ABSTRACT

A REAL TIME FREQUENCY ANALYSIS OF THE ELECTROENCEPHALOGRAM USING LABVIEW

by
Rupal Patel

The use of the Electroencephalogram (EEG) for diagnosis of brain related diseases is becoming a popular technique in the clinical and research environment. To achieve accurate reading of EEG, signal representation and classification becomes extremely important. The goal of this project was to develop a basic software program for acquiring and online processing of the electrical activity recorded from the brain. A program was developed using the LabVIEW programming software by National Instruments. Basic hardware components recorded the EEG signal and a software component divided the data into delta, theta, alpha and beta bands in the frequency domain. Emphasis was placed on critical programming parameters such as sampling rate, filtering, windowing and FFT.

The developed software was implemented in an already existing experimental paradigm that studies classical conditioning response. To prove the validity and accuracy of the system, a pilot experiment was conducted where EEG was recorded from six subjects. Data showed that as the subject learns, continuous theta activity is observed. Performance and testing of the EEG system demonstrated that the on line processing of EEG could be used in a variety of other applications where neural activity is involved such as classifying sleep stages in patients, discriminating various mental tasks, recording continuous EEG activity in neonatals with brain dysfunction etc.

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by

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TABLE OF CONTENTS

Chapter   **Page**

TABLE OF CONTENTS
(Continued)

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1 Objective

Neurophysiologic techniques such as electroencephalography are becoming increasingly important in various medical settings and in research areas. The Electroencephalogram (EEG) is a signal derived from the activity of complex neurons near the surface of the brain. Continuous recording of EEG can play a vital role in the detection of many central nervous system disorders such as seizures, epilepsy and brain damage. The aim of this project was to design an experimental apparatus to record and process the electroencephalogram from human subjects. The apparatus consisted of a hardware and a software component. The hardware component dealt with recording the physiological signals from the surface of the body through the electrodes and providing signal conditioning to make the signal suitable for recording. The software component recorded the data and processed further to obtain the frequency representation of delta, theta, alpha and beta bands of EEG. The main focus was on real time processing.

There can be many applications of on line recording of EEG. EEG can be used in categorizing sleep stages in patients[1], discriminating various mental tasks[2], demonstrating the effect of different visual perception on the brain[3], synchronizing and discovering information flow in epileptic patients[4] etc. This project focused on implementing this system in the currently used experimental paradigm that measures classical conditioning in humans.

Classical conditioning is a simple form of associative learning. The associative process involves learning theta stimulus and serves as a signal of an elicitor physiological

1

response. As you repeatedly signal the elicitation of the reflex, delivered, the subject learns to response in an anticipation of the reflex. Acquisition is apparent as a response to the signal. The system currently used for a classically conditioned experiment monitors the electrocardiogram (ECG), electromyogram (EMG) for eye blink detection and respiration. EMG measures the electrical activity of the muscles around the eye through which eye blink is identified.

Any type of learning in the brain can be viewed as temporary electrical activity in the neurons leading to persistent or permanent change. It has been showed that in animals during classical conditioning, increased theta activity of the EEG suggests more learning[5]. A similar phenomenon can be checked in humans through the recording of EEG and it can be explored if an unusual pattern exists during the learning process.

1.2 Background Information

1.2.1 Electroencephalogram

The human brain is an extremely important organ in the body through which a person is able to respond to various sensory stimuli as well as perform complex activities. Various activities such as cognition, language, sleep, wakefulness, emotions and memory are made possible by the brain. There are many aspects of the human brain that still remain unexplored. The measurement of EEG may provide some information to aid in our understanding of the brain.

The three main parts of the brain include the brainstem, cerebellum and cerebrum. The brain stem controls vital physiological functions such as breathing, blood pressure and heart rate[6]. The cerebellum receives sensory input from the cerebrum and

coordinates muscular movement according to motor messages. The cerebrum is an essential part of the brain that controls intelligence, learning, perception and emotion. The outer layer of the cerebrum in the brain is called the cerebral cortex, which contains sensory, motor and association areas. Association areas are located in the front of the frontal lobe and in parts of the occipital, parietal and temporal lobe[6].

There are two main types of cells in the cerebral cortex: pyramidal cells and nonpyramidal cells. Nonpyramidal cells do not contribute in the generation of EEG. However, pyramidal cells play a major role in producing the EEG signal. Pyramidal cells consist of a long apical dendrite, which is attached to the cell body on the outer layer of the cortex. Any potential changes within the dendrites create potential fields and generate the current. Since the apical dendrite and cell body are connected, the current also flows in the cell body and the entire cell is activated. The cell and the dendrites constantly shift their current dipole, strength and orientation, which produce large electric fields (wave like fluctuations) that can be recorded as EEG[7].

The electroencephalogram enables us to measure the electrical activity of the brain non-invasively. An EEG is a record of fluctuations of electrical potentials generated by nerve cell in the cerebral cortex. The inherent properties of the cerebral cortex such as understanding perceptual, motor and linguistic abilities can be monitored with this technique[6]. In addition, it is a major tool for diagnosis of many brain diseases and other diseases that are indirectly affected by the brain.

Scientists have been working on EEGs since the 1930s. In 1929, a psychiatrist named Hans Berger at the University of Jena, first made a scalp recording of brain activity in humans. Since then, a lot of research has been done to find better ways to

record and process the EEG for clinical purposes. Because the EEG uses a noninvasive method to detect the electrical activity of the brain, it is a very important tool in clinical assessment of cortical function.

Unlike intracellular recordings of individual neurons, the EEG records the activity of thousands of neurons through electrodes placed on the skull or scalp. Because it does not record the activity of a particular neuron, it provides regional performance of the brain instead the performance of a specific neuron. The most commonly used system for recording clinical EEG is the International Federation 10-20 system of electrodes. This system uses certain anatomical locations to standardize placement of EEG electrodes with earlobe electrodes as reference. The 10 and 20 refer to the 10% or 20% interelectrode distance. The electrodes are usually placed over the frontal, parietal, occipital and temporal lobes according to a conventional scheme (Figure 1.1).

Many diagnostic techniques such as MRI, PET and SPECT can be used in diagnosing brain related abnormalities. However, without strong evidence, the use of such techniques for research can be very costly. In addition, subject compliance may be difficult for conducting an imaging based research. An inexpensive and a relatively simple method to study the activity of the brain is through EEG. EEG is an important tool in monitoring brain dysfunction. A computer driven analysis of EEG patterns suggests a lot about brain activity. Thus, the purpose of the project was to develop an experimental paradigm to record and real time processing of EEG that will provide a diagnostic tool to the Neurobehavioral Research Laboratory at the Veterans Affairs NJ Health Care System (VANHCS) to carry out various brain related studies.
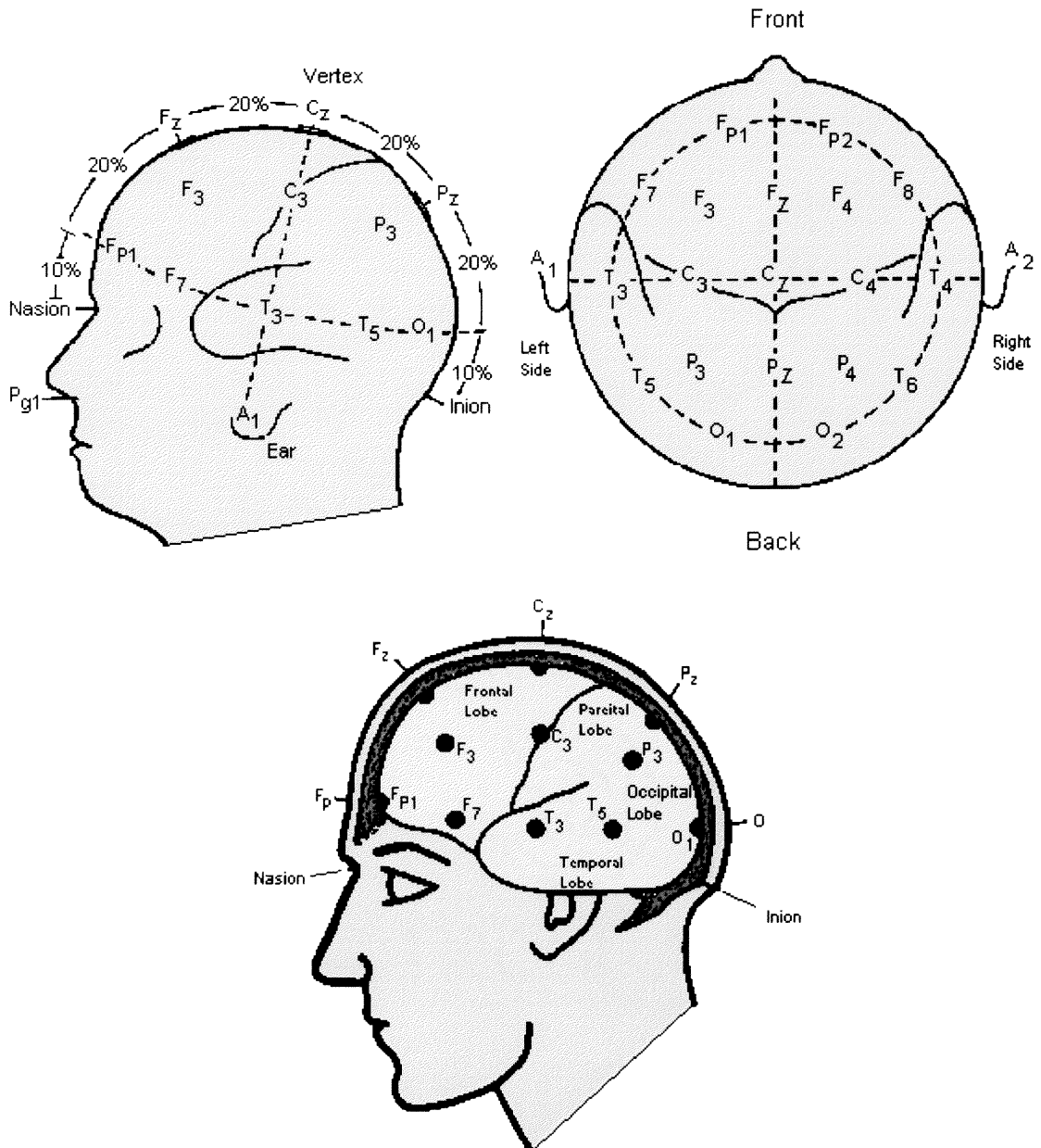
Figure 1.1 The 10-20 System of electrode placement

The EEG recorded from the scalp has intensities in the range of 50-200μV. Such

low amplitude is due to the attenuation of the EEG by meninges, cerebrospinal fluid,

skull and the scalp. The frequencies of these brain waves range from 0.5 Hz to 100 Hz

and are mostly dependent on the activity of the cerebral cortex. In a normal person, the brain waves can be classified in four groups: delta, theta, alpha and beta. Delta waves include all the waves in an EEG from 0.5 Hz to 3.5Hz. They tend to be the highest in amplitude and the slowest waves. They are normal and are dominant in infants up to one year and in stages 3 and 4 of sleep. They may occur in serious brain injury and in patients with subcortical lesions [8,9].

