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The Acute And Sub-Acute Effects Of Electrosurgical And Ultrasonic Surgical Devices On The Neurophysiologic Changes Of Sciatic Nerve Function

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THE ACUTE AND SUB-ACUTE EFFECTS OF ELECTROSURGICAL AND ULTRASONIC SURGICAL DEVICES ON THE NEUROPHYSIOLOGIC CHANGES OF SCIATIC NERVE FUNCTION

by

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DEDICATION

"Gurur Brahma Gurur Vishnu,
Gurur Devo Mahesh Varah.
Guru Shakshat Para Brahma,
Tasmai Shri Guruve Namah"

- A Shloka (Rhyme) from Sanskrit Literature

Translation

The Guru (teacher) is Brahma (The God of Creation)

The Guru is Vishnu (The God of Sustenance)

The Guru is Shiva (The God of Annihilation)

My Salutation to such a Guru, who is verily the Almighty

I would like to dedicate every bit of my work to the Guru (the teacher); to all the teachers who have bestowed knowledge in me for so many years and made me the person I am today. Nothing of what I have achieved would have been possible without their blessings.

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CHAPTER 1

INTRODUCTION

Since the dawn of medicine, blood loss has been one of the major concerns for the surgeons. Iatrogenic blood loss can have several harmful effects on the patient's recovery from surgery. With the rapid development in technology, the blood loss can now be significantly minimized by using a variety of vessel sealing instruments that use several energy sources to seal the blood vessels or tissue bundles instead of traditional sutures and clips. This technology results in around 40% to 60% lower blood loss than conventional cutting and sealing tools, thus reducing costs, hospital stays, and improved survival rate (Harold, Pollinger et al. 2003). Also, it leads to around 30% reduction in the surgery time and overall improved operating room throughput (Cronje and de Coning 2005).

The basic principle of these vessel sealing instruments is to use energy to force a phase change of the tissue proteins (primarily collagen and elastin) to form a seal. Thus, a single instrument can cut as well as seal the vessels and tissues, reducing the overall operational time. These instruments can be used in several general, colorectal, gynecological and urological surgical procedures.

The two types of vessel sealing techniques accepted worldwide are based on electrosurgical and ultrasonic technology. The electrosurgical technique uses electric energy while the ultrasonic technique uses ultrasonic energy. Electrosurgery was introduced in 1920's and even after 90 years it remains as a fundamental tool in the practice of surgery. An electrosurgery unit is an radio-frequency based system which

consists of a generator that powers the unit and converts the power supply into high frequency electric current between 500 kHz and 4000 kHz (Luciano, Soderstrom et al. 1994; Jones, Pierre et al. 2006). The generator produces different types of electrical waveform patterns which can produce different tissue effects like cut, coagulation, desiccation or fulguration. There are two types of electrosurgery devices monopolar electrosurgery (MES) and bipolar electrosurgery. Due to its effectiveness and versatility, MES is commonly used in clinical applications.

On the other hand, the ultrasonic energy-based surgical devices use ultrasonic energy to simultaneously cut and coagulate soft tissue and vessels. The ultrasonic energy is produced by a piezoelectric transducer that vibrates at its natural harmonic frequency of 55.5 kHz (Emam Tarek 2003). This vibration is transferred to the blade which in turn creates frictional heat when interacting with tissue. The ultrasonic devices cause minimal thermal tissue damage, charring and desiccation with improved visibility in the surgical field (Lee and Park 1999) . These devices help surgeons increase procedural efficiency and improve surgical outcomes in a wide range of surgical areas. There are different types of ultrasonic cutting and coagulating devices developed for a variety of applications.

Moreover, these instruments have a great application in laparoscopic surgeries. These surgeries employ a minimally invasive surgery technique by using fine instruments inserted through a small incision in the skin. This type of surgery is widely preferred over traditional open surgery as it facilitates increased efficiency, reduced complicated procedures and lower operational time. Moreover, laparoscopic surgery exposes the patient to reduced risks of infections and allows faster recovery.   However,

even with laparoscopy, complex surgeries involving a number of blood vessels can pose challenges. Sealing blood vessels using clips or ligatures can take much time as the surgeon cannot use his hands directly to carry out these procedures. Due to this issue, many complicated procedures might be performed as open surgeries instead of minimally invasive technique. However, the vessel sealing technology of energy-based instruments directs energy through fine instruments that allow surgeons to precisely seal and then cut blood vessels and tissues.

A variety of ultrasonic devices have been used for over 10 million procedures worldwide (www.ethiconendo.com). However, it is not clear if these devices have potential to cause nerve damage when applied in close proximity of the nerve. The safe distance to apply these devices from nerves has not been rigorously investigated.

1.1 HYPOTHESIS AND SPECIFIC AIMS

A hypothesis of this study is that conventional radio-frequency based cutting and coagulating devices may cause nerve injury and dysfunction because of radiant heat energy and electrical current. It is hypothesized that the ultrasonic energy based devices will produce less nerve injury and dysfunction as compared to the traditional electrosurgery devices. The purposes of this study were to determine the acute and sub-acute effects of monopolar electrosurgery unit, bipolar electrosurgery device, ultrasonically activated scalpel and ultrasonic shears on the sciatic nerve function in a rat model.

The specific aims of this study are stated below,

Specific Aim 1:

Monopolar electrosurgery and ultrasonically activated scalpel were used near the sciatic nerve to determine the acute effects of the energy sources on nerve conduction function. Also, these devices were applied at 1 mm, 2 mm, 3 mm and 4 mm from the nerve to determine a safe distance of application for minimal nerve injury.

Specific Aim 2:

Monopolar electrosurgery, bipolar vessel sealing device and ultrasonic shears were used near the rat sciatic nerve to establish a standard protocol that would mimic the actual clinical use of these devices in a controlled experimental setting. The acute effects of these devices on nerve conduction function were determined.

Specific Aim 3:

Monopolar electrosurgery, bipolar vessel sealing device and ultrasonic shears were used to surgically expose rat sciatic nerve as in specific aim 2. The sub-acute effects of these devices on nerve conduction function and behavioral changes were determined for 3 to 7 days after the application.

CHAPTER 2

SURGICAL DEVICES

In these studies, five surgical devices were tested in close proximity to the rat sciatic nerve. Two of these devices were radio-frequency based devices while other three were ultrasonically activated cutting devices. The devices used were:

- Monopolar Electrosurgery (MES) Cutting Device (Bovie) (ValleyLab, Boulder, CO)

- Ultrasonic Blade HK 105 Cutting Device (Ethicon Endo-surgery Inc., Cincinnati, OH)

- Harmonic Focus Cutting Shear Device (Ethicon Endo-surgery Inc., Cincinnati, OH)

- Harmonic ACE Cutting Shear Device (Ethicon Endo-surgery Inc., Cincinnati, OH)

- LigaSure Coagulating Device (ValleyLab, Boulder, CO)

Of these devices, MES is a radio-frequency (RF) based cutting blade and LigaSure is radio-frequency based coagulating shear device. The ultrasonic blade, as the name suggests, is an ultrasonically activated scalpel (blade) used for cutting tissues. Harmonic Focus and ACE are both ultrasonically activated shears for cutting tissues. The principles and working mechanisms for each of these devices are discussed in detail in the following sections.

2.1 RF BASED DEVICES

2.1.1 Electrosurgery Technique

The monopolar electrosurgery (MES) device is commonly referred to as 'Bovie'. This is because this technique was invented and developed by Dr. William Bovie, a Harvard graduate (McCauley 2003). Electrosurgery devices are based on the RF technique wherein there is an application of high frequency current to cut, coagulate, desiccate or fulgurate tissue during the surgical procedures (McCauley 2003). This device enables easy operation with minimum blood loss.

The RF device requires an electrosurgery unit (ESU) consisting of a generator, an active electrode and a return electrode. The generator powers the unit and converts the power supply into high frequency electric current between 500 kHz and 4000 kHz (Luciano, Soderstrom et al. 1994; Jones, Pierre et al. 2006). Also, the generator produces different types of electrical waveform patterns which can produce different tissue effects such as cutting, coagulation, desiccation or fulguration. Based on position and function of the return electrode, there are two types of electrosurgery techniques, monopolar electrosurgery (MES) and bipolar electrosurgery. Due to its effectiveness and versatility, MES is commonly used in clinical applications. LigaSure is a type of bipolar device.

Nerves and muscles can be stimulated by frequencies below 10,000 Hz (Luciano, Soderstrom et al. 1994). However, as the ESU generator produces electric current at much higher frequency, the current does not stimulate the neuromuscular

tissue. This allows passage of this current through the body tissue without a possibility of electrocution.

2.1.2 Monopolar Electrosurgery (MES)

2.1.2.1 Principle of Operation

High frequency alternating current is directly passed through the body tissue to cut, coagulate or desiccate the tissue. At the surgical site, the tissue ablation and desiccation is obtained by heating of the conducting tissue by an alternating electric current that causes tissue vaporization and ionization of water content in the area surrounding the blade tip (Brown 2005).

