ABSTRACT

The use of the classically conditioned eyeblink response, a form of associative learning, is a growing method of experimentation in modern science. This type of associative learning, has many features that make it useful for applications in the study of specific neurological functions. The goal of this project was to design and implement a software system for the automated control and on-line evaluation of a classical conditioning experiment for use with human subjects. A program was developed in the LabVIEW programming environment by National Instruments. Basic hardware components produce acoustic signals, deliver airpuffs, and sense and condition physiological responses. Safety features are utilized to eliminate hazards to test subjects. The apparatus is optimized for performance to reduce the cost of human experimentation. To verify the accuracy, reliability, and safety of the apparatus a series of tests was performed.

Chronic Fatigue Syndrome patients and healthy controls were tested in a sensory reactivity protocol, using white noise at three intensities, and a delay protocol for the evaluation of associative learning through the measurement of the classically conditioned eyeblink response. Though only a pilot study, the design of the experimental system has the reliability and sensitivity for the measurement of this type of experimentation.

COMPUTER AUTOMATED EXPERIMENTATION
FOR THE CONTROL AND ASSESSMENT OF
THE CLASSICALLY CONDITIONED EYEBLINK RESPONSE

by
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THE CLASSICALLY CONDITIONED EYEBLINK RESPONSE

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This thesis is dedicated to my wife and our families.
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TABLE OF CONTENTS

Chapter   **Page**

TABLE OF CONTENTS
(Continued)

Chapter   **Page**

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES
(Continued)

Figure   **Page**

CHAPTER 1

1 INTRODUCTION

1.1 Objective

Classical conditioning is a simple form of learning; the modification of reflex responding in anticipation of stimuli that alter homeostatic function. At the heart of this form of learning is the timing of the cue stimulus (conditioned stimulus (CS)) and the reflex-eliciting stimulus (unconditioned stimulus (US)). The goal of this project was to design and implement a software system for the automated control and on-line evaluation of a classical conditioning experiment for use with human subjects. The basic hardware components produce acoustic signals (CSs), airpuffs (US), and measure physiological responses. The main goal of this project was to develop software to coordinate and control these hardware components.

The requirements for the software were to: precisely control the initiation of the CS and US, evaluate physiological signal quality, predict problems, and record physiological responses. The software needed to be executable from a notebook computer. Overall, the experimental system was conceptualized as a computer/software control element coordinating two primary subsystems: a stimulus generation element and a physiological response element. Tests were performed to verify performance within the primary subsystems.

An important aspect of this experimental paradigm is a concern for safety. Basic hazards were identified. The use of electrodes with humans presented a hazard due to the possibility of ground-faults. A standard solution was implemented; patients were connected to battery-operated physiological amplifiers. A medically-rated isolation system was used between the physiological response elements and the stimulus generation element of the

1

primary subsystem to prevent ground faults from occurring. The experimental protocol involved the delivery of acoustic stimuli through headphones. A operator-controlled "panic button"-- a cutoff between the audio amplifier and the subject -- was developed. Lastly, the experimental protocol involved the delivery of airpuff stimuli to the eye of the subject. Two solenoids controlled the path of air to the eye. These solenoids were chosen such that their mechanical and electrical characteristics were opposite, that is, one solenoid was normally open, while the other was normally closed. Therefore, in the event of apparatus failure the path between the eye and air source was closed. A series of tests were performed to verify that each safety issue was successfully addressed.

Human experimentation can be very costly. Costs are attributable to the patient's time and effort, limitations of the patient pool, patient reimbursement and technician time and effort. To minimize these costs, the experimental system was designed to be efficient and reliable. A novel aspect of the software control system was an online evaluation of physiological signal integrity. The software predicted possible problems by strategically timed computer checks. In the unlikely event of a computer crash, a recovery method was implemented. To verify reliability and efficiency of the system a series of simulated system failures were performed.

For decades, neuroscientists have studied new motor learning in humans through classical conditioning of the eyeblink response. Technological advances enable a reassessment of how the conditioning experiment is controlled, how physiological signal integrity is verified. A software solution is offered for the coordination of stimulus generation and physiological response sub-systems. Through this software solution system reliability and signal fidelity were optimized to reduce the expense of human experimentation.

1.2 Background Information

1.2.1 Software and Instrumentation

Software and instrumentation technology has been consistently changing over the past 35 years. With the increases in computer power, software has made great strides in providing users with a continuous increase in performance at a lower cost. Also with the increases in computer power and the advances in semiconductor technologies, instrumentation has been able to provide new abilities to record and control an increasing array of instruments and applications.

In 1965, Hewlett-Packard designed the Hewlett-Packard Interface Bus (HP-IB) to connect their line of programmable instruments to their dedicated controllers. Because of its relatively high transfer rate this interface quickly became popular and became adopted as an industry standard by the Institute of Electronic and Electrical Engineers (IEEE) in 1975 called IEEE Standard 488-1975. Through the years this standard was advanced by Hewlett-Packard and then by others, like National Instruments (National Instruments - Austin, TX). National Instruments expanded the use of the IEEE-488 bus to computers manufactured by companies other than Hewlett-Packard. Thus, the development of the General purpose Interface Bus (GPIB). Other standards in computer interfacing have also been developed for a wide array of applications. In 1987 National Instruments introduced the VXIbus. This bus started its use in traditional test and measurement applications and later grew into a platform for data acquisition and analysis in research and control applications.

A growing instrumentation alternative is plug-in data acquisition. Plug-in data acquisition became possible because of advances in digitizers, computer power, and software power. Plug-in data acquisition boards are currently available for most types of computer

architecture buses, such as PC-AT, ISA, PCI, PCMCIA, Sun Micro systems, and the Macintosh NuBus. The user optimizes different capabilities of A/D conversion or D/A conversion alone, or in combination. Such features as sampling rate, resolution, data transfer methods, and channel capability are available. Different combinations of digital I/O and counter/timer operations can also be combined to allow a user to select a plug-in board to best suit an application.

Plug-in data acquisition boards are commonly used for data collection from transducers that are measuring physical phenomena from the real world. For example, thermocouples and thermistors are used to measure temperature, strain gauges to measure force, and other transducers to measure flow or pressure are also common. In medical research the use of plug-in data acquisition boards has allowed the measurement of multiple high speed channels from physiological sources of the human body.

With a continuing advancement of computer power, software capabilities have also increased performance. Where once many signal processing tasks required expensive electronics to condition a signals, like providing gain or a filter, today many of these tasks can be performed in software. This type of software application provides users with an increase in power and flexibility at a lower cost. Signals can be acquired at very fast sampling rates where algorithms can provide parameters that far exceed that of analog methods. Acquired signals can be filtered or amplified, mathematical operations like integration or differentiation can be performed in real time, jobs once done with pre-acquisition signal conditioning electronics. LabVIEW® by National Instruments is a complete data acquisition and control software package. Through the use of a graphical interface, this software give the user power and flexibility for instrument control, data acquisition, data analysis and connectivity.

LabVIEW for Windows 95® (Microsoft Corporation) takes advantage of the 32-bit architecture and flat memory model to improve speed for critical applications.

1.2.2 Classical Conditioning

A key component in learning is association. The mechanism by which associations occur have been debated since the seventeenth and eighteenth centuries. If we smell a particular food that we like, and eat some of it, then feel satisfied, then the next time we smell that same food, our previous experience will lead us to expect that eating this food again will also be satisfying. The same would hold true for something we would associate with a bad event. If we hear the screech of a car skidding, we cringe as we wait for a crash. We have learned through association that a crash is typically preceded by skidding. With the twentieth century, reflex physiology and the nature of associative learning were first described.

Ivan Pavlov, a Russian driven by a passion for research, performed many of psychology's most famous studies. Pavlov had been studying salivary secretion in dogs and previously determined that when food was put into a dog's mouth, the animal would invariably salivate. Pavlov noticed that when he worked with the same dog repeatedly, the dog would salivate with the presence of other stimuli. The mere site of food, the food dish, or even the person who regularly brings the food would make the dogs salivate. The dog, after the repeated delivery of these stimuli, learned to associate the one with the other. The dog would thus salivate even in the absence of the food. Because it interfered with his experiments on digestion, this phenomenon was considered a nuisance by Pavlov until he saw that it represented a simple but important form of learning. For the next three decades, up to his death at age 86, Pavlov devoted all his research to this form of learning.[1]

In classical conditioning the stimulus that will create an automatic or natural response is called the US. For Pavlov's initial experiments the US was food. In human eyeblink conditioning experiments the US is a puff of air that is delivered into the eye. The puff of air, through a natural response, causes the eyelid to close. This automatic reflex to the US is called the UR. Salivation in Pavlov's dogs is the UR. In Pavlov's experiments the dog learned to associate that the site of the food dish meant that food, the US, was being delivered. The food dish is called the CS or signal stimulus. Through successive pairings with the US, the CS comes to elicit a response in the absence of the US. This response to the CS is called the conditioned response (CR).

In modern eyeblink conditioning experiments, a tone or visual stimulus is used for the CS. To provide unambiguous evidence of associative learning, the CS should not elicit reflex responses in the absence of training. Pavlov called these responses orienting responses (ORs). Sokolov, a student of Pavlov, spent a great deal of time and effort describing the reflex physiology of ORs. In Sokolov's view, the OR was produced as a consequence of attention, increasing the organism's contact with the CS. However, the appearance of ORs complicates the interpretation of responding to the CS during training. A sensory reactivity test, preformed prior to training, is a simple test that can evaluate this responsiveness to a CS. Using different volume levels of an audio source, a subjects responsiveness can be measured. One would expect all subjects to respond to a loud and startling sound and not to a very soft sound. Different people however, will respond differently to sounds due to individual sensory threshold differences.

This type of associative learning, through the use of the classically conditioned eyeblink response, has many features that make it useful for applications in the study of

specific neurological functions. The CS is one that the subject has no control over. Associative learning is thought to have occurred if other reasons for responding have been accounted for. An advantage of Pavlovian learning is that these other reasons, like motoric impairments and sensory sensitivity, can be measured and accounted for.

Eyeblink conditioning is used in the study of Parkinson's disease, Alzheimer's disease, chronic fatigue syndrome, and aging.[2,3,4,5] Because eyeblink conditioning requires specific neural substrates to acquire the CR, it is being researched as a tool for gaining understanding of these diseases as well as increasing our knowledge of the complex mechanisms of the brain.[6,7,8] Utilizing other tools for looking at brain function in combination with eyeblink conditioning, even more can be learned about the brain and learning.[9,10] Parkinson's disease is a disorder of middle-aged and elderly people that is characterized by tremor, rigidity, and a problem of uncontrolled movements. This disease affects the basal ganglia, an area of the brain that plays an important role in the control of movement and posture and in more complex aspects of behavior. Alzheimer's disease is caused by a diffuse degeneration of the brain. Patients with chronic fatigue syndrome, an illness causing fatigue with unknown origin, have complaints of cognitive problems, such as impaired concentration, memory lapses, and confusion.

There are different types of protocols, for the evaluation of the classically conditioned eyeblink response. Delay of reinforcement conditioning, trace conditioning, and discrimination conditioning are all examples of classical
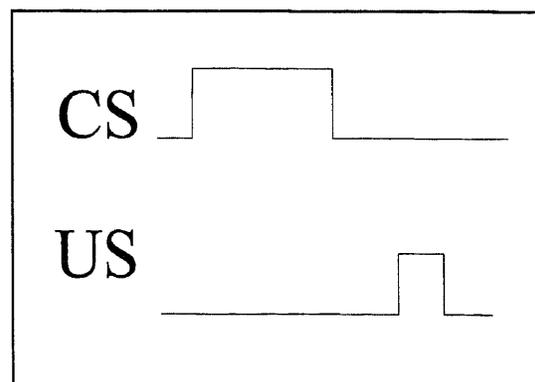
Figure 1.2 - Timing of the CS and the US for a trace type conditioning protocol.

conditioning. A delay type protocol (figure 1.1) is one in which the onset of the conditioning stimulus precedes that of the reinforcer, or US. This is in contrast to trace conditioning (figure 1.2) where the CS offset occurs before the onset of the US.

A delay-type protocol may have three different types of trials. A paired trial is one where the CS and the US are both presented. The CS is such that its duration is much longer in time and its onset precedes that of the US. The dotted line in the figure is indicating that the offset of each of the two stimuli occur at the same time. The time difference between the onset of the CS and the onset of the US is called the inter-stimulus interval (ISI). Another trial type for delay conditioning is when the CS is presented alone. This is called a CS-alone trial. This trial is used to test for the CR. If the subject has been
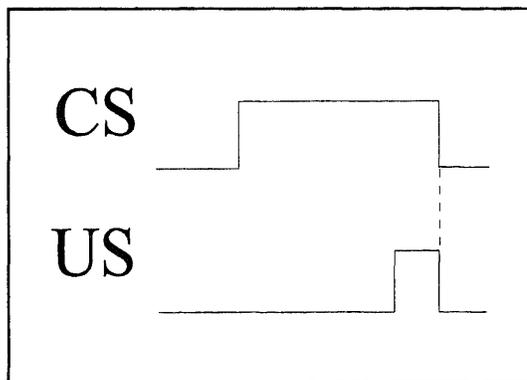
Figure 1.1 - Timing of the CS and the US for a delay type conditioning protocol.

conditioned, then the subject should blink even with the absence of the US. This response would be a blink at the time of the expected US, the airpuff. The last trial type is when the US is presented alone. This trial type called a US-alone trial is used to verify that the US is properly causing the unconditioned response (UR). By varying the ISI duration and the proportion of paired trials versus alone trials the rate of acquisition of the CR can be altered.

Another type of protocol used in conditioning is called a two-tone discrimination protocol. This type of protocol is an extension of the delay-type protocol in which two different frequency tones are used. Here one of the frequencies is paired with the US (CS+ trial). The timing of this trial is the same as the paired trial for a delay type protocol, that is

the offset of the two stimuli co-terminate. This protocol uses the same US alone trial as delay. The difference between these two types of protocols is the CS-alone trial. For two-tone discrimination, the CS-alone trial, here called a CS- trial, is a presentation of the other frequency tone alone. The subject should learn to discriminate between the two different tones and develop a CR to the frequency that is paired with the US, but not with the frequency that is not paired. This type of protocol typically requires more trials to obtain the desired response.

A useful second part to the two-tone discrimination protocol is called reversal. After a subject acquires a CR and discriminates between the two frequencies, the frequencies are reversed. The frequency that was previously presented alone is now paired with the US, and the previously paired frequency is now presented alone. By altering the two frequencies, the rate of acquisition and discrimination can be altered.