Theta waves have frequencies between 4Hz and 7Hz. These waves result during emotional stress in adults, particularly during periods of disappointment and frustration. Alpha waves are rhythmic waves occurring at a frequency between 8Hz and 13 Hz. Alpha is usually best seen in the posterior regions of the head on each side. It is found in EEGs of most all normal persons when they are awake, quiet and relaxed. It is the major rhythm in normal relaxed adults and present during most of life. Beta activity is "fast" activity and has a frequency of 14 Hz or greater. It is usually seen on both sides in symmetrical distribution in the frontal lobe. It appears during intense mental activity [8,9].

1.2.2 Classical Conditioning

In classical conditioning, a subject learns to respond in an ordinary way to a new stimulus. A new association forms between stimulus and response. The two types of stimuli involved in classical conditioning are the conditioned stimulus and unconditioned stimulus. The unconditional stimulus triggers reflex; for example, an air puff makes the eye blink. In order to initiate associative learning, another type of stimulus is provided called the conditional stimulus, which is delivered before the unconditional stimulus. When a conditioned stimulus (new stimulus) is repeatedly presented immediately before

the unconditioned stimulus, the person learns to associate a new stimulus to the response[10].

Ivan Pavlov, a Russian psychologist discovered this phenomenon in dogs. He noticed that the presence of food made the dog salivate. In this case, food is the unconditional stimulus. He rang a bell (conditioned stimulus) prior to food delivery. After repeating this continuously, the dog associated the sound of the bell with the presence of food. As a result of classical conditioning, the dog salivated at the sound of the bell, even when no food was present.

A similar method is followed in the eye blink conditioning experiment. A tone is used as the conditional stimulus and the air puff is presented as an unconditional stimulus. As a person is confronted with a tone and then the air puff for a certain time, he or she learns to associate one with another. Consequently, a subject blinks at the sound of a tone. As a person is learning, certain neurons in the hippocampus region of the brain increase their activity, which suggests a neuronal basis for the behavior. It has been shown in animals that during classical conditioning, animals who received a tone and air puff during theta range, exhibited enhanced learning. However, animals that were given a tone and air puff trials in the absence of the theta activity, learned much slower[5]. For this particular application on line processing of EEG is necessary to determine the occurrence of theta activity. The present system, built for on line processing of EEG, would enable one to carry out such research in human subjects. The first step in this particular application would be to figure out whether all the activities including theta are being recorded correctly and then try to find a correlation between the theta activity and learning.

1.2.3 Engineering Design of the Existing System

The system currently used at the VA to study classical conditioning is a sophisticated system consisting of hardware and software components. The hardware component created and delivered the stimuli (air puff and tone) regulated by the computer and the software component recorded and processed physiological responses[10] (Details about each component are presented in sections 1.2.3.1 and 1.2.3.2).

1.2.3.1 Output Hardware. The two parameters of hardware design included the audio delivery system and airpuff delivery system. The audio delivery system generated the audio signal, which can be transmitted to the subject using headphones. Two different audio stimuli were generated using the controlling software program and were transmitted to a digital to analog (D/A) converter. The output from the D/A converter was transmitted to an audio amplifier (DC-CON Model, Pioneer Electronics Corp.) for the final gain and impedance matching for delivery to the headphone worn by the subjects. Specific type of aviation headphones (Model H10-50 David Clark, Worchester, MA) were used to reduce any noise interference from the environment. The audio system sound levels were calibrated using a sound meter (Model 33-2050-Radio Shack/Tandy Corporation, Fort Worth, TX) [10].

The air puff was produced using an air tank with a high precision regulator. The output of the regulator was set to 5.0 PSI. This pressurized air was passed through a filter (Model 9933-11-BX/Baxton Inc Lexington, MA) to remove very small particles. It was then passed through the computer-controlled solenoid valve (Model ET-2-12-H Clippard Instrument Laboratory Cincinnati, OH) and collected into a reservoir of 0.0007 cu ft.

From the reservoir, the air passed to the second computer-controlled solenoid and then to the tygon tubing through which the air puff was delivered into an eye. This particular kind of airpuff generation arrangement was chosen because it produced a consistent and reliable stimulus[10].

1.2.3.2 Physiological Responses. Physiological measurements such as EMG, ECG and respiration were recorded at the same time the stimulus was delivered. The eyeblink was measured by an EMG using electrodes placed above and below a subject's eye. The EMG is a recording of electrical potentials generated by muscle fibers. The EMG signal was amplified using a battery-operated differential amplifier (Model 2283FT, UFI-Morro Bay, CA). The amplifier gain was set at 1000 with a low pass filter at the cut off frequency of 100 Hz and high pass filter at the cut off frequency of 0.1 Hz. During this experiment, cardiovascular activity was recorded by the ECG. An ECG is a measure of electrical changes on the body's surface in response to contraction and relaxation of the heart. The ECG measurement was made using an amplifier (Model RESP-I UFI- Morro Bay, CA) similar to EMG. The gain for the ECG amplifier was set to 1000 and high cut off frequency at 100 Hz[10].

Respiration was measured using impedance pneumography. The two electrodes were placed on the lower rib cage approximately 6 to 8 inches from the center and the ground electrode was placed on the low abdominal region. These were the same electrodes that were used for recording the ECG. A constant current source ($1\mu A$ at 30 KHz) was applied between the two electrodes and the differential voltage was measured. The impedance created due to the voltage changes represented a waveform of the breathing pattern of the subject. The respiration amplifier was AC coupled to eliminate

DC shifts. The low pass filter in the respiration amplifier was set to 1 Hz for signal conditioning. In addition to these measurements, two channels for the tone and air puff were added to measure the accurate timing of each stimulus during the experiment. The difference in each voltage level demonstrated which stimuli or combination of stimuli were present[10].

CHAPTER 2

METHODS

2.1 System Introduction

This experimental system was set up to measure, process, display and store the EEG data in real time in human test subjects. To check its performance in a scientific paradigm, it was applied in a pre-existing classical conditioning experiment. Two computers were used for processing the data collected from two different systems. One computer was used for the classical conditioning system, which delivered two stimuli (an unconditional stimulus (air puff) and a conditional stimulus (tone)) and recorded EMG. The other computer was used for the EEG system, which recorded the electrical activity of the brain and differentiated it into four normal bands (theta, delta, alpha and beta) of EEG.

Subject	$\Rightarrow$	Isolation Amplifier	$\Rightarrow$	Physiological Amplifier	$\Rightarrow$	Computer
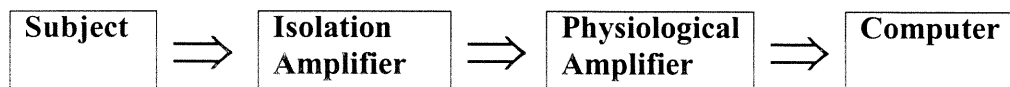
Figure 2.1 – Block Diagram of the EEG system

Figure 2.1 shows the block diagram of the EEG system. The detailed description of each component is described later in this section. EEG signals would be picked up through the electrodes from a subject and would be passed from an isolation amplifier to the physiological amplifier where the signals were conditioned according to the selected parameters (specification of the amplifier parameters are in section 2.2). The Analog output of the amplifier was fed into the A/D converter of the computer. In the computer, the analog input was converted to a digital output with the aid of the A/D (analog to digital) conversion card. Using software, signal processing was conducted on the collected data and the data were displayed and stored in the computer. The classical

conditioning experiment was 56 minutes long; therefore, Lab VIEW parameters such as sampling rate, filters and FFT were selected based on this time length (Details will be discussed later in this chapter).

2.2 Hardware Parameters

The intensity of the brain waves recorded from the scalp have a small amplitude of approximately 100μV. Thus, to analyze the EEG and calculate power in different frequency bands, amplification of the signal became necessary. The EEG signal was amplified using a physiological four channel differential amplifier (CWE, BMA- 830). A gain of 10,000 was selected for amplification of the EEG. Since the EEG ranges from 0.5Hz to 100Hz, the signal conditioning parameters of the physiological amplifier were set to meet this requirement. The high frequency cutoff of the amplifier was set to 0.1kHz and the low frequency cutoff was set to 1Hz.

The safe design and the safe use of medical instrumentation are very important in all medical procedures. Patients are usually exposed to more hazards in medical environments than home and workplace settings because in a medical scenario, a part of the medical instrumentation is directly attached to their skin. Through the use of electrodes, the body becomes a part of an electric circuit. Current enters the body at one point and leaves at some other point. The magnitude of this current is equal to the applied voltage divided by the sum of the series impedances of the body tissues and the interfaces at the contact points. The impedance at the skin-electrode interface is high; therefore, the purpose of the recording electrode is to reduce this impedance and improve the signal quality. As the impedance is reduced, the ability for the current to pass through

the electrodes is increased. When the electric current flows through the biological tissues, it can stimulate excitable tissues, cause resistive heating of the tissues and create electrochemical burns and tissue injuries.

To protect the patient from a possible electric current, an isolation amplifier was used between the electrodes and amplifier. An isolation amplifier usually consists of an instrumentation amplifier at the input followed by a unity gain isolation stage. It includes different supply voltage sources and different grounds on each side of the isolation barriers. This barrier prevents the passage of the current from the power line. The isolation amplifier used was (CWE, Iso-Z) with pre amplification gain of 10. Leakage current was limited to $2\mu A$ at 600 volts. This isolation met the specifications of the Underwriter's Laboratory standard for safety, medical and dental equipments as established under UL-544.

Once the signal was recorded via electrodes and passed through the amplifier, it was transferred to the computer via an interface. In the computer, the A/D conversion card converted the analog signal into a digital signal. The A/D conversion card chosen for this system was a National Instrument 12 bit A/D card. This card used a successive approximation method of conversion with a resolution of 12 bits over a range of $\pm10V$. Once the signal is in digital format, complete processing of the signal can be achieved.

