surface of the skin to the respiration and ECG monitors as analog signals. (top of figure 2.1)

As the lungs expand they fill with air changing the electrical resistance of tissue between electrodes. This registers on the Resp I device as a voltage change which increases during inhalation and decreases during exhalation. The ECG signal is produced as electrical impulses from within the heart create contractions or a heartbeat. The different portions of an ECG signal represent particular stages or contractions over the course of a heartbeat. The first deflection or P wave represents an atrial depolarization. The second, the QRS wave, represents a ventricular contraction. The third and final portion of the signal, the T wave represents a ventricular repolarization[5]. (Figure 1.12)

Four electrodes, one on the left clavicle, one on the right clavicle, one below the left underarm and one below the right underarm were used for the ECG monitor while three electrodes, one on the right clavicle and two below the left underarm (one ground) were used for the respiration impedance pneumograph. (Bottom of figure 2.1)

27

ECG Machine

Electrode wires

Blue Box (Instrument/PC interface)

Personal Computer

Electrode wires

Respiration Monitor

Black Box

Electrode wires

AgCl Electrode

Right Side

Left Side
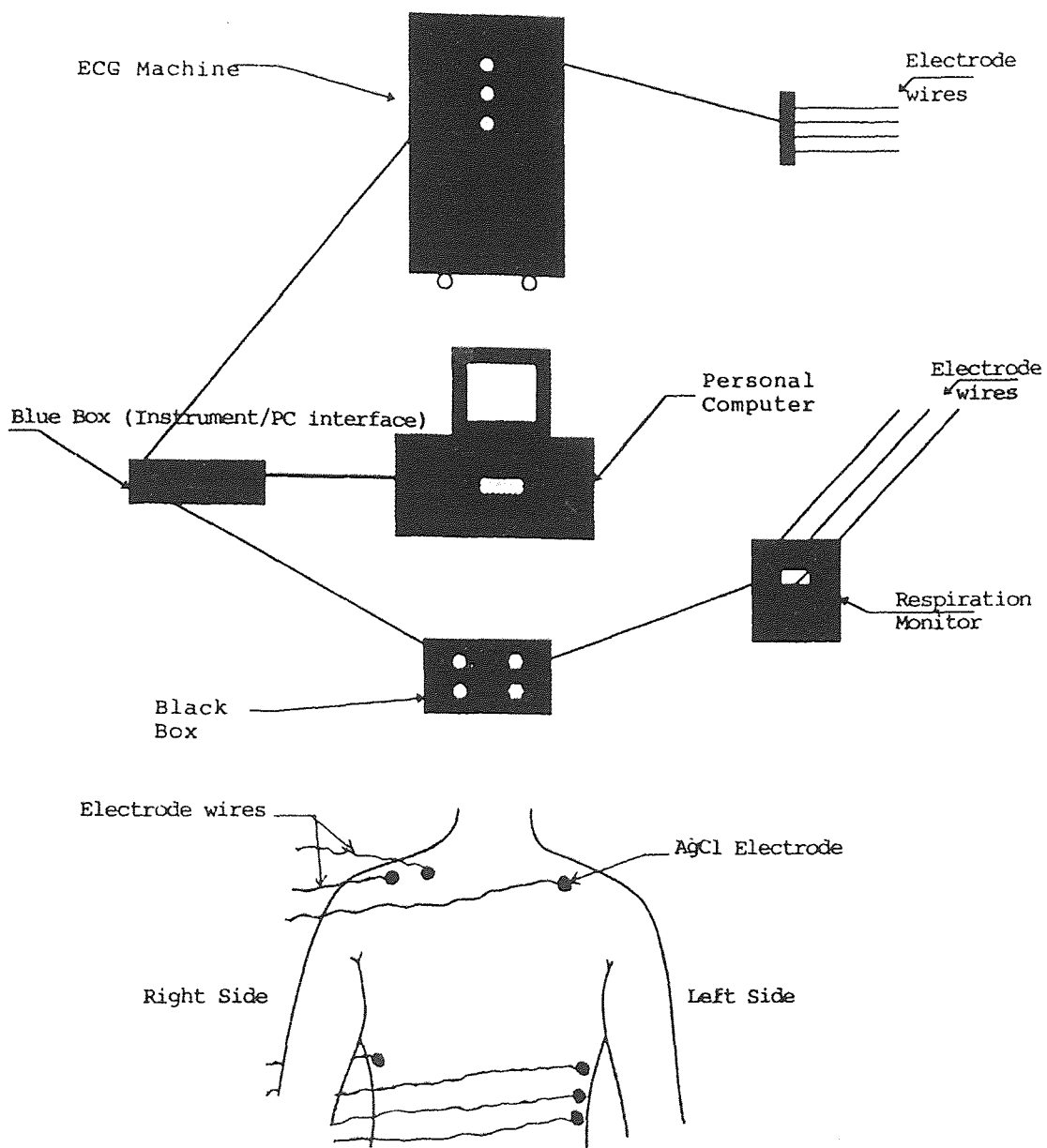
Figure 2.1   Data aquisiton setup. In the top of this figure is the manner in which the medical devices are connected to a personal computer. The bottom of this figure shows the positioning of the silver chloride electrodes upon the subject.

In order to insure proper conductance between the skin and electrodes the skin was

scrubbed thoroughly with an alcohol pad and then wiped with a paper towel to remove the

outer layer of skin. The analog signals from the respiration monitor and ECG machine were fed through a DAS-1601 analog-to-digital converter (Keithley MetrByte/Asyst, Taunton, MA) into an IBM compatible 286 computer with 1 Megabyte of RAM and a 230 Mb of hard drive space via Streamer v3.25 software(Keithley MetraByte/Asyst, Natick MA). Before beginning the experiment a program called Primplot(J.F. Andrews) was used to graphically insure that the equipment was properly acquiring data. If not, all connections and gain settings were checked and then adjusted accordingly. For illustrations of the data acquisition setup refer to the top of figure 2.1. For the detailed data acquisition procedures used refer to appendix A.

2.2 Protocol

The experiment was designed to test if the osteopathic manual medicine techniques called the CV4 and sacral/iliac holds evoked a parasympathetic response in healthy subjects. The protocol was set up so that rest intervals, during which no manual medicine interventions occurred, preceded and followed interventions. In this way measurements taken during the manual medicine techniques could be compared to those taken during non-intervention periods to help test for effects. In addition, carryover and order effects between interventions would be reduced and opportunities to measure delayed responses would be created. Refer to tables 2.1 and 2.2 for examples of the protocols used.

Three interventions were studied over the course of the experiment; the sacral/iliac hold, the CV4 hold and the "shoulder squeeze", a sham intervention that acted as an

object for comparison and a placebo. At the beginning and end of each intervention period, for one minute, a more mild form of the manual medicine techniques and the shoulder squeeze was administered: the cranial vault hold for the CV4 technique, the sacral hold for the sacral/iliac hold and a light touch on the shoulders for the shoulder squeeze.

Table 2.1   Example of the 30 minute protocol. The order in which the interventions took place was chosen randomly before testing began.

Time Interval (Min.)	Duration(Min.)	Event	Intervals Used for Statistics
0-2	2	Rest	0-2
2-3	1	Sacral Hold	not used
3-6	3	Sacral/Iliac Hold	3-5
6-7	1	Sacral Hold	not used
7-9	2	Rest	7-9
9-10	1	Light Touch	not used
10-13	3	Shoulder Squeeze	10-12
13-14	1	Light Touch	not used
14-16	2	Rest	14-16
16-17	1	Cranial Vault Hold	not used
17-20	3	CV4	17-19
20-21	1	Cranial Vault Hold	not used
21-30	9	Rest	21-23

Table 2.2   Example of the 48 minute protocol. The order in which the
interventions took place was chosen randomly before testing began.

Time Interval (Min.)	Duration (Min.)	Event	Intervals Used for Statistics
0-10	10	Rest	1-5, 6-10
10-11	1	Cranial Vault Hold	not used
11-15	4	CV4	11-15
15-16	1	Cranial Vault Hold	not used
16-21	5	Rest	17-21
21-22	1	Light Touch	not used
22-26	4	Shoulder Squeeze	22-26
26-27	1	Light Touch	not used
27-32	5	Rest	28-32
32-33	1	Sacral Hold	not used
33-37	4	Sacral/Iliac Hold	33-37
37-38	1	Sacral Hold	not used
38-48	10	Rest	38-42, 43-47

These acted as a buffer zones between the rest periods and intervention periods to reduce

the rate of change and allow the subject's autonomic nervous system to stabilize during

transitions. This was important since sudden changes would have been more likely to

evoke an autonomic response that could interfere with the activities of interest.

The order in which the interventions occurred was randomized to reduce any order

effects that may have occurred. The protocol used was initially 30 minutes but was later

changed to 48 minutes with extended rest periods at the beginning and end to allow the

heart to stabilize and to help look for delayed effects at the beginning and end of the

experiments, respectively. During the 30 minute protocol the rest periods were two minutes long (except for the last which was 9 minutes) and the intervention intervals were 5 minutes long. Each 5 minute intervention began and ended with the more mild forms of intervention for periods of one minute. (Table 2.1) During the 48 minute protocol all rest periods were five minutes long and interventions were 6 minutes long . Each of these 6 minute interventions began and ended with their more mild forms for periods of one minute. Each 48 minute experiment began and ended with two rest periods 5 minutes in length. (Table 2.2)

Before the experiment began the subjects were given the necessary instructions and positioned appropriately to help them avoid or adjust to any mental or physical stresses that they could experience during the test. This included pacing their breathing before the test began, coaching them to lift their hips when signaled to allow placement of the experimenter's hand below the sacrum during the sacral hold, and answering any questions that they may have had concerning the test to reduce anticipation. During the experiments a subject would lay flat on an examination table with a pillow underneath their head. Lying on a pillow helped keep the subject comfortable and mimicked the position of their skull during the CV4 procedure to reduce any changes in positioning as the CV4 hold began that could shift their equilibrium. Breathing was paced at ten breaths per minute with the aid of a moving light mounted within the subject's field of view to insure that the assigned high (0.15Hz to 0.4Hz) and low frequency (.05Hz to .15Hz) ranges would correspond to parasympathetic and a mixture of sympathetic and parasympathetic activity respectively. (as described in section 1.4.1)

As cranial manipulation began the experimenter performed the cranial vault hold by cradling the subject's skull with open hands. (Figure 1.3) After a minute of applying the cranial vault hold, the CV4 (compression of the fourth ventricle) maneuver was applied. This involved taking the back of one hand and placing it in the palm of the other with thumbs together and pointing in the direction of the subject's feet. Then the experimenter placed her hands, in the manner described, under the base of the subject's skull as the subject lay flat on his/her back. This put the lower portion of the experimenter's thumbs about the occipitomastoid suture, (Figure 1.1,1.4) a cranial bone joint, so that the flexion or outward widening of the skull was restricted and extension, an inward contraction and narrowing of the skull, was encouraged. This eventually resulted in a pause of the cranial rhythmic impulse known as the still point[1,2,3]. After a number of seconds or minutes the cranial rhythmic impulses resumed and were accompanied by a possible increase in autonomic activity. This was continued until the allotted time period (three minutes for the thirty minute protocol or four minutes for the forty-eight minute protocol) had expired whether or not a still point was detected. After the period had ended, the cranial vault hold was applied for one minute before the next rest interval began.

The sacral hold was performed by first placing a hand, fingers together and palm upward, under the sacrum of the subject as the subject lay flat on his/her back for the first minute of the intervention. The sacrum has been reported to be an alternative site for monitoring and regulating cranial rhythmic impulses since activities there are concurrent with those in the cranium. Then the investigator performed the iliac bridge hold by lightly pressing the front of the subject's iliac bone, often on the left side, with her fingers as she

performed the sacral mobilization. This was done for an allotted time period (three minutes for the thirty minute protocol and four minutes for the forty-eight minute protocol) after which the experimenter's fingers were removed from the subject's iliac so that only sacral mobilization was being performed for the last minute.

During the shoulder squeeze the investigator first placed her fingertips lightly on the subject's shoulders for one minute. For the next three or four minutes depending on the protocol used, the experimenter placed her hands on the shoulders of the subject and gently squeezed them while being careful not to apply pressure to the subject's neck with her fingers to avoid a baroreceptor response by the carotid artery. After the period had ended the experimenter placed only her finger tips on the subject's shoulder for one minute as before.

2.3 Data Processing

2.3.1 Time Frequency Analysis vs. Power Spectrum Analysis

Frequency spectrum and time-frequency analysis were used to measure autonomic activity over the course of the experiment. Both are based on heartrate variability measurements but frequency spectrum analysis gave us a single measurement for an extended time period while time frequency analysis gave us a series of instantaneous measurements over the course of a time interval. Although spectral analysis was the more conventional method of the two, time frequency analysis could better detect rapid changes in autonomic activity. This was important because it was not clear when and in what manner changes took place if they did occur.

2.3.2 Steps for Power Spectrum and Time Frequency Analysis

Data were collected from an analog Q4000 Monitor (Quinton Instrument Co., Seattle,

WA) ECG machine and a Resp I (UFI, Morrow Bay CA) impedance pneumograph

respiration monitor and fed into a DAS 1601 analog-to-digital converter (Keithley

MetrByte/Asyst, Taunton,MA) into an IBM compatible 286 computer with 1 Megabyte of

RAM and a 230 Mb of hard drive space via Streamer v3.25 software (Keithley

MetraByte/Asyst, Natick MA). The files were then transferred to 486 and Pentium based

personal computers where the analysis and processing of data were performed.

After the files were transferred they were converted from binary to ASCII format

using a software named Unpack (Keithley MetraByte/Asyst, Natick MA). These ASCII

files were then scanned into the software called S-plus (Statistical Sciences, Inc). In S-

plus a program called pslws (refer to appendix B for the program) was used to calculate

the time intervals between consecutive R-wave detections (or beats) using the synch pulse,

a signal provided by the Q4000 monitor which consists of a short pulse signal each time an

R wave is present. (Figure 2.2) These time intervals were then placed in a time series and

the resulting inter-beat interval signal was interpolated using a backward step function.

The Fourier transform was then performed upon the interpolated IBI signal to calculate

the frequency components present. The results of the Fourier transform were then placed

in a frequency distribution to illustrate the frequency components and their relative

contributions.
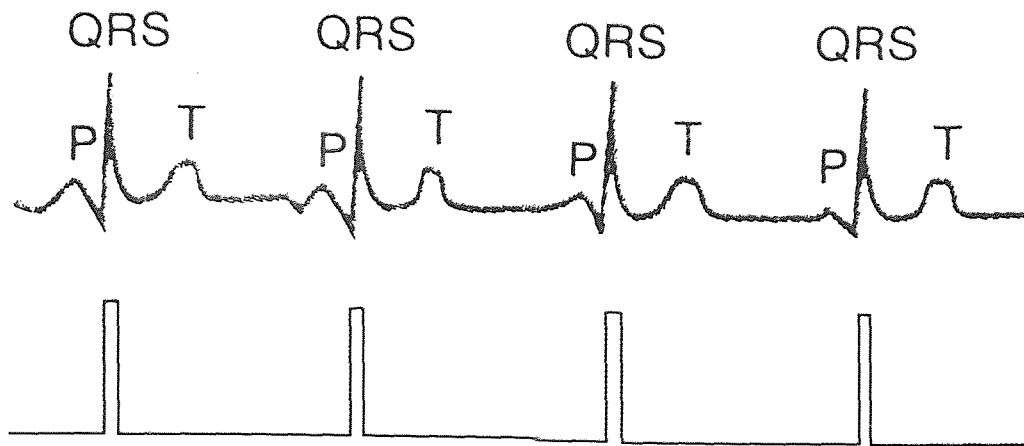
Figure 2.2   The ECG (top of figure) and synch pulse (bottom of figure). The synch pulse serves as an R or QRS wave detector giving off a square wave pulse each time the R/QRS wave is present. (Top of figure from Vander, A.J., Sherman, J.H., and Luciano, D.S., Human Physiology, New York, Applicon & Lange, 1993.)

The low frequencies in the range of 0.05Hz to 0.15Hz represented a mixture of sympathetic and parasympathetic activity while the high frequencies in the range of 0.15Hz to 0.4Hz represented parasympathetic activity. When measuring autonomic activity using spectral analysis the area under the frequency spectrum curve between the low and high frequency was taken to give us a measurement of autonomic activity. This area was in units of power giving this form of spectral analysis the name power spectrum analysis

To perform time-frequency analysis a continuous IIBI signal was fed into a software named Matlab(The Mathworks, Inc. Natick, MA). In Matlab (The Mathworks, Inc. Natick, MA) a program written by Sanjay Fernando[13] called sympar (see appendix B for program) was used to generate the time-frequency data . Sympar utilized the Wigner distribution upon the IIBI signal to generate results representing the sum of high

and low frequency activities over time. (as described in section 1.4.2) Time frequency

analysis gave us measurements of magnitude representing the cumulative activity of all

signals within the high and low frequency ranges over time so that we had two results, a

high frequency and a low frequency time-frequency curve. These high and low frequency

results were put into normalized form using a program called symparq1 (see appendix B

for program) to help us get a clearer picture of the activities taking place. These

normalized values were calculated according to the equations below for each time t.

$$\text{Normalized High Frequency} = \frac{\text{High Frequency}}{\text{High Frequency} + \text{Low Frequency}} \quad \textbf{(2.1)}$$

$$\text{Normalized Low Frequency} = \frac{\text{Low Frequency}}{\text{High Frequency} + \text{Low Frequency}} \quad \textbf{(2.2)}$$

Normalized values are relevant forms of information because they represent the fraction of

parasympathetic and sympathetic activity that often dictates the amount of activity that

will take place within an innervated organ[5]. The time frequency data was calculated

and then fed into graphing and statistical softwares called Excel(Microsoft, Seattle, WA)

and JMP (SAS Institute, Cary, NC). A program called symparpc was used to create files

that could be exported from Matlab.(see appendix B for program) For a detailed

description and instructions on the data processing performed, the difficulties encountered

and their solutions, refer to appendix A. Once the smoothed normalized high frequency

graphs were generated, reviewed, and analyzed qualitatively the next question was how to

quantify the patterns observed in the time-frequency plots. Since a convention for quantifying time-frequency analysis results had not been established, a number of different experimental measurements were taken. These included the average and maximum values of the time intervals chosen for comparison, the slopes of linear regression lines drawn through the intervals of interest, the curvatures of polynomial regression lines drawn through the intervals of interest, and detrended values of the intervals of interest. (These methods will be described in further detail in section 2.3.4)

2.3.3 Choosing Intervals of Interest

When extracting information from the analysis results, measurements were taken from the same time intervals for both frequency spectrum analysis and time-frequency analysis so that we could best compare the two methods. When comparing measurements between two different time periods within subjects it was most appropriate to extract information from equal periods of time. In order to get equal time periods, intervals were cut from the original rest and intervention periods. The last two minutes of the sacral/iliac hold, the CV4 maneuver, and the shoulder squeeze, the entire two minute rest periods, and the first two minute portion of the final nine minute rest period were used to take measurements for the 30 minute protocol. (table 2.1) The last four minutes of each intermediate 5 minute rest interval, the entire four minutes of the sacral/iliac hold, the CV4 maneuver, and the shoulder squeeze, and the last and first 4 minute portions of the first and last pairs of 5 minute rest periods respectively were used for the 48 minute protocol. (table 2.2) The last 4 minutes of the intermediate rest periods were chosen so that carryover effects

from previous intervals would be reduced so that each intervention could be analyzed independently.