1.2.3 Physiological Responses

An eyeblink response is detected through the detection of the EMG. This is the measurement of the muscles that surround the eye and are responsible for the contraction that causes the eye lash to close. The EMG is a bipolar electrical potential that is recorded on the skin surface. Muscle fibers are made up many motor units. The motor unit is the smallest unit that can be activated by a volitional effort. The motor unit creates a field potential from its active fibers that has a duration of 3-15 ms and an amplitude of 20-2000µV. The ranges in the duration and the amplitude are due to size differences of the motor unit. The muscle fiber motor units are interspersed with other motor units throughout the muscle group. The electric potentials of each of the individual motor units add together to create a larger potential. This

combination of potentials for active units creates the EMG signal that is recorded from the muscle.

There are two different electrode methods for use with muscles. A fine wire electrode is use to obtain signals from small units of muscles, even down to a single motor unit. This type of electrode, however, is invasive, because it penetrates the skin to measure the electrical activity directly from the muscle. When using surface electrodes, the measurement is made at the skin surface. This signal will be the sum of a much larger area, or number, of muscle fibers. Because of the ease of use, this type of electrode is commonly used for the measurement of the eyeblink response for human subjects.

Another important physiological system that gives the investigator an additional diagnostic tool into the effects and causes of this form of associative conditioning is the cardiovascular system. During classical conditioning, it is common for other types of responses to be acquired. Measuring the functions of the cardiovascular system provides insight into nonspecific learning that accompanies eyeblink conditioning. Through the measurement of ECG and respiration an evaluation about the autonomic nervous system can be made. The autonomic nervous system is made up of the sympathetic and parasympathetic nervous systems. These two parts of the autonomic nervous system contribute to the control of heart rate. The sympathetic nerves, for example, increase heart rate, while the parasympathetic nerves decrease it. Data suggest that the measurement of the autonomic nervous system correlates with a subject's ability to acquire an eyeblink response.[11]

The heart, a muscular organ enclosed in a fibrous sac called the pericardium, is located in the thorax. The walls of the heart are composed primarily of cardiac muscle cells called the myocardium. The heart, consisting of four chambers, circulates blood throughout the

body by contracting these muscles in a precise manner to pump the blood. The cardiac musclecontraction, like that of other muscle types, is triggered by depolarization of the plasma membrane of the cells making up the muscle. A cardiac cycle of the heart begins with an initial depolarization beginning in a small group of conducting system cells called the sinoatrial (SA) node. The SA node is the normal pacemaker of the heart. After this electrical initiation by the SA node the action potential is carried through a specific sequence of excitation. Gap junctions are connections between myocardial cells that allow these action potentials to spread from one cell to another. From the SA node the excitation spreads throughout the right atrium, and then to the left atrium. The spread throughout the two atria is rapid enough that they are both depolarized at essentially the same time. The ventricles are next in the sequence of excitation. The link between the atrium and the ventricles is a special part of the conduction system called the atrioventricular (AV) node. This node provides some very important functions. The AV node, like the SA, node is small in relative size. This forces the electrical excitation to pass from the atria to the ventricles at only one place. This sets the stage for a specific distribution of the excitation to take place. Another important function of the AV node is that it is a secondary pacemaker should the SA node fail. The third, and most important characteristic, is that the AV node provides a strategic time delay in the propagation of the action potential from the atria to the ventricles. This time delay, lasting about 100 ms, allows the atrial contraction to add additional blood to the ventricles before ventricular excitation occurs. After leaving the AV node the impulses are distributed throughout the ventricles via a conducting-system of fibers, called the bundle of His, and then the Purkinje fibers. Like the atria, both the left and the right ventricles contract at essentially

the same time. The ventricles, however, begin their contraction at the bottom and proceed up the ventricle; this allows for more efficient pumping by the heart.

The SA node controls the fundamental, or base, heart rate. Without any influence by the nervous system or hormones, the heart would beat at approximately 100 beats/min, due solely to the SA node. Normally however, the heart is under constant influence by hormones and the central nervous system. Epinephrine, for example, speeds the heart by acting on the beta-adrenergic receptors of the heart. Receptors that are abundant in the SA node. The heart rate is also influenced by body temperature, plasma electrolyte concentration and other hormones which influence, but to a smaller degree, heart rate. The largest influence to the increase or the decrease of heart rate is due to the parasympathetic and sympathetic nervous systems, which make up the autonomic nervous system. In the central nervous system, the autonomic nervous system has a large number of postganglionic fibers that end on the SA node. Heart rate increases due to activity by the sympathetic nerves and decreases due to activity by the parasympathetic nerves. The parasympathetic nerve to the heart is also called the vagus nerve.

The electrocardiogram (ECG) is a measurement that is recorded at the surface of the skin, on the chest, and is generated by electrical currents from the cardiac muscle action potentials that causes the contraction of the heart. The ECG is used primarily as a diagnostic tool for evaluating these electrical events within the heart. The ECG represents the sum of the action potentials, many of which are occurring simultaneously. The first deflection within the ECG signal is called the P-wave as seen in Figure 1.3. This wave corresponds to atrial depolarization. Since the atrial muscle mass is relatively small, the associated electrical activity only produces a small deflection on the ECG. The second deflection is called the QRS complex. This characteristic wave is a result of ventricle depolarization. The deflection of the QRS complex is large because of the muscle mass of the ventricles. The peak of this wave occurs approximately 150 ms after the P-wave. The very small deflection due to atrial repolarization is not usually seen on the ECG, as it is lost in the much larger QRS complex.
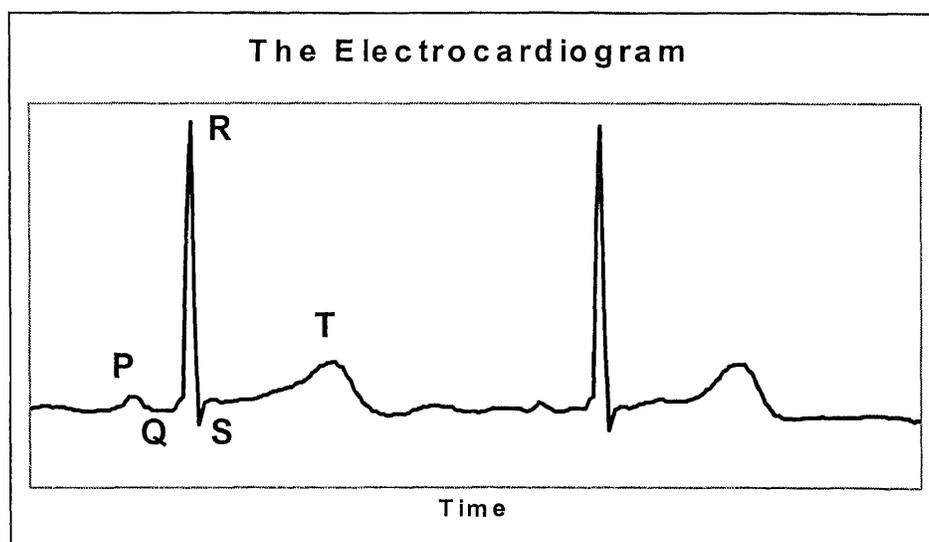
Figure 1.3 - Characteristic electrocardiogram

The final deflection, the T-wave, is a result of ventricular repolarization. As seen in Figure 1.3, the time for the ventricles to repolarize is relatively slow. It takes time for all the ionic movements associated with cardiac cell depolarization to recover and for the membrane potential to return to its normal state. The interval during which the cell cannot be depolarized is called the refractory period. The absolute refractory period in a normal heart is 250 ms.

The measurement of the ECG signal is similar to that of the EMG discussed earlier. Using a pair of surface electrodes and a ground electrode, the differential voltage signal can be measured on the surface of the skin. Locations for the two electrodes differ depending on the desired emphasis in the signal. Because of the size of the QRS complex, if one is only interested in heart rate, then almost any position of the two electrodes will be sufficient for this detection. Since the QRS complex is the easiest component of the ECG wave to detect, it is used as the calculation point for the determination of heart rate.

The respiratory system provides many functions for the body. Through the vagus nerve of the autonomic nervous system, the respiratory system and the cardiac system are linked together. This communication link provides feedback from the respiration to the control of heart rate. During inspiration heart rate increases and during exhalation heart rate decreases. This phenomena is commonly called heart rate variability. Through the measurement of the ECG to determine an instantaneous, or beat by beat, heart rate and the respiration waveform to determine the respiration rate a calculation of the heart rate variability can be made. This non-invasive measurement method attempts to quantify the amount of communication that is taking place through the vagus nerve.

CHAPTER 2

2 IMPLEMENTATION

2.1 System Introduction

The specific function of this experimental system was to provide a test apparatus for the application of measurement of the classically conditioned eyeblink response in human test subjects. This eyeblink experimental system consisted of three main sections: a computer, hardware sub-systems, and patient interfacing. Figure 2.1 is a block diagram of the eyeblink experimental apparatus. A software program that ran on a computer utilized a digital output port to control electronic hardware instruments for the generation and delivery of an audio stimuli to the patients ear and a puff of air to the patients eye. Electronic amplifiers measured physiological signals from the patient, conditioned them, and transferred the data to the
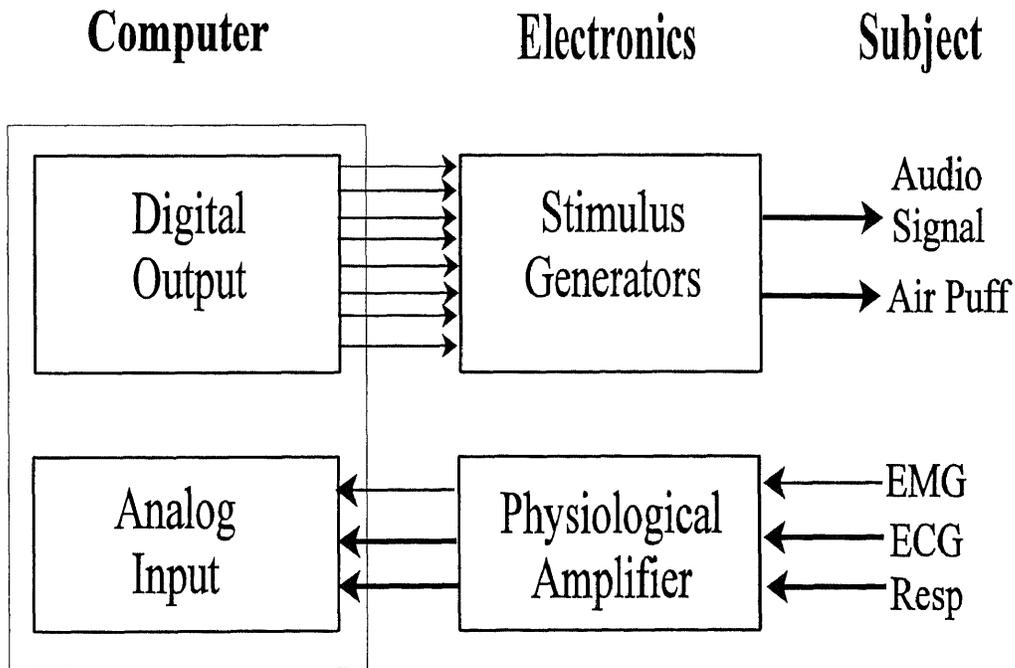
Figure 2.1 - Block diagram of eyeblink conditioning system

15

software, via an A/D converter, for storage on the computer. The coordination of all of these systems required a sophisticated software approach. The software needed to be powerful enough to control these subsystems while maintaining a level of user friendliness.

2.2 Software, Computer, and Acquisition Card Selection

The software for the automated control of the system was the brain of the experimental apparatus. The selection of software was crucial to meet the requirements of the experiment. The software needed to control the output stimuli, record and display physiological signals, monitor the status of the electronics, and provide to the user necessary feedback during the execution of the experiment.

The software chosen for this experimental application was LabVIEW® software by National Instruments. This multitasking data acquisition and control software package running on a multitasking computer operating system (Windows 95 - Microsoft Corporation), provided a programming environment that controlled each of the hardware sub-systems, with precise coordination, to properly execute the experiment. Using a programming method called virtual instrumentation, a user friendly interface was developed that provided the experimenter useful feedback during the experiment.

Though not fully implemented in the scope of this thesis, portability is a future design specification. It is usually desirable to have patients come to the laboratory. Most experimentation uses equipment and personnel that would be prohibitive and costly to make portable. With some learning paradigms, however, it is beneficial to bring the laboratory to the patient. With the patient in his or her own environment, the patient is more comfortable and relaxed for the long experiment ahead.

To fulfill this goal the computer and acquisition card were chosen primarily for their physical size. The notebook computer used for this system, was an IBM Thinkpad 760C (International Business Machines - Armonk, New York). The Thinkpad contained a Pentium® 133 MHZ processor with 40 Megabytes of RAM. For improved performance the computer had a PCI bus with a PCI to PCMCIA interface. The PCI bus gave the operating system improved multitasking capabilities. The PCI bus was the main communication pathway between the processor, memory and other main computer devices. The PCI bus was a system bus for adapters requiring fast access to memory or each other. The PCI bus was a high-performance synchronous bus operating at 66MHz. The addressing on the PCI bus can be set up to be either 32 bits or 64 bits. The PCMCIA bus was a branch of the PCI bus that was used to interface to external or auxiliary devices.

The A/D conversion card chosen for the system was a DAQ700 card also from National Instruments. The DAQ700 was chosen to utilize the PCMCIA interface in the notebook computer. This card had 16 channels of A/D conversion with 8 bits of digital input/output (I/O). The card had two 16 bit onboard counter/timers. The detailed specifications for the DAQ700 card are in Appendix C and will be discussed in detail in the hardware section of this chapter.

2.3 Software Design

The program was composed of two main sections: configuration and calibration, and protocol execution. Figure 2.2 is a block diagram of these steps for the complete execution of the experimental protocol.