2.3 Software Development and Evaluation

2.3.1 Introduction to Lab VIEW

The major part of the project was concerned with creating a program with the software parameters that were ideal for processing EEG. Since the frequency difference between

each desired band of the EEG is only 1 Hz, extra care had to be taken in choosing the programming paradigm.

Laboratory Virtual Instrumentation Engineering Workbench (LabVIEW) software by National Instruments was chosen for this processing application. It is a graphical programming language with three important components: data acquisition, data analysis and data visualization. This programming paradigm has been widely adopted by industry, academia and research laboratories over the world as the standard for data acquisition and instrument control software. The functions in LabVIEW are called Virtual Instruments (VIs). VIs perform small tasks such as mathematical functions, recording, configuration etc. There is a function palette, which lists all the functions available with categories and sub categories. These VIs have inputs and outputs, which gives the user the necessary information needed in the selection of the parameters. VIs in a block diagram manner simplify the use of classical digital signal processing. Signal processing is required to extract the useful information from the original signal by removing noise and other non-related frequencies[11].

There are two basic components in LabVIEW: the front panel screen and the block diagram screen. On the front panel, the running of the program and its performance can be viewed, but the actual programming takes place on the block diagram screen. The front panel is usually regarded as the user interface through which the user can visualize the performance of the program. On the block diagram screen, different VIs are selected and connected to carry out functions such as reading the data, filtering the data, storing the data etc.

2.3.2 Software Development
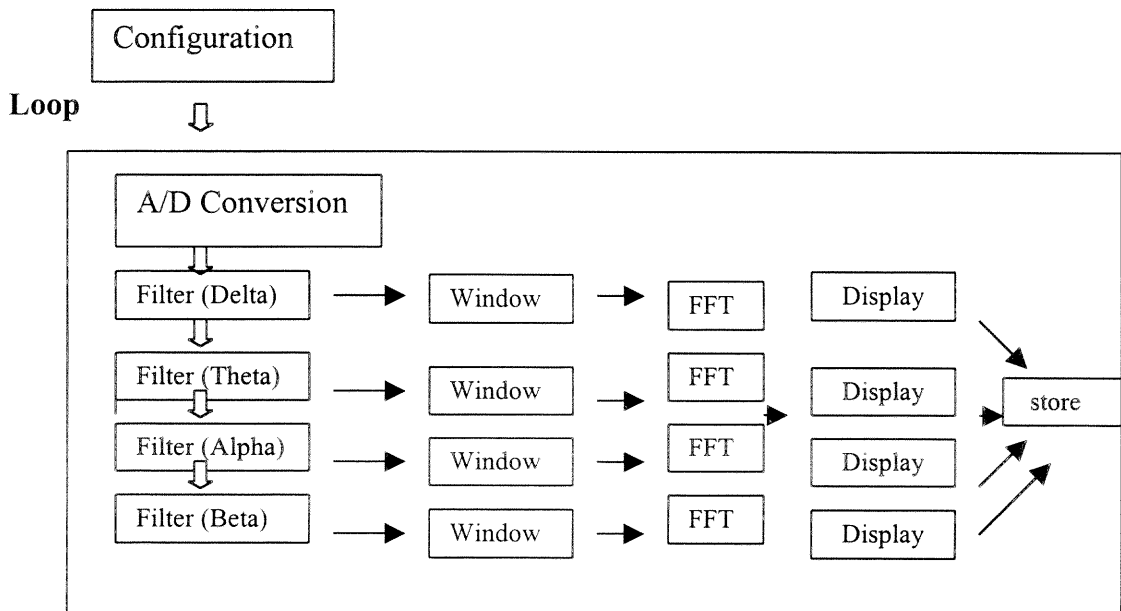
Figure 2.2 Block Diagram of the Software Development

Figure 2.2 shows a synopsis of the software development. First, the data collected from the amplifier were transmitted to the computer for the configuration. Then, the data were passed through four filters and windows and the FFT spectrum was plotted for each band and the data were stored in the computer. A thorough description of each step is discussed below.

The first few steps of the program set the parameters for proper communication between the software and the hardware. The "AI configuration" VI sets up the channel specification and the buffer size (refer to Appendix A for detailed LabVIEW program code). Three channels were used for this system. The first channel was for air puff delivery, the second for tone delivery and the third for recording of the EEG. The Buffer is the storage site in RAM where the data can be continuously acquired and retrieved. Two types of problems might occur where the buffer is involved in data acquisition. The

data might be retrieved slower from the buffer than it is placed into it; as a result, LabVIEW over writes the data[12]. Therefore, the size of the buffer should be decided very carefully to avoid any such dilemmas. In this particular system, a sampling rate of 1024 samples/sec was selected (more details regarding the selection of the sampling rate will be discussed in the next section). Since the sampling rate was very high, the buffer was set to 100,000 to provide enough storage of the data. The rate at which the data can be put in to the buffer is decided by the next VI.

2.3.2.1 Sampling Rate. According to the sampling theorem, the sampling rate should be at least twice the highest frequency to eliminate aliasing. Aliasing occurs when the requirements of the sampling theorem are not satisfied; consequently, the original signal is not recovered[13]. The sampling rate chosen for this particular system was 1024 samples/sec meaning every second, 1024 samples were stored into the buffer. The EEG ranges to 100Hz as discussed earlier in the amplifier section; therefore, a sampling rate of 250Hz would be adequate to satisfy the sampling theorem. However, in EEG, the frequency bands of interest are very narrow; as a result, good filter characteristics are required to separate each band (filters will be discussed in the next section).

Experimenting with different sampling rates proved that the sampling rate affected the output of the filter. At the lower sampling rate, due to lack of samples, the filter was unable to perform the sharper cut off needed for distinguishing the four bands of EEG. By increasing the order of the filter, a sharper roll off can be obtained; however, in order to increase the order, more samples were needed to do the required calculation. The sampling rate was chosen to be a power of 2 to allow the fast fourier transform (FFT) to be performed instead of a discrete fourier transform (DFT) (FFT will be discussed

below). By selecting a sampling rate of power of 2, the processing step of zero padding would be eliminated; therefore, the programming would become more efficient. Initially, it was hypothesized that the FFT would be performed on 1024 samples, and good frequency resolution would be obtained. However, after further testing, it was determined that zero padding was required for better frequency resolution (Δf) (this will be discussed below).

Thus, the sampling rate of 1024 samples/sec was chosen. There was no need to choose a higher sampling rate than 1024 because it would require more storage capacity and more processing time. The LabVIEW program code for this application was the "AI start VI", which initiated the background data acquisition and specified the sampling rate. Next, the data were transferred to the "AI Read", inside the for loop, where the data were taken out of the buffer every 2 seconds via number of scans to read. Initially the data were passed through a butterworth filter using a band stop filter between frequencies 58Hz and 62Hz to remove 60Hz line noise and the signal was plotted on the front panel (refer to Appendix A).

2.3.2.2 Filter Evaluation. The next step was to determine an ideal filter and its characteristics to produce the four common frequency bands of EEG from the raw signal. The evaluation of the filter was extremely critical for the EEG measurement. Since each band of the EEG is only separated from the next by 1Hz, a filter with a sharp slope had to be selected. A disadvantage to an increased slope is that it requires more processing time. Since the EEG had to be recorded continuously for 56 minutes, a longer processing time would affect the data collection and in turn slow down the experiment. Thus, a

balance was necessary where a sharper slope of the filter would not affect the processing time causing the data buffer to overflow.

In order to achieve this, a sub program was created to experiment and test different filters and their slopes within the LabVIEW environment. In this program, a "multi tone generator" VI from LabVIEW was used to produce sine waves of different frequencies. These sine waves were passed through various filters and the frequency representation for each sine wave was plotted. Sine waves of frequencies from 0.25Hz to 4Hz at 0.25 Hz increments were produced (see Figure 2.3 for the frequency generation and the filter specification). Various filters such as butterworth, chebyshev, elliptic and bessel (see Figure 2.4 for the general responses of the four filters) were tested. Further, the amplitude of each sine wave in the frequency domain was recorded (refer to Table 2.1 for the data).

Elliptic filters have the sharpest transition between the stop band and the pass band; however, they have ripples in its pass band and stop band[11]. Ripples in the pass band would eliminate the desired frequency and the ripples in the stop band would pick up the undesired frequency (table 2.1). The ripples in the pass band cause fluctuations in the frequency amplitude (0.00222, 0.00187, 0.00217, etc). Because of the ripples, frequencies within the four bands of the EEG can be altered and the elliptic filter would not be an ideal filter.

Chebyshev filters have sharper roll off than butterworth (butterworth will be discussed below) but they also have ripples in the pass band. Because of the ripples, all the frequencies within the pass band would not be treated equally and the power of some frequencies might be reduced. From the data in table 2.1, it can be observed that the

amplitude of the frequencies in the pass band is different (0.0022, 0.00184, 0.00223, etc). The power of all the frequencies does not remain homogeneous. If this filter was applied to separate the frequency bands in the EEG, uniform power within all bands of the EEG would not be obtained. Fluctuation of frequency power in the EEG due to filter type would not be tolerable. Therefore, Chebyshev would not be the best choice for this application.

Bessel filters, on the other hand, have a flat response but the filtering starts well before the specified cut off frequency compared to the other filters. This would result in the loss of the original signal. This was clearly seen from the data collected in table 2.1. At the frequency of 1.75Hz, the amplitude was 0.001, and consequently from that frequency at each increment of 0.25Hz, the amplitude kept decreasing (at the frequency of 2.75Hz, the amplitude=0.00022). Once some part of the original signal is lost, it would affect the final frequency output and all the frequencies present in the original signal might not be present in the FFT signal. The goal was to choose the best filter that would preserve most of the original signal and separate each band of EEG according to their cut-off frequencies. Thus, Bessel would not fulfill this requirement.