2.1.2.2 Working Mechanism of MES

MES unit consists of a generator, an active electrode and a patient return electrode. The active electrode is placed at the surgical site while the patient return electrode is placed at some other position on the body. The high frequency current, produced by generator, flows from the active electrode to the tissue, spreads through the body and then exits through the return electrode (Luciano, Soderstrom et al. 1994). Thus, the electrical circuit is completed with patient being part of the circuit. A typical set up of MES is shown (Figure 2.1). The active electrodes are available in different styles and shapes. In this project, we used the MES blade as active electrode (Figure 2.2).
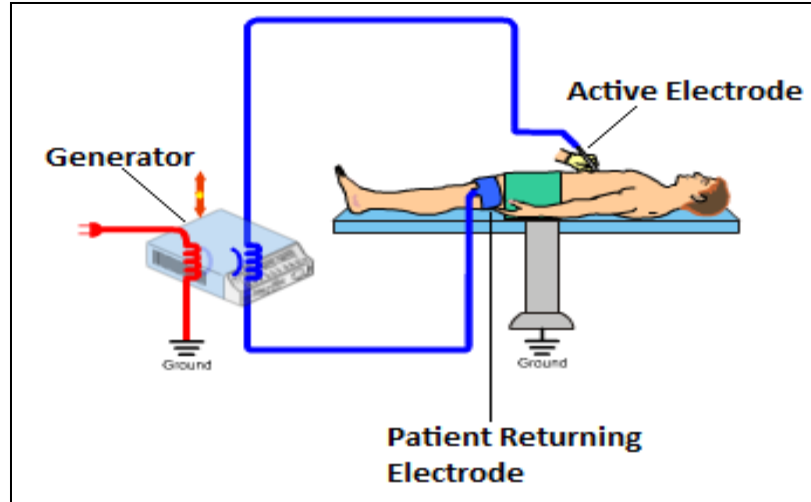
Figure 2.1: Operational set-up for MES showing the generator, active electrode and patient return electrode

(Modified from http://www.valleylab.com/education/poes/poes_03.html)
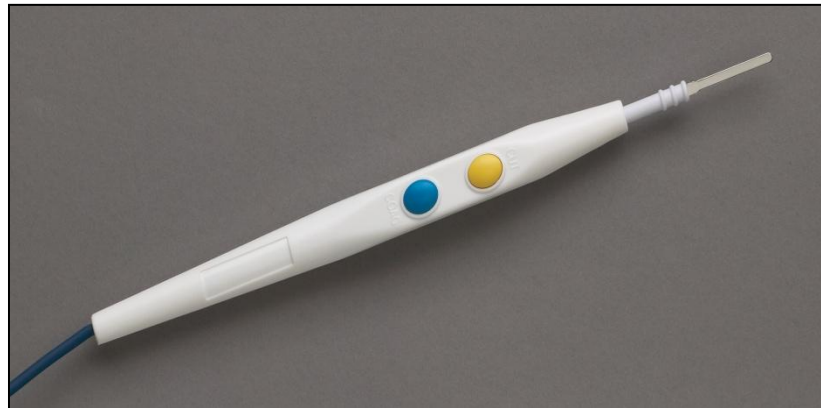
Figure 2.2: MES Blade (active electrode) (modified from http://www.ambercity.com/vega-series-electrosurgical-pencils-esrk3002.html)

2.1.3 LigaSure Coagulating Device

LigaSure is a radio-frequency based bipolar coagulating device (Figure 2.3). This is a vessel healing device which can seal vessels up to 7 mm diameter as well as

lymphatics and tissue bundles (http://www.ligasure.com). The LigaSure device can be used for surgical procedures such as thyroidectomy and parotid lobectomy.

2.1.3.1 Principle of Operation

In this device, bipolar technique is used. The high frequency alternating current is passed just through the part of tissue caught between the grasp of the LigaSure device (Figure 2.3). At the surgical site, there is no cutting of the tissue. When used on the vessels, a permanent seal is produced that completely seals the lumen of vessel (Giulio 2004)

Figure 2.3: LigaSure coagulating device
(http://www.ligasure.com/ligasure/pages.aspx?page=Products/Open/64430)

2.1.3.2 Working Mechanism

This device unit consists of a generator, an active electrode and a return electrode. A typical set up in shown in Figure 2.4. The current generated by the generator is passed on the required tissue through the active electrode. This current passes through the tissue and is returned to the generator via return electrode. Thus, the patient is not a part of the circuit.
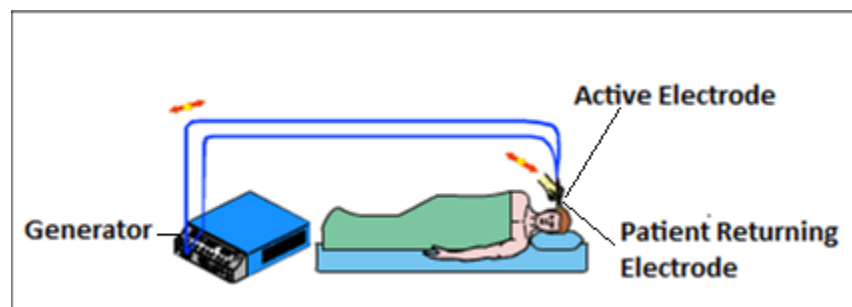
Figure 2.4: Basic operational set-up for bipolar technique application
(Modified from http://www.valleylab.com/education/poes/poes_05.html)

This device has automatic settings incorporated as Mode 1, Mode 2 and Mode 3. In most clinical procedures, Mode 2 is preferred. During activation, a feedback system switches off the device once the tissue is sealed. In Mode 2, the activation duration lasts for 2 seconds followed by an automatic shut off.

2.2 ULTRASONIC BASED DEVICES

2.2.1 Ultrasonic Technique

Ultrasonic (US) energy surgical cutting devices are surgical devices that help surgeons increase procedural efficiency and improve surgical outcomes in a wide range of surgical areas. The device uses ultrasonic energy to simultaneously cut and

coagulate soft tissue and vessels. The cutting speed of these devices is proportional to the power level, tissue traction and counter traction, and device tip sharpness (McCarus 1996).

2.2.1.1 Basic Principle

The basic principle of these devices is that the body tissue is exposed to the ultrasonic energy results in frictional heat being generated in the tissue. This causes a phase change within the tissue as unbound and bound water is driven out of the tissue proteins resulting in a coagulative or melting effect. Thus, in this technique the patient is not exposed to any kind of electric current directly or indirectly.

2.2.1.2 Working Mechanism

The system consists of a generator, hand piece and blade. Within the hand piece, the transducer consists of piezoelectric crystals which convert the electrical energy into mechanical vibration. The generator is a microprocessor controlled, high frequency switching power supply that drives this acoustic system within the hand piece by an AC current. The transducer thus vibrates at its natural harmonic frequency of 55.5 kHz (Emam Tarek 2003). This mechanical vibration is transmitted to the extender and the blade (Lee and Park 1999). Thus, the blade tip vibrates within the distance range of about 50 to 100µm (Emam Tarek 2003). There are two cutting mechanisms which can take place due to these mechanical waves.

- Cavitation Effect

When the device tip vibrates, continuous pressure changes take place within the tissue which causes cellular and intra cellular water to evaporate (Figure 2.5) (Lee and Park 1999). This causes rupturing of cells at low temperatures, enabling precise cutting.
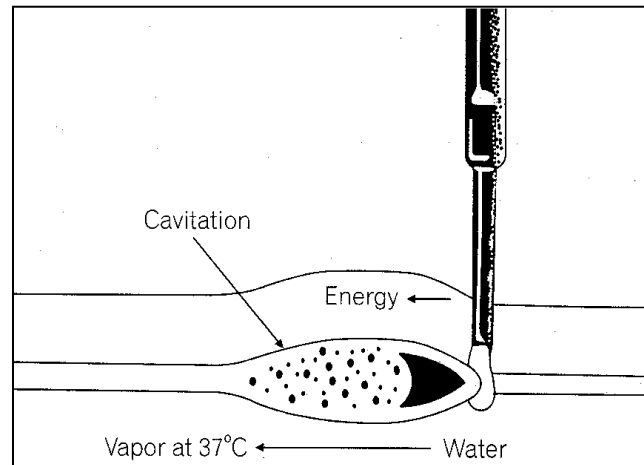
Figure 2.5: Cavitation effect (Lee and Park 1999)

- Power Cutting

When ultrasonic waves come in contact with the tissue, the tissue protein is converted into a sticky coagulum which seals the blood vessels. During this process, the mechanical energy breaks the hydrogen bonds within the protein resulting in the formation of sticky coagulum which seals the blood vessels without causing tissue desiccation or charring (McCarus 1996). Thus, the tissue cut is carried out by stretching the tissue higher than its elastic limit (Lee and Park 1999).

As a result of this mechanism, there is reduction in the area of thermal injury and minimized generation of smoke, resulting in greater visualization of the procedure site .

Any kind of ultrasonic cutting device, either blade or shear, works on the same principle as explained above. The different types of ultrasonic cutting devices used in this study are explained in the following sections. The power setting for each of these devices was set to level 5 in the generator, which is a common setting in clinical procedures.