2.3.4 Proposed Methods for Evaluation and Measurement

2.3.4.1 Averages: Averages were calculated for each interval by summing the magnitudes of all the high frequency normalized values and dividing by the total number of points. Averages were chosen because the normalized values often appeared relatively large during interventions, and this(Figure 2.3) could have resulted in higher average values relative to the surrounding rest intervals. Averages were also chosen because they seemed to be analogous to the power data that was derived from frequency spectra. Comparing averages for intervals seemed to be one of the most conclusive measurements to take. However, averages did not account for order, carryover, or trending effects that may have been caused by a period before an interval of interest.

2.3.4.2 Maximums: Maximum values were also chosen since it seemed likely that an interval that had greater values relative to others would also have a greater maximum value. In addition, it was felt that a peak value could represent a turning point for significant physiological activity. On the other hand, an outlier that did not properly represent the interval as a whole could have been chosen.
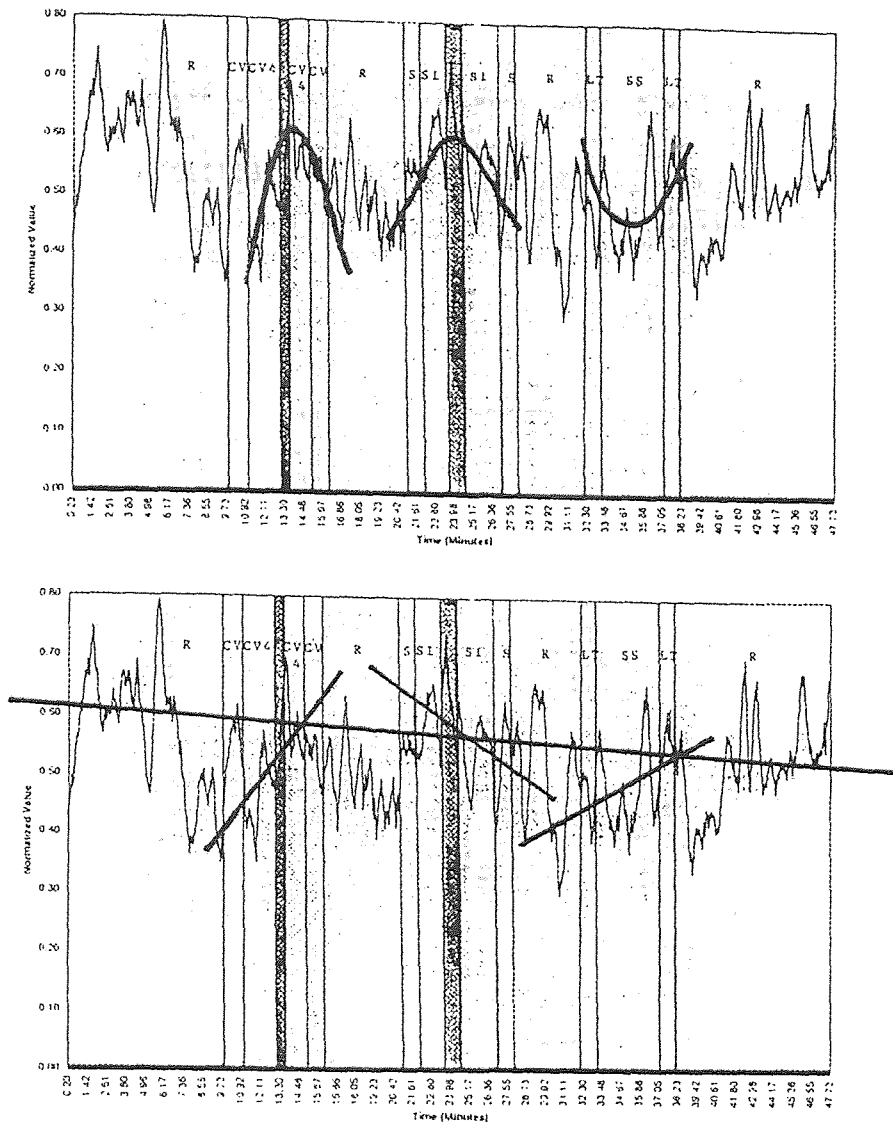
Figure 2.3   Top half: Normalized high frequency time-frequency results after
exponential smoothing. Second order polynomial regression lines were
drawn through the intervals of interest to give an appearance like the ones
above. Note the upside-down "U" shape of the graph during the CV4 and
sacral/iliac holds. The shaded time intervals represent the occurrence of
still points. Intervals are separated by the vertical lines and the
abbreviations are as follows: R=rest, CV=cranial vault hold, CV4=CV4
maneuver, LT=light touch, S=sacral hold, SS=shoulder squeeze,
S/I=sacral/iliac hold combination. Bottom half: Same data as the one
above. This example has linear regression lines drawn through some of the
intervals of interest and through the entire experimental length. The one
drawn through an entire experimental trial was averaged within the time
intervals of interest and then subtracted from the average value for that
interval to generate a detrended value.

2.3.4.3 Linear Regression: Linear regression lines were drawn through the intervals of interest. (See the bottom of figure 2.3) This was done to check for trends that may have occurred as a result of activities during the experiment. A linear regression line can be represented by the formula:

$$Y = B_o + B_1 x \qquad (2.3)$$

where B_o represents the Y intercept of the regression line and B_1 represents the slope[16]. The slope is calculated according to the formula:

$$slope = \frac{\sum (x - t)(y - m)}{\sum (x - t)^2} \qquad (2.4)$$

with x representing time, t representing average time, y representing the magnitude of a time frequency value and m representing the average magnitude of time frequency values[16]. The disadvantage to using linear regression lines is that they do not represent some of the more detailed trends present. On the other hand, a linear regression line still serves its purpose by dampening oscillations or noise.

2.3.4.4 Exponential Smoothing and Polynomial Regression: Exponential smoothing was used to give a more detailed trending picture than that of straight line regression.(Figure 2.3) Exponential smoothing reduces oscillations in a time series to help

visualize trends in the data. This is done by projecting values for each point along a time series by weighting the current value and the error from the previous forecast to obtain a new forecast value. The formula for exponential smoothing is:

$$F_t = A_{t-1} + \omega(F_{t-1} - A_{t-1}) \qquad (2.5)$$

F_t represents is the new forecast value while A_{t-1} represents the current actual figure and F_{t-1} represents the prior forecast. Thus $(F_{t-1} - A_{t-1})$ represents the most recent forecast error. The damping factor is represented by ω and affects the rate at which forecasted values react to changes in trends[17]. A small damping factor will cause the process to adjust rapidly to trends while a large damping factor causes forecasts to react more slowly thus having a more prominent smoothing effect[17]. In the experiments described a factor of 0.9 was used for the time-frequency data.

Polynomial regression lines, generated through individual intervals, seemed to be a good way to represent the upside-down "U" effect that took place during interventions. On the top of figure 2.3 this method is represented on the exponentially smoothed normalized high frequency data graph. (discussed in further detail in section 3.2). The benefit behind fitting a polynomial to intervals was the fact that the second derivative of a polynomial would be a measure of concavity. A positive second derivative would represent a curve that is concave up while a negative derivative would represent a curve that was concave down. The magnitude of this second derivative is a measure of the rate at which the slopes of tangents about the curve change[18]. Therefore a larger magnitude

would represent a line with greater curvature. Second order polynomials were fit to the different intervals since they would indicate the concavity overall for the entire interval of interest. In addition the second derivative of a second order polynomial would always be equal to twice the coefficient of the second order term (as seen in equations 2.6 and 2.7)

$$y = ax^2 + bx + c \qquad\qquad (2.6)$$

$$y^{''} = 2a \qquad\qquad (2.7)$$

allowing one to simply take the coefficient of the second order term as a measurement of concavity.

2.3.4.5 Average Minus Regression Line: To help filter out carryover and trending effects, the average value of a linear regression line fit through the entire curve was calculated during an interval of interest and then subtracted from the average normalized high frequency value of that interval. On the bottom of figure 2.3 there is an illustration representing a linear regression line for the full experimental length. Reduction of trending effects was a useful tool for analyzing data. For example, if a value was taken at the end of a time frequency curve on an upward trend it would be higher in magnitude than one taken in the beginning. If this upward trend was caused by an intervention that took place in the beginning or was due to a change in the subject's equilibrium it would interfere with the comparison of intervals that we were interested in.

2.3.4.6 Statistics: The measurements above were automatically calculated and placed into matrices as ASCII files by modified versions of sympar called sympar302 and sympar482 (as seen in appendix B) so that they could be imported into statistical software.

The next step was to draw conclusions from the measurements taken using statistics. Initially it made sense to compare time-frequency and frequency spectrum data using a standard t-test. But the samples that are compared must be taken from populations that are independent of one another. For the experiments done this was not the case since measurements were taken from the same time series. Therefore a paired two tailed t-test was used to compare sample means. Such a test is based on the idea that samples are taken from the same group or groups that are nearly identical[16]. When performing a paired t-test the hypothesis that two means are identical is tested. This is done by calculating the probability that the range of differences between two means would not include zero in a sample population[16]. (Figure 2.4) Normally an arbitrary value of ninety to ninety-nine percent is chosen as a threshold for statistical significance. The end result of a t-test will give a value of one minus this probability, otherwise known as the degree of confidence. If the value given exceeds the limit (.10 in our case) then it can be said that difference between means is not statistically significant. If it is less, then the difference between means is statistically significant. The paired t-test described works under the assumption that only one pair is being compared[16]. But in our set of circumstances there were multiple pairwise comparisons.
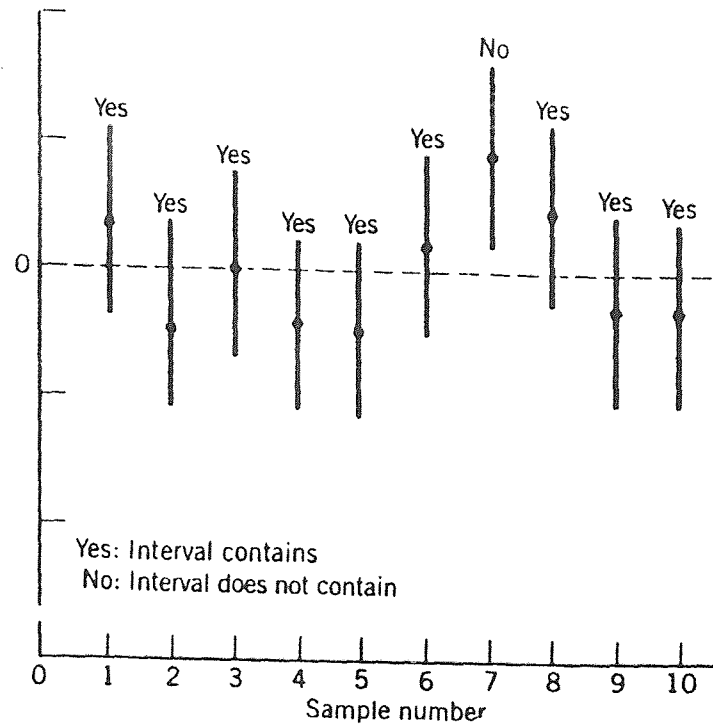
Figure 2.4 Illustration on the meaning of the confidence interval. In the case above, the data samples contain zero nine out of ten time giving us a ninety percent confidence level corresponding to $\alpha = 0.90$ (From Johnson, R., Statistics - Principles and Methods, New York, 1992.)

This required us to use the Bonferroni method and divide our confidence limit of .10 by the number of pairwise differences and then compare it to our confidence result to test for statistical significance[16]. When making pairwise comparisons between the interventions and average of all rest intervals the confidence limit is divided by three since we are making three pairwise comparisons: CV4 technique vs. rest, sacral/iliac hold vs. rest and shoulder squeeze vs. rest. When making comparisons between the interventions and adjacent rest intervals the confidence limit is divided by six since we have six comparisons being made: CV4 technique vs. rest interval before, CV4 technique vs. rest

interval after, sacral/iliac hold vs. rest interval before, sacral/iliac hold vs. rest interval

after, shoulder squeeze vs. rest interval before and shoulder squeeze vs. rest interval after.

CHAPTER 3

RESULTS

3.1 Observations

Observations of the power spectrum data indicated that there were no significant differences between intervals and no consistent pattern associated with the manual medicine techniques. (Refer to appendix C, table one, for the power spectrum data.)

The normalized time-frequency graphs were more helpful than the raw high frequency graphs but trends were masked by oscillations in the normalized time-frequency data. To reduce the oscillations and to help detect trends, exponential smoothing was used. The exponentially smoothed results indicated an increase in normalized high frequency values around the time that manual medicine interventions began, followed by values relatively high in magnitude, and then a decrease in normalized high frequency values around the time the interventions ended giving the appearance of an upside down "U" during interventions. (as seen in figure 2.3) (Refer to appendix C for the remainder of the graphs.) Such a pattern illustrates that the parasympathetic effects may be short term and most pronounced as the manual treatments are applied.

Normalized high frequency time-frequency values reached relatively large values during still points that occurred as the CV4 and sacral/iliac holds were administered. Refer back to figure 2.3 for exponentially smoothed normalized high frequency time-freqeuncy data for one trial run of the experiment. Note that peak values occur during still points as indicated by the graph. (See appendix C for the remaining graphs which illustrate this point.)

3.2 Statistical Results

Intervals were compared so that the rest intervals before and after interventions were compared to the interventions themselves. In addition, each intervention was compared to the average value for all rest intervals. When comparing values between intervals, the averages for all subjects were compared. Then the differences between means were calculated to test the probability that they were not equal to zero. Such a test would indicate whether or not there was a significant difference between means. To do this a specialized version of a two-tailed paired t-test introduced by Bonferroni was used. [16,21]. This test works by dividing the critical α value by the number of pairwise comparisons being made before comparing the calculated α value to it.

When using a paired t-test to compare the high and low frequency power spectrum values of the rest intervals before and after interventions to the interventions themselves, no significant increases or decreases were present. This was also the case when the average power spectrum value of all rest intervals for all subjects was compared to the averages of the interventions for all subjects. See Table 3.1 for power spectrum statistical results.

When calculating time-frequency results for the intervals of interest the data was as seen in Table 3.2. Each intervention was compared the adjacent rest intervals and the average of all rest intervals. When paired t-tests were performed upon time frequency analysis results there were no statistically significant increases from rest intervals to interventions or significant decreases from interventions to rest intervals. However, the probability levels for significant differences were greatest for the average and detrended

values for the CV4 and sacral/iliac holds so that the shoulder squeeze had less significant

differences from the adjacent rest intervals compared to the CV4 and sacral/iliac holds

(Table 3.2).

Table 3.1   Mean differences (+/- standard deviation) between the normalized and
standard power values for the intervals of interest. In the column next to
the mean differences are the α (alpha) values for each pairwise
comparison. None of the differences above are statistically significant.

STATISTICAL RESULTS						
POWER SPECTRUM DATA						
INTERVENTIONS VS. REST INTERVALS BEFORE AND AFTER						
	NHFPower	alpha	HFPower	alpha	LFPower	alpha
CV4 - Rest Before	0.024+/-0.153	0.5960	-0.874+/-46.090	0.9488	5.075+/-57.086	0.7638
CV4 - Rest After	0.033+/-0.118	0.3581	5.111+/-42.807	0.6871	7.963+/-29.775	0.3747
Sacral/Iliac - Rest Before	0.048+/-0.164	0.3325	-1.222+/-35.580	0.9074	-7.595+/-17.092	0.1520
Sacral/Iliac - Rest After	0.042+/-0.207	0.4940	-23.443+/-52.626	0.1511	-24.405+/-33.180	0.0271
Shoulder Sqz - Rest Before	0.041+/-0.196	0.4841	-9.283+/-41.020	0.4496	-7.102+/-37.300	0.5231
Shoulder Sqz - Rest After	0.051+/-0.126	0.1855	-9.856+/-32.730	0.3193	-17.216+/-26.461	0.0456
	NHFPower	alpha	HFPower	alpha	LFPower	alpha
CV4 - All Rest	0.021+/-0.103	0.4915	9.768+/-45.704	0.4746	1.593+/-39.020	0.8901
Sacral/Iliac - All Rest	0.069+/-0.120	0.0722	0.670+/-17.123	0.8947	-20.251+/-57.529	0.2482
Shoulder Sqz - All Rest	0.028+/-0.174	0.5894	3.283+/-30.050	0.7123	-8.714+/-18.727	0.1353

The mean average normalized high frequency value of the sacral/iliac hold was

significantly greater (98.7% probability) than the mean average normalized high frequency

value of all rest intervals. Bonferroni's paired t-test results also indicated that the average

detrended normalized high frequency value for the sacral/iliac hold and CV4 techniques

were significantly greater (91.6% and 99.7% probabilities respectively) than the average

detrended value of all rest intervals.