Butterworth filters have a maximally flat response at all frequencies; consequently, the loss of the original signal due to ripples would not be of concern. It has monotonic decrease from the specified cutoff frequencies[11]; thus unlike the Bessel filter, the pass band frequencies would not be affected. This was proved in the collected data from table 2.1. The amplitude of all the frequencies from 0.25Hz to 2.75Hz remain constant at 0.00224. This is different from any of the filters described above. This would be desirable for the EEG because by using this filter, one can obtain uniform power

among all the frequencies within each band. The Butterworth does not have a sharper slope than elliptic and chebyshev filters but at a higher order, sharper cut off can be achieved. For the test program, 10th order was selected (this is specified in the filter specification in figure 2.3). From the data in table 2.1, one can see that with order 10, at the frequency of 3.50Hz (cut off frequency) the amplitude was 0.00112 and at the frequency of 3.75Hz the amplitude decreased to 0.00043 (refer to table 2.1). This was almost a 50% reduction in the amplitude after the cut off frequency. In addition, at 4Hz frequency, the amplitude was 0.000. The undesired frequency (4Hz) was eliminated which was only 0.5Hz away from the cut off frequency (3.5Hz). This meant that the sharper cut off needed for separation of EEG bands can be achieved using this filter. Thus, the Butterworth filter was selected keeping flat response and desired sharper cut off at the forefront in the EEG processing. The LabVIEW VI to perform this function was "Digital IIR filter" VI. Four filters for four different bands were used with the same filter characteristics (the details about high and low cut off frequency for each band are shown in Appendix B).
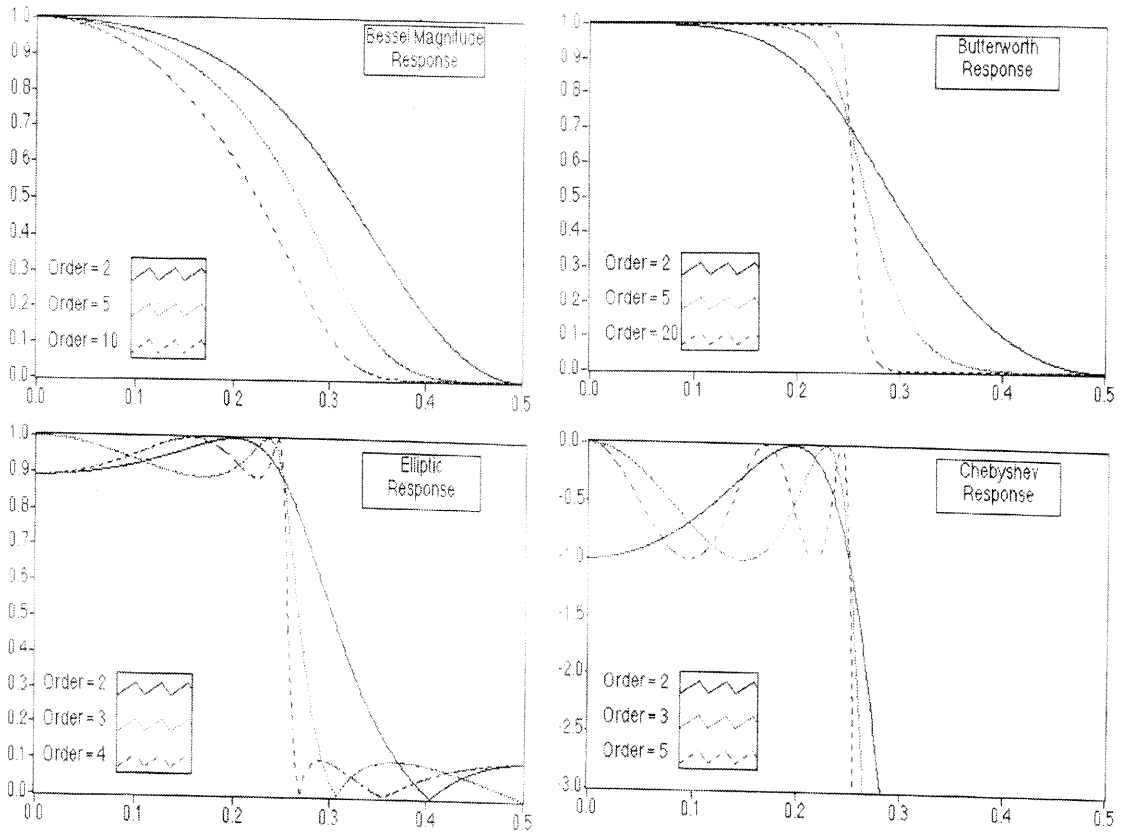
Figure 2.4 Responses of Bessel, Butterworth, Elliptic and Chebyshev filters

In order to check how the order affects the processing time, a timer was put in the LabVIEW test program mentioned earlier. Table 2.2 shows the different filters and the processing time for order 10, 15 and 20.

Table 2.2 Processing time for different filters at different order

	Order 10	Order 15	Order 20
Chebyshev	795ms	867ms	884ms
Butterworth	620ms	730ms	755ms
Bessel	716ms	741ms	761ms
Elliptic	810ms	873ms	867ms

Since the EEG had to be recorded continuously, longer processing time would affect the data collection and in turn slow down the experiment. Since the data were taken out in 2 seconds blocks from the buffer, data collection, real time processing and storage of the data had to be done in 2 seconds. Therefore, higher order than the order of 10 would not be advisable because that would slow down the processing. Based on table 2.2 the lowest processing time was achieved at order 10 with the butterworth filter. At order 10, the desired sharper slope was also achieved as mentioned earlier. Therefore, a butterworth band pass filter with order 10 was selected for processing of all bands. The order could not be increased to more than 10 because slowing down of the experiment was observed.

2.3.2.3 Windowing, FFT and Zero Padding. After the filter, the signal was passed through a windowing function before the FFT was performed. Since the data are processed every 2 seconds, there can be discontinuity at the conversion edges of each period. Applying windowing minimizes this discontinuity and separates smaller amplitude signals from larger amplitude signals. Passing a signal through a window simply means multiplying the original signal by the window function. It changes the shape of the signal in the time domain[12]. Various windows such as rectangular, exactblackman, Bartlett, hamming and hanning were tested. Every window has a main lobe width and a side lobe height.

Table 2.3 –Properties of four window functions

Window function	Main lobe width	Side lobe height
Rectangular	2/N	-14dB
Bartlett	4/N	-25dB
Hanning	4/N	-32dB
Hamming	4/N	-44dB

The purpose of the window is to reduce the side lobe height, which can make the conversion of the next period smoother. The Hamming window has the lowest lobe height (-44dB)[12], which was used in this program. It has a sinusoid shape and the edges do not get close to zero. The programming VI used for this application was "scaled window" VI.
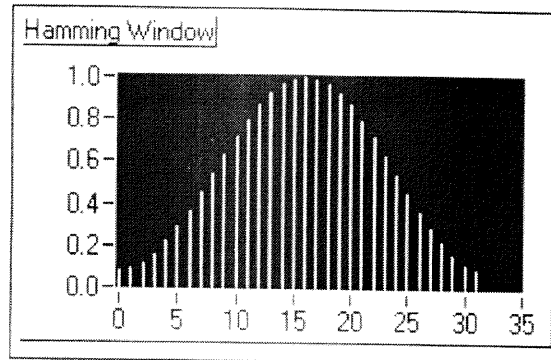

Figure 2.5 Hamming Window

The next step of the programming was to obtain a frequency representation of the four bands. The Fourier transform establishes the relationship between a signal and its representation in the frequency domain. There are number of issues related to the Fourier transform such as the number of points in the resulting transform and the distance between each point. The distance between each point in the frequency domain in the resulting transform is referred to as the frequency resolution. Mathematically, frequency resolution(Δf) is the reciprocal of the length of the time signal (L) in seconds ($\Delta f = 1/L$)[12]. The epoch length for this particular EEG system was 2 seconds, which produced a frequency resolution of 0.5 Hz.

For the EEG processing, the frequency resolution of 0.5Hz was not satisfactory; consequently, the time signal needed to be lengthened. One way to increase the time signal is by using a method called zero padding. In zero padding, zeros are added at the

end of the original signal at the same time spacing as the signal. As a result, the frequency resolution will be the reciprocal of the length of the zero padded signal. This does not affect the actual signal but its resolution increases. For the EEG system, 2^{13} zeros (8192) were added and since the sampling rate was 1024 samples/sec, 8 seconds (8192/1024) of zeros were added to the original signal yielding the total time length of 10 seconds (original 2 seconds+ 8 seconds of zeros). This produced a frequency resolution of 0.1Hz(1/10sec). A FFT was performed on this input signal by using "power spectrum" VI in the LabVIEW. The output of the FFT for the four bands was plotted on the front panel (See Appendix A).

To carry out this complex processing, computer power turned out to be extremely essential. Initially the program was built on a Pentium II IBM with a 550MHz processor. This worked fine for the initial stage of the program but when the physiological signals from the electrodes were input to this computer, it stopped processing. As the program became more sophisticated, a better computer was needed. At that point, a faster computer was required for real-time processing and a Pentium III IBM with a 700MHz processor was used for running the experiment, data collection and processing.

The software provided the tools to accomplish real time processing of the EEG. "AI configuration", "AI start" and "AI read" laid out the basic parameters of acquiring the data at 1024 samples/sec and taking out every 2 seconds from the buffer for further processing. The butterworth filter with order 10 provided the frequency differentiation necessary to separate the four bands of the EEG. Then, the FFT was performed to convert the signal into the frequency domain. The next and final step of the configuration was to open a file and store the A/D data. A file dialog box VI was chosen to carry out

this particular function. When the user ran the program, he or she had to enter the file name for the storage of the A/D data. Once the file was entered, the experiment was ready to begin (refer to Appendix D for the block diagram of the LabVIEW code for each VI).

2.4 Protocol and Artifact Concern

The protocol section of the program was set up to perform two different tasks using two different computers. One computer was used to control the conditioning experiment and the other was used for the acquisition and the real time processing of the EEG. In the eye blink conditioning system two types of protocols; delay 1 and delay 2 were created. In the delay 1 protocol, there were 120 trials of 20 or 30 seconds duration. During each trial there was an air puff followed by a tone. The tone was 500ms long and co terminated with a 50ms long air puff given at 5psi. Every 10 trials, there was one trial with just a tone and another with just an air puff. In the delay 2 protocol, everything remained the same except the tone was changed from 500ms to 1 second. This change was executed to see whether the longer stimulus has any effect on the EEG.

Over 56 minutes of the experiment, the subject is expected to acquire the associative relationship of the stimuli by correlating the air puff with the tone. The continuous recording of the brain activity during this time would demonstrate whether the associative learning has any impact on the normal rhythms of the brain.