2.2.2 Ultrasonic Blade (HK 105)

The HK 105 blade is an ultrasonically activated scalpel. It has a hook shaped blade at the tip of the hand piece (Figure 2.6).
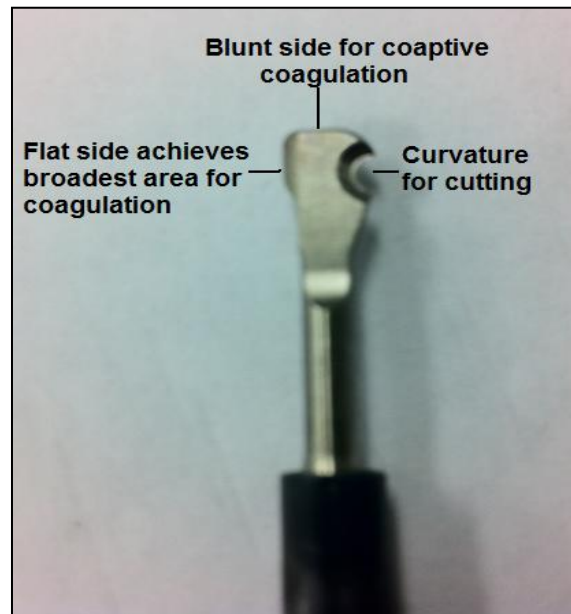
Figure 2.6: HK 105 blade, Blade hook design characteristics

The hook shape of the blade has specific design characteristics (Figure 2.6). The blade hook has sharp inner curvature for cutting purposes. The flat side opposite to the curvature serves broader area for the coagulation purpose. Also, the tip of the hook is a blunt surface so as to assist coaptive coagulation (http://www.ethiconendosurgery.com).

The applications of blade can be difficult around areas where there is no support for the tissue to be cut. Thus, getting a grip of the tissue can be a concern. This limitation can be overcome with the shear devices that assist in carrying out dissections for unsupported tissues without difficulty (Lee and Park 1999). The shear devices have one vibrating blade which is ultrasonically active and a tissue pad which is not ultrasonically active (Lee and Park 1999). The tissue pad presses tissue against the vibrating blade thus providing the grip.

2.2.3 Harmonic Focus

Harmonic Focus is an ultrasonically activated shear device (Figure 2.7). The length of the curvature tip is around 17-18 mm. This device facilitates cutting, coagulating, grasping and dissecting of tissue all with one device. It can seal blood vessels up to diameter 5 mm and lymphatic vessels up to 1 mm diameter (www.ethiconendosurgery.com).

Figure 2.7: Harmonic Focus cutting shear

(www.timecompression.com)

(www.ethiconendosurgery.com)

2.2.4 Harmonic ACE

The Harmonic ACE is an ultrasonically activated shear device with a pistol grip (Figure 2.8). The device has a curved tip but with relatively less curvature compared to the Harmonic Focus. It is used for laparoscopic as well as open surgical procedures. ACE has an added advantage over other devices. It has an overall length of 23 cm, thus providing additional distance for performing excessively inward surgeries without unnecessary procedures (http://medgadget.com). It can seal blood vessels up to 5 mm in diameter as well as lymphatic vessels up to 1 mm diameter (www.ethiconendosurgery.com).

Figure 2.8: Harmonic ACE cutting shear
(http://www.healthcarejobs.co.uk/blog/index.php/2010/09/page/2/)

Each of these devices was tested in muscle tissue near the sciatic nerve in a rat model. The time of activations for each application of the device was measured. The acute and sub-acute effects of these devices on the nerve conduction function were

neurophysiologically assessed. Also, the sub-acute effects on peripheral nerve function in terms of behavioral changes of the rat were studied.

CHAPTER 3

NERVE ANATOMY

3.1 OVERVIEW OF NERVOUS SYSTEM

The nervous system is the most complicated and highly organized of the various systems (Gray 2000). It is a major control system that regulates all the internal organs. It basically coordinates every function in the body.

3.1.1 Classification

The nervous system is basically divided into two parts:

3.1.1.1 Central nervous system (CNS): It consists of brain and spinal cord.

3.1.1.2 Peripheral nervous system (PNS): It consists of nerves that connect the CNS to the muscles and other sensory organs. Basically, it consists of all the nerves within the periphery of the body.

The nervous system is composed of nerve cells which are distributed in a network throughout the brain, spinal cord and periphery. These cells communicate with each other via nerve impulses. These nerve impulses are electrical and chemical signals. An individual nerve cell is called as 'neuron'.

3.1.2 Neuron

A neuron is the basic fundamental and structural unit of the nervous system. It is an electrically excitable cell that processes and transmits information by electrochemical signaling (Eric P. Widmaier 2006). The nerve impulses travel through the neurons, thus influencing different parts of body. There are neurons with different shapes and sizes. However, all the neurons have basic features that allow cell to cell communication.
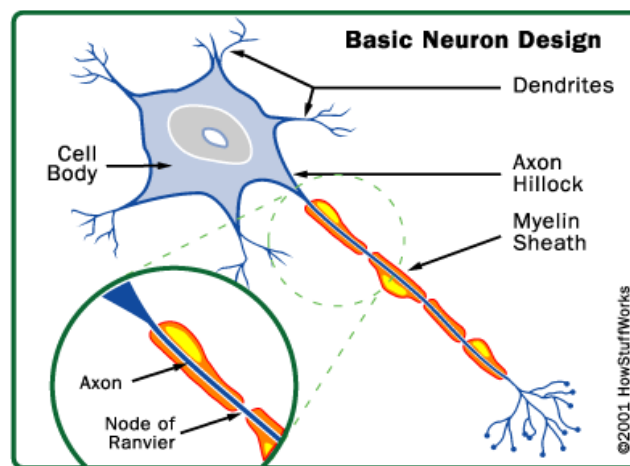
Figure 3.1: A typical neuron (http://pustaka.ictsleman.net/how/b/brain/brain.htm)

Each neuron consists of three basic parts, the cell body (soma), an axon and the dendrites (Figure 3.1). The cell body contains the DNA and typical nuclear cell organelles. Dendrites are the branches from the cell body that receive the signals. Each neuron has one axon that takes the signal away from the cell body. However, there can be axon collaterals which are the branches from the main axon. Axons are also termed as 'nerve fibers'. Thus, the transmission of the signals through the neuron starts from dendrites to the cell body and finally moving out through the axon. A bundle of axons together is called as 'nerve'.

3.1.2.1 Structure of Axon

The axonal membrane is also called as the 'axolemma'. It basically encloses the cytoplasmic extensions of the cell body. The origin of the axon near the cell body is called the axon hillock; it is the trigger zone where most of the signals are generated. These signals then propagate away from the cell body along the axon to the target cells or sometimes back along the dendrites (Eric P. Widmaier 2006). The axons of many neurons are covered by 'myelin sheath'. The myelin sheath is made up of approximately 20 to 200 layers of plasma membrane wrapped around the axon by a supporting cell. In the central nervous system, these myelin forming supporting cells are 'oligodendrocytes', which can provide myelin to around 40 axons. In the peripheral nervous system, the supporting cells are the Schwann cells. The spaces between adjacent myelin sheaths on an axon are termed as 'Nodes of Ranvier' wherein the axon is exposed to the extra cellular fluids (Figure 3.1) (http://psych.athabascau.ca/html/Psych289/Biotutorials/1/nodes.shtml). The myelin sheath acts as insulation which helps increase the rate of transmission of signals.

3.1.2.2 Types of Neurons

The basic function of a neuron is signal transmission. However, depending upon the direction of the transmission and the target areas the neurons can be functionally divided into the following types: afferent neurons, efferent neurons and interneurons (Figure 3.2).
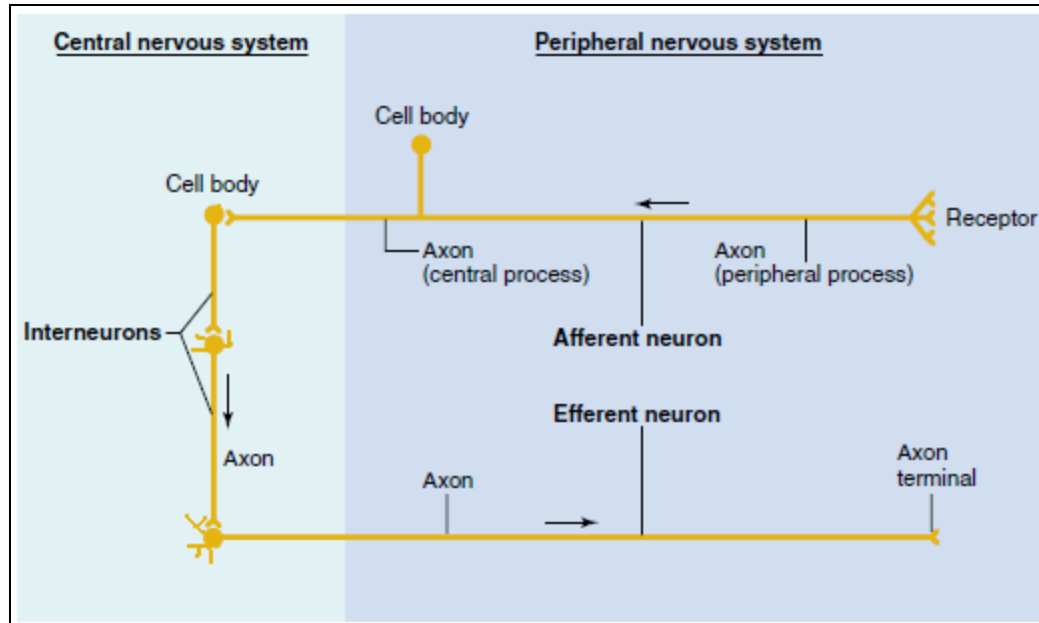
Figure 3.2: Types of neurons (arrows indicate the direction of signal transmission)

(Eric P. Widmaier 2006)

- Afferent Neurons

These are the neurons that transmit the information from the various organs and tissues of the body to the CNS. The cell body and a long process of axon are present within the PNS. A small process of axon enters the CNS. The afferent neurons do not have dendrites. At the peripheral ends of the axons of the afferent neurons, there are sensory receptors. The sensory receptors are specialized cells that respond to any kind of physical or chemical changes by generating electrical impulses in the neurons. These neurons are also called as sensory fibers.