When performing paired t-tests on the results for all other forms of time-frequency

measurements including maximum values, "U" effect, and linear regression line slope, no

significant effects were detected. (See table 3.2)

Table 3.2 Mean differences (+/- standard deviation) between the average, detrended, maximum, slope, and "U" effect values for the normalized high frequency time-frequency data. In the column next to the mean differences are the α (alpha) values for each pairwise comparison. Note that the statistically significant mean differences are in bold print and italicized.

STATISTICAL RESULTS						
TIME-FREQUENCY DATA						
INTERVENTIONS VS. REST INTERVALS BEFORE AND AFTER						
	Avg NHF	alpha	Detrended NHF	alpha	Maximum	alpha
CV4 - Rest Before	0.033+/-0.065	0.0886	0.036+/-0.065	0.0732	0.034+/-0.062	0.0749
CV4 - Rest After	0.029+/-0.055	0.0830	0.026+/-0.052	0.0926	0.017+/-0.055	0.2775
Sacral/Iliac - Rest Before	0.032+/-0.069	0.1183	0.035+/-0.068	0.0925	-0.002+/-0.015	0.9318
Sacral/Iliac - Rest After	0.050+/-0.090	0.0660	0.048+/-0.084	0.0618	0.026+/-0.097	0.9772
Shoulder Sqz - Rest Before	0.015+/-0.100	0.5952	0.017+/-0.099	0.5372	0.018+/-0.078	0.4288
Shoulder Sqz - Rest After	0.012+/-0.063	0.5200	0.009+/-0.070	0.6357	0.003+/-0.069	0.8847
	Slope	alpha	U effect	alpha		
CV4 - Rest Before	0.004+/-0.052	0.8110	-0.058+/-0.133	0.1384		
CV4 - Rest After	-0.001+/-0.054	0.9629	-0.023+/-0.058	0.1798		
Sacral/Iliac - Rest Before	0.023+/-0.049	0.1214	-0.025+/-0.102	0.3975		
Sacral/Iliac - Rest After	0.008+/-0.053	0.6061	-0.011+/-0.117	0.7334		
Shoulder Sqz - Rest Before	0.006+/-0.044	0.6451	-0.012+/-0.115	0.7014		
Shoulder Sqz - Rest After	0.008+/-0.061	0.6269	-0.025+/-0.096	0.3583		
INTERVENTIONS VS. AVG. OF ALL REST INTERVALS						
	Avg NHF	alpha	Detrended NHF	alpha	Maximum	alpha
CV4 - All Rest	0.038+/-0.068	0.0694	*0.032+/-0.046*	*0.0280*	0.019+/-0.073	0.3605
Sacral/Iliac - All Rest	*0.054+/-0.056*	*0.0044*	*0.038+/-0.032*	*0.0010*	0.011+/-0.085	0.6388
Shoulder Sqz - All Rest	0.025+/-0.071	0.2338	0.021+/-0.068	0.2792	0.015+/-0.068	0.4353
	Slope	alpha	U effect	alpha		
CV4 - All Rest	-0.018+/-0.55	0.2727	*0.032+/-0.046*	*0.0280*		
Sacral/Iliac - All Rest	-0.020+/-0.057	0.2266	-0.007+/-0.054	0.6357		
Shoulder Sqz - All Rest	-0.011+/-0.034	0.2719	0.022+/-0.077	0.3333		

CHAPTER 4

DISCUSSION

4.1 Interpreting The Time-Frequency Data

Normalized high frequency values were used to account for the balancing effects between parasympathetic and sympathetic activities. These normalized values were calculated by dividing the high frequency values by the sum of the high and low frequency values at each point in time. After exponentially smoothing the normalized high frequency time-frequency data it became apparent that an increase in activity occurred around the time that the CV4 and sacral/iliac holds began followed by elevated values and then a decrease in activity as the manual medicine techniques ended. (As seen in figure 2.3 and Appendix C)

4.2 Quantifying Patterns

Time-frequency results were quantified to give a more concrete assessment of the phenomena that were taking place. To quantify results, methods of measurement had to be developed, because an established process for quantifying time-frequency results had not been established. First, the intervals of time that were used to take measurements had to be determined. To make comparisons it was important to compare activities during manual medicine interventions to that of the adjacent baseline rest intervals and the average of all rest intervals.

Comparing intervals of equal time-length was an important consideration. This was because a summation measurement in the form of area was used for power spectrum analysis. As time progresses, the sum of autonomic activities accumulated to give a larger

area under the frequency spectrum curve. This can present a problem if power was compared for two different intervals that cover different lengths of time. As a means of compensating it may be tempting to multiply the power accumulated over the course of one time interval by the proportion of both time intervals. However this is not acceptable because it would be assumed that the activities of the shorter interval would remain constant if prolonged.

When developing some of the techniques for time-frequency measurement an objective was to quantify the observed patterns. The proposed measurements taken were averages, maximums, detrended values, slopes and curvatures.

Averages seemed to be a sensible way to quantify an increase or decrease in activities between intervals of time. If there was an increase in activity from one interval to another, it seemed likely that the average normalized high frequency value would also increase. Taking averages seemed sensible for another reason. When looking at the time frequency graphs it was hard to say that one interval of time was different from any other. This was because the normalized high frequency time-frequency graphs oscillated so that the different intervals had many values in common. However, some intervals appeared to oscillate about higher values, so logically, it seemed that they would have greater average values. Averages also appeared to be analogous to the conventional power value for the frequency spectrum analysis. Power is the result of a summation of many measurements taken over time and so is an average.

Taking the maximum value of a time interval also seemed to have uses. A maximum value would give the peak value that occurs during a particular intervention and this would be of primary importance for evaluating the physiological effects that a technique had.

Most often the time-frequency data would undergo an upward or downward trend over the course of an experiment. This could have been due an increase in baseline activities or the effects from interventions. If there are baseline changes or carryover effects, then comparisons between intervals are more difficult to make. For example, suppose one had a time-frequency graph on an upward trend with the shoulder squeeze as the first intervention and the sacral/iliac hold as the third. When comparing the sacral/iliac hold to the shoulder squeeze a greater activity measurement for the sacral/iliac hold may be present because of trending effects. This called for a measurement that reduced a gradual upward or downward trend in the data. A value for every point in time equal to each normalized high frequency measurement minus the value of a linear regression line drawn through the entire data set was calculated. Then the average detrended value for each interval of interest was used to make comparisons.

Slopes of linear regression lines drawn through the intervals of interest were also calculated to help detect the presence or absence of upward or downward trends. Polynomial regression lines were also used for the intervals of interest to help quantify non-linear trends and the upside-down "U" effect that was apparent for the normalized high frequency-time frequency data during manual medicine techniques. This upside-

down "U" effect represented the increase that took place as manual medicine techniques

began followed by relatively high values and then a decrease around the time that manual

medicine techniques ended.

4.3 Paired t-test

Quantifying some of the observed patterns would allow the use of statistics to perform

effect tests. A two-tailed paired t-test was used to measure the effects of the manual

medicine techniques. When using a paired t-test the means of two sample sets, taken from

the same or related populations/individuals, are compared to measure significant

differences between them. When performing a paired t-test a critical α-value is calculated.

This critical α value is representative of an assigned probability value that the two

populations being compared are distinct. For a paired t-test that makes only one pairwise

comparison an α-value of .10 indicates a probability of 90% that there is a significant

difference between populations. This is usually the cutoff point for a difference that is

considered statistically significant. When more than one pairwise comparison is made,

then Bonferroni's version of the paired t-test is most appropriate. This method is

performed by dividing the critical α value for statistical significance by the number of

pairwise comparisons. In the experiments done here there were six (as described in

section 2.3.6.6) when comparing interventions to their adjacent rest intervals. This gives

a critical α value of 0.0166 for a 90% probability of difference which is the critical value in

this paper for statistical significance. When comparing interventions to the average values

for all rest intervals there were three pairwise comparisons (as described in section

2.3.6.6) making the critical α value 0.033 for a 90% probability of difference. As long as the calculated value does not exceed the critical value then we know that the probability of a difference is at least a great as the probability assigned to the critical value. In the experiments done some subjects were tested more than once and on different days. Therefore the statistical values collected were grouped according to subjects before performing a t-test.

4.4 Repeated Measures ANOVA

Another excellent means of comparison was repeated measures analysis of variance (ANOVA) on multiple regression models. Although it was ultimately not used for the statistical analysis done here, it can serve as a valuable statistical tool when more data is collected and for future experiments. Repeated measures multiple regression accounts for the fact that several measurements are taken from each subject and then compared to one another. In addition, it assumes that multiple sample sets of data are taken for each subject over the course of many trial runs of an experiment[16,19]. This was not done for most subjects at the time the preliminary study had ended so repeated measured ANOVA was not used. Multiple regression, accompanied by analysis of variance tests, allows the effects of more than one independent variable (like intervention, subject, order, etc.) upon the dependent variable (like an autonomic activity measurement) to be measured. If a standard multiple regression ANOVA test was used to compare the effects for the intervals of interest it would tell us that any interval may have been different from any other. For example, it would only indicate whether or not the effects of the CV4

technique compared to the sacral/iliac hold or the shoulder squeeze or the average rest interval would be significantly different. By utilizing contrast tests the desired comparisons between intervals can be made. For example, contrast tests could be used to measure the effects of the CV4 and sacral/iliac holds and how they measure up against the rest intervals only.

Analysis of variance effect tests measure the effects that independent variables have on the variation of dependent variables. It does this by calculating an F value which is represented by the degree of variation caused by the known independent variables divided by the degree of variation that cannot be accounted for, also known as the noise or error term. If the F value exceeds the known critical value dictated by the degree of confidence desired, then the effects of the independent variables and their differences would be considered significant[16].

Among the models that could have been generated, had the guidelines for repeated measures been met (as described above), are univariate models with normalized high frequency time-frequency values as the single dependent variable and intervention, subject, etc. as the independent variables. A univariate model can be arranged so that individual average values are estimated for the CV, sacral, shoulder and rest periods. By measuring values during rest periods we could then compare them to periods during which treatments were administered. It could then be seen if effects were taking place as a result of manual techniques. Such a model would initially tell us that the average rest period and any type of intervention may be different from any other period. To compare particular intervals to one another we would then do a contrast test. A benefit to including all time

periods in our model as opposed to a select few with each comparison is the large number of degrees of freedom that we are working with when making comparisons. A larger number of degrees of freedom is beneficial because it could increase the power and significance of results.

The tests described above are not limited to a single dependent variable. In the work performed measurements from rest intervals before and after interventions along with the interventions themselves were of great importance. This was because the average normalized high frequency time-frequency values of interventions would often be greater than that of the surrounding rest intervals so that an upside-down "V" shaped plot would be formed (Figure 4.1) when lines were drawn between the average normalized values. This upside down "V" apparently characterized an activity that took place as a result of interventions so it was useful to use all three points when comparing interventions. By using three points for each autonomic activity measurement the sample size and degrees of freedom could be increased giving the calculation more power and significance.

4.5 Weeding Out Outlier Samples and Invalid Data

Much of the data collected had a substantial amount of variation. This led to the question as to whether or not some data should be eliminated before it was used to draw conclusions. Variation was probably caused by the fact that there was a difference in baseline activities between individuals and within individuals on different days. Baseline activities are the responses present without any interventions. Depending on the individual

they may have different levels of autonomic activity during rest. This can be dictated by physiological or psychological factors. In addition to a variety of baseline activities there may have been a difference in reactivities. Reactivity is a measure of how a person's autonomic nervous system may react to things like olfactory, tactile, audible, pressure and chemical stimuli and to what extreme they would react.
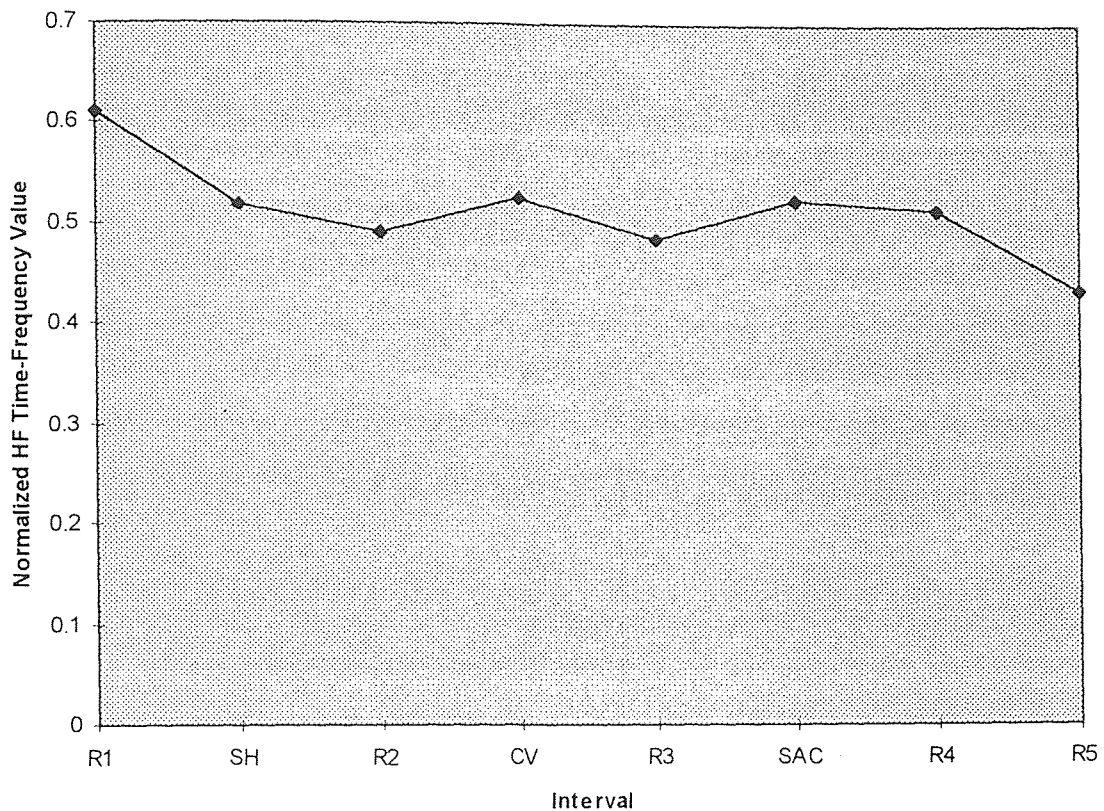

Figure 4.1   Average normalized high frequency time-frequency values vs. intervals of interest. Average values for each interval are marked with a diamond. Note the upside-down "V" shape when going from rest interval to manual technique to rest interval. This data is from one trial run of the experiment.

Although it seemed wise to remove some data from the sample sets it was ultimately decided that all data should be included. This was because a large portion of the data collected was affected by unavoidable outside influences and a criterion for acceptable data had not been established. In addition, the repeated measured ANOVA, if used in the future, would help account for the variation due to noise.

CHAPTER 5

CONCLUSIONS

5.1 Effects of the CV4 Maneuver and Sacral/IliacHold

Exponentially smoothed normalized high frequency time-frequency results indicated that

parasympathetic activity increased around the time that the CV4 and sacral/iliac hold

began followed by an elevated parasympathetic response and then a decrease in activity

around the time the manual medicine techniques ended. These measurements also reached

relatively large values for the still points compared to the other values during the CV4 and

sacral/iliac holds. Bonferroni's paired t-test results showed that the average

parasympathetic activity levels for the sacral/iliac hold and CV4 technique were

significantly greater than the average parasympathetic activities for all rest intervals. The

above results suggest that increased parasympathetic effects are present during the CV4

technique and sacral/iliac hold and are most pronounced as the manual techniques are

performed.

5.2 Suggestions for Future Work

Average values for time frequency analysis results produced data with statistical

significance most often while detrended values produced results with statistical

significance only once. All other forms of experimental measurement including maximum

values, slopes of linear regression lines, and the coefficients of second order polynomial

regression lines ("U" effect measurements) did not show any statistically significant results

in favor of a parasympathetic increase or decrease. This may prompt the idea to further

investigate the use of these measurements since they generate results that bring the

investigator to a similar set of conclusions produced by the power spectral data. On the other hand, future investigators may want to continue to use average and detrended values since they may be capable of recognizing effects that power spectrum analysis or the other proposed time-frequency measurements do not detect.

As stated earlier, this study was preliminary. Since results indicated an increase in parasympathetic activity as a result of the manual techniques it would be worthwhile to continue pursuing the effects of manual therapy on autonomic activity using heart-rate variability. Future work should also use more subjects to increase the power of the statistical results. This may also reduce the amount of variation experienced. When testing subjects each individual should be tested more than once. This will enable the use of repeated measures ANOVA which is a more powerful statistical method that Bonferroni's paired t-test. Also, the development of new programs or software that will allow fewer steps should be pursued in order to consuming less time.

Since this study suggests an increase in parasympathetic activity as a result of manual medicine techniques, it would also be worthwhile to pursue its benefits for headache and anxiety patients since they experience an imbalance of autonomic activities. Testing the effects of other alternative medicine techniques that have been reported to evoke relaxation or other health benefits would also be of use. This way it can be verified whether or not there is a true physiological response as a result of alternative treatments.