While recording any type of electrical potentials from the body, artifacts are a major concern. Artifacts originate from the surrounding electrical equipment, motion artifact and other physiological signals. If the artifacts are not removed from the signal,

they mask the original signal and thus could result in inaccurate readings. Filters used in the EEG system are optimal in getting rid of motion artifact and the artifact created by 60Hz cycle noise. 60 Hz noise can be created due to poor electrode preparation, a poor common mode rejection ratio (CMRR) between the inputs and other electrical equipment in the vicinity of the patient.

However, artifact created by an eye blink is still an issue of concern. Eye movements produce electrical activity on the scalp due to muscle firing and interfere with the EEG. The electromyogram frequency of an eye blink is approximately 2Hz. Thus, this possible artifact due to an eye blink can affect the delta band of the EEG, which has a frequency ranging from 0.5Hz to 3.5Hz. Within the scope of the project, every possible effort was made to design extremely tight filters, which would remove most of the artifact due to eye movement. In the eye blink conditioning experiment, the theta band is of interest to observe the relationship between learning and EEG[5]. Therefore, artifact in the delta band would not affect the band of interest (theta band) and its correlation with learning. However, removing this artifact completely is for future work. Many methods exist in the literature to remove the artifact of an eye blink from the EEG signal. One method describes subtracting a fraction of the eye blink signal from the EEG[13]. The subtraction fractions in this particular paper were obtained individually for each electrode site on the basis of the regression coefficient derived from an analysis of 50 blinks[13]. Another way to find a solution for this problem is to average a number of eye-movements. As the number in the average increases, eye blink magnitude increases relative to the EEG and it can be removed[14].

Table 2.1 FFT Amplitude of the frequencies generated in the test program for filter evaluation

Frequency (Hz)

	0.25	0.5	0.75	1	1.25	1.50	1.75	2
Chebyshev (Amplitude)	.0022	.00184	.00223	.00195	.00178	.00207	.00219	.00183
Butterworth (Amplitude)	.00224	.00224	.00224	.00224	.00224	.00224	.00224	.00224
Bessel (Amplitude)	.00211	.00222	.00207	.00184	.00156	.00128	.00100	.00074
Elliptic (Amplitude)	.00181	.00211	.00222	.00187	.00198	.00178	.00187	.00217

Frequency (Hz)

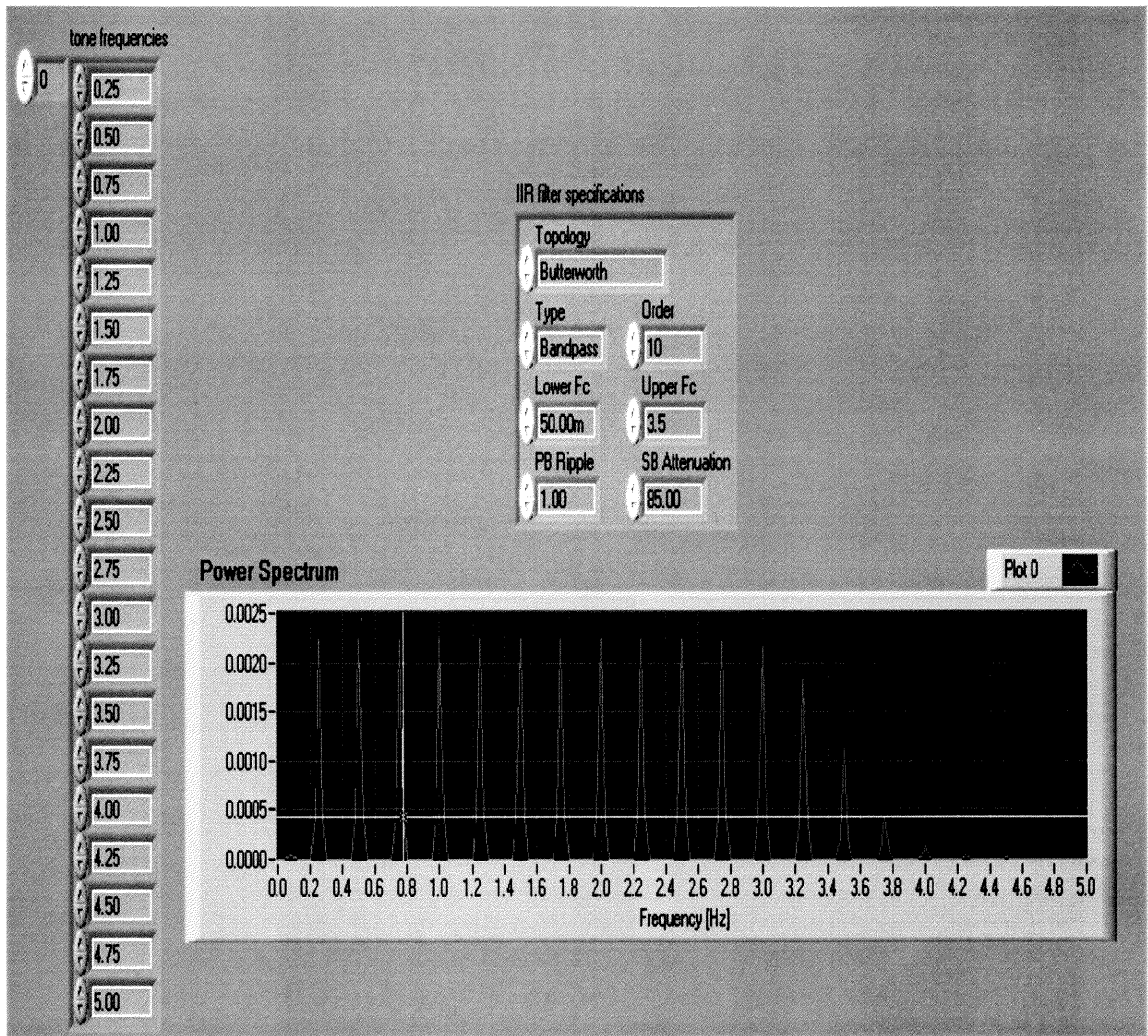|	2.25	2.50	2.75	3.00	3.25	3.50	3.75	4.00
Chebyshev (Amplitude)	.00187	.00223	.00186	.00198	.00195	.00175	.00020	0.000
Butterworth (Amplitude)	.00224	.00223	.00022	.00215	.00184	.00112	.00043	0.000
Bessel (Amplitude)	.00035	.00022	.00022	.00013	.00007	0.000	0.000	0.0000
Elliptic (Amplitude)	.00214	.00180	.00198	.00210	.00201	.00111	0.00020	0.0001

Figure 2.3 Front Panel User Interface for the Test Program

CHAPTER 3

SYSTEM UTILIZATION

3.1 Procedure

Six healthy control subjects, three males and three females with ages ranging from 18 to 29 were asked to participate in this experiment. All subjects upon arrival were asked to remove contact lenses if they wore them and then they were instrumented. Two physiological signals (EMG and EEG) were recorded during the experiment using two different computers. The international federation of 10-20-electrode system set up by the International Federation of EEG societies was used to determine the placement of electrodes for measuring the EEG. For recording one channel of EEG, the placement of the two electrodes had to be determined. As mentioned earlier, the cerebrum controls learning and the outer layer of the cerebrum, the cerebral cortex contains the association areas. The Cerebral cortex originates at the frontal lobe and covers some areas of the temporal and the parietal lobe. Thus, it would be adequate to measure the EEG from the frontal lobe where learning is thought to take place. Further, at the frontal positions, the artifact due to hair would be greatly reduced. As a result, Fp1 and Fp2 positions were selected for the placement of two electrodes and the earlobe was selected as the ground electrode for recording EEG. The electrodes used were standard surface silver/silver chloride (Ag/AgCl). To reduce the impedance at the electrode-skin interface, the subject's skin was cleaned with a sterile wipe.

The EMG was measured for the assessment of classical conditioning through the evaluation of the eye blink response. One electrode was placed above the eye and the

other was placed below the eye to measure the signal generated from the eye muscles while blinking. The ground electrode for EMG was placed on the side of the neck. The next step was to place the headphones on the subject's head to deliver the tone. The eyepiece for the air puff delivery was mounted on the headphone. Both the headphones and the eyepiece were adjusted by the subject to make them feel comfortable. Three subjects (subjects 1, 2 and 6) were run with delay 2 protocol (1000ms tone) and other three subjects (subjects 3, 4 and5) were run with delay 1 protocol (500ms tone).

3.2 Data Collection and Processing

3.2.1 Data Collection

As mentioned earlier, one computer recorded the EEG and the other computer recorded EMG for the detection of the eye blink. The goal was to divide the EEG data into delta, theta, alpha and beta bands according to their frequency. Once the four bands were collected, the purpose was to determine if there is any correlation between the onset of the stimulus and recorded EEG. The EEG signals from the amplifier were A/D converted at a sampling rate of 1024 samples/sec.

The software program developed for the EEG system processed the data in 2 second blocks; however, this parameter needed to be changed for this particular application. In order to observe the effect of a tone on EEG, a 2 second block is a long time to draw any conclusion. Since the tone is only 500ms and 1000ms in duration, information stored in that 1 second block would provide valuable information instead of 2 seconds block which will also include an air puff. An air puff is a forced stimulus and it will not give the correct measure of a person's learning. To extract the effect of the

tone on the EEG, the data were processed and stored in 1-second blocks. For the data analysis, a one-second block at the onset of tone in each trial was used.

The EMG was processed to determine if, and when, an eye blink occurred during the stimulus trial. The program written in S-plus language[10] was used for processing the EMG. First, marker channels (tone and air puff) were identified in each trial and a 1-second duration at the onset of the conditional stimulus (tone) was analyzed to determine if the blink occurred. If the blink occurred in the CS duration prior to the US (air puff), it was considered as the conditioned response. This meant that the subject learned to blink at the onset of the tone. The eye blinks recorded in the conditioned response period and unconditioned response period were plotted for 120 trials. Since the learning behavior of the subject is determined from the conditioned response, a graph of all the conditioned responses (learning curves) of the six subjects was plotted. Figure 3.1 shows these learning curves that provide the information regarding a person's learning activity throughout the experiment. Subjects 2, 4 and 5 acquired the conditioned response while subjects 1, 3 and 6 did not acquire the conditioned response. A patient has acquired the conditioned response if there are 60% conditioned responses in any block of 12 trials.