- Efferent Neurons

Generally, these neurons transmit the information from the CNS to the effector organs like the muscles, skin, glands or even other neurons. The cell body and an initial

part of the axon lie in the CNS while the major part of the axon lies within the PNS. There neurons include the s motor neurons and the autonomic neurons.

- Interneurons

These neurons lie completely within the CNS. They act as connection between the afferent and the efferent neurons within the central nervous system and are called association neurons (http://users.rcn.com). The interneurons connect to other neurons through synapses.

A synapse is an anatomically specialized junction between two neurons wherein the electrical activity in the 1st neuron (pre-synaptic neuron) affects the electrical activity in the 2nd neuron (post-synaptic neuron). The activity of synapses can alter the activation of the post-synaptic neurons (Eric P. Widmaier 2006). There are two types of synapses namely electric and chemical. At electric synapses, the plasma membranes of pre and post synaptic cells are joined by gap junctions which allow local currents resulting from action potentials to directly through the connecting channels in either direction. The electric synapses are relatively rare in the mammalian nervous system (Eric P. Widmaier 2006). In case of chemical synapses, an extracellular space called the synaptic cleft separates the pre and post-synaptic neurons. This cleft prevents the direct propagation of the current from the pre-synaptic neuron to the postsynaptic neuron. In the chemical synapse, the signals are transmitted across the synaptic left by means of a chemical messenger called as a neurotransmitter released by the pre-synaptic axonal terminal.

The afferent neurons, efferent neurons and the interneurons function together so as transmit information from various parts of the body to the CNS and from CNS to the periphery. Thus, all of these neurons together form the nervous system with both CNS and PNS.

The PNS consists of 12 cranial nerves which originate from the brain and 31 spinal nerves which branch from the spinal cord. The roots of the spinal nerves originate from the spinal cord. Thus, the spinal nerve roots act as a bridge between the central nervous system (brain and spinal cord) and the peripheral nervous system (all nerves of the periphery).

3.2 SPINAL NERVE ROOT

3.2.1 Spinal Nerve Root Anatomy

A spinal nerve is attached to the spinal cord by means of two roots; an anterior or ventral root and a posterior or dorsal root. The ventral root emerges from the anterior side of the spinal cord while the dorsal root emerges from the posterior side of the spinal cord (Gray 2000). The motor fibers of spinal nerves begin at the ventral side of the spinal cord i.e. at the anterior horn of the gray matter. The root of sensory fiber is located at the dorsal side of the spinal cord i.e. at the dorsal root ganglion (Figure 3.3 A). These motor and sensory fibers exit through the spinal column through the inter-vertebral foramen. After this point, they combine to form a mixed nerve called the spinal nerve (Figure 3.3 B) (http://www.laesieworks.com/spinal/SCIinfo01.html).
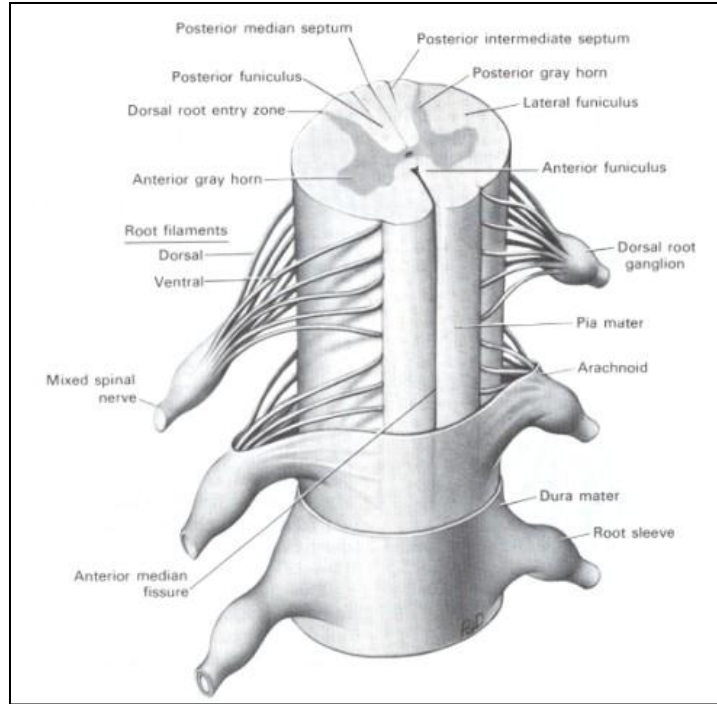
A.
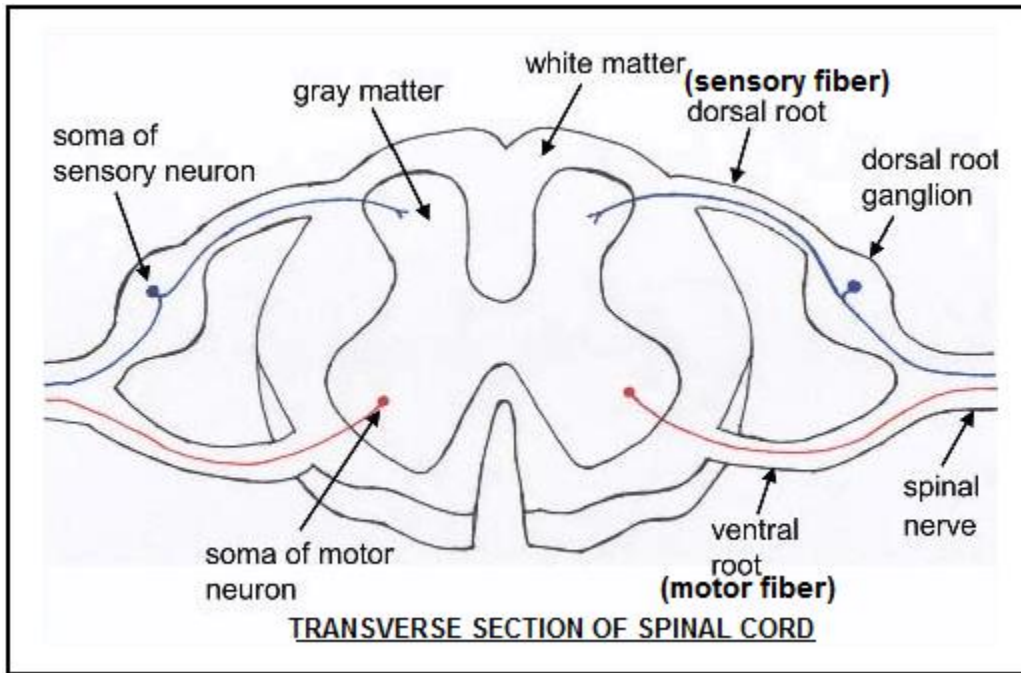
Figure 3.3: Spinal nerve roots

A. Spinal cord (Heimer 1983)

B. Transverse section of spinal cord

(Modified from http://www.acceleratedcure.org/msresources/neuroanatomy/)

Eight pairs of spinal nerves originate at the cervical level of the spinal cord, twelve pairs origin at thoracic level, five pairs each originate at lumbar and sacral levels while one pair originates at the lowermost coccygeal level (http://www.laesieworks.com/spinal/SCIinfo01.html).

3.2.2 Fiber Population of Lumbar Roots

A detailed correlation between the nerve composition and corresponding functional characteristics was studied by Prodanov. This study was performed on four female Wistar rats. Various parameters were analyzed including number of fibers, density of fibers, cross sectional area of the nerve and fiber diameter. Actual numbers of fibers obtained from this study were tabulated for L4-L6 lumbar roots (ventral and dorsal) along with sciatic nerve and its branches and are listed in Table 3.1 below (Prodanov and Feirabend 2007).