2.3.1 System Configuration

Software on a computer that is utilizing a peripheral, needs to set parameters for proper

communication between the software and the hardware. The first few steps of the program

set necessary parameters on the acquisition card for the A/D conversion and the digital I/O

used during the experiment (refer to Appendix A for detailed LabVIEW program code). For
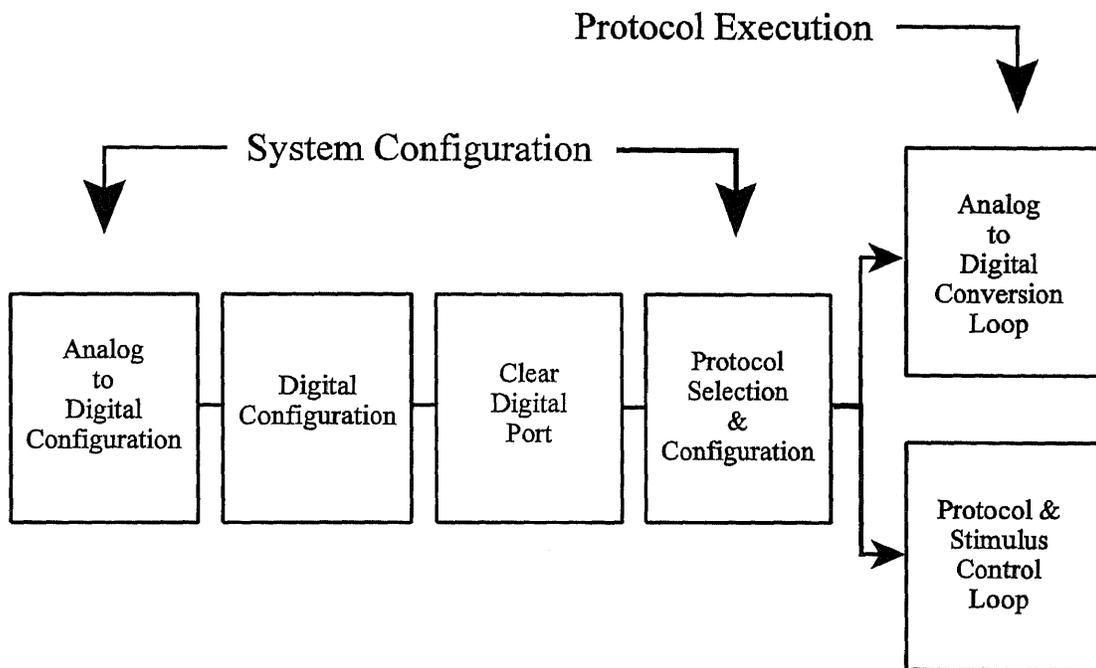
Figure 2.2 - Block diagram of system configuration and experiment execution.

A/D conversion the setting were used to match the characteristics of the signals recorded. The

A/D configuration step involved setting the computer base address of the card (used for

addressing the card by the computer), the number of active A/D channels, the sampling rate

of the conversion, the analog signal polarity (bipolar or unipolar), the analog input voltage

range, the analog input mode (referenced single-ended or differential). The settings for the

configuration of the A/D card activated analog channels 0 thru 3, set them all to be single ended (referenced to ground), bipolar (having positive and negative voltages), with a maximum voltage range of ± 10 V.

During the protocol execution section of the program there was a necessary time period where all non-essential functions of the program were inactivated. During this special time period, hard drive access was disabled and a temporary data storage method was established. This data buffer was set to 10,000 bytes. This buffer was a special memory allocation in the random access memory (RAM) of the computer. Utilizing the direct memory access (DMA) feature of the acquisition card, data was written directly from the A/D conversion to the RAM. DMA is a feature of many computer peripherals where data can go into RAM with out going through the computers main processor.

The next configuration step was to configure the digital section of the card. The digital I/O port base address was set and the I/O port mode was configured as output. Once the card was configured by the software a self-test was performed by the configuration software. If either one of these two configuration steps, the analog or the digital, fails an error was generated and the user was prompted to the problem. Examples of computer errors are: the DAQ700 card was not seen by the computer, an address conflict with another peripheral.

To help identify problems before the execution of the protocol tests of the A/D conversion and the digital I/O configuration were performed after the configuration steps. First, an output code to clear the hardware is sent to the digital I/O port. This tests the card and its interfacing and clears all hardware devices turning them "off". When this was complete, the pressure monitoring circuit was sampled to obtain a pressure measurement. In the airpuff delivery system, described in greater detail in the hardware section of this chapter,

a high pressure was considered to be dangerous to the patient. If the pressure in the air reservoir was outside of an acceptable tolerance value then the user was prompted with a visual warning so that an adjustment can be made.

The protocol section was for the user to select which protocol, ie. experiment, was going to be performed, Sensory Reactivity, Delay, or Two-Tone Discrimination. The LabVIEW front panel display can be seen in Figure B.2 of Appendix B. The user was simply prompted with a display of each protocol and was required to point to one of the three choices before the program continued. This selection loaded a file that determined the inter-trial intervals (ITI) and codes for the trial types. The next, and final, step of the configuration section was the user was prompted to enter a filename for the storage of the A/D data. The programming step to perform this function was the use of a file dialog box virtual instrument (VI). Once a file was entered the experiment was ready to begin.

2.3.2 Protocol Execution

The protocol execution section of the program was made up of two parallel tasks. One task was a continuous A/D conversion. This section of the program performed: the A/D conversion, checked for signal quality, and stored data. The other task was a section to control the timing of the experiment and to control digital output. Although Windows 95 and LabVIEW are promoted as multi-tasking they are really task-swapping. The system required concurrent high speed A/D conversion and digital I/O control with millisecond accuracy. If the task-swapping is not carefully controlled then either one or both of the two tasks could be inaccurate. To perform task swapping control a communication link was established between the two concurrently running sections. Using an inter-loop variable as a logical (either true

or false) to enable nonessential functions of the program, one section of the program could put other sections of the program to "sleep" when the first section needed extra computer resources.

The A/D conversion section was in a repeating loop where the A/D converter was read, the data transferred to a buffer, displayed in a graph, and written to disk. Figure 2.3 is a block diagram of the A/D conversion loop (see figure A.2 of appendix A for detailed LabVIEW program code). The first step was a continuous A/D read from the port to a buffer. This was a continuous operation with DMA. As discussed earlier, DMA was used

```
┌──────────────┐   ┌──────────────┐
│  Continuous  │   │              │
│   Analog     │   │    Read      │
│     to       │   │  Data from   │
│   Digital    │───│   Buffer     │
│    Read      │   │  (in blocks) │
│  to buffer   │   │              │
└──────────────┘   └──────────────┘
```

```
┌────────────┐  ┌────────────┐  ┌────────────┐
│ Data Write │  │    Data    │  │    Data    │
│    to      │  │  Display   │  │  Quality   │
│   Disk     │  │    to      │  │   Check    │
│            │  │   Graph    │  │            │
└────────────┘  └────────────┘  └────────────┘
```
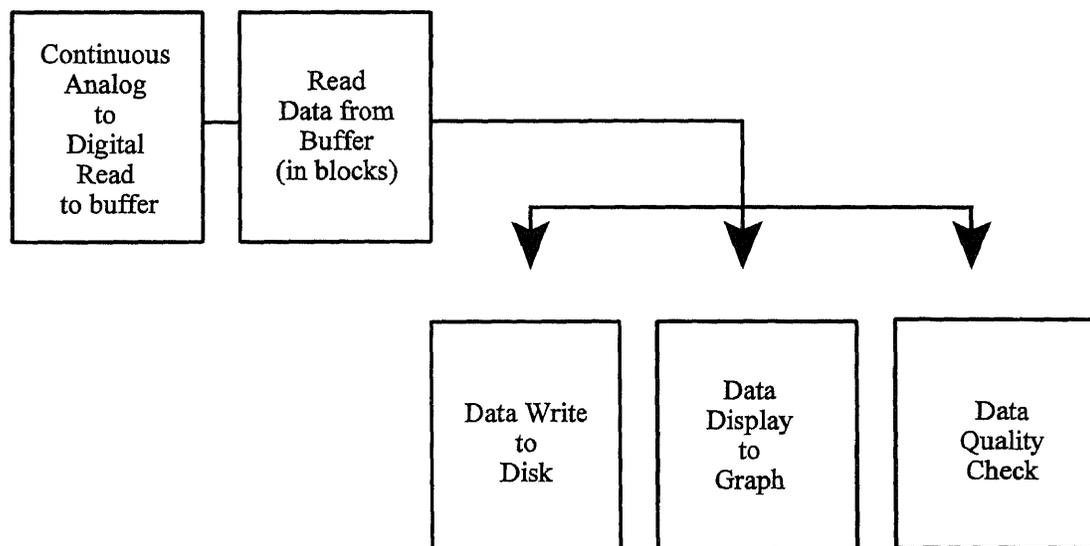
Figure 2.3 - Block diagram of A/D conversion loop.

because the computer peripheral, in this case the A/D converter, can send data directly to RAM without going through the computer processor. This capability of the DAQ-700 card

saved processor resources for other tasks. The A/D conversion was sampled at a rate of 200 samples/sec for each channel. For program efficiency, data was read from the buffer in blocks of 100 samples. The software and the computer can move data and perform functions on data more efficiently when the data is in blocks. The data, after being read was displayed in a scrolling plot window for feedback to the experimenter, stored to disk in an continuous ASCII file, and then compared to threshold variables for a signal quality check.

The quality of the analog signals being recorded are essential for the processing of the data. During the protocol the signals were constantly being evaluated by both the software and the experimenter to keep costs to a minimum by reducing unnecessary data loss. While the experimenter was viewing the A/D converted data in a real time plot window, the program compared the data to two window comparisons. The first checked that the data was not approaching the maximum range of the A/D converter. The range used for the this window was ±9 V, 1 V less than the full range of the acquisition card. The second windows for comparison of the data is to check for a signal that is too small. To prevent signal loss due to a disconnected electrode or a weak battery in the physiological amplifiers, the A/D data maximum range is checked. If the A/D data was less than 1 V peak to peak (p-p), then their was a potential problem. If either of these window comparisons fail, the user was prompted with a visual signal.

The second main task, parallel to the A/D conversion loop, was the timing of the event sequence. This task was also a loop. In this loop, each iteration of the loop culminated in the delivery of a stimulus event to the subject. The timing loop had two parts, the ITI and the digital I/O. A timer was used to accurately perform the ITI. During the ITI, the computer performed the A/D conversion (as mentioned earlier) and monitored critical systems. When

the ITI was complete, all system resources, except the A/D conversion to buffer, were paused. This was performed by setting the inter-loop logic variables to true, thus putting all nonessential functions (the real time plot windows, the data storage to disk, and the signal quality check comparisons) to "sleep".

The event sequence for the output of stimuli to the patient was programmed into different subroutines, one for each of the different stimulus trial types. Using a case statement, the trial type code from the protocol file was compared, thus selecting a different routine. Each one of these routines contained a sequence structure. In LabVIEW, a sequence is a structure that forces a series of instructions to occur one after another. This is similar to the way a command line programming language, such as BASIC or C++ would be executed.

The first three trial types was for the delivery of the 3 different volume levels of the white noise generator for the sensory reactivity protocol. The sequence for these trial types was simply to output a code, wait for 50 ms, then output a code of 0 to clear the port. As seen in Table 1.1, each bit on the port, controlled a function of the stimulus equipment. The specific functions will be discussed in the hardware section of this chapter. Combining the bits created the ability to generate the different stimulus types at different levels. For

Table 1.1 - Output bits for stimulus control

D0	Tone Frequency #1
D1	Air Puff (solenoid #2)
D2	Tone Frequency #2
D3	White Noise
D4	Supra - Threshold
D5	Threshold
D6	Sub - Threshold
D7	Air Refill (solenoid #1)

example, the codes for the creation of white noise is 8 with the volume levels being 16, 32, 64 for supra-threshold, threshold, and sub-threshold respectfully. To output white noise at a particular volume level the codes are simply added and then output to the digital port. To create the stimulus types for the delay conditioning protocol a similar sequence of events was used. After the corresponding code for the trial type was output to the port, a wait time of 500 ms was used before the output port was cleared. The US alone trial also used this same structure with a wait time of 50 ms. The sequence for the CS paired trial was more complex. First the code for the audio was output to the port. Next was a wait command for the CS duration minus the US duration, called the CS-US interval. After this wait time the code for the desired audio signal plus the code for the US was output to the port. Next was a wait command for the US duration and then finally a code of 0 to clear the port. When either of these sequences were complete, the buffer monitor, plot window, and disk write tasks were all re-enabled and the next ITI began.

2.4 Hardware Design

The hardware design of the system was made up of two main sections. The output section, addressed by the digital I/O, created stimuli and delivered them to the patient while the input section sensed and conditioned physiological signals from the patient and recorded them on the computer.

2.4.1 Output Hardware

The sensory reactivity study utilized white noise at three intensities. White noise, or broadband noise, is a uniform distribution of energy for all frequencies in the human audio

range (20 Hz to 20 KHz). The delay protocol for the classical conditioning experiment utilized pure tones of 800 Hz and 2300 Hz. as a conditioning stimulus and a puff of air to the eye as an US. The function of the output section of the hardware was to create, control and deliver these stimuli to the experimental subject.

2.4.1.1 Audio Delivery System : The first stimulus type was the development of the audio signals which can be seen in the block diagram of Figure 2.4. The two different audio stimuli types were generated using signal generators from Coulbourn Instruments LLC,

```
┌───────────┐      ┌───────────┐      ┌───────────┐
│ Attenuator│ ◄──  │ Attenuator│ ◄──  │ Generator │
└─────┬─────┘      └───────────┘      └───────────┘
      │
      ▼
┌──────────────┐      ┌───────────┐
│Rise/Fall Gate│ ──►  │ Amplifier │ ──►
└──────────────┘      └───────────┘
```
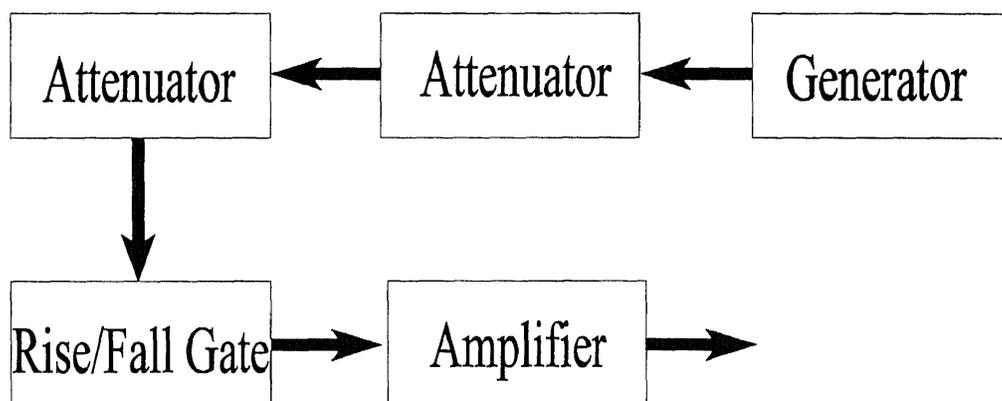
Figure 2.4 - Block diagram of audio generation section

Allentown, PA. (Precision Signal Generator - Model S81-06 & White Noise Generator - Model S81-02). The three intensities of this signal, supra-threshold (102 dB), threshold (92 dB), and sub-threshold (82 dB), were created by passing the raw audio signal through one of two programmable signal attenuators (Model S85-08 - Coulbourn Instruments). The original signal, with out going through an attenuator, would produce a signal at 102dB. When the

signal passed through one attenuator the signal was attenuated by 10 dB. A signal of 92dB was then produced. When the signal passed through the second attenuator, the signal output was 82dB. The activation of the attenuators was controlled by the computer to an enable gate control input on each of the attenuators. To prevent a popping sound in the audio signal, due to an instantaneous onset of the signal into the audio amplifier, the signal was passed through a rise/fall gate (Model S84-04 - Coulbourn Instruments). The rise fall gate created a ramped onset and offset to the audio signal. The white noise stimulus was triggered for a duration of 50 ms with a 5 ms rise and fall time provided by the rise/fall gate.