3.2.2 Data Processing

Once the EEG data were collected, the next step was to figure out various ways to analyze these data and organize it in a way that would be easy to examine. One method was to observe the overall activity of the EEG throughout the experiment. In order to achieve this, the EEG activity within a 1 minute block was summed for each band (delta, theta, alpha and beta) for all the subjects. The plots for all six subjects can be seen in

Figure 3.2. For all the subjects, there were major fluctuations in the EEG power of all bands. There was no pattern observed that can be correlated to learning. The second technique was to separate the subjects according to their protocols (500ms tone vs 1000ms tone) which would suggest whether the longer or shorter stimulus has any effect on learning. The final scheme was to compare a 1-second interval in the EEG for each trial at the onset of the tone and compare the theta activity of the EEG between the subjects who acquired the conditioned response and the subjects who failed to acquire the conditioned response. To accomplish this, a one-second interval at the onset of the tone was extracted from the theta band of the EEG in each trial and its response was plotted for all six subjects (see figure 3.3). This figure showed that out of the three subjects (2, 4 and 5) who acquired the conditioned response, subjects 4 and 5 showed more theta activity. On the other hand, subject 2 did not show any theta activity. Subjects 1, 3 and 6 who did not acquire the conditioned response, showed minimal to no theta activity. The quantification of all the data is described in the next section.

3.3 Results

Before running the subjects, it was necessary to check whether the filters and the FFT selected for processing EEG performed the functions accurately. To verify this, the program created initially to do filter evaluation was run several times to find out the output of the filter and the FFT. Digitally created test signals consisting of sine waves of frequencies from 0.25Hz to 4Hz at 0.25Hz increments were passed through different filters and their output in the frequency domain were plotted (refer to figure 2.3 for the visual display and table 2.1 for the data). The plot showed that frequencies produced in

the power spectrum were the same as in the original signal and changing the pass band of the filter, the program was able to eliminate the undesired frequencies. From the plot, it can be seen that sine waves of frequencies from 0.25Hz to 4Hz at 0.25Hz increments were produced and that in the power spectrum the same frequency representation of each sine wave was achieved (frequency peak at 0.25Hz, 0.5Hz, 0.75Hz, etc). This program did not involve any analog to digital conversion. Therefore, the next step was to confirm whether the final program generated to collect data from the subject did all the processing parameters correctly and stored the data.

The program was run ten times and no errors were observed while recording or processing. No changes were made in the program in each run. Once it was confirmed that the program was running well with this simulated input, six subjects were run and the recorded data were analyzed. Delta, theta, alpha and beta bands were plotted and showed continuous recording of all bands over the entire range of the experiment. The plot of one of the bands from one of the subjects can be seen in figure 3.4. This proved the validity of the software program and its purpose of processing and differentiating the brain activity into four bands of EEG based on frequency.
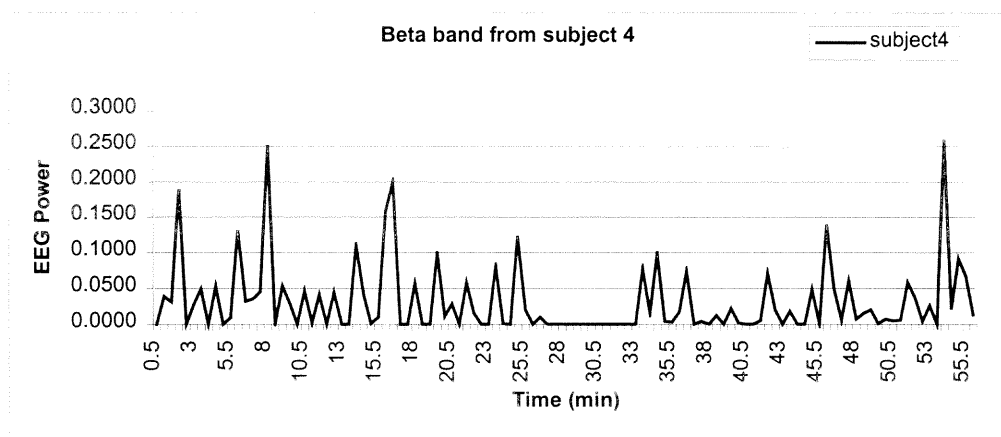

Figure 3.4 Frequency representation of Beta band from one of the subjects

The collected data were examined in various ways to determine if there is any correlation between learning and EEG. The overall activity of all bands for six subjects for the entire duration of the experiment was plotted (see Figure 3.2). For all the subjects, there were major fluctuations in the EEG power of all bands No pattern was observed between the EEG bands and learning.

Next, the EEG power in the theta band was plotted related to the conditional stimulus trials (see Figure 3.3). There were 110 trials where the tone was delivered and 1 second of the theta activity at the onset of the tone for each trial was plotted for the six subjects. The goal was to compare the theta activity of the subjects who acquired the conditioned response to the subjects who did not acquire the conditioned response. The following table divides each subject by their learning ability, the type of the protocol they received and the sum of the theta activity.

Table 3.1 Division of the subjects according to the CS duration and learning behavior

Subject	CS Duration(ms)	Learned	theta activity
1	1000	NO	0.015
2	1000	Yes	0
3	500	NO	0.117
4	500	Yes	0.48
5	500	Yes	2.04
6	1000	NO	0.262

Subjects 2, 4 and 5 acquired the conditioned response and thus fell in the category of subjects who developed associative learning (refer to figure 3.1). However, subjects 1, 3 and 6 did not acquire the conditioned response as the experiment progressed and did

not develop associative learning (refer to figure 1). Subjects 1 did not satisfy the requirements of acquiring the conditioned response (refer to figure 3.1). Actually, in the beginning of the experiment, subject 1 showed learning and as the program progressed, learning subsequently decreased. This is very unusual and the responses achieved in the beginning of the experiment can be treated as coincidental. The EEG data showed that subjects 4 and 5 who developed learning also had more theta activity than any of the other subjects (refer to table 3.1). The theta activity was calculated by acquiring the area under the curve of the frequency bands. However, subject 2 who developed learning, did not show any theta activity. Thus, it cannot be concluded that there is a coorelation between learning and theta activity when looking at over the whole time period. The above discussion shows that the EEG system had the capability of recording the data continuously and distinguishing it into four common bands of EEG.
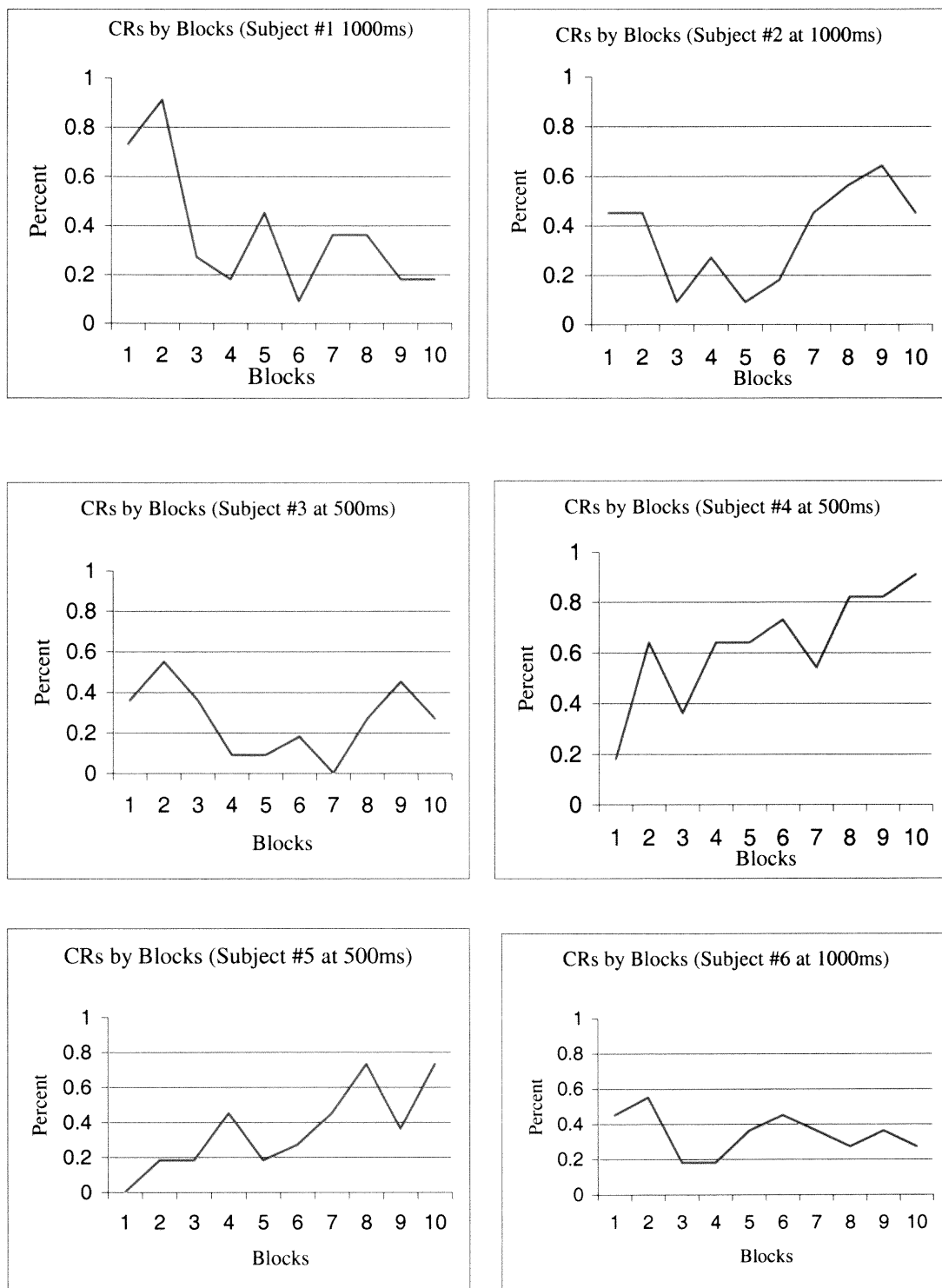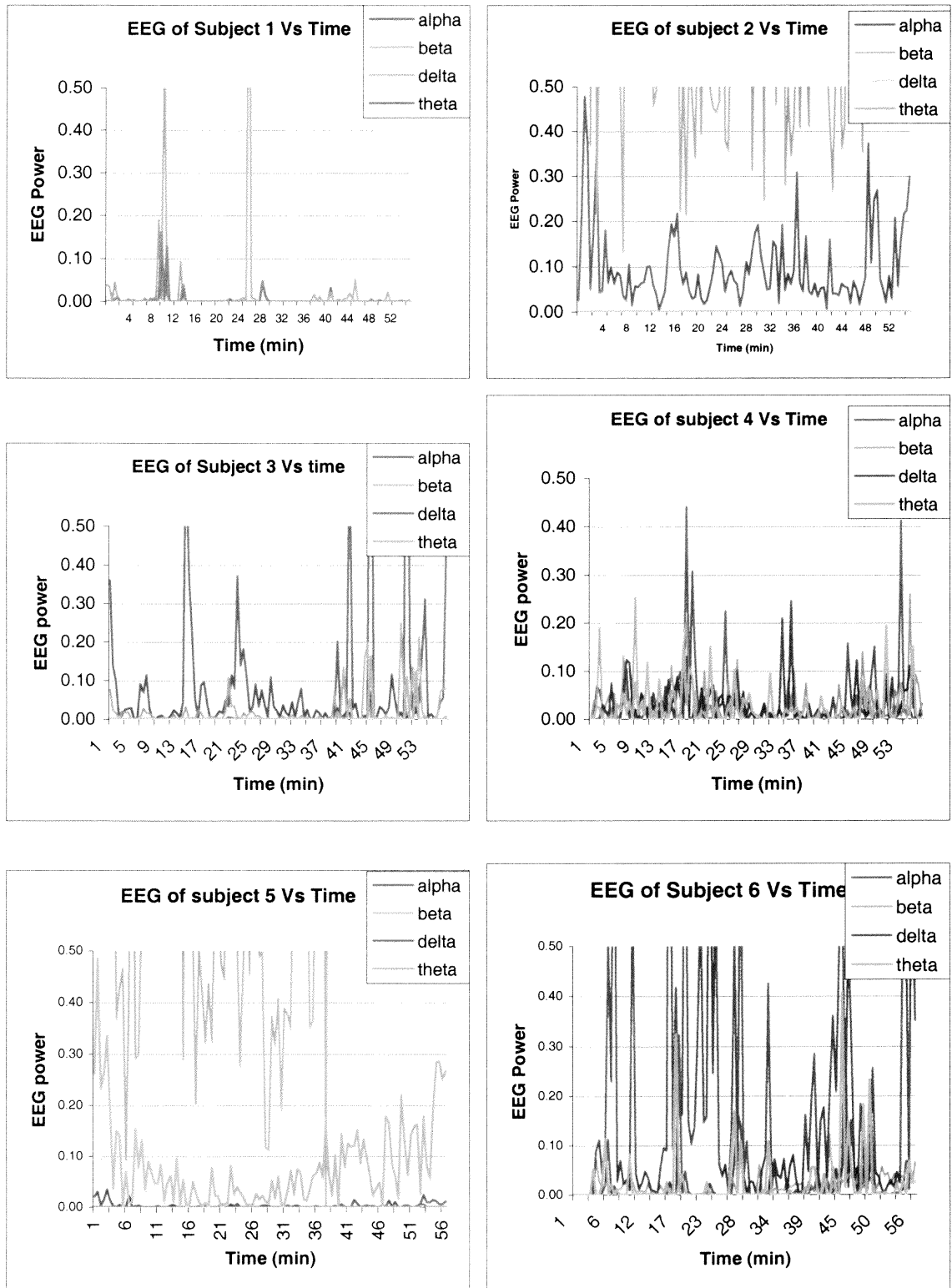
Figure 3.1 Learning Curves of Subjects