APPENDIX A

DATA ANALYSIS AND PROCESSING

A.1 Obstacles Encountered and Their Solutions

During the course of data processing difficulties occurred that created the need for investigation and modifications in programs and data processing techniques. When converting from binary to ASCII format there were instances when unpack would indicate that a bad point occurred in an interval of interest near a particular data point. As a result unpack would process only up to the bad data point and not beyond so that the entire interval of interest would not be converted to ASCII format. In addition signals would be out of synch and not assigned to their proper channels even if attempts were made to unpack about the bad points. As a result pslws, one of the S-plus program used, would not be able to process the data properly since it expects to see each signal in particular channels; the respiration signal in channel one, the synch pulse or the R-wave detection signal produced by the ECG machine in channel two, and the ECG signal in channel three. To remedy the problem intervals would be unpacked from the originally desired first data point to the bad data point location indicated minus two. Then from the bad point location minus one to the original number marking the end of the interval minus one. Following this interval one is subtracted from the first and last numbers of the next intervals that were originally intended to beunpacked. For each additional bad point the same procedure as above is performed and an additional point is subtracted from the first and last points of the remaining intervals. The first number in the interval must be a respiration data point, which is then followed by a synch pulse data point and then an ECG data point. (Figure A1) This pattern repeats itself and forms a continuous row of points.

The cause of the problem as indicated by a shift in channel assignments is most likely the result of a synch pulse point being skipped when converting from binary to ASCII format three data points behind the number indicated by unpack. This is what causes all points to be shifted back one point from their expected locations. (Figure A.1)

123 123 123

Unpack program reads in this direction.

Ch.1 = Respiration, Ch.2 = Synch Pulse, Ch.3 = ECG

A bad point is encountered.

↓

123 1ₓ3 123

Unpack program reads in this direction.

↓

The bad point is skipped and the Unpack program reads:

123 13 123

Unpack program reads in this direction.

After a bad point the Unpack program looks for the synch pulse at the next point but unknowingly finds the ECG signal. As a result the channels are read improperly (Ch.1 = Synch pulse, Ch.2 = ECG, Ch. 3 = Respiration) and are scanned out of synch.

Figure A.1   The manner in which Unpack (Keithley MetraByte/Asyst, Natick, MA) scans a binary file when converting it to ASCII format. When a bad point is encountered the binary file is scanned so that the channels within the ASCII file are out of synch.

In order to perform time-frequency analysis a continuous inter-beat interval signal was needed. Unpack could only process files up to about four minutes in length. In addition, the personal computers used could only process files up to five minutes in length

within S-plus. This forced the binary data files to be broken into four minute pieces and then processed within S-plus to get the inter-beat interval data. Once the inter-beat interval data was obtained the four minute segments were rejoined. However, each time a binary data file was cut an inter-beat interval was lost at the location where the cut was made. This was because the inter-beat interval forming program only detected and saved the time intervals *between* beats. As a result, the portions of the time that came before and after the first and last beats of each time interval were not kept. (FigureA.2)

CUT
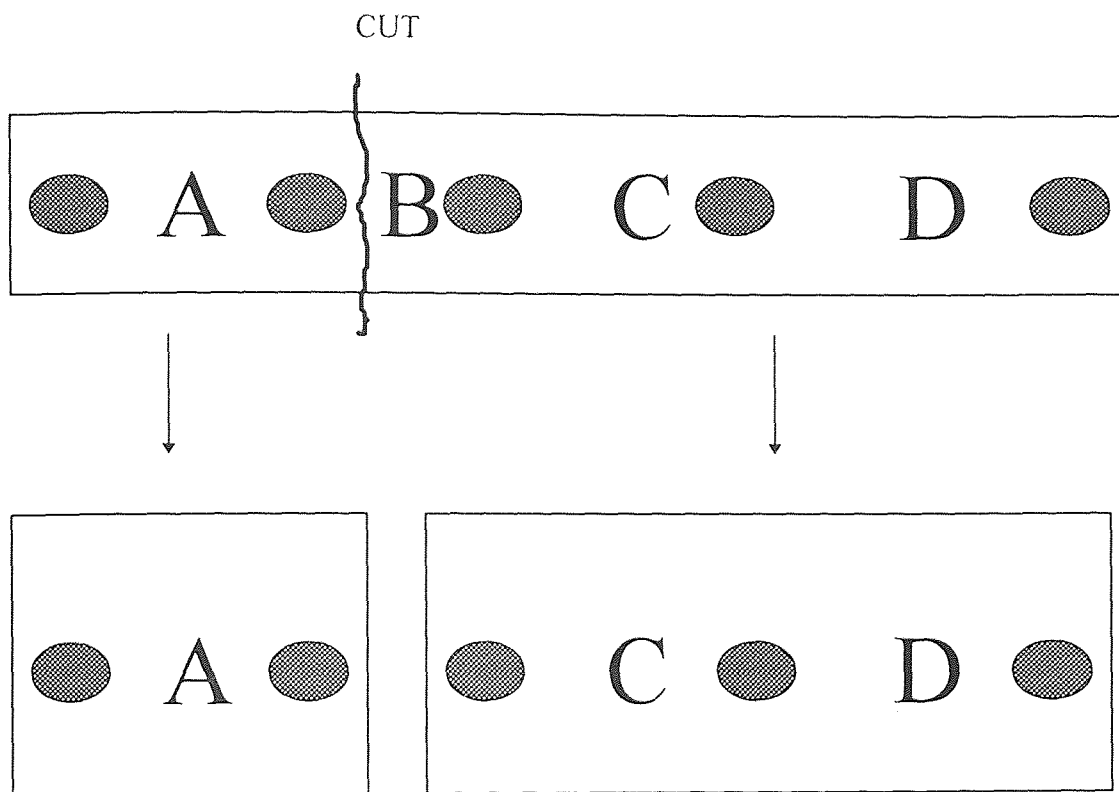
Figure A.2   When the III signal is cut two segments are produced. In the process of making the cut an interbeat interval, like B, is lost.

This made it impossible to preserve all of the inter-beat interval data before pasting the segmented inter-beat intervals back together. To remedy this problem the first beat of each interval was included in the previous interval as the last and the last beat of each interval

was included in the next interval as the first beat. This prevented the loss of inter-beat intervals and preserved the full file length of the signal so activities would be charted at the proper points in time. Refer to the following pages in Appendix A for a more detailed account and instructions on how to perform such functions within S-plus (Statistical Sciences, Inc.).

A.2 Steps for Data Analysis

A.2.1 Data Collection

•Refer to figure 2.1 for the equipment setup.

•Within DOS create a file for Streamer das16 (Keithley MetraByte/Asyst, Natick, MA) by typing:

mkfile Xmmddyy.dat ####

X represents the first initial of the subjects last name. mmddyy represents the month, day and year of the experiment,. #### represents the file length in kilobytes. The file length in kilobytes is calculated using the following equation: [sampling rate(in my case 200)*number of seconds(in my case 48*60)*number of channels(in my case 3)*2]/1000.

•Make sure the signals from each piece of medical equipment are feeding into the computer into their appropriate channels by using primplot. Respiration should be in channel one, Synch pulse in channel 2 and EKG in channel 3.

•Use Primplot (J.F. Andrews) by typing the following within DOS:

primplot

Press F5 to analyze and switch between channels. When you are sure that everything is

connected properly you are ready to use streamer das16 (Keithley MetraByte/Asyst,

Natick, MA). Hit control c to get out of Primplot (J.F. Andrews) and to the c prompt

within DOS.

•Use streamer das16 (Keithley MetraByte/Asyst, Natick, MA) by typing:

streamer das16

Fill in the filename, the range of channels you wish to monitor(in my case 1 to 3), and the

sampling rate [equals ((200 * number of channels)/(1000)) Khz] (in my case it is 0.6)

When you are ready to start press F1. When finished press F10 to quit.

•After the data file has been collected copy the file to your S (network) drive directory by

typing the following within DOS:

copy *filename*.dat, *network drive name:\your directory\your subdirectory*

A.2.2 Converting Data File to ASCII Format

•Make sure that unpack.exe and the binary data file are in the same directory.

•IN DOS Type:

unpack *filename*.dat,*filename*.asc,*first number in interval of interest-last number in*

interval of interest

•The spacing must be identical to the above example (in other words there are no spaces

between characters). It is also important that you follow my examples for spacing in other

programs for other types of commands.

•A desired point in an interval is calculated as the number of seconds*sampling rate(200 in my case)*number of channels(3 in my case). The beginning of intervals must be divisible by the number of channels to insure that channels are in the desired order.

•If you encounter a bad point you must break the interval into two pieces and then join them later in S-plus (Statistical Sciences Inc.). When a bad point is encountered unpack from the originally desired first point of the interval to the bad point minus 2. Then unpack from bad point minus1 to the originally desired last point in the interval minus1. Subtract 1 from the beginning and ends of all following intervals to insure that channels will remain in synch. With each additional bad point you encounter you subtract an additional point from the beginning and ends of each of the following intervals.

A.2.3 Working with Data in S-Plus

•Attach to the directory containing your data and programs by typing:

attach(*"drive containing data:\your directory name\your subdirectory name"*)

•To convert ASCII files into S-plus (Statistical Sciences Inc.) files type:

filename_matrix(scan(*"drive containing data:\your directory\your subdirectory\filename*.asc",sep=",",skip=1),ncol=3,byrow=T)

The ncol command tells S-plus (Statistical Sciences Inc.) how many columns to create to accommodate the respective number of channels you have in your data file.

•You may view your data within S-plus (Statistical Sciences Inc.) and Matlab (The Mathworks, Inc., Natick, MA) to insure that the channels are in their proper place. When

using the programs for power analysis and time frequency in S-plus (Statistical Sciences

Inc.) and Matlab (The Mathworks, Inc., Natick, MA) respectively the channels must be as

follows: ch.1=respiration,ch.2=synch pulse, ch.3=EKG.

Data can be viewed by typing the following commands:

win.graph()   (creates a graphics window)

plot(*your filename[beginning of interval:end of interval,channel number]*

Interval limits can be calculated by multiplying sampling rate(200 in my case) by time in

seconds

●If the signals are not in in their appropriate channels you can transfer them to the correct

channels using cbind.   Example: Let's say we are supposed to have ch. 1=respiration,

ch.2=synch pulse,ch.3=EKG but we have ch.1=synch pulse, ch.2=EKG, and

ch.3=respiration. We can assign the signals to their appropriate channels by typing:

new filename=cbind(*filename[,3],filename[,1],filename[,2]*)

●To calculate spectral data, including interbeat intervals within S-Plus (Statistical Sciences

Inc.) type:

filename.m_pslws2(*filename*)

 If an error occurs your channels may not be in the appropriate places. Use cbind as

described above.

●View the power spectra by typing:

win.graph()

stdgraf(*filename*.m,title="*your desired title*",sr=20)

This will illustrate interbeat intervals,interpolated interbeat intervals, and respiration. Hit return and you will then get the power spectrum graph. If you have outliers on your interbeat interval graph that appear to be errors you can use the programs called dqrs or mqrs to correct the ibi. If outliers are large in magnitude use mqrs, if they are small in magnitude use dqrs.

•To correct outliers that are small in magnitude type within S-plus (Statistical Sciences Inc.):

filename.d_dqrs(*filename before pslws2*[,2],*filename*.m$pk)

Click on the outliers w/ the left mouse button. When you are done press the right mouse button. Next, click on each misdetection line with the left mouse button several times. When you are finished hit the right mouse button.

•To correct outliers that are large in magnitude type:

fiename.q_mqrs(*filename before pslws2*[,2],*filename*.m$pk,pout(diff(*pslws filename*.m$pk)))

Click to the left and right of each peak not detected with the left mouse button. Then click on the right mouse button when finished. You must click on all undetected peaks on each screen because you will not be able to go back and will be forced to rerun the program. If misdetected or undetected beat errors are not corrected, repeat the above again.

•To prepare data for time frequency analysis you will need a continuous interbeat interval sequence. This is done by taking the interbeat interval sequences from consecutive intervals and pasting them together. But it is important to preserve interbeat intervals that

may be lost each time the total ibi sequence is cut. This is done by overlapping the ends of each interval so that an interval includes the last beat of the previous interval and the first beat of the next.

•You can do this by viewing the synch pulse at the ends of an interval.

Do this by typing:

plot(*filename*[0:300,2]) (for the detection of the first beat)

plot(*filename*[*end of interval* - 300:*end of interval*,2] (for the detection of the last beat)

If you do not see a beat you should use a number higher than 300 because this represents only the first and last 1.5 seconds in each interval (if your sampling rate is 200 as it is in my case). Keep a record of where the beats are located so that you can recut your data file appropriately. In order to recut you can unpack new intervals from your data file or add beats from the intervals before and after to the interval you wish to modify.

When cutting you must make a cut before or after a beat but not within the timeframe that the beat detection occurs. This is because it may lead to the misdetection of a beat resulting in the loss of an interbeat interval.

•Paste intervals or portions of intervals together using rbind as follows:

*filename.r*_rbind(*filename of interval before*[*pt. just before last beat:last pt. in interval,*],*filename of interval,filename of interval after*[*first pt. in interval:pt. just beyond first beat in interval,*])

•Now perform pslws2 on all modified intervals. This will calculate the ibi sequences for your intervals.

● Join ibi's using c (the concatenate funtion) to get a continuous ibi sequence that can be

used for time frequency analysis in Matlab (The Mathworks, Inc., Natick, MA).

Connect intervals by typing the following:

filename.ibi_c(*filename of interval1*.m$ibi,*filename of interval2*.m$ibi,*filename of interval3*.m$i

The .m files are the files created by pslws2.

●Write the continuous ibi file in S-plus (Statistical Sciences Inc.) form to an ASCII file

that can be used in Matlab (The Mathworks, Inc., Natick, MA) by typing:

write(*filename*.ibi,file="*filenamen*.ibi",ncol=1)

This file will probably be stored in an unexpected place so do a search on the filename to

help yourself find it.

A.2.4 Time Frequency Analysis in Matlab

●In Matlab (The Mathworks, Inc., Natick, MA) you need to attach to the directory

containing your ibi file in ASCII format and the time frequency programs for Matlab (The

Mathworks, Inc., Natick, MA).

To attach to your directory within Matlab (The Mathworks, Inc., Natick, MA) type:

cd *drive name*:

cd *directory name*:

cd *subdirectory name*:

If you have a problem entering your directory you may need to type cd .. a few times and

then enter the above commands.

●Load your file by typing within Matlab (The Mathworks, Inc., Natick, MA):

load filenamen.ibi

●To insure that the file has been loaded and it is the appropriate length type:

whos

Divide the file size by 20 to get the length of the file in seconds.

●You can perform time frequency analysis by typing within Matlab (The Mathworks, Inc.,

Natick, MA):

sympar(*filenamen,'desired title'*)   (exclude the .ibi extension)

Order of your filter is five,sampling rate is 20,low frequency index numbers are 2:8, high

frequency index numbers are 8:20.

This program will give you the high and low frequency values.

●If you are interested in normalized values use sympar1 instead of sympar. Symparq1 also

allows one to select the interval of interest that he/she wishes to view. When running the

program it will ask what segment of the file needs to be graphed. A response is given by

typing *time-frequency point marking the beginning of interval:time-frequency pt marking*

the end of the interval. Since a point is plotted every 1.25 seconds by symparq1 it is

necessary to divide time in seconds by 1.25 to calculate the points along the time series

that will be used.

●If you wish to transfer your data from Matlab (The Mathworks, Inc., Natick, MA):

to a statistical or graphics software you can modify sympar1 to save your data to an ascii

file using the save command. This file can then be imported into outside software.

 Add a line to sympar1 that goes as follows:

save *variable name desired filename* -ascii

●Other programs written to make data calculation and transfer easier and faster were

sympc302, and sympc482. These programs generate the time frequency results used for

statistical analysis and place them into an ASCII file that can be imported into statistical

software. But, these were written exclusively for the purposes of generating data for the

manual medicine protocols. Sympc302 was made for the thirty minute protocol and

sympc482 was designed for the forty-eight minute protocol.

Before running the program it is neccesary to modify the second to last line within the

sympc302 or sympc482 program. The line reads: save *filename*.mat matrix -ascii. The

filename in this line must be given the appropriate name so the ASCII file created can be

identified. To run these programs you type within Matlab (The Mathworks, Inc., Natick,

MA):

sympc*XX*2(*filenamen*.ibi)

After this program is used one can retrieve the result file and transfer it to statistical

software.