In this type of experimental application noise from the environment must be carefully controlled. Aviation headphones (Model H10-50 - David Clark, Worchester, MA) were chosen for their large attenuation characteristics to ambient noise (21dB). A common application of these headphones is for helicopter pilots during flight and also for military personnel on aircraft carriers. The output of the rise/fall gate was fed into an audio amplifier (Model VSX-5000, Pioneer Electronics Corp). This provided the final gain and also had an output that was impedance matched with the headphones. The audio system sound levels were calibrated using a sound meter (Model 33-2050 - Radio Shack/Tandy Corporation, Fort Worth, TX) measured at the ear's position in the headphones as they were during experimentation.

2.4.1.2 Airpuff Delivery System : In a classical conditioning experiment, the US, must meet a critical requirement. The stimuli must be aversive, that is the US must be one that causes an UR, ie. an eyeblink, every time. The creation of this US needed to be consistent and reliable. The US, a puff of air, was delivered through a small armature located 1 cm

directly in front of the patient's eye. This airpuff, when it hit the surface of the eye, causes an eyeblink to occur.

Figure 2.5 is a block diagram of the airpuff generation system. The airpuff began by using an air tank with a high precision regulator. The output of the regulator is set to 5.0 PSI. This regulated pressure was passed through a filter (Model 9933-11-BX / Balston Inc. - Lexington, MA) to remove very small particles, and then to a computer controlled solenoid
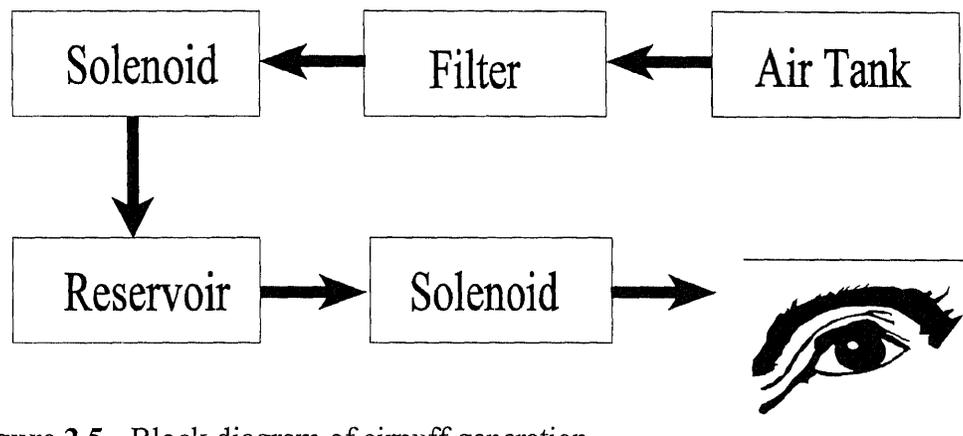
Figure 2.5 - Block diagram of airpuff generation

valve (Model ET-2-12-H - Clippard Instrument Laboratory - Cincinnati, OH). This solenoid valve was used to fill a small reservoir of a fixed volume (.0007 cu.ft.). The airpuff was delivered to the eye by a second computer controlled solenoid valve that released the air from the small reservoir through a 0.06 in ID tygon tubing. Prior to an airpuff stimulus being delivered, the first solenoid valve was opened for 4 s. This allowed the small reservoir to pressurize to 5.0 PSI. The stimulus was delivered to the patient for 50 ms using the second solenoid.

2.4.2 Input Hardware

The second main section of the hardware system was for the measurement of the signals that determined conditioning, both of the eyeblink response and the cardiac response. The recording of physiological signals occurred in parallel with the delivery of the output stimuli.

The occurrence of an eyeblink was measured by an EMG. Surface electrodes, placed above and below the subject's eye, were used to sense the EMG. The EMG electrodes were placed on specific muscles, the characteristics of surface electrodes were such that the measured signal was a broad (non-selective) detection of many motor units that correlate with the overall level of contraction of muscle groups near the electrodes. The EMG signal was amplified using a battery-operated differential amplifier (Model 2283FT, UFI - Morro Bay CA). The amplifier had a high common-mode rejection ratio (>70dB) to help remove noise. The amplifier gain was set at 10,000 and a low-pass anti-aliasing filter with a corner frequency of 20 Hz was used to condition the signal prior to recording. To remove DC drift a high-pass filter at .15 Hz was used.

The EMG amplifier utilized a Fetrode®, consisting of a field effect transistor (FET) at the electrode. To reduce the effects of motion artifacts and noise that are associated with physiological data acquisition, reducing the skin impedance by abrasion of the skin was used. The Fetrode electrode artifact reduction system also helps to remove motion artifact. The FET is also a pre-amplifier which reduces artifacts due to electrostatic potentials and noise in the cabling between the patient and the amplifier.[12]

The ECG measurement was made using a differential amplifier similar to the EMG (Model RESP-I, UFI - Morro Bay CA). This amplifier also used 2 differential inputs and a ground reference. Because the ECG signal from the skin was at a greater natural amplitude

compared to the EMG the gain for the ECG amplifier was set to 1000. An anti-aliasing low-pass filter with a corner frequency of 100 Hz was used.

Respiration was measured using impedance pneumography. A constant current source (1 μA @ 30 KHz) was applied and flowed between the two electrodes. At the same time, a differential voltage measurement was made between these same electrodes. Voltage changes developed across the two electrodes were due to impedance changes across the trans-thoracic and trans-abdominal region of the body. These impedance changes were mostly due to chest expansion from breathing, thus a waveform representing a breathing pattern was recorded. Some other physiological changes, such as blood movement, also caused impedance changes across the chest. However, the frequency range of these changes are higher and were easily filtered out by the amplifier. The respiration amplifier was AC coupled to eliminate DC shifts. The respiration waveform was filtered with a low-pass filter at 1 Hz. The electrodes were placed in a non-standard configuration. The two electrodes were located on the lower rib cage approximately 6 to 8 in. from center. This configuration was used to improve the respiration signal while causing no change in the desired signal for the ECG.

Since the accuracy of the timing of the stimulus and the recording of the response by the patient are critical, the marker channel was used to aid in the processing. This marker channel was derived by using the digital output control bits as feedback into the A/D converter. Using an 8 bit digital to analog converter, the output digital bits, that were used to control the different stimuli, were used as the digital input. The analog output was recorded into the A/D converter of the computer to synchronize with the physiological signals. This 8 bit digital to analog converter created as an output one of 256 different voltage levels. Using the different levels generated by the marker channel, an Splus function was developed

that scanned the marker channel to find and identify each trial type and its exact timing. The output of this function was an index, that identified the onset and offset of each stimulus during the trial. Since the raw data file is very large, this index was used to create smaller subsets of the raw data to aid in the speed and efficiency of the processing.

Figure 2.6 is a representation of a recording of the marker channel for a trial in the delay protocol. The y-axis was a voltage from the A/D converter as discussed earlier. The difference in each voltage level indicated which stimuli or combination of stimuli were active at each moment of time. Through a simple calibration procedure where each output is turned on sequentially, the voltage of the output of the digital to analog converter was measured and used for processing. As seen in Figure 2.6 during the baseline period the voltage was at one
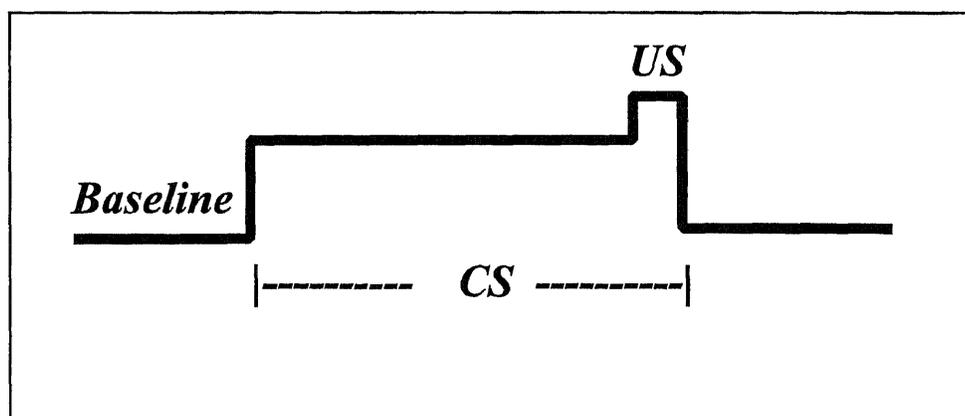
Figure 2.6 - Timing diagram of marker channel for stimulus output during delay protocol

level. When the voltage changed to another level it marks that one of the stimuli turned on. In either the delay or two-tone paradigms this would indicate the onset of the CS. The voltage now changed to a third level, indicating the US onset. During this period the CS was on, but now the US, the air puff, was also on.

2.5 Reliability and Safety

The development of any sophisticated electronic system, for use in medical research, requires that the system be both safe and reliable. When connecting a human patient to electrodes for recording of physiological signals, there exists a risk of electric shock. When a person is connected to equipment, such as an electrocardiograph machine or electromyograph amplifier, the person, through the skin electrode interface, is now part of an electric circuit. The degree of a physiological event to occur is dependant on the quantity of current through the body. Applying Ohm's law to this system, the magnitude of the current is equal to the applied voltage divided by the sum of the series of impedances of the body tissues and the interfaces at the contact points. Because the largest impedance is located at the electrode-skin interface, the main function of recording electrodes is to reduce this impedance between the skin and the recording device. Though its design is to improve signal strength and quality, this reduction in impedance increases the ability for current to pass between the two electrodes directly through the person. This situation can cause injury or even death to the patient.

Three types of phenomena can occur as a result of electrical current flowing through biological tissue. The first is electrical stimulation of excitable tissues. At higher levels current can cause resistive heating of tissue and then even electrochemical burns. The cardiac muscles of the heart contract because of electrical conduction. This makes the heart particularly susceptible to electrical currents. If the hearts own cardiac electric activity altered by disrupting the normal propagation, then the hearts pumping action is compromised.

To protect the patient from a possible electrical ground fault an isolation transformer was used in line between the battery-operated physiological amplifiers connected to the patient and all electrical devices. This isolation transformer (Model AD284J / Analog

Devices - Norwood, MA) complies with leakage requirements for the Underwriters

Laboratory (UL) standard for safety, medical and dental equipment as established under UL-

544 for type A and B patient connected equipment. Leakage current was limited to 2.0 μA

rms at 115 V AC, 60Hz. This transformer was powered by a ±15 V power supply. The

transformer was placed on a split circuit board where the isolated side of the transformer chip

was separate from the non-isolated side of the chip and the power source. The circuit board,

power supply with switch and fuse, was placed in a small plastic project box. Three panel

mount phono plugs were used as the input from the output of the physiological amplifier.

Three panel mount BNC connectors were used as the output to feed the signals to the A/D

converter card interface.

One session of the experiment lasted for up to one hour. During this hour, 120 total

trials were delivered to the subject. Computers are not 100% reliable, they can (and do) crash

on occasion. In the event of a computer crash two points needed to be addressed. The first

relates to the reliability of the system. The ability to restart the experiment at the same place

that it accidentally ended, was required. To solve this problem, at the end of each trial that

was delivered to the subject, a trial counter was written to a file called "gamma.log"on the

hard drive. This file was updated at the end of each trial and if the program finished normally,

the file "gamma.log" was deleted. When restarting after failure, the last value in

"gamma.log" was read , ie., the last completed trial, and jumped forward to that location in

the ITI counter loop. The name "gamma" is taken from a problem that NASA has with

computers on board the Space Shuttle. Uncontrolled rebooting of computers is caused by

gamma rays that penetrate the shuttle and cause electronic interference.

An important concern should a computer crash is patient safety. As discussed in the output hardware section, the airpuff delivery system utilized two solenoid valves. The use of two solenoid valves was a patient safety feature to limit the total quantity of air that could have been delivered continuously to the patient's eye to the volume of the reservoir. The valves were chosen that in a mechanical or electrical failure within the valves, the valves would close. In an the event of a computer failure, however, the valves would open causing a continuous flow from the tank. To prevent this occurrence, the valves are setup for one to be normally open and the other to be normally closed, thus only one could fail open.

CHAPTER 3

3 - PERFORMANCE AND SAFETY EVALUATION

3.1 Introduction

To evaluate the system, for its ability to perform an experiment in an accurate, reliable, and safe manner, two preliminary evaluations of the system were performed. The first measured the accuracy of the digital I/O of the program. The second used simulated computer and electronic failures to evaluate the safety features of the system.

3.2 Output Accuracy Test

The first test of the system was to evaluate the accuracy and reliability of the timing of the digital I/O signals. These signals were the signals that were used to control the triggers for the output stimuli electronics. To evaluate this I connected the output of the conditioning system computer (IBM Think-pad with DAQ700 card) to a second test computer. This second computer was an IBM compatible desktop computer with a Pentium 233 MHZ processor with an A/D converter card (DAS-1602 - Keithley/Metrabyte, Taunton MA). A custom cable was created to connect the digital I/O port of the DAQ700 card installed in the IBM Think-PAD computer to the analog input connector of the DAS-1602 card installed in the desktop PC. While the conditioning protocol program ran in LabVIEW, the digital output signals were recorded by the A/D converter in the PC at 1000 samples/s for each of the 8 digital output bits. The data were sampled and recorded to disk by Snap-Master (HEM Data Corporation, Southfield, MI). Because of the very large file sizes that this rate and duration

of sampling can create, sampling was limited to two seconds before and after each of the trials. This saved file space but allowed for enough data for the accuracy testing.