TABLE 2. Quantitative Parameters of the Roots and Nerves

Material	n[1]	Mean diameter (d[f])	Total fiber number estimate ($N[f]_{total}$)	Relative area of interspace ($A[i]_{rel}$)	Area of nerve profile ($A[n] \cdot 10^{-4}$)	Relative sampling area ($A[s]_{rel}$)
Ventral roots						
L4	8	7.97 ± 1.07	1,163 ± 385	34.4 ± 6.3	12.01 ± 3.58	58.8 ± 26.5
L5	7	7.65 ± 0.88	1,611 ± 636	32.8 ± 4.7	11.93 ± 2.03	55.1 ± 21.9
L6	6	8.52 ± 0.79	1,400 ± 70	32.1 ± 5.2	13.98 ± 2.32	45.3 ± 7.3
Mean		8.05 ± 0.44	1,391 ± 224	33.1 ± 1.2	12.64 ± 1.16	53.1 ± 7.0
Dorsal roots						
L4	3	5.69 ± 0.73	3,302 ± 1,345	31.2 ± 3.5	12.02 ± 3.62	39.6 ± 14.2
L5	4	5.75 ± 0.26	3,645 ± 725	35.0 ± 5.7	18.41 ± 4.99	35.6 ± 9.7
L6	6	6.00 ± 0.83	4,425 ± 835	34.1 ± 8.4	22.72 ± 2.00	29.0 ± 4.5
Mean		5.81 ± 0.16	3,791 ± 576	33.4 ± 2	17.72 ± 5.38	34.7 ± 2.4
Proximal sciatic	8	6.61 ± 0.68	7,599 ± 1,331	48.1 ± 9.8	59.03 ± 11.81	14.6 ± 3.9
Distal sciatic	8	5.76 ± 0.87	8,270 ± 3,705[2]	50.3 ± 7.3	44.45 ± 7.32	21.1 ± 3.9
Cutaneous	2	4.42 ± 0.11	439 ± 4	57.0 ± 1.1	1.80 ± 0.03	100.0 ± 0.0
Peroneal	7	6.29 ± 0.41	1,629 ± 140	53.8 ± 5.8	12.68 ± 1.71	49.6 ± 6.7
Tibial	8	6.36 ± 0.92	4,262 ± 936	46.2 ± 6.7	30.65 ± 5.53	26.4 ± 10.4
Sural	6	5.26 ± 0.63	920 ± 572	49.3 ± 8.4	4.19 ± 2.02	100.0 ± 0.0

[1]n, Number of samples.
[2]Large variation due to one abberant case with the highest density and moreover the largest area of the transected nerve profile, each time more than twice the smallest value for this nerve.

Table 3.1: Quantified parameters for nerve roots and nerves (Prodanov and Feirabend 2007)

3.2.3 Number of Fibers

It was seen that dorsal lumbar roots had larger number of fibers as compared to the ventral lumbar roots (Table 3.1). In dorsal roots, there was a direct proportionality between fiber number and the dorsal root level number i.e. the number of fibers in the root increased as the level of root increased. For example, dorsal root L6 had higher number of fibers as compared to dorsal root L5. However, ventral roots showed no such relationship (Prodanov and Feirabend 2007).

3.2.4 Fiber Density

There was no significance difference in fiber density (axons per unit area) between the individual ventral roots or individual dorsal roots. However, the density of fibers in the sensory dorsal roots was higher than that in the motor ventral roots (Prodanov and Feirabend 2007).

3.2.5 Types of Fibers

The nerve fibers in the L5 ventral roots were found to be Aγ and Aα (motor) fibers. Also, the dorsal roots consisted of Aδ, Aβ and Aα (sensory) fibers (Prodanov and Feirabend 2007).

In this study, the neural activity was recorded from the left lumbar nerve root at level L5. This is the nerve root that mainly contributes to the left sciatic nerve in the rat model. Thus, the neural activity was recorded from the dorsal L5 nerve root consisting of approximately 3,645 A (δ, β and α) sensory fibers (Prodanov and Feirabend 2007).

3.3 SCIATIC NERVE

The sciatic nerve is the thickest and the longest nerve in the body. It originates in the sacral plexus in the lumbo-sacral spine and runs through the hip region and down the lower limb. The sciatic nerve supplies sensation and strength to the leg as well as the reflexes of the leg. It connects the spinal cord with the outside of the thigh, hamstring muscles in the back of thighs, and muscles in the lower leg and feet. As such, when the sciatic nerve is impaired it can lead to muscle weakness in the leg and/or numbness or tingling.

The rat sciatic nerve is used as an experimental model for many studies. In terms of histological studies, the sciatic nerve tissue of the rat is similar to the human sciatic nerve (Mackinnon, Hudson et al. 1985).

3.3.1 Sciatic Nerve Anatomy

Figure 3.4 shows location of a typical rat sciatic nerve and its branches. The sciatic nerve originates in the minor pelvis and travels into the groove in between the dorsal side of the ischeum and the sacral bones. It further passes through the anterior side of piriformis muscle after leaving the sciatic notch. The nerve passes over quadratus femoris muscle approximately 5 mm away caudal to the piriformis muscle (Uysal, Mizuno et al. 2009). This nerve branches at the distal end approximately 5 mm from the knee joint. The two main branches of the sciatic nerve are peroneal nerve and the tibial nerve (Uysal, Mizuno et al. 2009).
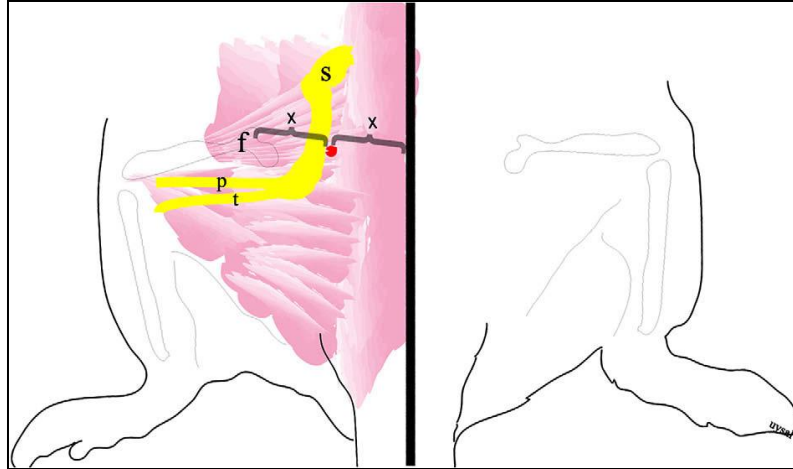
Figure 3.4: Rat sciatic nerve location S: sciatic nerve, f: femoral head, p: peroneal nerve, t: tibial nerve. x: represents the equal distance from the femur head and dorsal midline

(Uysal, Mizuno et al. 2009)

There have been several past studies related to the parent innervations of the sciatic nerve. The results from some of these studies are discussed in detail in the following sections.

3.3.2 Parent Innervations

The rat sciatic nerve originates from fusing of the lumbar nerve roots L5 and L6 and sacral nerve root S1 (Uysal, Mizuno et al. 2009). There are alternate findings that suggest that the sciatic nerve originates from the fusing of only lumbar nerve roots L4, L5 and L6 (Klusakova and Dubovy 2009). Alternate studies also showed that parent innervations of the sciatic nerve were the L4, L5 and L6 lumbar nerve roots (Schmalbruch 1986; Prodanov and Feirabend 2007). It was also found that L4 and L5 spinal nerves fuse together to form sciatic nerve while L6 spinal nerve only had one thin branch going to sciatic nerve (Asato 2000).

In any case, both the motor (ventral root) and the sensory (dorsal root) fibers from each level of the spinal cord combine to form the corresponding mixed spinal nerves. These spinal nerves further combine to form the main trunk of the sciatic nerve (Figure 3.5).
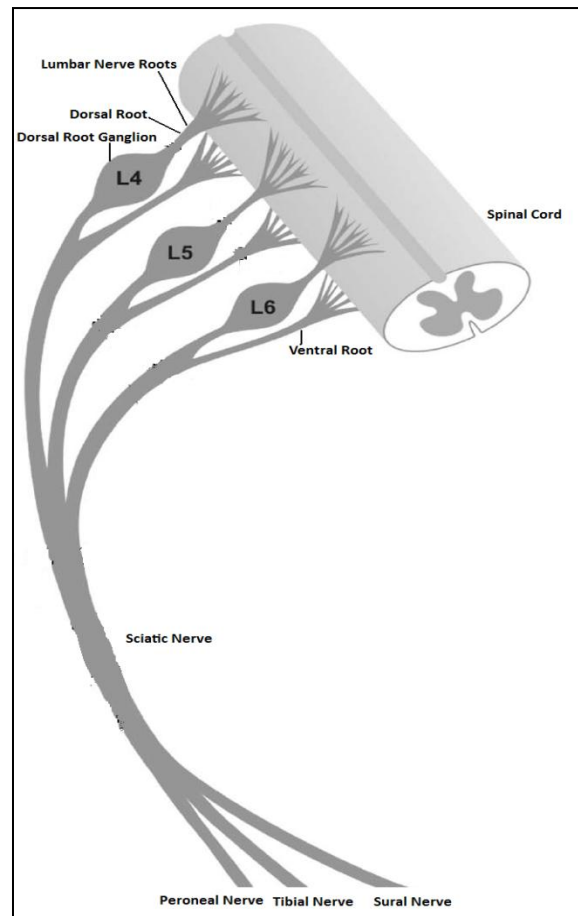
Figure 3.5: Parent innervations and distal branches of sciatic nerve
(Modified from (Klusakova and Dubovy 2009)