APPENDIX B

PROGRAMS

B.1 IIBI and Spectrum Programs for S-Plus

B.1.1 Pslws

```
funcion(x)
{
jks <- x[,2]
aa<- grep(T, diff(jks) > 400)
ljs <- lsbp(x, aa)
ljs
}
function(x, x.pk, nt = 8192, ns = 6, decimate = 10, tooruff = 0.8, f = 0.1, ld = 10, sd = 10)

{
```

B.1.2 Lsbp

```
mruff  <- max(abs(ruff(diff(x.pk))))
if(mruff > =  tooruff)
print(paste("ibi's may be too ruff", tooruff, sep = ""))
x.ecg <- iibi(diff(x.pk))
x.iu <- iibi(diff(x.pk))
x.i <- x.iu{seq(1, len(x.iu), decimate)]
x.sq <- sqdt(x.i, f = f, ld, sd)
x.isp <- spect(x.i, nt = nt, ns = ns)
x.rpd <- x[seq(1, len(x[, 1]), 10), 1]
x.rlw <- lowess(1:len(x.rpd), x.rpd, f = 0.3, iter = 2, delta = ceiling((length(x.rpd) *
0.3)/8))$y
x.rsp spect(x.rpd - x.rlw, nt = nt, ns = ns)
z list(pk = x.pk, ibi = x.i, ibiu = x.iu, sq = x.sq, rpd = x.rpd, rlw = x.rlw, isp = x.isp, rsp =
x.rsp, ecg = x.ecg,  )
z
}
```

B.1.3 Iibi

```
function(x, sr = 1)
{
        y <- NA
        for(i in 1:length(x)) {
                y <- c(y, rep(x[i], round(x[i]/sr)))
        }
        y  <- y{2:length(y)]
        y
```

B.1.4 Sqdt

```
function(x, f, ldelta, sdelta)
{
        ltr <- lowess(1:length(x), x, f = f, iter = 2, delta = ldelta)$y
        xs <-  (1:length(x))[c(F, diff(x) ! = 0)]
        xs <-  (xs)[seq(1, length(xs), sdelta)]
        ys <- sqwv(xs, ltr)
        ys
}
```

B.1.5 Spect

```
function(z, nt = length(z), taper = 0.2, ns = 20)
{
        if(any(is.na(z)))
                stop("NA's not allowed")
        if(taper < 0 | taper > 1)
                stop("taper must be between 0 & 1")
        if(nt < length(z))
                z z[1:nt]
        z <- z - mean(z)
        z <- spec.taper(z, taper)
        print(paste("Windowing length per side is ", nt * taper, sep = ""))  #z <-
        .Fortran("spcos",
#       x = as.double(z),
#       y = as.double(z),
#       as.integer(length(z)),
#       as.double(taper))$y

if(nt > length(z))
        z <- c(z, rep(0, nt - length(z)))
z <-  (Mod(fft(z))^2/(2 * pi *nt)) [1:(nt %/% 2 + 1)]
if(ns >0)  {
        z <- spec.smo(z, span = 7)              #z <- .Fortran("trsmooth",
s = as.double(z),
as.integer(length(z)),
as.integer(ns))$x
        z <- rev(z)
        z <- spec.smo(z, span = 7)              #z <- .Fortran("trsmooth",
x = as.double(z),
as.integer(length(z)),
as.integer(ns))$x
        z <- rev(z) }z
```

B.2 Matlab Programs

B.2.1 Sympar
```
function sympar(rawdata,top)
% SYMPAR(iibi,gtitle)
%
% Program calculates the Vagal tone and Sympo-vagal ratio.
% It is an implementation of time-frequency analysis
% using the Wigner distribution.
%
% iibi is the interpolated interbeat interval calculated
% by pslwsu, either in Matlab or in S-plus. Gtitle is the
% title of the output graphs.

% Written by Sanjay Fernando.
%
% last revision: 7/96 by Michael Zullow
%

rawdata=rawdata(:);
order=input('Please enter the order of the lowpass filter. ');
freq=input('Please enter the cuttoff frequency for LPF. ');
sample=input('Please enter the sample rate of the data. ');
nfreq=freq/sample;
[b,a]=butter(order,nfreq);
dtrend=filtfilt(b,a,rawdata);
dtrendata=rawdata-dtrend;
[row,col]=size(dtrendata);
I=1:row;
I=I(:);
A=(I/sample)/60;

% This  part is for testing Janse Kaiser Wigner calculation
% algorithm with no window
fs=1000;
m=512;                  % The size of the fft we will be computing.
skip=25;         % Number of points we skip to get the next segment.
p=129;          % The number of freq vals we will be plotting
k=fix((row-m)/skip);    % the number of spectra we compute
w=ones(size(1:m));      % window specification. Can be changed.
w=w(:);
x=hilbert(dtrendata);   % Forms the analytic funcion of x
L=m/2;
l=-(L-1):(L-1);
```

```
n=L;
Z=zeros([p,k]);
for i=1:k
        % Here I think 0 lag is at the Lth point, hence the first
        % time location is at LT=128*.001 or .128 sec; hmmm.
        g=x(n+l).*conj(x(n-l));
        g(2*L)=0;
        y=w.*g; % Apply window to g or kernal.

        Y=2/m*abs(fft(y,m)); % evidently because it's analytic
                            % we only need 2/N
        Z(:,i)=Y(1:p);
        n=n+skip;
end


Z1=Z;
Z=Z(1:30,1:k);


LFC = input('Please enter the low frequency range in index numbers. ');
symvag=sum(Z(LFC,1:k));
HFC=input('Please enter the high frequency range in index numbers. ');
vagal=sum(Z(HFC,1:k));
symtopar=symvag./vagal;




% This section is for determining the instantaneous frequency
% from the Wigner distribution.




n=128;        %The number of frequency values.
[r,c]=size(Z);   %Determines the size of the matrix created by wgjka4.m.
for i=1:c;       %Repeation based on number of columns.
  W=Z(:,i);       %W is assigned the values of the column.
  Y=(1:r)';       %Y represents the frequencies.
  M=W.*Y;
  S=sum(M);
  F=sum(W);        %Sum of the Wigner Distribution values.
  E(i)=S/F;
end




% Plotting commands
```

```
J=[12.8+(1:k)*1.25]/60;

subplot(3,1,1);
plot(A,rawdata,'r',A,dtrend,'g');
title(top);
xlabel('time')
ylabel('amplitude')

subplot(3,1,2);
plot(A,dtrendata);
title('IBI detrended');
xlabel('time');
ylabel('amplitude');

subplot(3,1,3);
plot(J,E);
xlabel('time');
ylabel('frequency');
title('Instantaneous frequency');

print -dwinc -v

%subplot(1,1,1);

subplot(2,1,1);
mesh(Z);
xlabel('time');
ylabel('frequency');
title(top);

subplot(2,1,2);
contour(Z);
xlabel('time');
ylabel('frequency');
title('Contour plot of WD');

print -dwinc -v


subplot(3,1,1);
plot(J,symvag);
gtext(top);
title(' Mixture of Sympathetic and Parasympathetic');
xlabel('time');
```

```
ylabel('amplitude');

subplot(3,1,2);
plot(J,vagal);
title('Parasympathetic range');
xlabel('time');
ylabel('amplitude');

subplot(3,1,3);
plot(J,symtopar);
title('Ratio of Low Frequency to High Frequency')
xlabel('time');
ylabel('amplitude');
print -dwinc -v
end
```

B.2.2 Symparpc

```
function sympar(rawdata,top)
% SYMPAR(iibi,gtitle)
%
% Program calculates the normalized values and places
%them in ASCII files to be exported
% It is an implementation of time-frequency analysis
% using the Wigner distribution.
%
% iibi is the interpolated interbeat interval calculated
% by pslwsu, either in Matlab or in S-plus. Gtitle is the
% title of the output graphs.

% Written by Sanjay Fernando.
%
% last revision: 9/22/94 by Christopher King
%

rawdata=rawdata(:);
order=5;
freq=.03;
sample=20;
nfreq=freq/sample;
[b,a]=butter(order,nfreq);
dtrend=filtfilt(b,a,rawdata);
dtrendata=rawdata-dtrend;
[row,col]=size(dtrendata);
I=1:row;
```

```
I=I(:);
A=(I/sample)/60;


% This part is for testing Janse Kaiser Wigner calculation
% algorithm with no window
fs=1000;
m=512;                   % The size of the fft we will be computing.
skip=25;        % Number of points we skip to get the next segment.
p=129;          % The number of freq vals we will be plotting
k=fix((row-m)/skip);    % the number of spectra we compute
w=ones(size(1:m));      % window specification. Can be changed.
w=w(:);
x=hilbert(dtrendata);   % Forms the analytic funcion of x
L=m/2;
l=-(L-1):(L-1);
n=L;
Z=zeros([p,k]);
for i=1:k
        % Here I think 0 lag is at the Lth point, hence the first
        % time location is at LT=128*.001 or .128 sec; hmmm.
        g=x(n+l).*conj(x(n-l));
        g(2*L)=0;
        y=w.*g; % Apply window to g or kernal.

        Y=2/m*abs(fft(y,m)); % evidently because it's analytic
                         % we only need 2/N
        Z(:,i)=Y(1:p);
        n=n+skip;
end

Z1=Z;
Z=Z(1:30,1:k);

LFC=2:8;
symvag=sum(Z(LFC,1:k));
HFC=8:20;
vagal=sum(Z(HFC,1:k));
symtopar=symvag./vagal;
%Normalized values are calculated here
nvlf=symvag./(symvag+vagal);
nvhf=vagal./(symvag+vagal);


% This section is for determining the instantaneous frequency
% from the Wigner distribution.
```

```
n=128;         %The number of frequency values.
[r,c]=size(Z);  %Determines the size of the matrix created by wgjka4.m.
for i=1:c;     %Repeation based on number of columns.
 W=Z(:,i);      %W is assigned the values of the column.
 Y=(1:r)';      %Y represents the frequencies.
 M=W.*Y;
 S=sum(M);
 F=sum(W);       %Sum of the Wigner Distribution values.
 E(i)=S/F;
end


v=1:k
J=[12.8+(v)*1.25]/60;
Min=fix(J);
Sec=(J - Min).*60;

nvhf = rot90(nvhf,3);
nvlf = rot90(nvlf,3);
J = rot90(J,3);
Min = rot90(Min,3);
Sec = rot90(Sec,3);

save s0701hf nvhf -ascii
save s0701lf nvlf -ascii
save s0701tm J -ascii
end
```

B.2.3 Symparq1

```
function sympar(rawdata,top)
% SYMPAR(iibi,gtitle)
% Program calculates the normalized values for a chosen interval
% It is an implementation of time-frequency analysis
% using the Wigner distribution.
%
% iibi is the interpolated interbeat interval calculated
% by pslwsu, either in Matlab or in S-plus


% Written by Sanjay Fernando.
% revision: 9/22/94 by Christopher King
% revision: 7/96 by Michael Zullow
```

```
rawdata=rawdata(:);
order=5;
freq=.03;
sample=20;
nfreq=freq/sample;
[b,a]=butter(order,nfreq);
dtrend=filtfilt(b,a,rawdata);
dtrendata=rawdata-dtrend;
[row,col]=size(dtrendata);
I=1:row;
I=I(:);
A=(I/sample)/60;


% This  part is for testing Janse Kaiser Wigner calculation
% algorithm with no window
fs=1000;
m=512;                   % The size of the fft we will be computing.
skip=25;          % Number of points we skip to get the next segmen
p=129;          % The number of freq vals we will be plotting
k=fix((row-m)/skip);    % the number of spectra we compute
w=ones(size(1:m));    % window specification. Can be changed.
w=w(:);
x=hilbert(dtrendata);   % Forms the analytic funcion of x
L=m/2;
l=-(L-1):(L-1);
n=L;
Z=zeros([p,k]);
for i=1:k
        % Here I think 0 lag is at the Lth point, hence the first
        % time location is at LT=128*.001 or .128 sec; hmmm.
        g=x(n+l).*conj(x(n-l));
        g(2*L)=0;
        y=w.*g; % Apply window to g or kernal.

        Y=2/m*abs(fft(y,m)); % evidently because it's analytic
                            % we only need 2/N
        Z(:,i)=Y(1:p);
        n=n+skip;
end

Z1=Z;
Z=Z(1:30,1:k);

LFC=2:8;
```

```
symvag=sum(Z(LFC,1:k));
HFC=8:20;
vagal=sum(Z(HFC,1:k));

%The Normalized Values are Calculated Here
symtopar=symvag./vagal;
nvlf=symvag./(symvag+vagal);
nvhf=vagal./(symvag+vagal);

% This section is for determining the instantaneous frequency
% from the Wigner distribution.


n=128;          %The number of frequency values.
[r,c]=size(Z);  %Determines the size of the matrix created by wgjka4.m.
for i=1:c;      %Repeation based on number of columns.
  W=Z(:,i);       %W is assigned the values of the column.
  Y=(1:r)';       %Y represents the frequencies.
  M=W.*Y;
  S=sum(M);
  F=sum(W);       %Sum of the Wigner Distribution values.
  E(i)=S/F;
end

v=input('Please enter the points you wish to graph.(?:?) ');

J=[12.8+(v)*1.25]/60;

% Plotting commands
subplot(3,1,1);
plot(J,vagal(v));
gtext(top);
title('Parasympathetic Range');
xlabel('time (minutes)');
ylabel('amplitude');

subplot(3,1,2);
plot(J,symvag(v));
title('Mixture of Sympathetic and Parasympathetic' );
xlabel('time (minutes)');
ylabel('amplitude');

subplot(3,1,3);
plot(J,symtopar(v));
```

```
title('Ratio of Low Frequency to High Frequency');
xlabel('time (minutes)');
ylabel('amplitude');

print -dwinc -v

subplot(2,1,1);
plot(J,nvlf(v));
gtext(top);
title('Normalized Value for Low Frequency');
xlabel('time (minutes)');
ylabel('(lf)/(lf+hf)');

subplot(2,1,2);
plot(J,nvhf(v));
title('Normalized Value for High Frequency');
xlabel('time (minutes)');
ylabel('(hf)/(lf+hf)');

%Saves Normalized Data to ASCII Files
save filename nvhf  -ascii
save filename nvlf -asii
save filename J -ascii
print -dwinc -v

end
```

B.2.4 Sympc302

```
function sympar(rawdata,top)
% SYMPAR(iibi,gtitle)
%
% Program calculates the normalized statistical values for the 30 minute profile.
% It is an implementation of time-frequency analysis
% using the Wigner distribution.
%
% iibi is the interpolated interbeat interval calculated
% by pslwsu, either in Matlab or in S-plus.

% Written by Sanjay Fernando.
% revision 9/22/94:  by Christopher King
% last revision: 8/96 by Michael Zullow
```

```
rawdata=rawdata(:);
order=5;
freq=.03;
sample=20;
nfreq=freq/sample;
[b,a]=butter(order,nfreq);
dtrend=filtfilt(b,a,rawdata);
dtrendata=rawdata-dtrend;
[row,col]=size(dtrendata);
I=1:row;
I=I(:);
A=(I/sample)/60;


% This  part is for testing Janse Kaiser Wigner calculation
% algorithm with no window
fs=1000;
m=512;                  % The size of the fft we will be computing.
skip=25;        % Number of points we skip to get the next segment.
p=129;          % The number of freq vals we will be plotting
k=fix((row-m)/skip);    % the number of spectra we compute
w=ones(size(1:m));      % window specification. Can be changed.
w=w(:);
x=hilbert(dtrendata);   % Forms the analytic funcion of x
L=m/2;
l=-(L-1):(L-1);
n=L;
Z=zeros([p,k]);
for i=1:k
        % Here I think 0 lag is at the Lth point, hence the first
        % time location is at LT=128*.001 or .128 sec; hmmm.
        g=x(n+l).*conj(x(n-l));
        g(2*L)=0;
        y=w.*g; % Apply window to g or kernal.

        Y=2/m*abs(fft(y,m)); % evidently because it's analytic
                             % we only need 2/N
        Z(:,i)=Y(1:p);
        n=n+skip;
end

Z1=Z;
Z=Z(1:30,1:k);

LFC=2:8;
```

```
symvag=sum(Z(LFC,1:k));
HFC=8:20;
vagal=sum(Z(HFC,1:k));

%Calculation of Normalized Values
symptopar=vagal./symvag;
nvlf=symvag./(symvag+vagal);
nvhf=vagal./(symvag+vagal);

r1=nvhf(1:86);
i1=nvhf(182:278);
r2=nvhf(326:422);
i2=nvhf(518:614);
r3=nvhf(662:758);
i3=nvhf(854:950);
r4=nvhf(998:1094);
r5=nvhf(1094:1190);

% Averages
ar1=mean(r1);
ar2=mean(r2);
ar3=mean(r3);
ar4=mean(r4);
ar5=mean(r5);
ai1=mean(i1);
ai2=mean(i2);
ai3=mean(i3);

%Maximums
maxr1=max(r1);
maxi1=max(i1);
maxr2=max(r2);
maxi2=max(i2);
maxr3=max(r3);
maxi3=max(i3);
maxr4=max(r4);
maxr5=max(r5);

% This section is for determining the instantaneous frequency
% from the Wigner distribution.
```

```
for i=1:c;        %Repeation based on number of columns.
  W=Z(:,i);        %W is assigned the values of the column.
  Y=(1:r)';        %Y represents the frequencies.
  M=W.*Y;
  S=sum(M);
  F=sum(W);        %Sum of the Wigner Distribution values.
  E(i)=S/F;
end
v=1:k;
J=[12.8+(v)*1.25]/60;


[p,S]=polyfit(J,nvhf,1);
nvhfit=polyval(p,J);
[p20,S20]=polyfit(J,nvhf,20);
nvhfit20=polyval(p20,J);

Jr1=J(1:86);
Ji1=J(182:278);
Jr2=J(326:422);
Ji2=J(518:614);
Jr3=J(662:758);
Ji3=J(854:950);
Jr4=J(998:1094);
Jr5=J(1094:1190);

%Linear Regression
[pr1,Sr1]=polyfit(Jr1,r1,1);
[pi1,Si1]=polyfit(Ji1,i1,1);
[pr2,Sr2]=polyfit(Jr2,r2,1);
[pi2,Si2]=polyfit(Ji2,i2,1);
[pr3,Sr3]=polyfit(Jr3,r3,1);
[pi3,Si3]=polyfit(Ji3,i3,1);
[pr4,Sr4]=polyfit(Jr4,r4,1);
[pr5,Sr5]=polyfit(Jr5,r5,1);

%Second Order Polynomial Regression
[cpr1,cSr1]=polyfit(Jr1,r1,2);
[cpr2,cSr2]=polyfit(Jr2,r2,2);
[cpr3,cSr3]=polyfit(Jr3,r3,2);
[cpr4,cSr4]=polyfit(Jr4,r4,2);
[cpr5,cSr5]=polyfit(Jr5,r5,2);
[cpi1,cSi1]=polyfit(Ji1,i1,2);
[cpi2,cSi2]=polyfit(Ji2,i2,2);
```

```
[cpi3,cSi3]=polyfit(Ji3,i3,2);

%Average NHF Value Minus Average Linear Regression Line
nvhfitr1=nvhfit(1:86);
nvhfiti1=nvhfit(182:278);
nvhfitr2=nvhfit(326:422);
nvhfiti2=nvhfit(518:614);
nvhfitr3=nvhfit(662:758);
nvhfiti3=nvhfit(854:950);
nvhfitr4=nvhfit(998:1094);
nvhfitr5=nvhfit(1094:1190);
avgfitr1=mean(nvhfitr1);
avgfiti1=mean(nvhfiti1);
avgfitr2=mean(nvhfitr2);
avgfiti2=mean(nvhfiti2);
avgfitr3=mean(nvhfitr3);
avgfiti3=mean(nvhfiti3);
avgfitr4=mean(nvhfitr4);
avgfitr5=mean(nvhfitr5);

dr1=(ar1-avgfitr1);
di1=(ai1-avgfiti1);
dr2=(ar2-avgfitr2);
di2=(ai2-avgfiti2);
dr3=(ar3-avgfitr3);
di3=(ai3-avgfiti3);
dr4=(ar4-avgfitr4);
dr5=(ar5-avgfitr5);

%Results Placed In Matrix
matrix=[cpr1(:,2) cpi1(:,2) cpr2(:,2) cpi2(:,2) cpr3(:,2) cpi3(:,2) cpr4(:,2)
cpr5(:,2);cpr1(:,1) cpi1(:,1) cpr2(:,1) cpi2(:,1) cpr3(:,1) cpi3(:,1) cpr4(:,1)
cpr5(:,1);pr1(:,1) pi1(:,1) pr2(:,1) pi2(:,1) pr3(:,1) pi3(:,1) pr4(:,1) pr5(:,1);dr1 di1 dr2 di2
dr3 di3 dr4 dr5;maxr1 maxi1 maxr2 maxi2 maxr3 maxi3 maxr4 maxr5;ar1 ai1 ar2 ai2 ar3
ai3 ar4 ar5];
Matrix = rot90(Matrix,3);

%Matrix Saved to ASCII File
save file.mat matrix -ascii
end
```