After three runs of the protocol, the data were converted to ASCII. The processing to determine the accuracy of the digital I/O timing was programmed in S-Plus (MathSoft, Seattle WA). The processing of the data was performed in a few simple steps. The sampling rate of the collected data was 1000 samples/s. This creates samples at 1-ms intervals. The program consisted of a large loop that read in each data file in order. Each data file consisted of 8 columns, one for each of the digital bits. The numbers of rows in the data file was 5000 (1000 samples/sec x 5 s of recording for each trial). The digital output from the DAQ700 card is a TTL compatible output. When the output bit was "off" or "low" the voltage was 0 V and when the output bit was "on" or "high", the output was 5 V. The program stored the row (or sample) that the first number was greater that 4.5 V and then summed the number of consecutive points that remained greater than 4.5 V for each of the columns. The first number represented the onset of the stimulus and the second number represented the duration that the digital output bit was on for that trial.

The results of the output accuracy test showed that the duration for each of the digital I/O was "on"and the difference between the onset of the CS and the onset of the US was accurate to within 1 ms of the parameter setting within the conditioning setup. These results are within the desired specifications for the application of stimuli to a subject.

3.3 Safety Features Test

To perform a test of the safety features, transducers were used, in place of the patients ears and eye, to measure the output of each of the stimulus devices. A plastic mannequin head was outfitted with a microphone transducer to measure the sound from the headphones for each ear and a transducer to measure the air flow from the air puff delivery system. In the ear of the mannequin head, located at the same approximate location of the human ear, the microphone transducer was mounted from the sound meter as described earlier. This meter has an analog output that was recorded using an A/D converter (DAS-1602 as described in section 3.2). Located at the location of where the subjects eyeball would be, the flow transducer was also mounted in the mannequin.

A series of system failures were simulated to verify that stimulus durations were not in excess for the safety of the patient. There were two types of system failures. The first is a failure by the computer. The second failure is a problem with the hardware. The hardware could experience a power loss or a cabling problem in the interfacing and distribution of signals.

A test was performed by running the experimental protocol, as it would be run in a normal experiment, but using the mannequin as the test subject and the second test computer as discussed earlier. Using the same recording procedure, the output of the microphone and flow meter were recorded for each trial at 1000 samples/s. System failures simulations were created both between each trial, and during delivery of each trial. Below is a chart containing a list of the failure simulations that were created and a summary of how the simulation was performed:

Table 3.1 - System failure simulations

Type of Failure	Simulation Method Summary
Computer AC power loss	With the DC computer battery uninstalled, the AC power cord was removed.
Computer DC power loss	With the AC power disconnected, the computer battery was removed.
Computer to interface cable disconnection	Cable connecting the DAQ700 card to the hardware interface was disconnected.
Electronics power loss (complete) Electronics power loss (ground) Electronics power loss (+12 V) Electronics power loss (-12 V) Electronics power loss (+5 V)	A switch box was installed between the DC power supply and the hardware. Using switches, each of the power lines could be disconnected independently.
Pressure regulator failure	Simulated by forcing an over pressure situation.
Software error: hard drive full	Filled hard drive except for enough for a few trials to complete
Software error: A/D buffer full	Created a small buffer that would easily fill on the first trial.

The results of the safety tests were mixed. On the positive side, when the software had an error, it did not cause a safety problem for the "patient". For all of the simulated software errors, none of them caused the outputs to respond by turning the output bits "on". If the hard drive filled up, failed, or the A/D buffer filled during the protocol, LabVIEW created an error and the program execution halted. The only time that a safety problem occurred was when the hard drive or the A/D buffer filled during the delivery of the digital I/O such that the point in the program that cleared the stimuli was never be reached. This caused what ever stimulus was "on" at the time to remain "on". This possibility was avoided by the inter-loop communication variable, as described earlier, that halted all nonessential processing functions

during output stimulus delivery. If the LabVIEW or the operating system crashed or halted the digital I/O also responded in a predictable manner.

The tests showed that if the airpuff delivery system had a mechanical or an electrical failure, the system never had an open path from the pressurized air source to the subject's eye. Using solenoids that were mechanically opposite, that is one is normally open and the other is normally closed, provided an acceptable margin of safety.

Simulations of the electronics showed that power problems did create a situation where the audio stimuli was "on" for an unsafe period of time. The testing of the electronic systems showed that there was a safety hazard if the power that powers the electronics was compromised or when the interface between the DAQ700 card and the digital TTL to 12 V converter in the electronics was disconnected. When the cable became inadvertently disconnected, the inputs to the converter did not have a reference and thus were "floating" or in a tri-state. By default, the outputs of the converter turn "on" causing all of the output stimuli to turn on. The subject did not receive an air puff as discussed earlier but did receive the audio signals. This same situation did occur when the 5 V power or the +12 V power were did connected from the electronics. Because of this negative result of the testing of the electronics, a method to prevent this needed to be implemented. The Occupational Safety and Health Administration (OSHA) sets the limit of sound for the human ear at 90 minutes per day at a volume level of 102 dB. Even without any modifications to the system this safety limit would never be reached.

However, a simple upgrade for the benefit of the patient was still implemented. By installing a switch within the immediate reach of the experimental operator, an unnecessary sound would easily be limited. This "panic button" was a large button, approximately 3

inches in diameter and enclosed in a small plastic project box. The box had an input connector to connect the audio line from the audio amplifier, and an output connector to connect to the headphones for the subject. The switch was a normally closed switch, that when pressed open the connection between the audio amplifier and the subject, turning off all sounds.

3.4 Conclusions

Patient safety was of the utmost concern. By installing a UL544 approved isolation transformer in the path between the electrodes and all electronic components the risk of electric shock due to ground faults was eliminated. Though the simulation tests of different system failures showed that there were some safety deficiencies, they was easily remedied to provide a safe solution. The tests of the measurements of the timing and reliability of the digital output clearly showed that the system had the accuracy to perform the desired experiments.

CHAPTER 4

4 - SYSTEM USAGE

4.1 Introduction

Patients with chronic fatigue syndrome (CFS), an illness causing fatigue with unknown origin, have complaints of cognitive problems, such as impaired concentration, memory lapses, and confusion. This illness is characterized by an onset of severe fatigue persisting for more than six months and accompanied by infectious, rheumatological, and neuropsychiatric complaints.[13] The deficits in attention and concentration, combined with central fatigue, should translate into poorer acquisition of the classically conditioned eyeblink response, therefore, we designed a pilot study to evaluate acquisition of this type in CFS.

As discussed earlier in section 1.2.1 on the background of classical conditioning and seen here in Figure 4.1, a tone was used as a CS and an air puff was used as the US for a delay type protocol. The conditioning parameters were chosen from the literature. The CS duration was set to 500 ms, and the US duration was set to 50 ms. The inter-stimulus interval (ISI) which influences the speed of acquisition, is calculated as the time between the onset of the CS and the onset of the US. A test of sensory reactivity was performed to evaluate each subjects
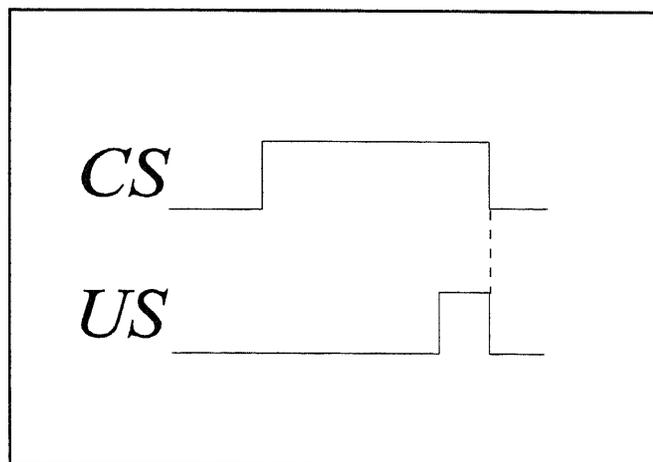
Figure 4.1 - Timing of the conditioned stimulus and the unconditioned stimulus for a delay type conditioning protocol.

startle responsiveness and sensitivity to the acoustic stimuli. We then evaluated new motor learning.

4.2 Materials and Methods

4.2.1 Subjects

CFS (n=7) and healthy control subjects (n=10) were obtained from The Chronic Fatigue Syndrome Center, located at The New Jersey Medical Schools' Newark campus. The Chronic Fatigue Syndrom Center is supported by NIH Center Grant #U01AI-32247. Intake of patients into the center for research purposes involves a complete medical evaluation which included a history and physical exam, a standard blood work-up, and a psychiatric diagnostic interview (Q-DIS, Washington University Department of Psychiatry, St. Louis, MO) to rule out any known illnesses or psychiatric disorders. Subjects were also excluded if they report facial tics, Bell's palsy, report a loss of consciousness for more than 25 minutes, or are taking medications which affect cardiovascular tone. All subjects were females ranging in age from 22 to 50 years of age. The mean ± S.E.M. for CFS was 34.6 ± 4.2 and for controls was 37.3 ± 2.3.

4.2.2 Procedures

Prior to the arrival of the patient, the system was calibrated and environmental conditions were measured. The volume level of each of the audio stimuli used for the experiment was calibrated using the sound meter and measurement procedure described earlier. The ambient noise and temperature of the room was measured and documented on a work sheet to be logged into an experiment log (see appendix E). All subjects on arrival signed an Informed

Consent approved by the Institutional Review Boards of both the University of Medicine and

Dentistry of New Jersey and the Department of Veterans Affairs New Jersey Health Care

System. Subjects then filled out a brief questionnaire. This questionnaire, which was part of

the experiment work sheet, asked questions about factors that may affect the subject's ability

to acquire the eyeblink CR. Though the informed consent tells the subject not to wear contact

lenses, question #8 of the questionnaire is used as a double check point. If the subject

responds "yes" to this question, then the subject is asked to remove them. After filling out

this questionnaire, the subjects were instrumented.

To assess the acquisition of the classical conditioning through the evaluation of the

eyeblink response, two electrodes and a ground were used to measure the differential (bipolar)

signal generated from the muscles that surround the eye and cause the eyelid to blink. One

electrode was placed just above the right eyebrow on the lateral frontalis. The electrode lies

on an imaginary vertical line 1 cm lateral to the vertical that traverses the pupil of the eye

during center gaze. The other electrode was placed below the eye, high on the cheek, on the

orbicularis oculi.[14] The electrodes used were standard infant surface silver-silver chloride

(Ag-AgCl). Infant electrodes were used because they are smaller and lighter than adult

electrodes and thus make it more comfortable for the patient to wear on the face for the

duration of the experiment.

To measure the ECG and the respiration waveform from the subject, electrodes were

placed on the left and right side of the subject's lower rib. A ground or reference electrode

was used on the subject's abdomen. To reduce noise, the impedance of the electrode-skin

interface was lowered. The subject's skin was first cleaned with an alcohol wipe and then

lightly abraded. The last step in the patient's preparation was to place the headphones on the subjects head and allow them to adjust them for comfort.

To verify that the subjects had a normal sensitivity and responsivity to the acoustic stimuli, the subject was first given a sensory reactivity, or startle test. This protocol, as discussed earlier, uses 3 intensities (102 dB, 92 dB, and 82 dB) of white noise over a series of 24 trials. This protocol, with a 2 min baseline recording period before and after the sequence of trials, required 18 min.

Once this was complete, the eyepiece for the airpuff delivery was placed in front of the subject's eye. The subjects were given an option to rest or stretch before the conditioning began. The conditioning protocol used was the delay-type protocol as discussed earlier. This protocol was a series of 120 trials, delivered with a pseudo random ITI between 15 and 25 s. This protocol, with a 2 min baseline recording before and after the trials, was 50 min in duration. To reduce boredom, the subject was invited to watch a video tape (The Bear, Space Jam, The Wizard of Oz, Free Willy, Free Willy 2, or Grease) of their choice during the test session.

4.2.3 Data Collection and Processing

The physiological signals from the EMG, ECG, and respiration amplifiers were A/D converted at a sampling rate of 200 samples/s for each signal. The highest frequency component of interest in the signals that were being collected was approximately 40-50 Hz. This was the frequency range of the R-wave component of the ECG signal. As discussed earlier, the ECG amplifier has a low-pass filter at 100 Hz to filter out high frequency noise. To prevent aliasing, a sampling rate of twice this value was used for the A/D conversion. The

respiration was filtered with a low-pass at 1 Hz and the EMG at 20 Hz, well below a potential

aliasing problem. Sampled at this same rate, the marker channel was also collected. This

marker channel is derived from the digital output as discussed earlier. That data was stored

by the LabVIEW program in ASCII format. The post acquisition digital signal processing

and analysis statistics were performed in Splus.

The EMG was processed to determine if, and when, an eyeblink occurred during the

stimulus trial. Appendix G is the Splus function written to do the first steps of the EMG

processing. This function was used to scan the marker channel file to identify the stimulus

times and then split the raw EMG file into subsets of files representing each trial. For each

trial a 1-s window of the raw EMG waveform was stored. Using the onset of the CS as the

starting point, the one second window started from 50 samples (250 ms) before the CS onset,

through 150 samples (750 ms) after the CS onset. For each patients session in the

conditioning experiment, a matrix was created where the file contains 120 rows, where each

row is a trial, by 200 columns (1 s of data). For the sensory reactivity, the file contains 24

rows.

After the large EMG file was split into the matrix of trials, each trial was processed

to evaluate the EMG response. The first step in this processing was to clean the signal using

a low-pass filter. This "lowess" filter, uses robust locally linear fit, and was used to remove

unwanted noise. A window was placed about each sample value; points that are inside the

window are weighted so that nearby points get the most weight.[15] Appendix F is a summary

of the lowess function reproduced from the Splus help manual by StatSci. The component

of the signal that we wish to keep or to enhance, the eyeblink, was in the frequency range of

5 to 20 Hz. Figure 4.2 is a sample recording from a conditioning trial that has a 60-Hz noise

problem. It can be seen from the figure that the lowess low-pass filter did a good job at

filtering out the noise from the desired signal. This plot is the raw signal as recorded from

the A/D card, where there is approximately a 0.2 V peak to peak level of 60-Hz noise on this

signal. The darker line through the center of the wave is the filtered output signal from the

lowess filter. The noise in the signal can come from a variety of sources. Quality of the

electrode preparation, placement of the ground for the amplifiers differential input, a poor

common mode rejection ratio (CMRR) between the inputs, and other electrical equipment

near the patient or physiological equipment are just a few of the reasons for 60-Hz line noise.