3.3.3 Fiber Composition of Sciatic Nerve

Schmalbruch studied the number and types of nerve fibers within the sciatic nerve and its branches. This was a detailed study carried out 40 male Wistar rats. This

study showed that the entire sciatic nerve consisted of 7,800 myelinated fibers. Out of these fibers, 4,500 fibers formed the tibial nerve and 1,900 fibers formed the peroneal nerve. Also, 1,050 fibers formed the sural nerve branch and 350 fibers formed the cutaneous branch of the sciatic nerve (Figure 3.6). The myelin sheath had diameters varying from 1.5 μm to 12.5 μm. The number of unmyelinated fibers within the sciatic nerve trunk was around 19,000 (Schmalbruch 1986).
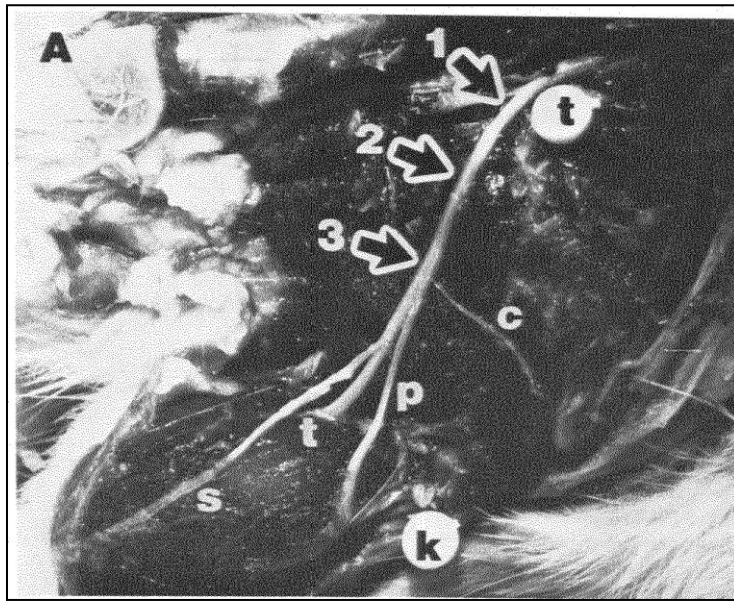
Figure 3.6: The branching of sciatic nerve. (t): tibial nerve, (p): peroneal nerve, (s): sural nerve, (c): cutaneous nerve. The positions of trochanter and knee joint are indicated by **t** and **k** respectively. 1 and 2: Complete sciatic nerve trunk; 3: Nerve begins to branch into tibial and peroneal portions. (Schmalbruch 1986)

A study by Prodanov showed that there were no major differences between the number of fibers within the proximal and distal sciatic nerve. In this study, it was found that the tibial nerve had 58.8%, the peroneal nerve had 22.5% and the sural nerve had 12.7% of the total nerve fibers in the sciatic nerve (Table 3.1). The sciatic nerve

consisted of myelinated fibers Aδ, Aβ, Aγ, Aα and unmyelinated C fibers (Prodanov and Feirabend 2007).

To summarize, the dorsal L5 spinal nerve root consists of fiber types Aδ, Aβ and Aα (sensory) and the unmyelinated C fibers. The sciatic nerve is a mixed nerve consisting of myelinated sensory nerves fibers Aδ, Aβ and Aα (sensory) along with motor fibers Aγ, Aα (motor) and unmyelinated C fibers. In this study, the sciatic nerve was stimulated and corresponding activity was recorded from dorsal L5 nerve root. Thus, neural activity was recorded from Aβ fibers in dorsal L5 nerve root following a stimulation of Aβ fibers in sciatic nerve. The basic characteristics and the neural activity generated by each fiber type are discussed in detail in the following chapter.

CHAPTER 4

NEUROPHYISIOLOGY

4.1 RESTING MEMBRANE POTENTIAL

If a voltmeter is used to measure voltage across the cell membrane, i.e. the voltage of inside of cell relative to the outside, it can be seen that inside of the cell is more negative with respect to the outside of cell. Thus, the membrane potential can be defined as the electrical potential that exists across the membrane of a cell. When measured under the resting state of the cell, it is called as the resting membrane potential.

The membrane potential can be determined by the following factors,

- Concentration of ions inside and outside the cell

- Permeability of the cell membrane with respect to these ions

- Activity of the sodium potassium pump that regulates the ion concentrations across the membrane (Arthur C. Guyton 2002).

The sodium potassium pump is a trans-membrane electrogenic pump which causes transport of sodium and potassium ions across the membrane. Three sodium (Na^+) ions are pumped to the exterior of the nerve cell while two potassium (K^+) ions are pumped to the interior of the cell. Thus, there is larger number of positive ions pumped out of the cell as compared to those pumped inside the cell. Also, the inside of the nerve cell consists of a large number of negatively charged ions which cannot or very poorly

diffuse through the membrane. Thus, the inside of the cell membrane becomes electro-negative while the outside becomes electro-positive. As more and more Na$^+$ ions are pumped to the exterior by the Na$^+$ K$^+$ pump, these ions begin to diffuse back into the cell as a result of diffusion caused by,

- Sodium concentration gradient that develops from the outside of the nerve fiber to inside.
- A negative membrane potential on the inside which attracts more positively charges sodium ions.

Ultimately, there occurs a stage where in the inward diffusion equals the outward pumping by the sodium potassium pump (Eric P. Widmaier 2006). This is when the membrane potential inside the nerve fiber is -90 mV. This is called as the resting membrane potential of a nerve fiber (Figure 4.1) (Arthur C. Guyton 2002).
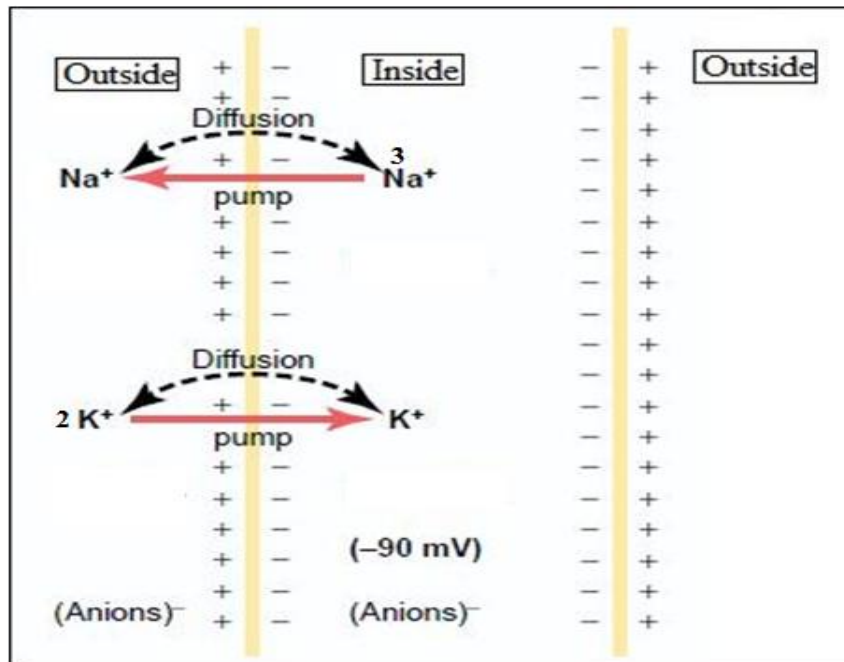
Figure 4.1: Resting Membrane Potential (Arthur C. Guyton 2002).

4.2 ACTION POTENTIAL

Action potential is a momentary reversal in the membrane potential when the nerve fiber is activated by a stimulus. These changes are responsible to transmitting the signals along nerve fibers. These can be elicited by any factor that suddenly increases the permeability of the membrane to Na^+ ions. The factors can be electrical stimulation, mechanical compression or any factor that disturbs the resting state of the nerve fiber (Arthur C. Guyton 2002).

4.2.1 Development and Propagation

At an onset of the action potential sodium ion permeability increases by 5000 times the normal state followed by immediate return of sodium permeability to normal and then a large increase in the potassium ion permeability. The successive stages in the development and propagation of an action potential are explained in Figure 4.2.
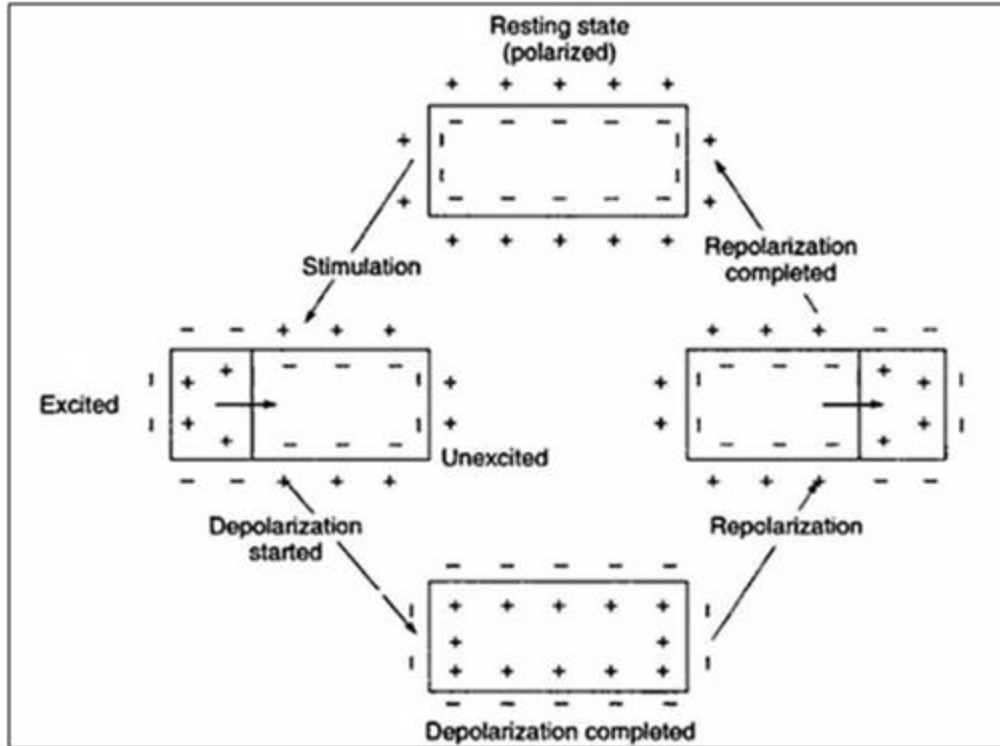
Figure 4.2: Sequential events during formation of action potential (Khandpur 2003)

4.2.1.1 Resting State

This is the state before the action potential begins. At this state, the membrane is called as 'polarized' as a result of the negative resting membrane potential (-90 mV) (Khandpur 2003).