B.2.5 Sympar482

```
function sympar(rawdata,top)
% SYMPAR(iibi,gtitle)
```

```
% Program calculates the normalized statistical values for the 48 minute profile.
% It is an implementation of time-frequency analysis
% using the Wigner distribution.
% iibi is the interpolated interbeat interval calculated
% by pslwsu, either in Matlab or in S-plus.

% Written by Sanjay Fernando.
%  revision: 9/22/94 by Christopher King
%  last revision: 8/96 by Michael Zullow

rawdata=rawdata(:);
order=5;
freq=.03;
sample=20;
nfreq=freq/sample;
[b,a]=butter(order,nfreq);
dtrend=filtfilt(b,a,rawdata);
dtrendata=rawdata-dtrend;
[row,col]=size(dtrendata);
I=1:row;
I=I(:);
A=(I/sample)/60;

% This  part is for testing Janse Kaiser Wigner calculation
% algorithm with no window
fs=1000;
m=512;                    % The size of the fft we will be computing.
skip=25;          % Number of points we skip to get the next segment.
p=129;          % The number of freq vals we will be plotting
k=fix((row-m)/skip);    % the number of spectra we compute
w=ones(size(1:m));    % window specification. Can be changed.
w=w(:);
x=hilbert(dtrendata);   % Forms the analytic funcion of x
L=m/2;
l=-(L-1):(L-1);
n=L;
Z=zeros([p,k]);
for i=1:k
        % Here I think 0 lag is at the Lth point, hence the first
        % time location is at LT=128*.001 or .128 sec; hmmm.
        g=x(n+l).*conj(x(n-l));
        g(2*L)=0;
        y=w.*g; % Apply window to g or kernal.
```

```
        Y=2/m*abs(fft(y,m)); % evidently because it's analytic
                    % we only need 2/N
        Z(:,i)=Y(1:p);
        n=n+skip;
end

Z1=Z;
Z=Z(1:30,1:k);

LFC=2:8;
symvag=sum(Z(LFC,1:k));
HFC=8:20;
vagal=sum(Z(HFC,1:k));
%The normalized values are calculated here
symptopar=vagal./symvag;
nvlf=symvag./(symvag+vagal);
nvhf=vagal./(symvag+vagal);

r0=nvhf(86:278);
r1=nvhf(278:470);
i1=nvhf(518:710);
r2=nvhf(806:998);
i2=nvhf(1046:1238);
r3=nvhf(1334:1526);
i3=nvhf(1574:1766);
r4=nvhf(1862:2054);
r5=nvhf(2054:2246);

%Averages
ar0=mean(r0);
ar1=mean(r1);
ar2=mean(r2);
ar3=mean(r3);
ar4=mean(r4);
ar5=mean(r5);
ai1=mean(i1);
ai2=mean(i2);
ai3=mean(i3);

%Maximums
maxr0=max(r0);
maxr1=max(r1);
maxi1=max(i1);
maxr2=max(r2);
```

```
maxi2=max(i2);
maxr3=max(r3);
maxi3=max(i3);
maxr4=max(r4);
maxr5=max(r5);


% This section is for determining the instantaneous frequency
% from the Wigner distribution.


n=128;        %The number of frequency values.
[r,c]=size(Z);  %Determines the size of the matrix created by wgjka4.m.
for i=1:c;      %Repeation based on number of columns.
  W=Z(:,i);       %W is assigned the values of the column.
  Y=(1:r)';       %Y represents the frequencies.
  M=W.*Y;
  S=sum(M);
  F=sum(W);        %Sum of the Wigner Distribution values.
  E(i)=S/F;
end

v=1:k;
J=[12.8+(v)*1.25]/60;


[p,S]=polyfit(J,nvhf,1);
nvhfit=polyval(p,J);
[p20,S20]=polyfit(J,nvhf,20);
nvhfit20=polyval(p20,J);

Jr0=J(86:278);
Jr1=J(278:470);
Ji1=J(518:710);
Jr2=J(806:998);
Ji2=J(1046:1238);
Jr3=J(1334:1526);
Ji3=J(1574:1766);
Jr4=J(1862:2054);
Jr5=J(2054:2246);

%Linear Regression
[pr0,Sr0]=polyfit(Jr0,r0,1);
[pr1,Sr1]=polyfit(Jr1,r1,1);
```

```
[pi1,Si1]=polyfit(Ji1,i1,1);
[pr2,Sr2]=polyfit(Jr2,r2,1);
[pi2,Si2]=polyfit(Ji2,i2,1);
[pr3,Sr3]=polyfit(Jr3,r3,1);
[pi3,Si3]=polyfit(Ji3,i3,1);
[pr4,Sr4]=polyfit(Jr4,r4,1);
[pr5,Sr5]=polyfit(Jr5,r5,1);

%Second Order Polynomial Regression
[cpr0,cSr0]=polyfit(Jr0,r0,2);
[cpr1,cSr1]=polyfit(Jr1,r1,2);
[cpr2,cSr2]=polyfit(Jr2,r2,2);
[cpr3,cSr3]=polyfit(Jr3,r3,2);
[cpr4,cSr4]=polyfit(Jr4,r4,2);
[cpr5,cSr5]=polyfit(Jr5,r5,2);
[cpi1,cSi1]=polyfit(Ji1,i1,2);
[cpi2,cSi2]=polyfit(Ji2,i2,2);
[cpi3,cSi3]=polyfit(Ji3,i3,2);


%Average NHF Value Minus Average Linear Regression Line Value
nvhfitr0=nvhfit(86:278);
nvhfitr1=nvhfit(278:470);
nvhfiti1=nvhfit(518:710);
nvhfitr2=nvhfit(806:998);
nvhfiti2=nvhfit(1046:1238);
nvhfitr3=nvhfit(1334:1526);
nvhfiti3=nvhfit(1574:1766);
nvhfitr4=nvhfit(1862:2054);
nvhfitr5=nvhfit(2054:2246);

avgfitr0=mean(nvhfitr0);
avgfitr1=mean(nvhfitr1);
avgfiti1=mean(nvhfiti1);
avgfitr2=mean(nvhfitr2);
avgfiti2=mean(nvhfiti2);
avgfitr3=mean(nvhfitr3);
avgfiti3=mean(nvhfiti3);
avgfitr4=mean(nvhfitr4);
avgfitr5=mean(nvhfitr5);

dr0=(ar0-avgfitr0);
dr1=(ar1-avgfitr1);
di1=(ai1-avgfiti1);
```

```
dr2=(ar2-avgfitr2);
di2=(ai2-avgfiti2);
dr3=(ar3-avgfitr3);
di3=(ai3-avgfiti3);
dr4=(ar4-avgfitr4);
dr5=(ar5-avgfitr5);

%Placing Results Into Matrix
Matrix=[cpr0(:,2) cpr1(:,2) cpi1(:,2) cpr2(:,2) cpi2(:,2) cpr3(:,2) cpi3(:,2) cpr4(:,2)
cpr5(:,2);cpr0(:,1) cpr1(:,1) cpi1(:,1) cpr2(:,1) cpi2(:,1) cpr3(:,1) cpi3(:,1) cpr4(:,1)
cpr5(:,1);pr0(:,1) pr1(:,1) pi1(:,1) pr2(:,1) pi2(:,1) pr3(:,1) pi3(:,1) pr4(:,1) pr5(:,1);dr0
dr1 di1 dr2 di2 dr3 di3 dr4 dr5;maxr0 maxr1 maxi1 maxr2 maxi2 maxr3 maxi3 maxr4
maxr5;ar0 ar1 ai1 ar2 ai2 ar3 ai3 ar4 ar5];
Matrix = rot90(Matrix,3);

%Saving Matrix to an ASCII File
save file.mat  Matrix -ascii
end
```