All of the EMG waveforms were passed through this filter regardless of the signal to noise

ratio of the waveform.
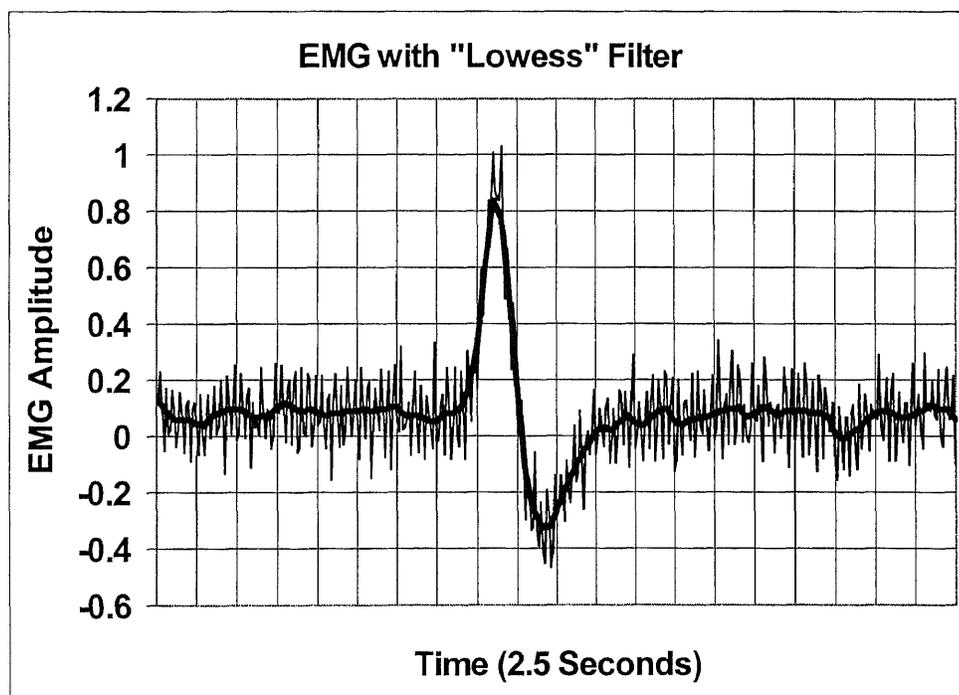
Figure 4.2 - A 1-s recording of an EMG with a 60-Hz noise problem. The
dark line is the output of the *lowess* filter in Splus removing noise.

The next step in the processing to determine if an eyeblink occurred was to create a threshold. This threshold, unique to each person, was used to adjust for differences in the amplitude of the EMG signal between subjects. At the beginning of a session of conditioning, 3 US alone trials were delivered to the patient. A US alone trial was a trial that delivers an airpuff without any preceding stimuli. This US elicited a blink, a UR. The EMG response from these 3 US alone trials were evaluated by using the amplitude of these 3 forced blinks for each patient. A threshold value of 25% of the mean of the 3 responses was used for the processing of the rest of the conditioning data.

For evaluation of conditioning, each trial was split into four windows of time as seen in Figure 4.3. The figure is a representation of the marker channel as discussed earlier. The first window, the baseline, was 250 ms in duration and was the signal prior to the onset of any stimuli. The second window, the orienting response (OR), was the first 50 ms that follows the onset of the CS. In the beginning of the experiment, the subject may have found the tone, the CS, to be startling. It is common for this to cause an eyeblink, however this was not due
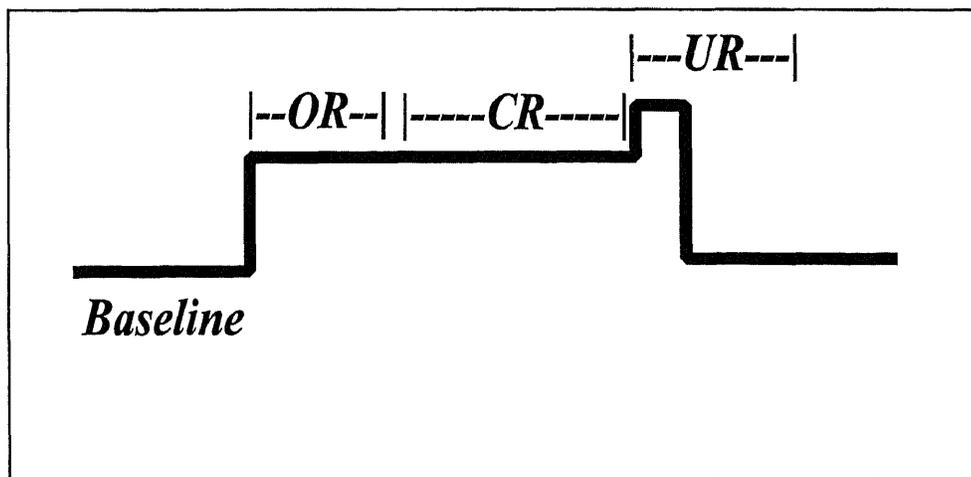
Figure 4.3 - Timing diagram of conditioning trial for data processing of eyeblink responses
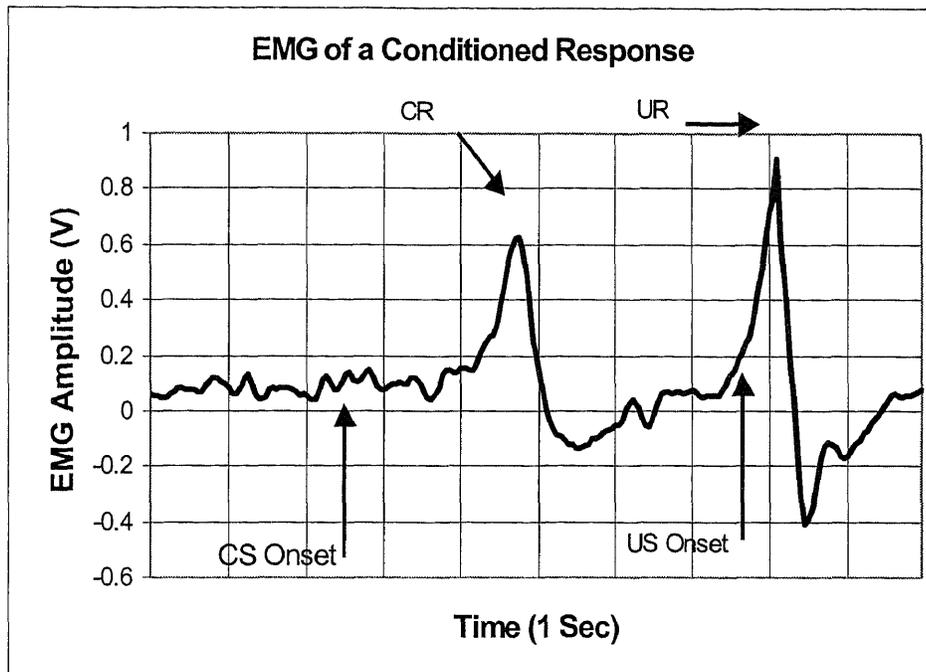
Figure 4.4 - Sample EMG of a typical conditioned response during a CS trial

to a learning of a CR. This response comes from orienting to the sound of the tone. The CR window (CR) was the 350 ms prior to the onset of the US, or air puff. Within this window a CR occurs. The last window was to evaluate the UR. This window was 100 ms in duration and begins at the offset of the US.

Looking at each of the 4 windows of a sample trial, the EMG was compared to the threshold value. If any sample of the EMG was greater than this threshold, the maximum value and its location in time was stored. The output file to the processing was a matrix that has 120 rows, (each row is a trail as in previous steps) and 8 columns. The 8 columns were 4 pairs of output values for each window. One pair was time and amplitude of a blink, if one occurred. If a window did not have any EMG signal greater than the threshold then a pair of zeros was stored as a place holder in the output file. If an eyeblink was present in either the baseline period, or the OR window, the trial was considered to be invalid. A blink in these

windows would be considered random, and not a function of the stimulus. An eyeblink here could cause a different response than normal if a random eyeblink carries into the trial. Using the same threshold the CR & UR windows were then also compared. Figure 4.4 is an example of how an eyeblink would look during a CS trial. We see that there is an eyeblink in the time window of a CR and the UR.

The ECG was used to calculate an instantaneous heart rate measure through out the protocol. The first step in the processing of the raw ECG waveform was to filter using the same lowess filter that was used to process the EMG. By changing characteristics of the algorithm, namely the window size, the resulting effective 3dB point of the filter can be changed to a new desired frequency. This same filter can also be used as a high pass filter. By subtracting a low-passed signal from the original signal a high pass filter is created. This technique was used to filter out low frequency components in the ECG thus enhancing the
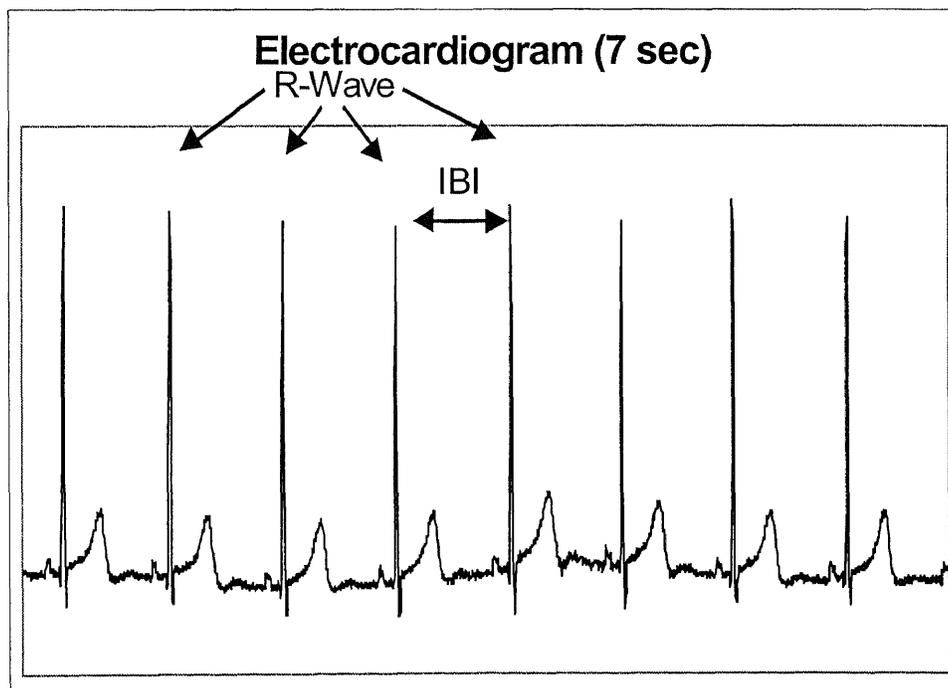
Figure 4.5 - 7-s sample of an electrocardiogram waveform.
R-waves are used to calculate each interbeat-interval

R-wave. DC levels, motion artifact, and the T-wave are all reduced by this high pass filter. The first processing step for the ECG waveform was to pass the raw signal through an R-wave detector. This function, developed in Splus (See appendix H), scanned the ECG for the QRS complex by using a sliding window to scroll through the waveform to identify localized maxima. Figure 4.5 is a plot of 7-s of an ECG for a patient. The output of the R-wave detector function is a 4 column file which contained the compiled information about the ECG. The first column was an index of the locations for each of the R-waves for the 50 minute record. Though not currently used in the analysis, the amplitudes of the R-waves were stored in the second column. The interbeat interval was the duration of time between each successive R-wave as indicated in the figure. Using the index values from each of the R-waves stored in column 1, the interbeat interval was calculated in samples between beats. The heart rate, in beats per minute, was then calculated by the following formula:

$$HeartRate = 60 * \frac{SamplingRate}{IBI} \tag{4.1}$$

Where IBI is the interbeat interval and the sampling rate was 200 samples/s, as discussed earlier. The IBI and the heart rate were stored into columns 3 and 4 respectively.

Respiration was processed by passing the raw signal though a peak detector. Here the first step was not to filter the signal because the electronic filters in the respiration amplifier were adequate to remove any undesired noise. Similar to the R-wave detector for the processing of the ECG, the respiration version of the peak detector function finds the peak of each breath which was then used to calculate the respiration rate. To save file space on the hard drive the raw waveform was decimated. As discussed earlier, the sampling rate for these

signals was set at 200 samples/s, however, the respiration only required 20 samples/s. By taking every tenth sample from the respiration signal and then storing the output file with the same name, an effective sampling rate of 20 Hz was obtained. An output file was created by the peak detector function. Similar in structure to the ECG output file, the respiration output file contained the same 4 column types. The first column of the file was the index, or sample value of the location of each peak. The second value was the amplitude of this peak. The third column was the interval between each pair of respiration peaks which was then used to calculate the respiration rate and stored in column 4.

4.3 Results

The sensory reactivity data were analyzed using a 2 x 3 (Group x Intensity) ANOVA split-plot model. Sensitivity was defined as the proportion of eyeblink responses. Responsivity is the magnitude of eyeblink responses when they occur. For sensitivity, the ANOVA revealed only a main effect of stimulus intensity, $F(2,30) = 13.8$, $p<.05$. Similarly, only the main effect of stimulus intensity was significant for the measure of responsivity $F(2,22)=3.6$, $p<.05$. Sensitivity and responsivity were the same between the two experimental groups and positively related to the stimulus intensity.

Conditioned Responses - CFS

Conditioned Responses - Control
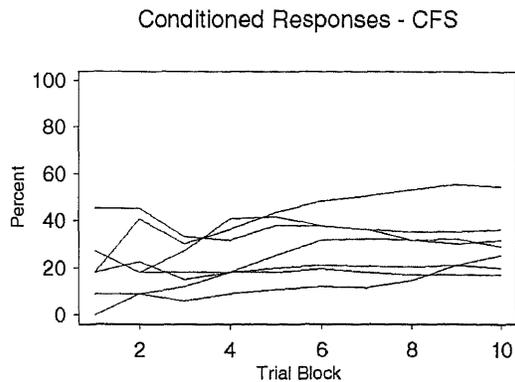
Figure 4.6 - CFS group acquisition curves. Shown as percent responses in blocks versus time
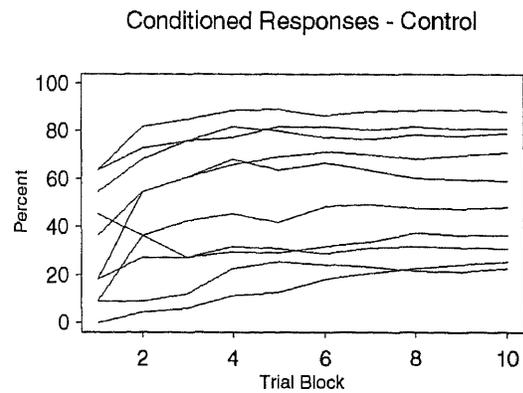
Figure 4.7 - Control group acquisition curves. Shown as percent responses in blocks versus time

Figures 4.6 and 4.7 show acquisition curves of the CRs from CFS and control subjects

respectively, from the delay type protocol. As discussed in the processing section, a CR was

indicated if an eyeblink occurred in the specified window for each trial. The data is shown

as a percentage of CRs in blocks of 10 trials versus time. In the delay protocol, each block

of 10 trials has 8 CS+ trials (CS paired with the US), 1 CS alone trial, and 1 US alone trial.