4.2.1.2 Depolarization State

During this state, the membrane suddenly becomes highly permeable to sodium ions as a result of any kind of stimulus. This allows a large number of positively charged sodium ions to permeate into the inside of the nerve fiber. As a result, the normal -90 mV membrane potential inside the fiber begins to neutralize due inflow of Na^+ ions

resulting in rise of membrane potential in positive direction. This is called as depolarization (Arthur C. Guyton 2002).

4.2.1.3 Repolarization State

During this state, the sodium channels begin to shut while the potassium channels begin to open more than they normally do. Thus, a rapid diffusion of the K^+ ions from inside of the fiber to the outside of fiber takes place resulting in the normal negative resting membrane potential inside the fiber. This state is thus called as repolarization of membrane (Arthur C. Guyton 2002).

4.2.2 Membrane Potential Changes

As the action potential is developed across the membrane, there are significant changes in the permeability of Na^+ and K^+ ions across the membrane. These changes are regulated by opening and closing of sodium and potassium channels within the membrane. The sodium and potassium channels are controlled by electrically charged gates which open and close the channels (Arthur C. Guyton 2002).

The change in the membrane potential during development of action potential, evoked by a current stimulus, with respect to time can be plotted as in Figure 4.3.
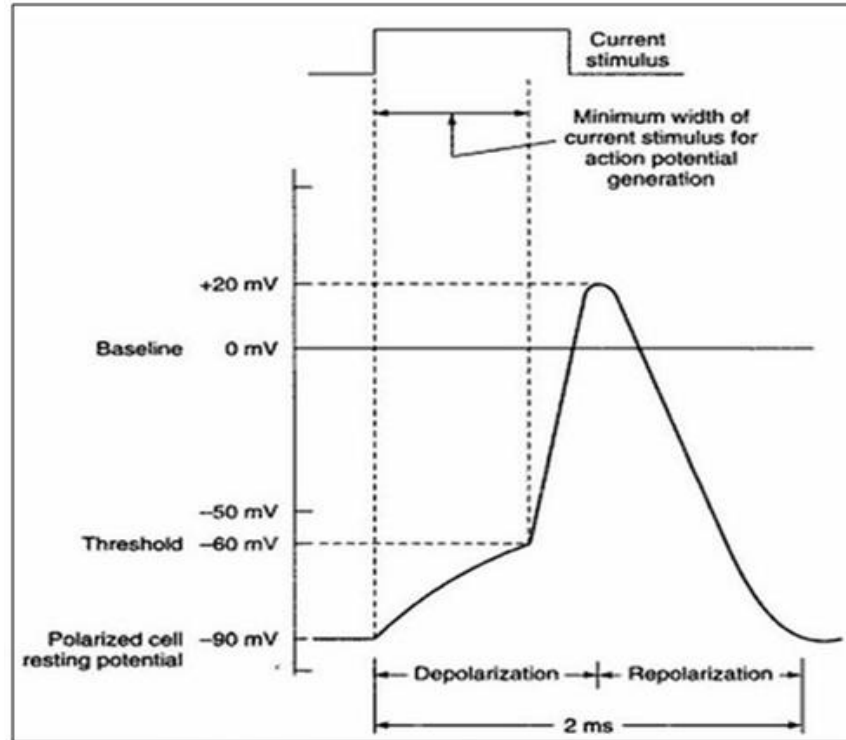
Figure 4.3: Action potential waveform (Khandpur 2003)

As seen from the Figure 4.3, with the onset of current stimulus, the membrane potential rises positively from -90 mV to about -60 mV. This rise in the potential is caused by highly increased permeability of Na^+ ions across the membrane. At this stage, the membrane is about 20 to 30 times more permeable to Na^+ ions than to K^+ ions (Arthur C. Guyton 2002). This change of potential in positive direction further increases the Na^+ ions permeability by opening the sodium gates within the membrane (positive feedback effect). This continues to occur until the sodium gates are completely opened leading to a positive potential within the membrane. This is called as the 'Reversal Potential' or an overshoot. It can also cause an overshoot wherein the membrane potential becomes a slight positive. The overshoot is usually seen in the

large nerve fibers. However, in case of small fibers as well as many CNS fibers, the maximum membrane potential just approaches zero value (Arthur C. Guyton 2002).

Eventually, this positive interior potential causes repulsion of the Na$^+$ ions by closing of sodium gates in the membrane. As sodium channels begin to close, the potassium channels begin to open to move the K$^+$ ions out to the exterior of the membrane. Loss of K$^+$ ions to exterior causes movement of positive charges to the exterior. Thus, the inner membrane potential of the nerve fiber returns to the initial negative potential.

4.3 COMPUND ACTION POTENTIAL

A compound action potential (CAP) developed by a nerve represents the sum of action potentials generated by different nerve fibers within the nerve trunk. Thus, it can also be called as a graded response of a nerve to a stimulus (Mann 2008). Electrical stimulation is commonly used to elicit and track CAP changes.

4.3.1 Electrical Stimulation

There are different types of electrical stimulation pulses used for different studies. A past study showing the distribution of conduction velocity on CAP waveform in a demyelinated frog sciatic nerves used an electrical stimulation with a square pulse of supra-maximal amplitudes and 0.1 ms duration at 10 Hz frequency (Kiziltan, Dalkilic et al. 2007). A study measuring the CAP in rat sciatic nerves using microelectrode array used an electrical stimulus, a constant current stimulus of 5V, 1 mA for 0.5 ms (Chungkeun Lee 2006). Another study with electrical stimulation for tracking CAP

changes in the rat sciatic nerve used the simulation voltages from 0.3 to 0.6 V, 5 µsec pulse duration (Wells, Kao et al. 2005).

4.3.2 CAP Development

CAP is produced in a nerve trunk consisting of a population of nerve fibers (axons) discharging as a result of a Stimulus (Mann 2008). The amplitude of CAP increases linearly with the magnitude of stimulation (Wells, Kao et al. 2005). The CAP waveform basically represents combined activity of the nerve fibers within a nerve trunk (Figure 4.4).
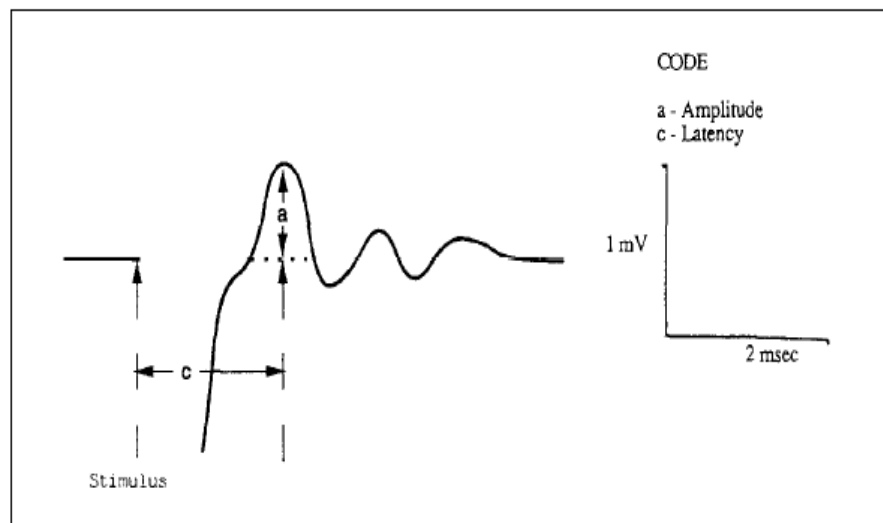
Figure 4.4: Compound Action Potential (CAP) (Nokes L D 1991)

The membrane potential values of different nerve fibers can be determined from the CAP waveform depending upon the conduction velocities of the corresponding nerve fibers.

4.3.3 Conduction Velocity

Conduction velocity of a nerve fiber can be defined as the speed at which action potentials are transmitted along the axon. The conduction velocity depends on the fiber diameter and whether the fiber is myelinated or not. As the diameter of axon increases the conduction velocity also increases, (Waxman 1980). The myelin acts like an insulator which reduces the flow of charge between inside of fiber and outside of fiber. Thus, reduced leakage of charge occurs through myelin-covered sections of the membrane of nerve fiber. Thus, propagation of the action potentials through myelinated fibers is faster than in unmyelinated fibers.

The fastest component of CAP represents the fastest and the largest nerve fibers. While the slower components of CAP represent the slower and smaller fibers.