APPENDIX C

TIME-FREQUENCY DATA

The following abbreviations represent the following intervals: R=rest, LT=light touch, SS=shoulder squeeze, CV=cranial vault hold, CV4=CV4 maneuver, S=sacral hold, SI=sacral/iliac hold. Shaded regions represent still points.
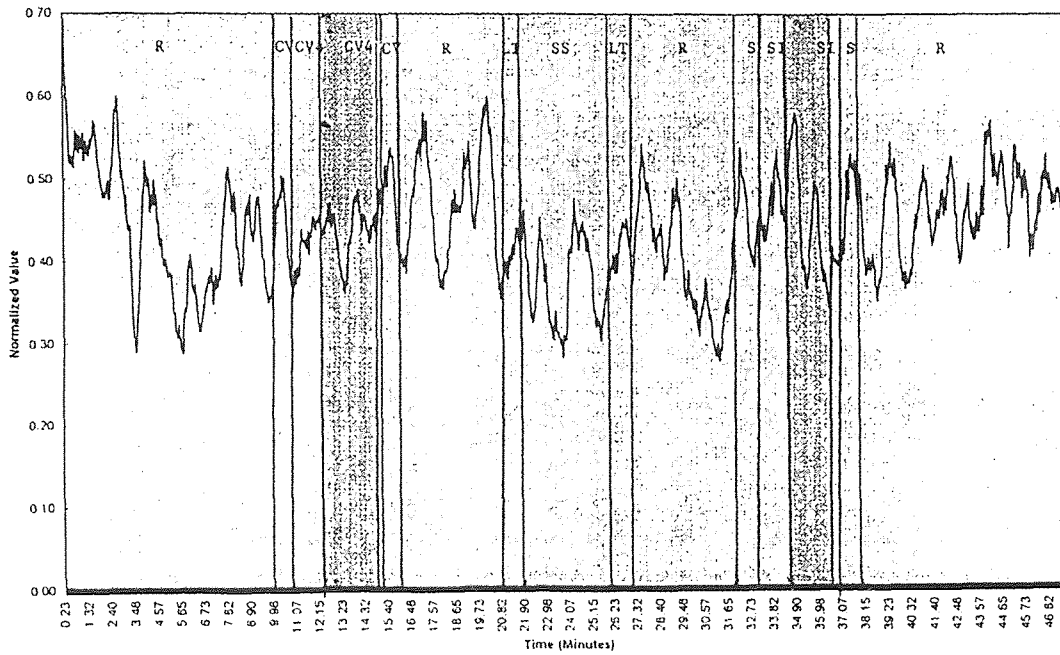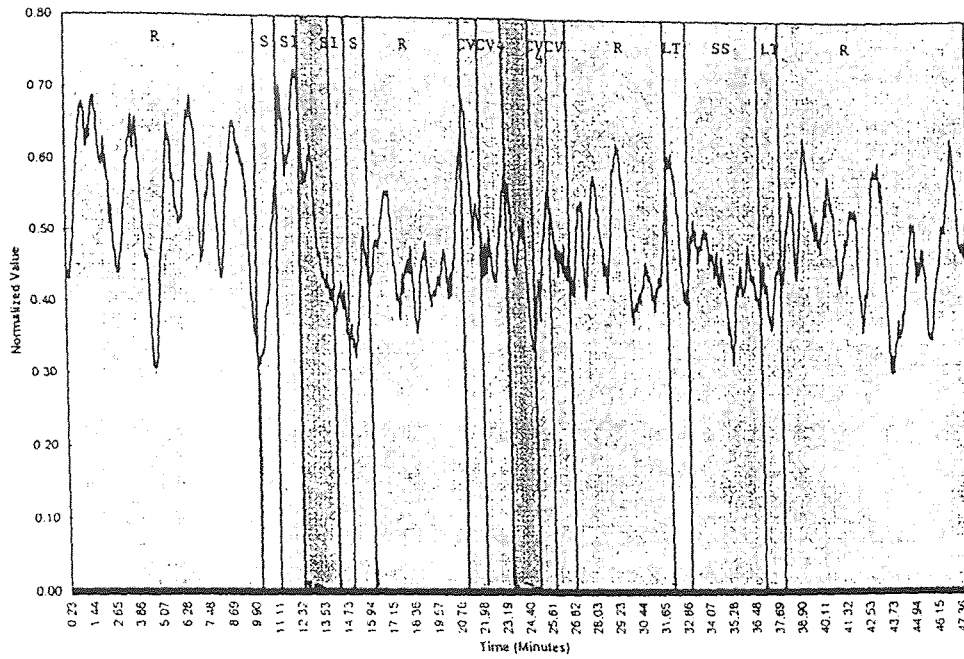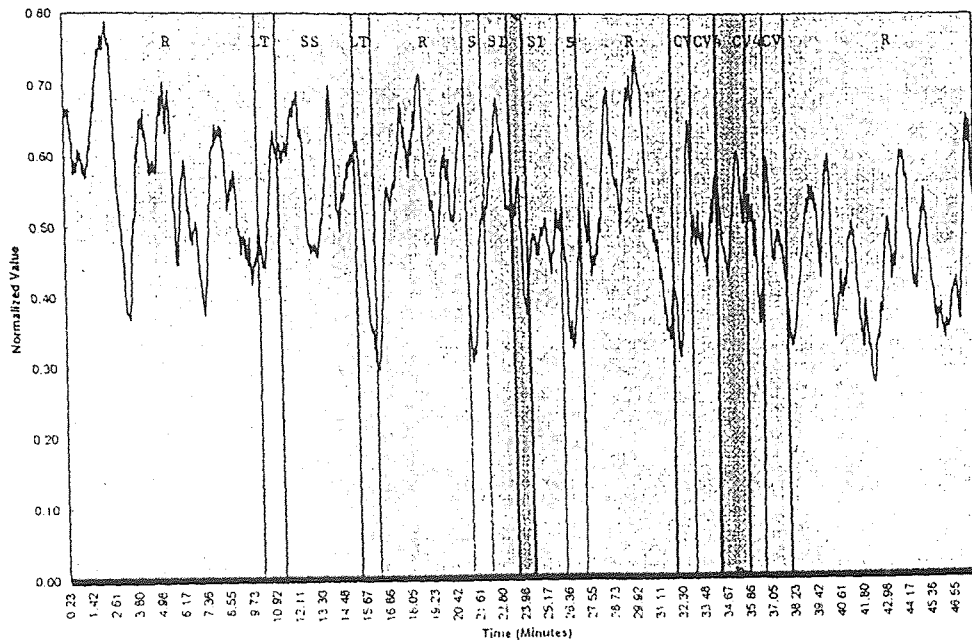
Exponentially Smoothed NHF Values for D051396
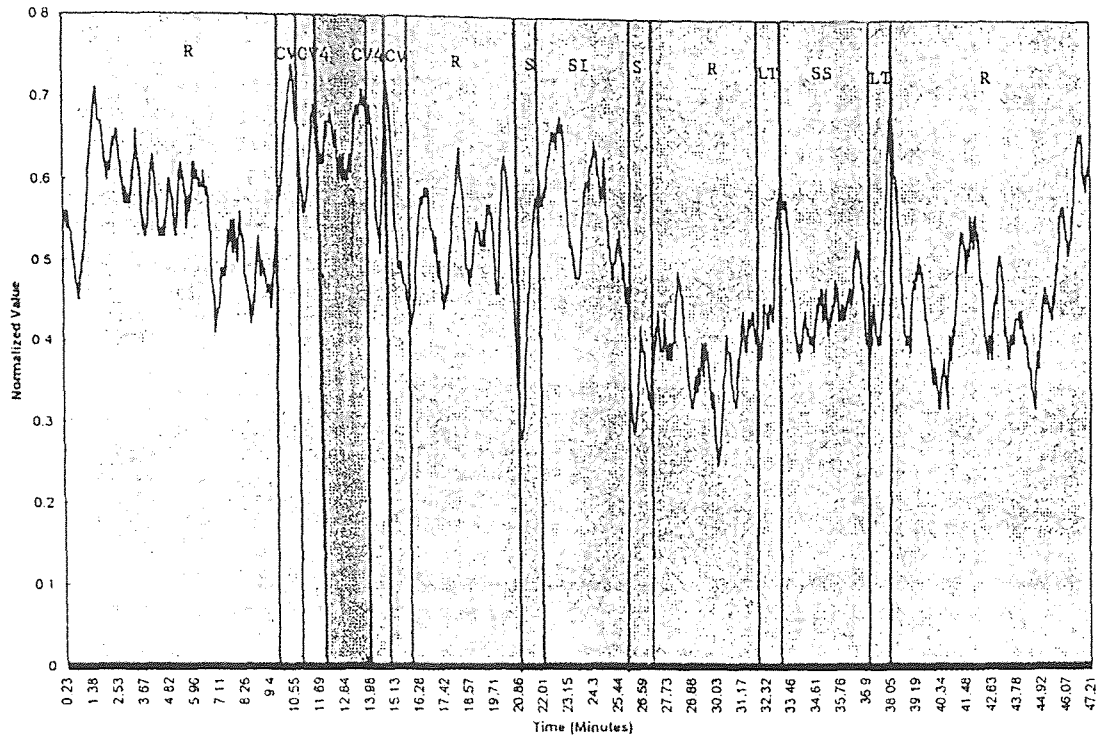
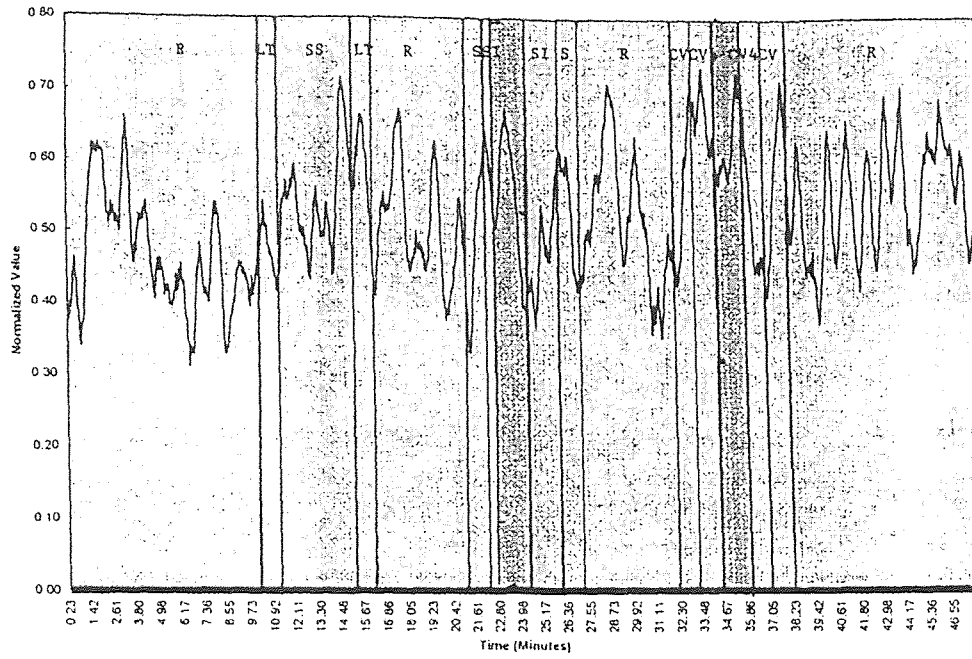Exponentially Smoothed NHF Values FOR A060596
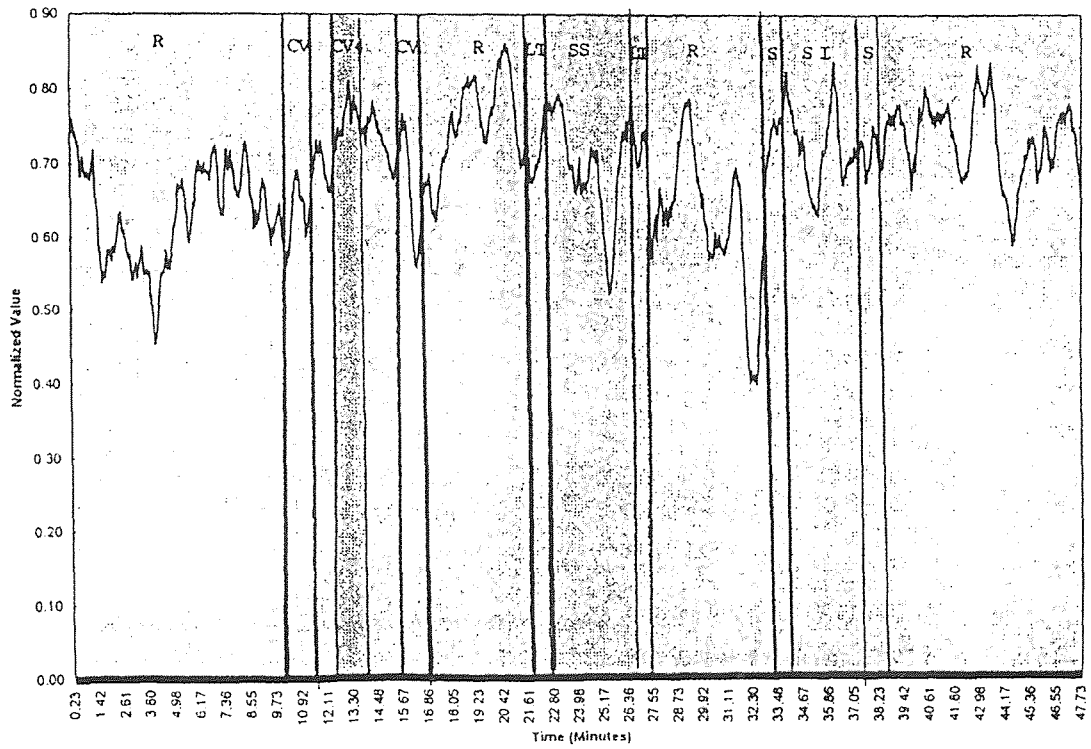
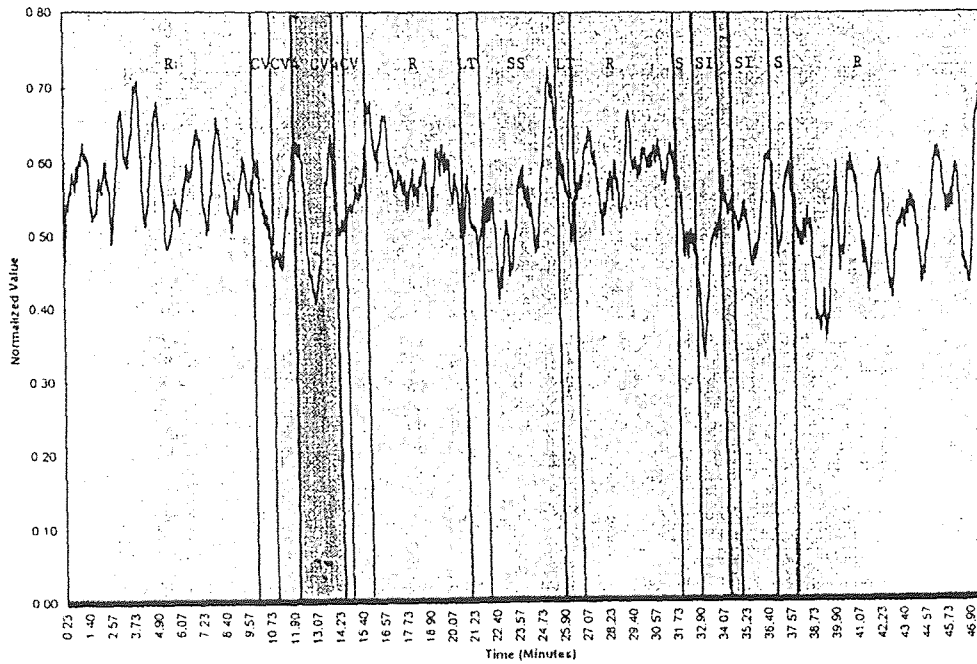Exponentially Smoothed NHF Values for S060396
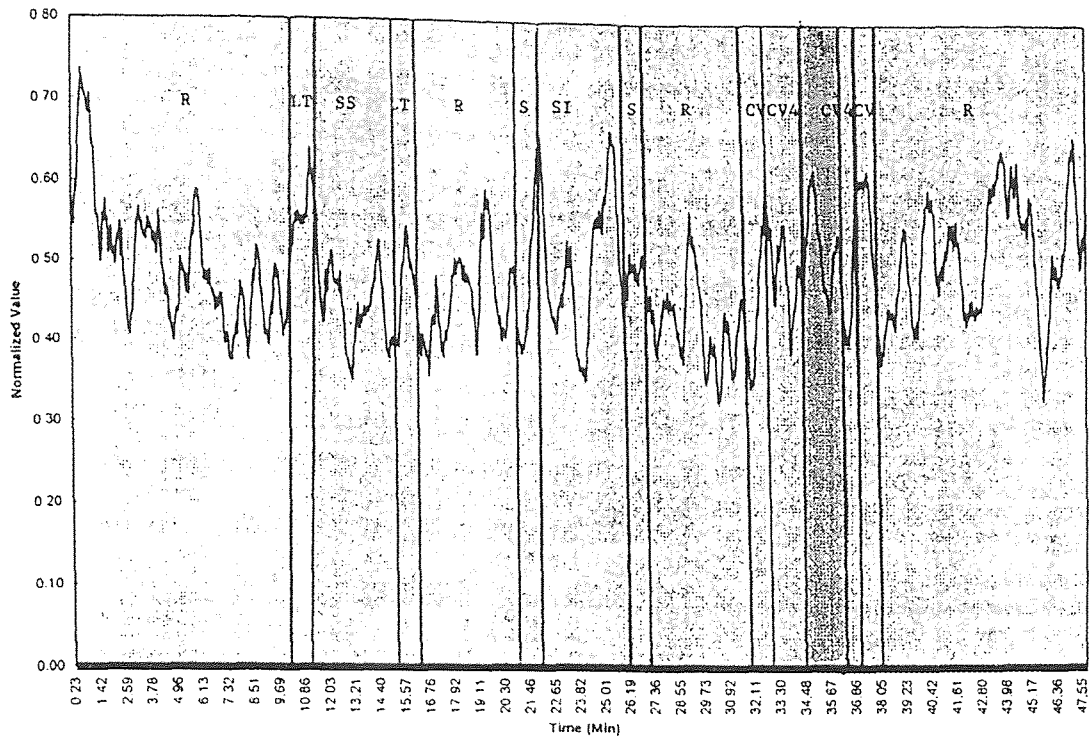
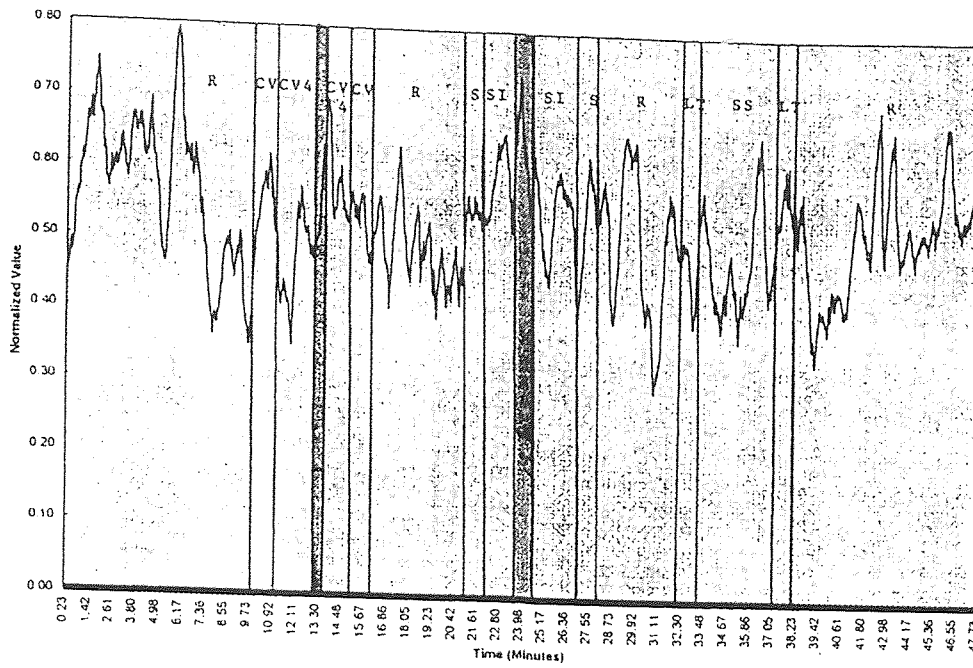Exponentially Smoothed NHF Values for K042596
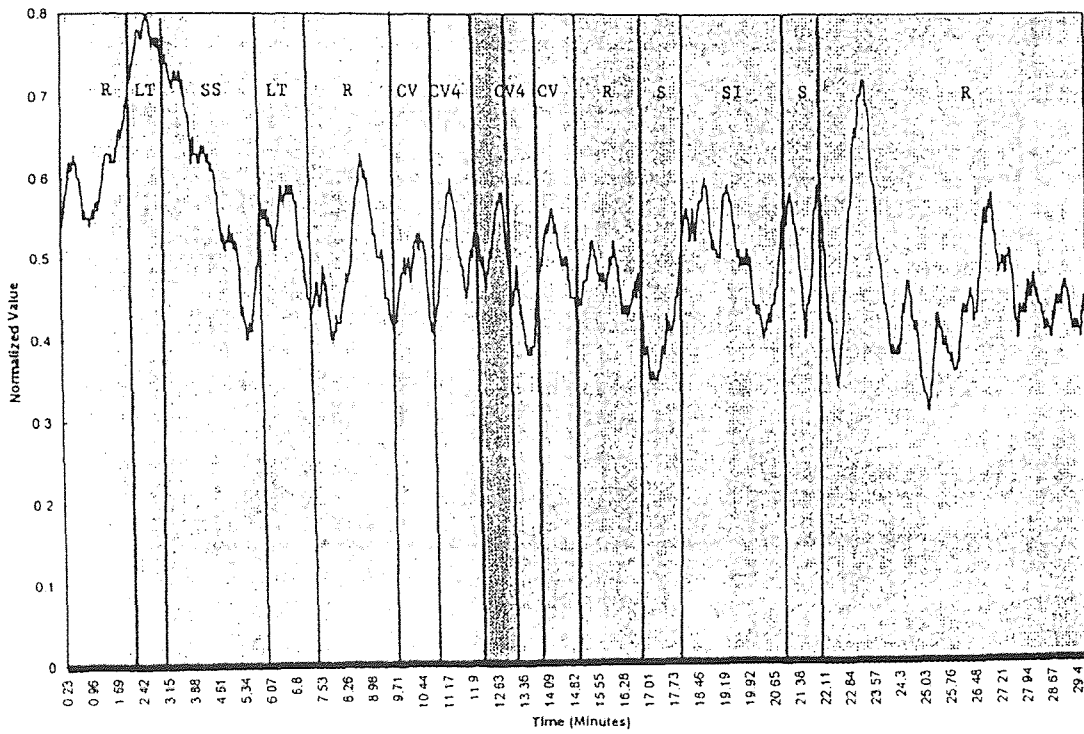
Exponentially Smoothed NHF Values for S052296
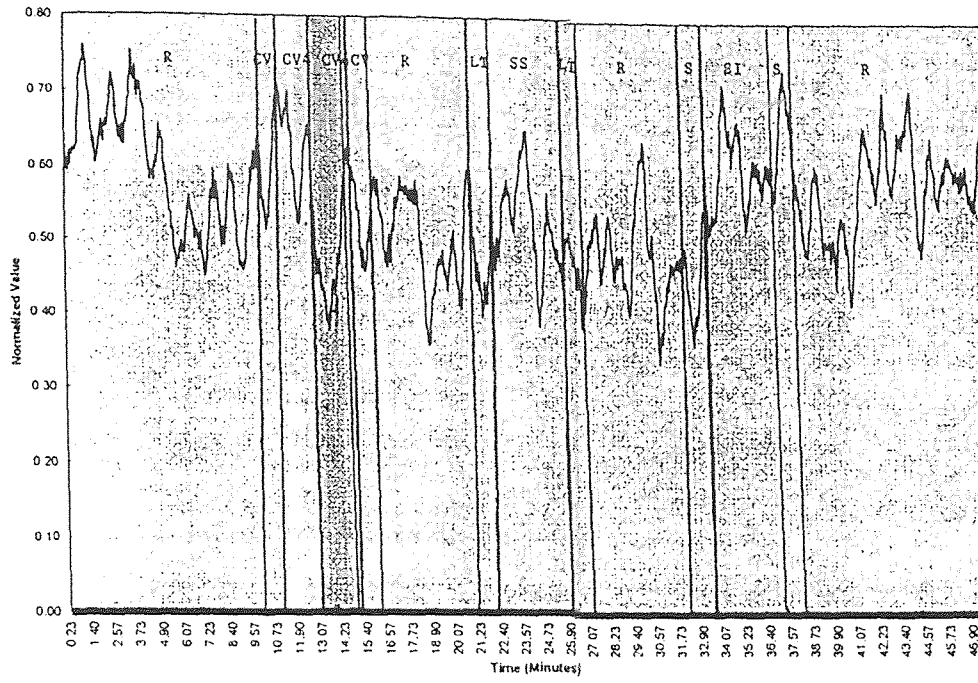
Exponentially Smoothed NHF Values for F060396
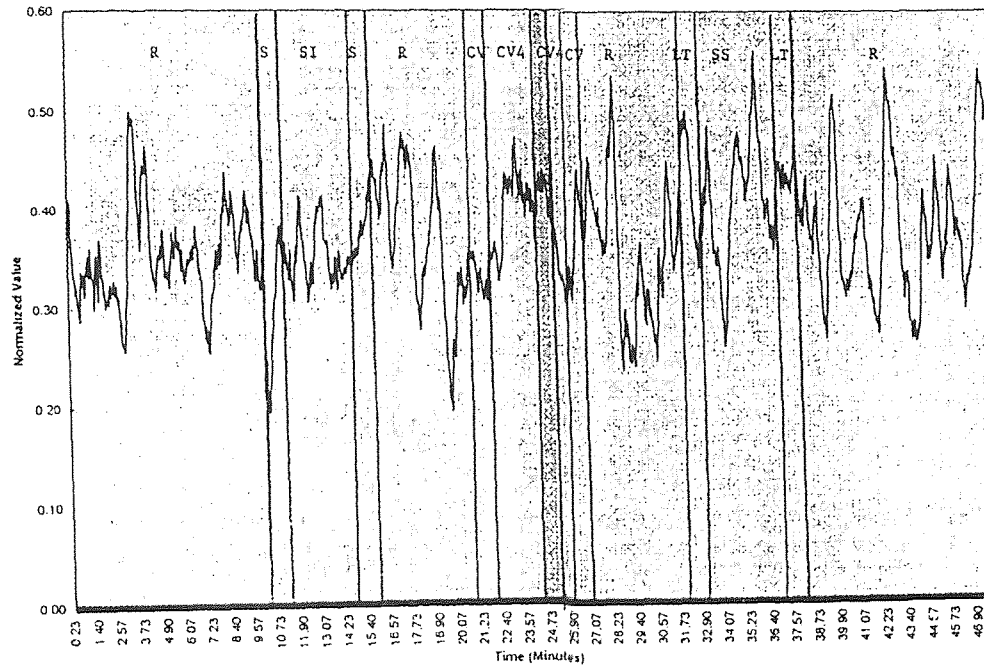
Exponentially Smoothed NHF Values for D041296
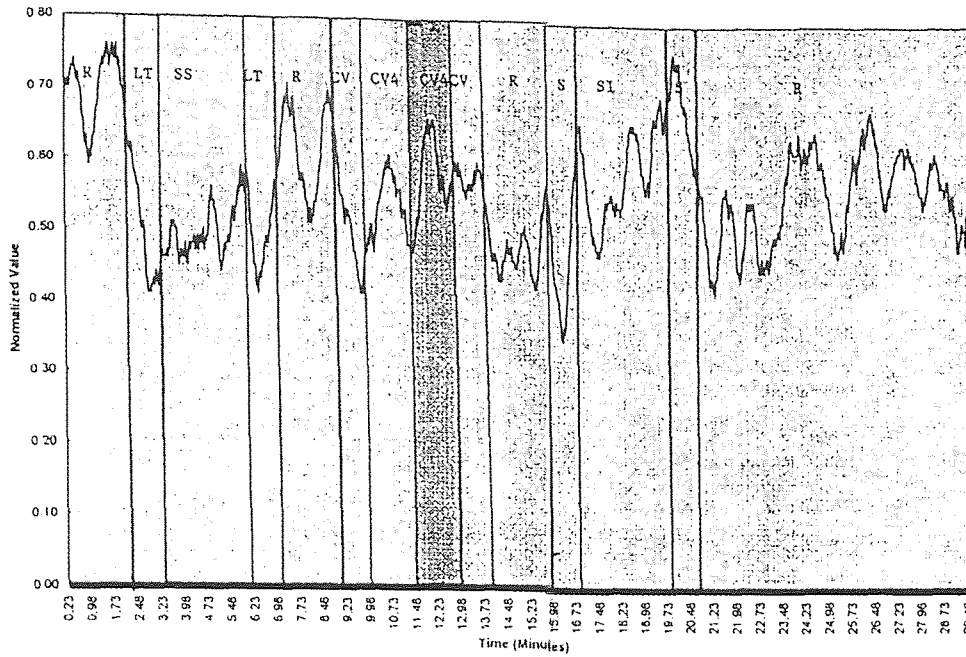
Exponentially Smoothed NHF Values for P042396