In each block of trials a score of 100% would be 8 out of the 8 CS+ trials. The characteristic

acquisition curve can be seen in the first few trial blocks of the controls. In the later blocks,

an asymptote is reached where 60% is normal for most healthy controls. It is common for

20% of health controls to not produce a conditioned response. Figure 4.8 is a plot of the

group acquisition curves. Data is shown as the mean ± standard error for controls (squares)

and CFS patients (triangles). The acquisition data were analyzed using a 2 x 10 (Group x

Block) ANOVA split -plot model. For acquisition the main effects of Group,

$F(1,15)=5.0$, and Block, $F(9,135)=14.3$, were significant, $p < .05$. These were qualified by

Percent Conditioned Responses by Trial Block
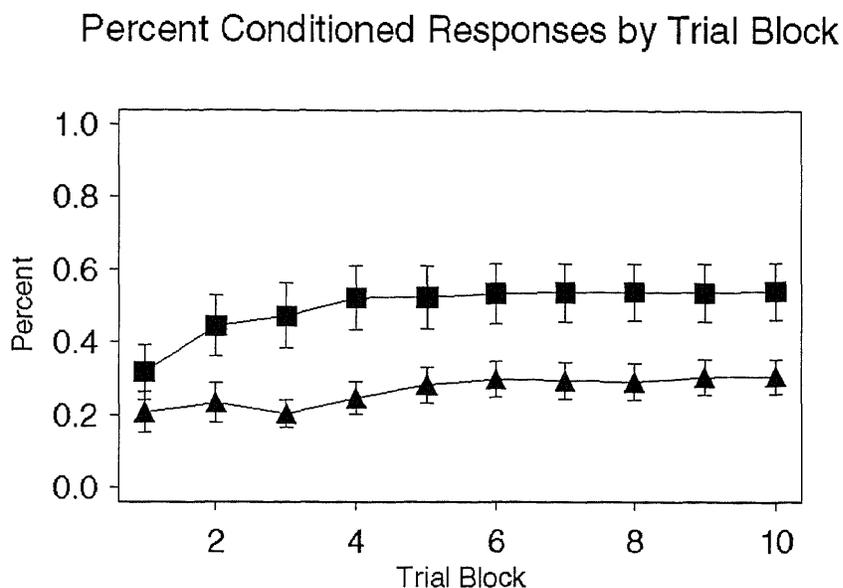
Figure 4.8 - Group acquisition curves of conditioned responses for delay type protocol. Shown as percent responses in blocks versus time

the significant Group x Block interaction, $F(9,135)=2.3$, $p < .05$. The CFS Group required more trials to learn the eyeblink CR.

Heart rates were calculated for each patient during the conditioning protocol. Using the output file described in the methods for the storage of each r-wave and its corresponding heart rate of that beat, the mean heart rate was calculated for each 2-min block of time for the experiment. The first two minute period was the base line and the last two minute period was the post experiment recording. Figure 4.9 is a plot of each of the two experimental groups. Controls (squares) and CFS (triangles) are plotted as heart rate in beats per minute versus time in two minutes periods.

4.4 Conclusions

The pilot experiment showed that the conditioning system has the capability to perform the desired protocols. The sensitivity and responsivity of the subjects were measured through the application of the sensory reactivity protocol. Conditioning of a subject, through the measurement of the classically conditioned eyeblink response was achieved by the application of the delay protocol. The system and parameters were such that conditioning took place in an acceptable duration of time, while not sacrificing the sensitivity of the measurement between experimental subjects.

CHAPTER 5

5 - CONCLUSIONS

The assessment of the eyeblink response, in a classical conditioning type of experiment, requires the precise coordination between stimulus output and physiological signal recording to measure a response. The main goal of this project focused on the computer software to control an entire experimental system. The system contained the development of specific hardware components to create and deliver stimuli and for the measurement of physiological responses. The software was able to simultaneously control desired output stimuli and record patient responses. Special safety features were installed in the system to provide a safe apparatus for use on human patients. To improve the systems performance the software also evaluated signal quality, and helped to predict problems.

Heart Rate / Delay Protocol - CFS vs. Control

Time in 2 Minutes Periods
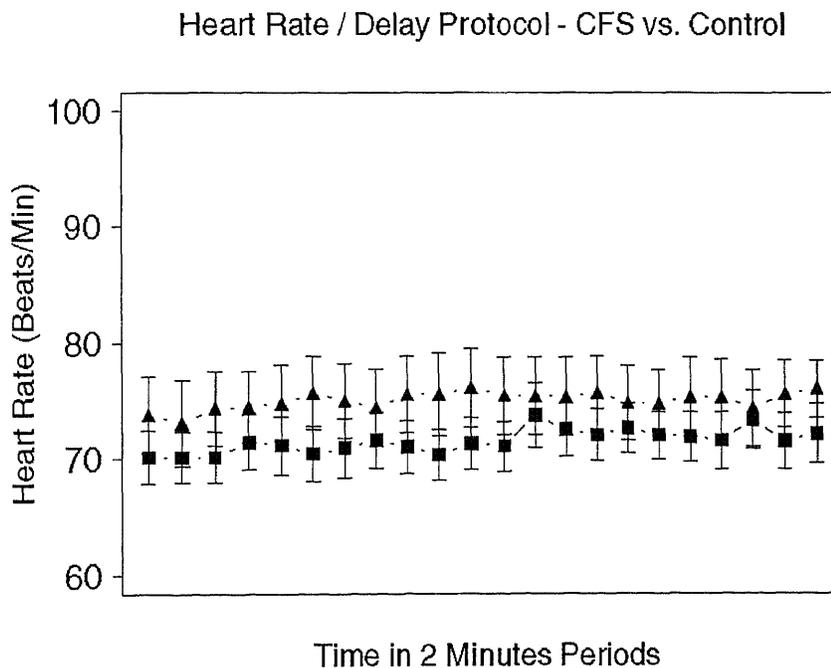
Figure 4.9 - Mean heart rate for CFS and control group per trial block

54

Through a series of tests, the system was shown to be reliable, accurate, and most importantly safe. Once tests that proved this were completed, experimental data was recorded from subjects. These tests clearly showed that the parameters selected properly produce the desired learning response from a conditioning protocol.

There are a few future directions for the continued improvement of this apparatus. In this current application, processing of the data was performed post-acquisition. Processing steps were optimized and a file management system was created to allow the data to be processed and summarized within 10 minutes after the end of the experimental session. A future step would be to add a software program, for data collection and protocol execution, to process the eyeblink response; that is, to evaluate the acquisition of the eyeblink response in real time. If the experimenter has this information in real time, then sessions could be adjusted as acquisition criteria are met. If the ECG was processed in real time then there could be a savings in file storage size. This would require an algorithm that the ECG would pass through and produce as its output a file that would contain the intervals between the R-waves. The same could also be done with the respiration signal.

The parameters of this protocol for the measurement of the eyeblink response did not provide an optimal system for the processing of cardiovascular responses. The recording and analysis presented here for the ECG and respiration signals focused on accurate recording methods and an algorithm for the detection of the R-wave for the calculation of a beat by beat heart rate of the patient during the experiment. The first pass analysis of the heart rate clearly showed a trend for one group to be different from the other, that type of processing it not very sensitive to small changes in the cardiovascular system. A future processing could look at the transient response from each of the stimuli to see if there is a CR in the cardiovascular system.

Another method might be to look at the response of the autonomic nervous system also as a function of conditioning.

In many forms of experimentation there can be logistical problems with the execution of the experiment in a traditional laboratory. In future experimental protocols; two-tone discrimination for example: patient compliance can be difficult. If the subject must travel a great distance, getting to the research center 5 days in a row, might pose a problem. There are also known psychological changes that can take place when a patient enters a research setting. It would be desirable to have a system that was portable, where the experimenter can bring the equipment to the subject. The software, computer, and A/D conversion card have already been designed to be portable. The electronic interface, however, could be designed to be smaller and more portable. More over, a software version of the audio generation system utilizing the DAQ700 acquisition cards digital to analog converter could replace the audio hardware.

ANALOG TO DIGITAL CONVERSION SECTION - LABVIEW CODE
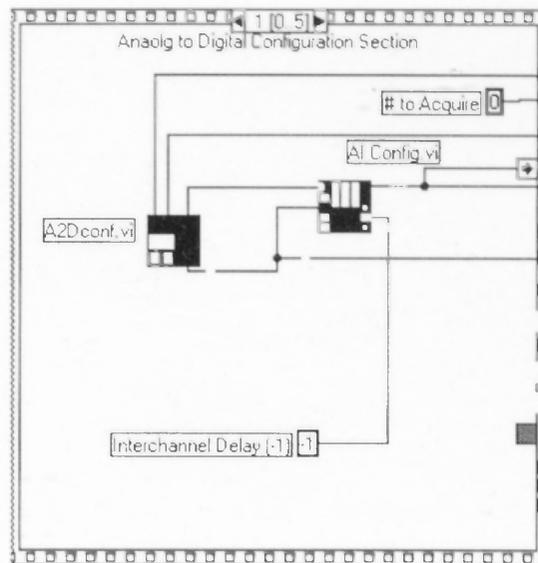
Figure A.1 - A/D Configuration VI
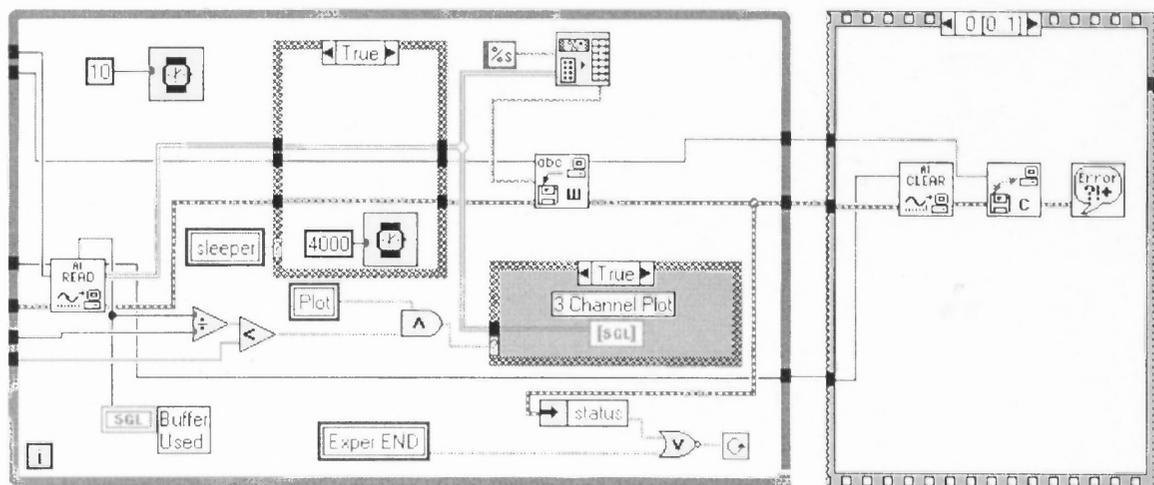
Figure A.2 - Analog to Digital Conversion loop

PROTOCOL CONTROL LOOP SECTION - LABVIEW CODE
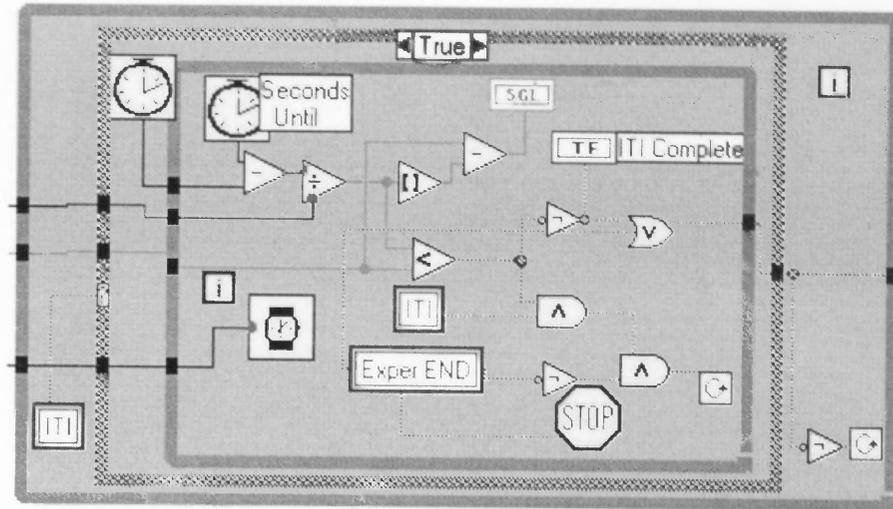
Figure B.1 - LabVIEW Code for Inter-trial Interval (ITI) section