Figure 3.2 EEG of the entire experiment for six subjects
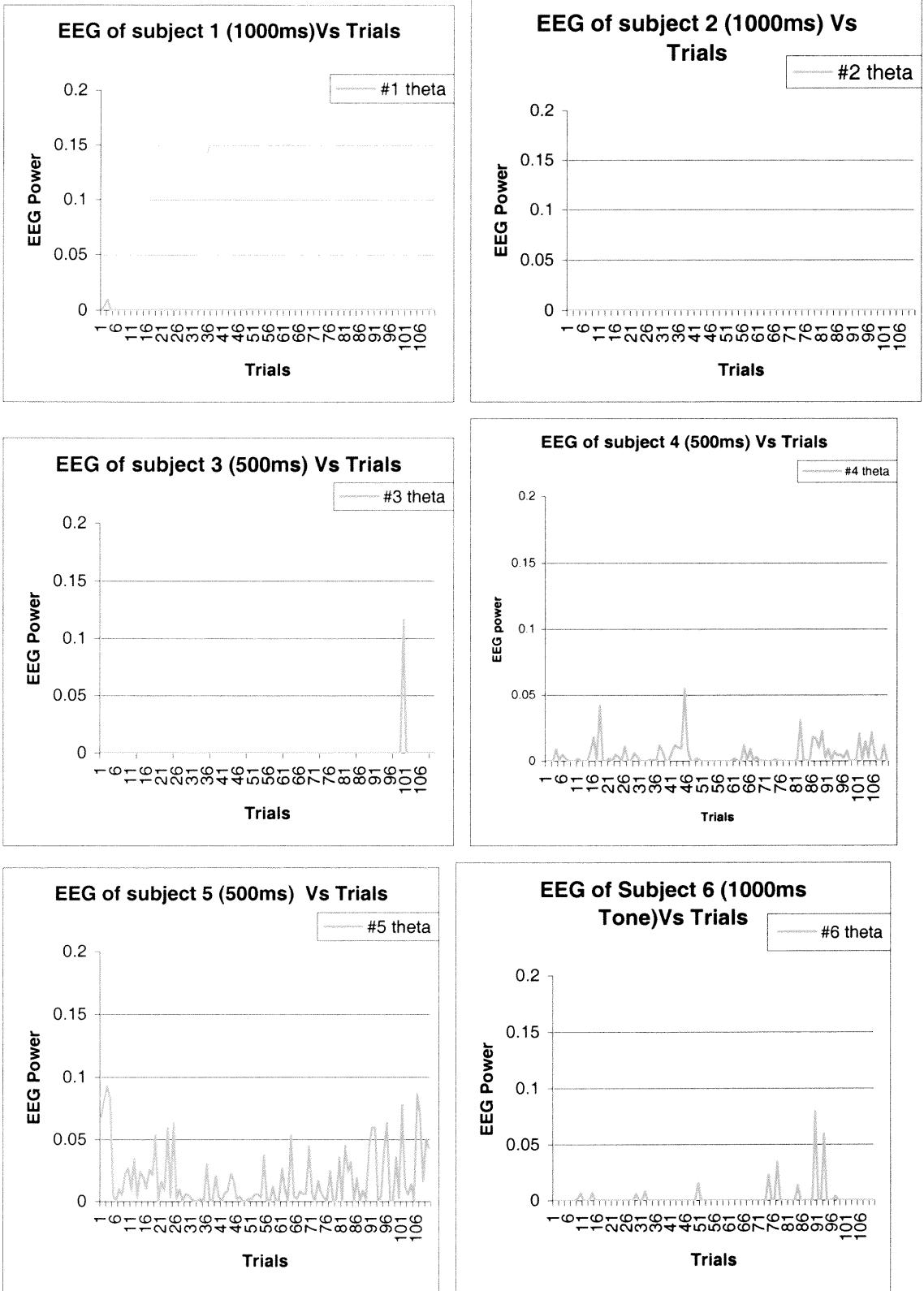
Figure 3.3 EEG of all subjects versus CS trials

CHAPTER 4

CONCLUSIONS

The main goal of this project focused on creating a generic software program to record and perform on line processing of EEG. Extra precision was required in selecting some of the important parameters of processing such as filtering, windowing and FFT. The software was checked many times to evaluate its signal quality, processing time and frequency resolution. The best possible parameters were chosen so that delta (0.5-3Hz), theta (4-7Hz), alpha (8-13Hz) and beta (14-25Hz) waves can be differentiated according to their frequencies.

Through a series of tests, the system was shown to be accurate and reliable. Once the tests were completed, to check this system in an experimental setting, an already existing classical conditioning protocol was used. The hypothesis was that learning may be correlated to EEG and some pattern may be revealed that can describe the relationship between associative learning and EEG. Based on a finding in animals, during classical conditioning, more theta activity suggests enhanced learning[5]. Based on this, an attempt was made to compare the theta activity of the subjects who achieved learning versus the theta activity of the subjects who failed to learn.

Subjects 4 and 5, who developed learning, showed theta activity of 0.48 and 2.04 respectively when observing prior to the US. On the other hand, subjects 1, 3 and 6, who did not develop learning showed much lower theta activity (subject 1=0.015, subject 3=0.117 and subject 6= 0.262). This supports the finding observed in animals and it can be taken further in future. More human subjects can be tested in the future and the theta

activity of the subjects who developed associative learning can be compared to the theta activity of the subjects who did not develop learning. This reference[5] also showed that learning can be enhanced by giving the stimulus during the theta activity of the EEG. Animals were given the stimulus as soon as the theta activity was observed in the EEG. The animals that received a stimulus during the theta activity learned faster compared to the animals that were given the stimulus randomly. With on line processing of the EEG, this type of experiment in the humans can be performed.