99
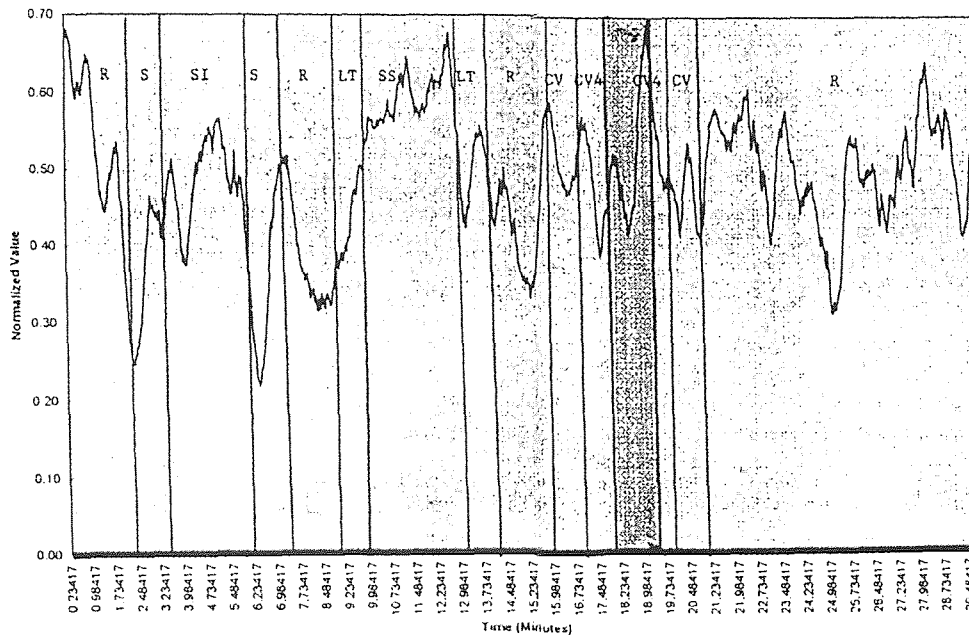
Exponentially Smoothed NHF Values for T042496
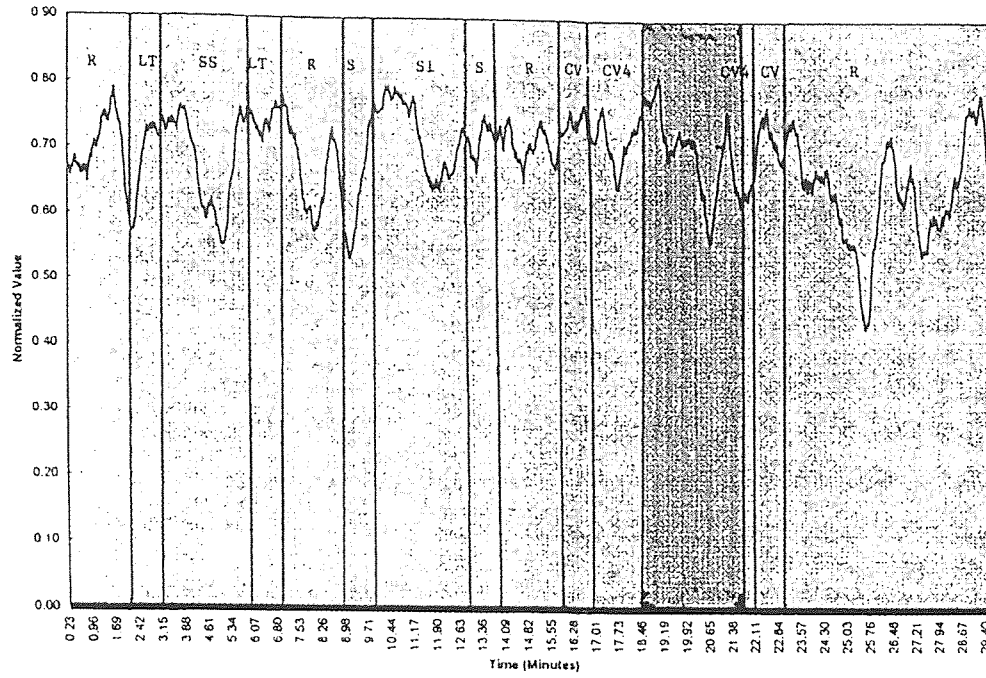
Exponentially Smoothed NHF Values for K032696
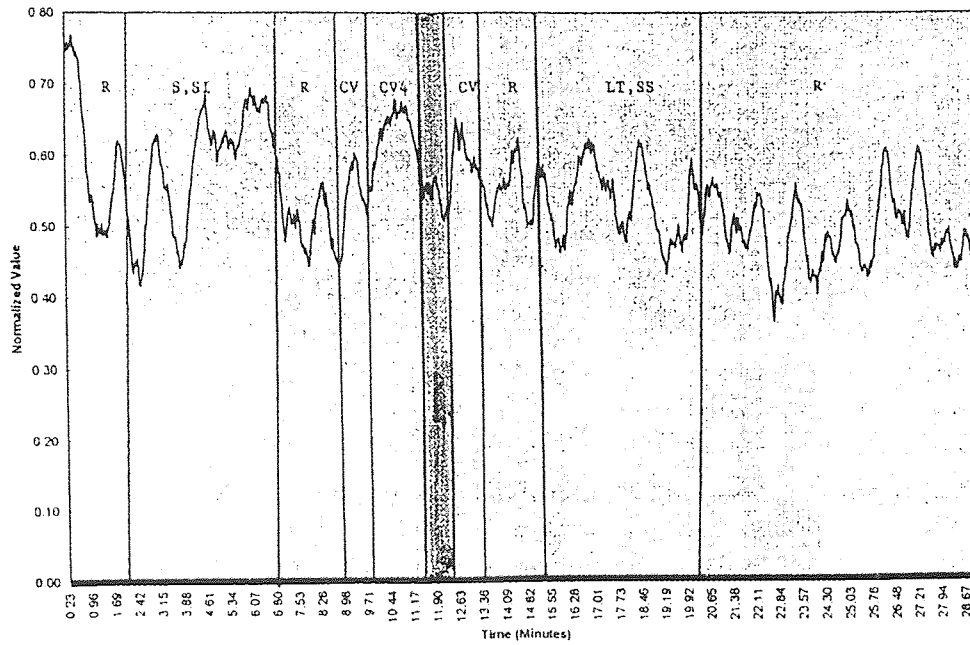
Exponentially Smoothed NHF Values for U082296
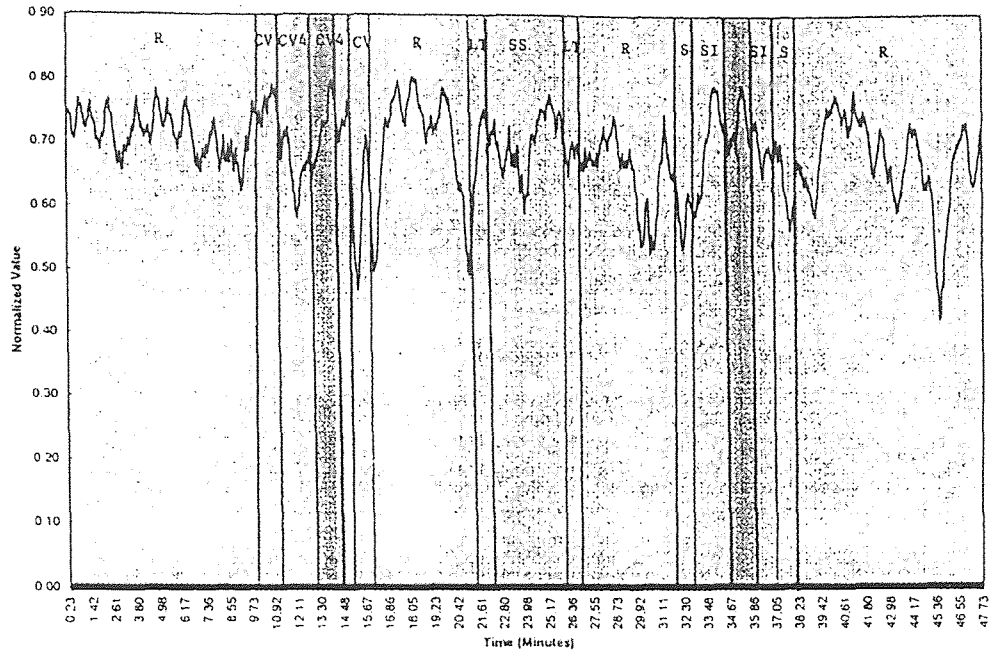
Exponentially Smoothed NHF Values for S061896

Exponentially Smoothed NHF Values for L082696

Exponentially Smoothed NHF Values for L082296
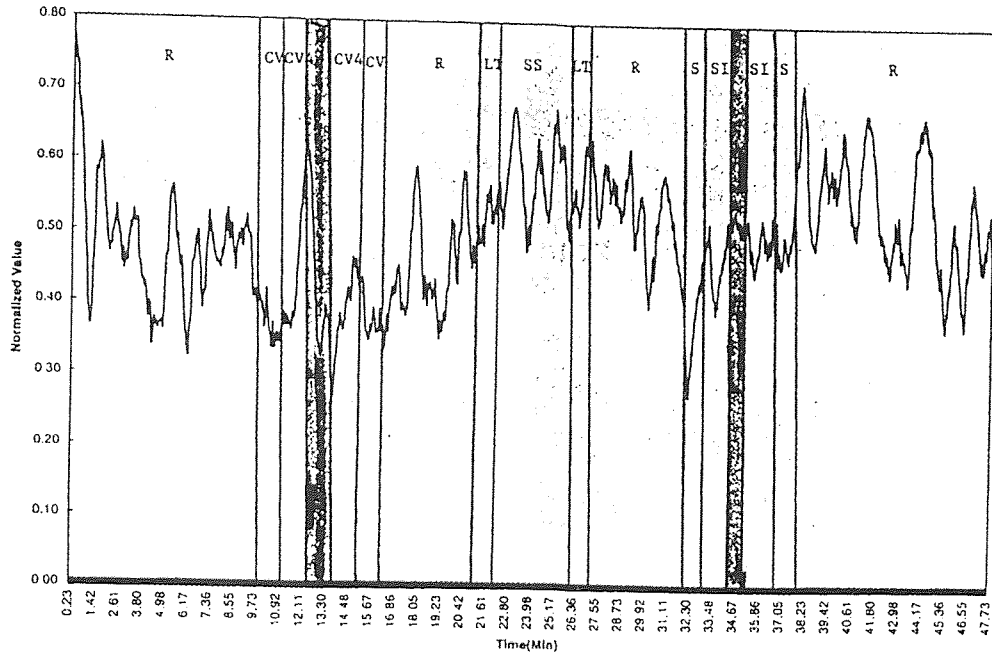
Exponentially Smoothed NHF Values for J072296
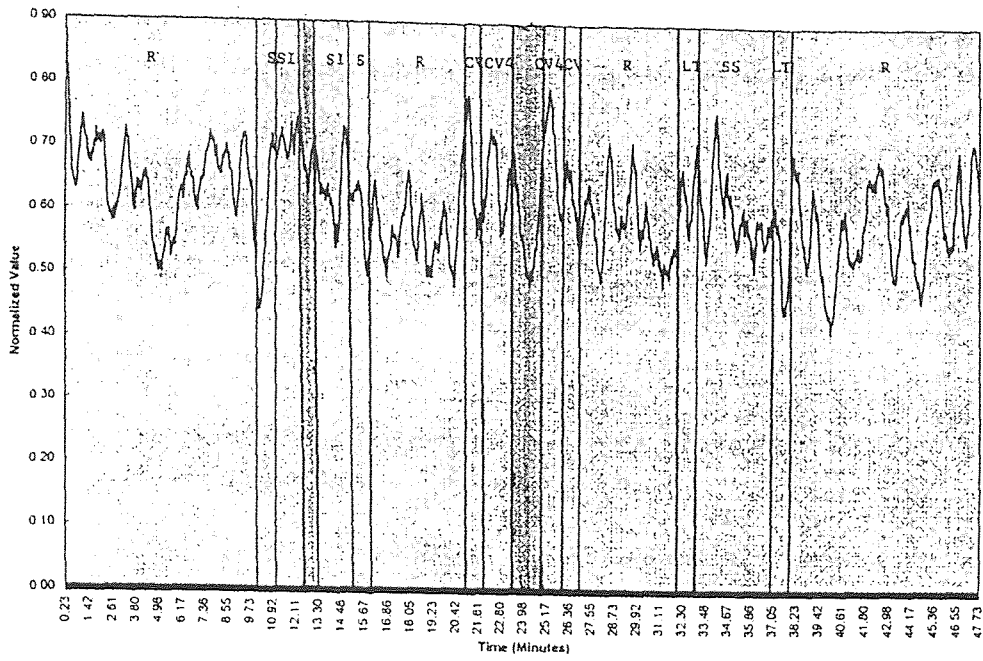

Exponentially Smoothed NHF Values for S061796

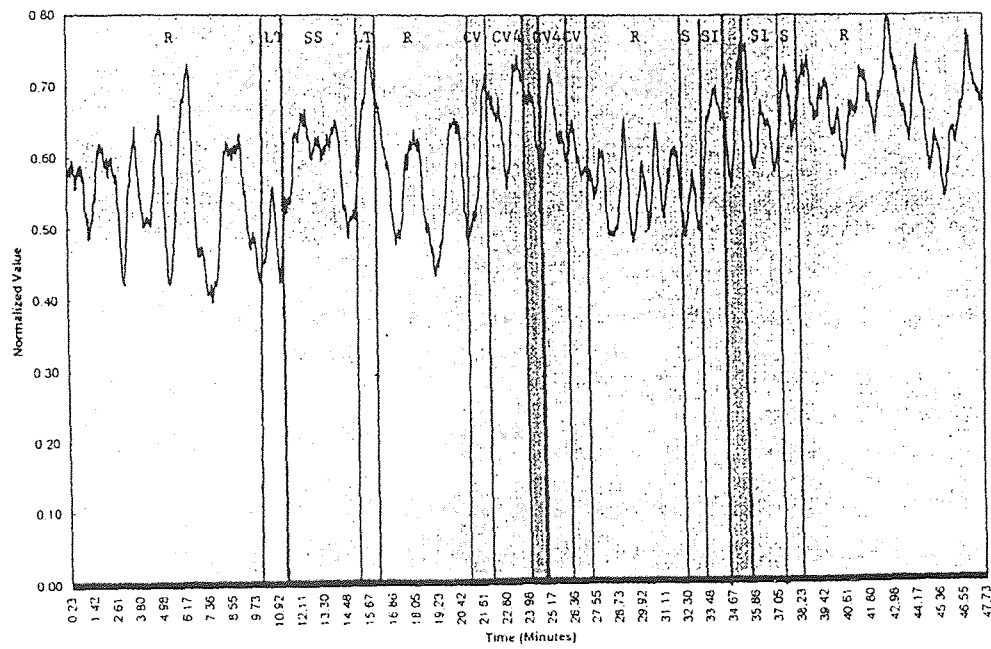Exponentially Smoothed NHF Values for Z082196

Smoothed Normalized High Frequency Values for s070196

Exponentially Smoothed NHF Values for K061796

Exponentially Smoothed NHF Values for J073196

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