FRONT PANEL USER INTERFACES FOR CONDITIONING PROGRAM
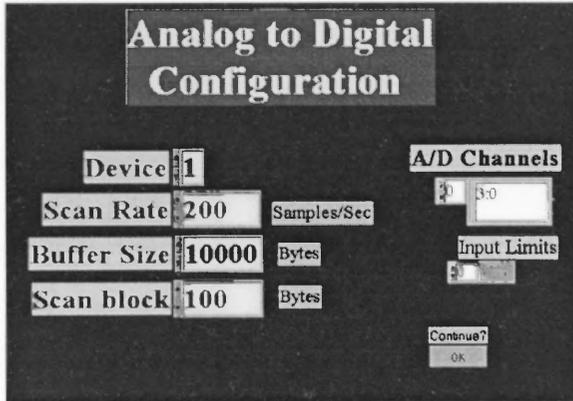
Figure C.1 - LabVIEW Front Panel A/D configuration specifications
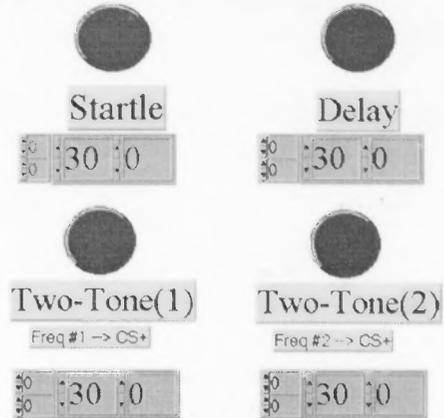
Figure C.2 - Front panel user interface for selection of experimental protocol.
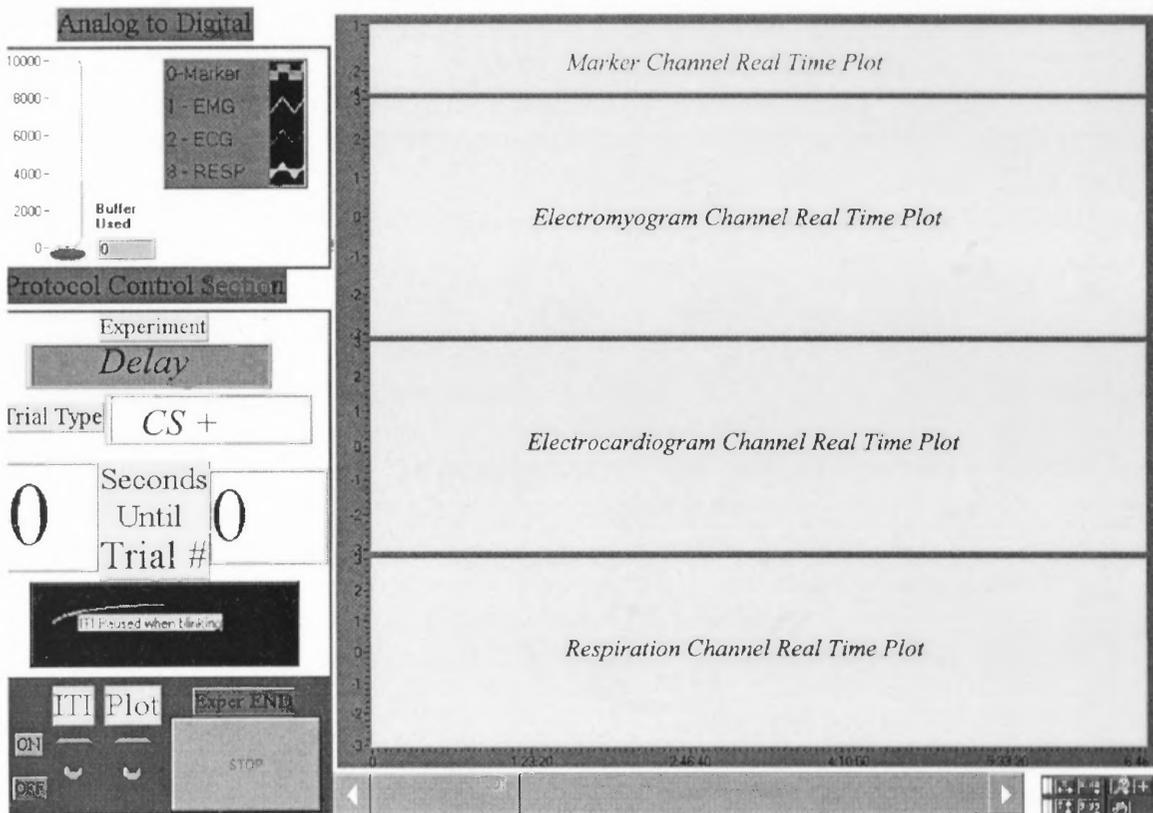
Figure C.3 - Front panel user interface for conditioning program during protocol execution.

APPENDIX D
SPECIFICATIONS OF THE NATIONAL INSTRUMENTS CORPORATION
DAQ-700 PCMCIA DATA ACQUISITION CARD

Analog Input Characteristics

Number of Channels	16 single-ended or 8 differential
Type of ADC	Successive approximation
Resolution	12 bits, code range -2024 to +2023
Max sampling rate	100 kS/s
Input signal ranges	±10V, ±5V, ±2.5V software selectable
Input coupling	DC
Over voltage	±30 Volts
FIFO buffer size	512 samples
Data transfers	Interrupts, programmed I/O

Transfer Characteristics

Relative accuracy	±1 LSB typ, ±1 LSB max
DNL	±0.5 LSB typ, ±1 LSB max
No missing codes	12 bits, guaranteed
Offset error	
After software calibration	±1 LSB
Before software calibration	±2 LSB typ, ±9 LSB max
Gain error	
After software calibration	±0.036% max
Before software calibration	±0.07% of reading typ, ±0.4% max

Amplifier Characteristics

Input impedance	1 GΩ in parallel with 40 pF
CMRR (all input ranges)	-72 dB, DC to 60 Hz

Digital Input / Output Characteristics

Number of channels	8 input and 8 output
Compatibility	TTL
Number of counter timer channels	3 counter/timers (1 dedicated to analog input)
Resolution	16 bits
Compatibility	TTL, gate and source pulled high with 100kΩ
Base clock	1 MHZ ± 0.01%
Max source frequency	10 MHZ

APPENDIX D
(Continued)

Min source/gate pulse duration .... 50 ns

Data transfers .... Programmed I/O

Miscellaneous Characteristics

Bus Interface type .... slave

PC Card type .... PCMCIA Type II

I/O connector .... 50-pin male

Operating temperature .... 0° to 70°C

Storage temperature .... -55° to 150°C

Relative humidity .... 5% to 90% noncondensing

APPENDIX E
SPECIFICATIONS OF PHYSIOLOGICAL AMPLIFIERS

Model Resp 1 / ECG - Impedance Pneumograph with Electrocardiogram output.

Case	Powder coated aluminum extrusion
Power	9 Volt alkaline battery - 100 hour life
Excitation	30 KHZ sine wave at constant current
Range	1Ω to 2KΩ
Output	Respiration - 1 to 5 Volts
	ECG - 1V/mV

Model 2283 FTi - Biopotential Amplifier

Power	9 volt alkaline battery
Input impedance	100MΩ
Output impedance	< 15KΩ
Amplification	x10, x100, x1,000, and x10,000
Frequency response	0.01 to 120 Hz (3dB)
Common mode rejection	> 70 dB
Noise	< 3µV (ref to input)

APPENDIX F
EXPERIMENT WORK SHEET

Experiment Work Sheet

Subject Name _____   Date _____

Subject Number _____   Session _____

Subject arrival time _____

Consent form signed and copied for subject   Y   N

Pre-Test Measurements:

Ambient Noise   Room Temp   Sub-threshold   Threshold   Supra-Threshold

_____dB   _____deg   _____dB   _____dB   _____dB

Startle   Began at: _____   File Name: _____

Delay   Began at: _____   File Name: _____

2 Tone   Began at: _____   File Name: _____

Pre- Test Patient Questions:

Do You ...
1)   Drink coffee   Y N   How much: _____   Last One: _____
2)   Caffeinated drinks   Y N   How much: _____   Last One: _____
3)   Exercise   Y N   How much: _____   Last One: _____
4)   Smoke   Y N   How much: _____   Last One: _____
5)   Drink alcohol   Y N   How much: _____   Last One: _____
6)   Take   Y N   What: _____How much:_____Last One: _____
        Medications   Y N   What: _____How much:_____Last One: _____
                      Y N   What: _____How much:_____Last One: _____
                      Y N   What: _____How much:_____Last One: _____
7)   When was your last period   _____
8)   Are you currently wearing contact lenses   Y   N
9)   When was your last meal: _____
10)   Age: _____
11)   Occupation _____

Experimental Notes:

APPENDIX G
DESCRIPTION OF SOFTWARE FOR LOWESS FILTER
(SPLUS HELP MANUAL)

DESCRIPTION   Gives a robust, local smooth of scatterplot data. Among other options is the fraction of data smoothed at each point.

USAGE   lowess(x, y, f = 2/3, iter = 3, delta = .01*range(x))

REQUIRED ARGUMENTS   x,y   vectors of data for a scatter plot.

OPTIONAL ARGUMENTS

f   fraction of the data used for smoothing at each x point. The larger the f value, the smoother the fit.

iter   number of iterations used in computing robust estimates.

delta   interval size (in units corresponding to x). If lowess estimates at two x values within delta of one another, it fits any points between them by linear interpolation. The default is 1% of the range of x. If delta=0 all but identical x values are estimated independently.

VALUE   list containing components named x and y which are the x,y points of the smoothed scatter plot. Note that x is a sorted version of the input x vector, with duplicate points removed.

NOTE   This function may be slow for large numbers of points; execution time is proportional to (iter*f*n^2). Increasing delta should speed things up, as will decreasing f.

DETAILS   This is a scatterplot smoother - it does not make any assumptions about the x values being evenly spaced. Lowess uses robust locally linear fits. A window, dependent on f, is placed about each x value; points that are inside the window are weighted so that nearby points get the most weight.

REFERENCES
Cleveland, W. S. (1979). Robust locally weighted regression and smoothing scatterplots. Journal of the American Statistical Association 74, 829-836.

Chambers, J. M., Cleveland, W. S., Kleiner, B. and Tukey, P. A. (1983). Graphical Methods for Data Analysis. Wadsworth, Belmont, California.

APPENDIX H
EMG TRIAL SPLITTING FUNCTION

```
function(basename = "", l1 = 0.21, l2 = 0.27, pre.window = (250/5), pos.window    = (750/5),
        ylimit = 2, split = F){
        print(paste("Searching for data files for patient:", basename, sep = " "))
        filename.emg <- paste(basename, ".emg", sep = "")
        filename.mark <- paste(basename, ".mark", sep = "")
        uncompress(filename.emg, pos = 1)
        uncompress(filename.mark, pos = 1)
        file.emg <- get(filename.emg)
        file.mark <- get(filename.mark)
        print("=================================")
        print(paste("EMG    file:", filename.emg, sep = " "))
        print(paste("Marker file:", filename.mark, sep = " "))
        print("=================================")
        print("Scanning marker file for trials...........")
        y <- grep(T, diff(file.mark) > 0.4)
        yy <- file.mark[y + 3]
        par(mfrow = c(1, 1), mar = c(3, 2, 1, 1))
        plot(yy)
        l1 <- (-3)
        l2 <- (-2.5)
        l1 <- (-2)
        z1 <- y[(yy > l1) & (yy < l2)]
        z2 <- y[(yy > l2) & (yy < l3)]
        par(mfrow = c(8, 15), mar = c(0.1, 0.1, 0.1, 0.1))
        zz <- rank(c(z1, z2))
        z <- rep(NA, length(zz))
        z[zz] <- c(z1, z2)
        print(paste("Found ", length(z), "marks", sep = ""))
        if(split == T) {
                col.tot <- pre.window + pos.window
                mark.outfile <- matrix(rep(NA, (120 * col.tot)), ncol = col.tot,byrow = 120)
                emg.outfile <- matrix(rep(NA, (120 * col.tot)), ncol = col.tot,byrow = 120)
        }
        for(i in 1:length(z)) {
                mark.piece <- file.mark[(z[i] - pre.window):(z[i] + (pos.window -1))]
                emg.piece <- file.emg[(z[i] - pre.window):(z[i] + (pos.window - 1))]
                plot(mark.piece, ylim = c(-4, 3), type = "l", ylab = "", xlab = "", axes = F,
                        lty = 2, err = -1)
```

APPENDIX H
(Continued)

```
                par(new = T)
                plot(emg.piece, ylim = c( - ylimit, ylimit), type = "l", col = 2, ylab = "",
                        xlab= "", err = -1)
                text(20, (-0.75 * ylimit), i, cex = 1, srt = 1)
                if(split == T) {
                        mark.outfile[i, ] <- mark.piece
                        emg.outfile[i, ] <- emg.piece
                }
        }
text(100, (0.75 * ylimit), basename, cex = 0.4, srt = 1)
if(split == T) {
        emg.name <- paste("acq", basename, ".emg.trial", sep = "")
        mark.name <- paste("acq", basename, ".mark.trial", sep = "")
        mark.index.name <- paste("acq", basename, ".mark.index", sep = "")
        assign(emg.name, emg.outfile, where = 1)
        assign(mark.name, mark.outfile, where = 1)
        assign(mark.index.name, z, where = 1)
}
compress(filename.emg, pos = 1)
compress(filename.mark, pos = 1)

}
```

```
function(filename = "", samplerate = 200, min.window = 90, filt = F, f = 15){
        ################################################################
        ##### This function finds the QRS complex in order      ####
        ##### to calculate heart rate.  The function outputs    ####
        ##### an index column for the location of the peaks     ####
        ##### and a column of the value at the peak             ####
        ##### and a column of ibi's (the diff between each  ####
        ##### peak and a column of heart rates (beats/min)      ####
        ################################################################
        ##### filename= data file name in ""                    ####
        ##### y = the column of the file that has the ECG   ####
        ##### samplerate = the A/D sampleing rate               ####
        ##### min.window = half of tis would be the minimum     ####
        ##### distance in samples that 2 R-waves can exist      ####
        ################################################################
        #####  filt --> set to T to detrend data file           ####
        #####  f --> # of points to smooth over, def>(f=15)     ####
        ################################################################
        print("<<<<<    ECG Processing Function     >>>>>")
        print(paste("Processing file > ", filename, sep = ""))
        file <- get(filename)
        if(filt == T){
                print("<<<<<    Filtering (Detrending) data file     >>>>>")
                f <- f/length(file)
                z <- lowess(1:length(file), file, f = f, delta = 0, iter = 2)$y
                file <- file - z
        }
        min.window <- min.window * 2
        print("Scanning data file for QRS Complex peaks")
        ecg.index <- getpeaks.ecg(file, span = min.window)
        ecg.values <- file[ecg.index]
        ecg.file <- cbind(ecg.index, ecg.values)
        print("<<<<<   Fine tunning Systolic points   >>>>>")
        for(i in 1:(length(ecg.file[, 1]) - 1)) {
                ecg.file[i, 1] <- tuneit(file = file, index = ecg.file[i, 1], span = 10,
                        domax = T, keep = 1)
        }
```

APPENDIX I
(Continued)

```
print("<<<<<       Calculating IBIs      >>>>>")
ecg.ibi <- c(NA, diff(ecg.file[, 1]))
print("<<<<<    Calculating Heart rate     >>>>>")
ecg.hr <- (samplerate/ecg.ibi) * 60
ecg.hr <- round(ecg.hr, digits = 2)
ecg.file <- cbind(ecg.file, ecg.ibi, ecg.hr)
ecg.file <- t(data.frame(t(ecg.file), row.names = c("index", "value", "ibi", "hr")))
ecg.out <- paste(filename, ".hr", sep = "")
assign(ecg.out, ecg.file, where = 1)
print("========    DONE   =================")
print("")
}
```

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