More data were analyzed for correlation between EEG and associative learning. No pattern was obtained by observing the EEG activity of all subjects during the entire experiment (see figure 3.1). When the EEG activity of all the subjects was observed according to the duration of the conditional stimuli (500ms vs 1000ms tone), the subjects who received the stimulus for 500ms, showed more theta, alpha and beta activities, while more delta activity was observed in the subjects who received tone for 1000ms. Out of three subjects who received the conditioned stimulus for 500ms, two of them developed learning. This suggests that by providing a shorter tone with the air puff, the associative learning ability of a person is increased due to a shorter stimulus duration. However, in response to a 1000ms tone, the subject is unable to develop a correlation because of longer duration of the conditioned stimulus followed by the unconditioned stimulus.
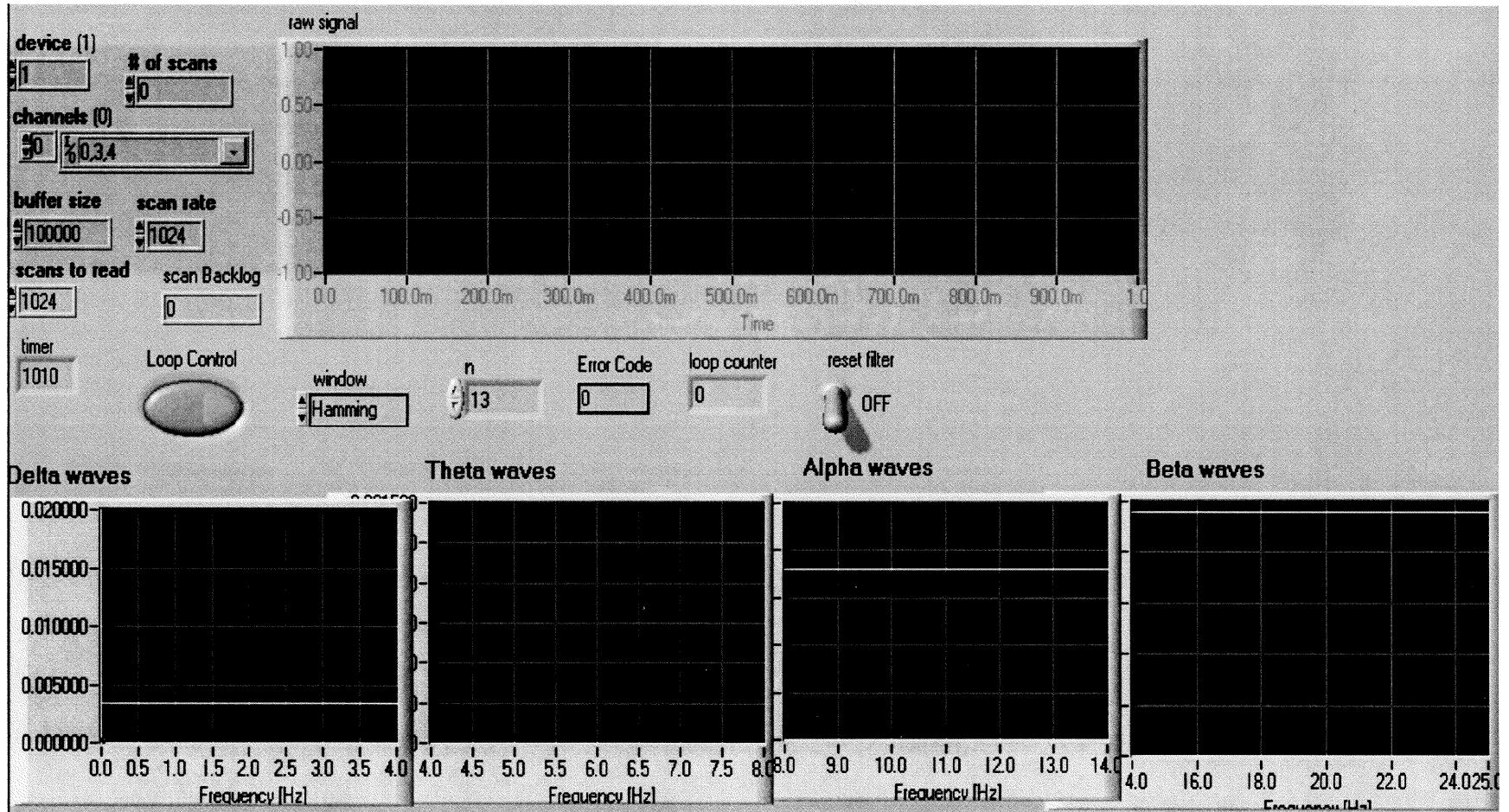
The testing of the program proved the accuracy of the developed software. The program differentiated each band of EEG and performed these calculations in real time. The real time processing of the EEG can open up more advanced research topics where on line processing of the EEG can be used to train mentally disabled person to communicate with rest of the world by their brain activity. The developed program can

be utilized in many other applications such as studying mental disorder, distinguishing mental tasks, classifying sleep stages etc. This program provides the basic foundation and according to individual experimental needs, program parameters such as sampling rate, filter type, window type and filter order can be changed.

There are several future directions for the continued improvement of this system. As mentioned earlier, artifact due to an eye blink can be an issue of concern. Further research is needed to find a method to eliminate the eye blink in a way that would not affect the programming of the existing EEG software. To explore the relationship between learning and the EEG and to test a similar observation obtained in animals, the experiment protocol needs to be changed as follows. This software performs real time processing; therefore, the presence of theta activity can be spotted during the experiment. As soon as the theta activity is observed, the stimulus can be given and its effect on learning can be analyzed.

FRONT PANEL USER INTERFACE FOR EEG PROGRAM

**FILTER CHARACTERISTICS OF THE FOUR BANDS OF EEG – LABVIEW CODE**

APPENDIX C

DETAILED DESCRIPTION OF BUTTERWORTH FILTERS

A smooth response at all frequencies and a monotonic decrease from the specified cutoff frequencies characterize the frequency response of Butterworth filters. Butterworth filters are maximally flat-the ideal response of unity in the passband and zero in the stopband. The half power frequency or the 3-dB down frequency corresponds to the specified cutoff frequencies.
The following illustration shows the response of a lowpass Butterworth filter. The advantage of Butterworth filters is a smooth, monotonically decreasing frequency response. After you set the cutoff frequency, LabVIEW sets the steepness of the transition proportional to the filter order. Higher order Butterworth filters approach the ideal lowpass filter response.
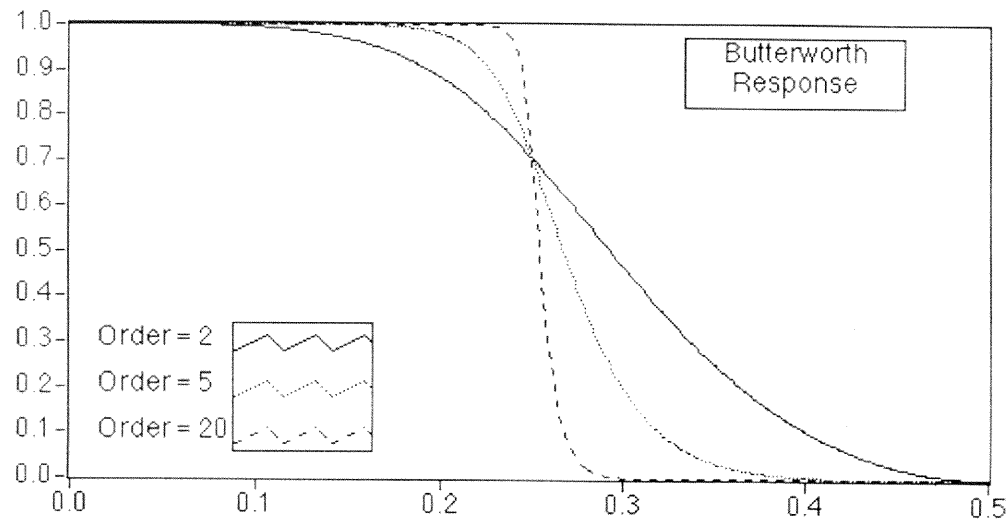
APPENDIX D

BLOCK DIAGRAM OF EEG PROGRAM- LABVIEW CODE
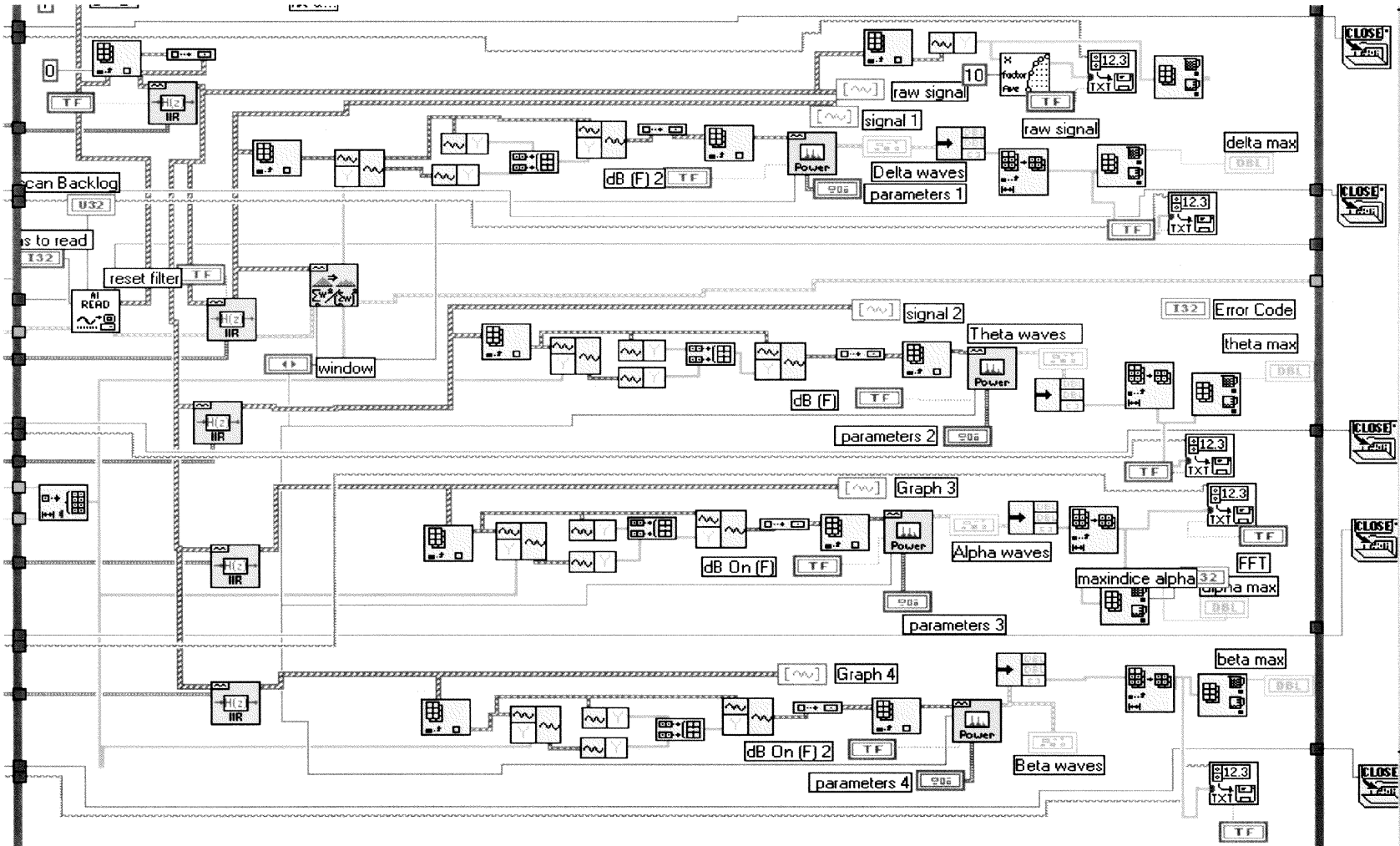
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