4.4 PHYSIOLOGIC CLASSIFICATION OF NERVE FIBERS

Based on the nerve fiber diameters, the conduction velocities and nerve function, nerves are classified into different groups (Figure 4.5).
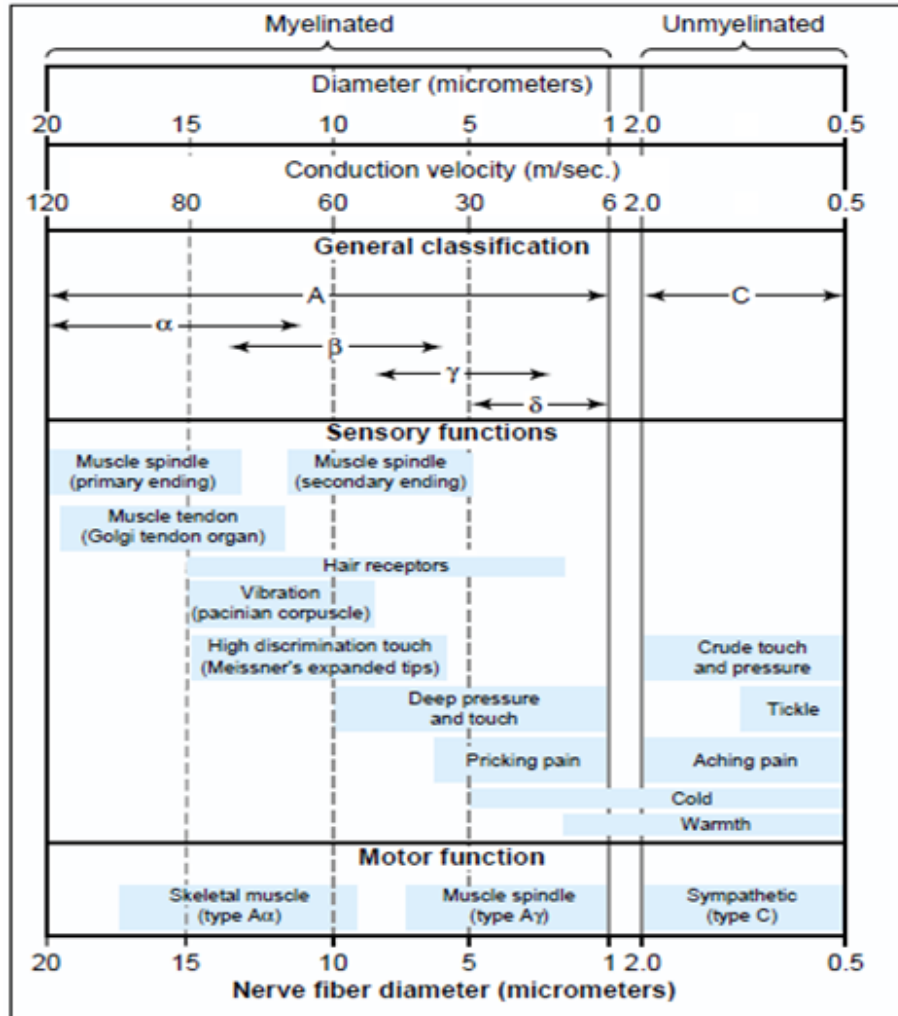
Figure 4.5: Physiological classifications and functions of nerve fibers. (Modified from

(Arthur C. Guyton 2002)

The range of conduction velocities of nerve fibers is 0.5 m/s to 120 m/s. Also, the larger the diameter, the greater is the conduction velocity. Figure 4.5 shows a general classification which includes the motor and sensory nerve fibers with their respective functions. The fibers are basically divided into types A and C.

4.4.1 Type A Fibers

These are the fastest conducting fibers (De Andres, Alonso-Inigo et al. 2005). The type A fibers are further divided into α, β, γ and δ fibers. Type A fibers are typical myelinated fibers of the spinal nerves. These fibers have conduction velocities ranging from 6 m/s to 120 m/s and diameters from 1 micron to 20 micron. The basic functions of the sensory nerve fibers falling in type A include sensing deep pressures, pricking pain, and innervating muscle spindles and tendons. The functions of motor nerve fibers within type A include those related to skeletal muscles (type Aα) and muscle spindle (type Aγ) (Arthur C. Guyton 2002).

4.4.2 Type C Fibers

Type C fibers are relatively smaller fibers which are unmyelinated. The type C fibers have lower conduction velocities ranging from 0.5 m/s to 2 m/s. Around two-thirds of the peripheral nerves consist of C fibers. The basic functions of type C sensory nerve fibers include responses to stimuli including pain, crude touch and pressure. Type C motor fibers include functions of the sympathetic nervous system (Arthur C. Guyton 2002).

4.5 ERLANGER / GASSER CLASSIFICATION

As discussed in earlier chapter, the rat sciatic nerve consists of different types of nerve fibers. A detailed classification for the sensory nerve fibers is shown by Erlanger and Gasser (Nokes L D 1991) (Table 4.1).

THE ERLANGER/GASSER CLASSIFICATION OF SENSORY NERVE FIBRES			
Fibre type	Function (examples)	Average fibre diameter (mm)	Average conduction velocity (m/s)
Aα	Primary muscle spindle afferents motor to skeletal muscle	15	100
Aβ	Cutaneous touch and pressure afferents	8	50
Aγ	Motor to muscle spindles	5	20
Aδ	Cutaneous temperature and nociceptive afferents.	<3	15
B	Sympathetic preganglionic	3	7
C	Cutaneous nociceptive afferents sympathetic postganglionic.	1	<2

Table 4.1: Erlanger/Gasser classification of sensory nerve fibers (Nokes L D 1991)

Table 4.1 shows specific fiber diameter and conduction velocity values for respective fiber types, showing that Aα fibers are the fastest sensory fibers while C fibers are the slowest. These fibers can be identified in a typical CAP depending upon the occurrence of onset peaks in CAP amplitudes (Figure 4.6).
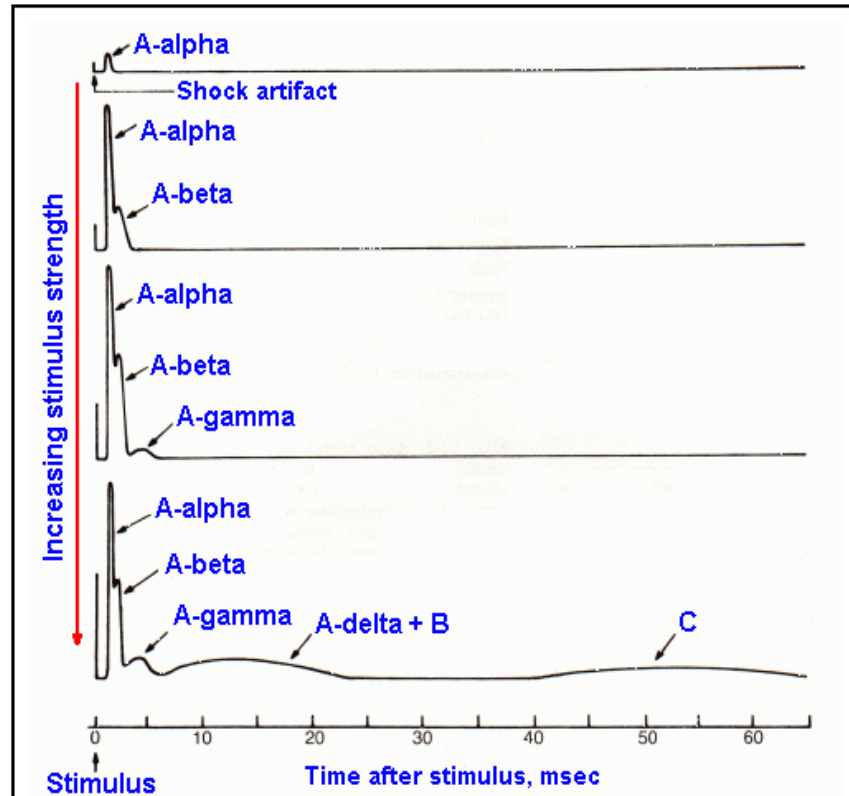
Figure 4.6: CAP from the sensory nerve (sciatic nerve) when stimulated by an electric impulse applied to the nerve 80 mm away. Increasing stimulus strength from top to bottom

(Mann 2008)

Figure 4.6 indicates that even with the smallest stimulus strength, the Aα nerve fibers result in CAP. As the stimulus strength increases, Aβ fibers contribute to the CAP along with Aα whose CAP amplitude is greater as compared to Aβ. Further, with the increasing stimulus strength, the CAP amplitudes of Aα and Aβ fibers increase steadily. Also, eventually nerve fiber types Aγ, Aδ + B and C contribute to the CAP generated. The C fibers are the ones which require highest stimulus strength to appear on a CAP waveform.

4.6 STIMULATION SPECIFICATIONS

The minimum intensity of electric stimulus required to trigger the action potential is specific for each type of nerve fiber. The minimum current intensity required to evoke action potential when applied to nerve fiber without time limitation is called as Rheobase (De Andres, Alonso-Inigo et al. 2005). The minimum current duration required to produce the stimulation intensity twice of the rheobase is called a Chronaxie (De Andres and Sala-Blanch 2001). The Chronaxie is specific for each type of nerve fiber. The chronaxie is inversely proportional to the extent of myelin sheath on a nerve fiber. Greater the extent of myelin lesser the intensity of stimulus is required to trigger an action potential. The chronaxie for myelinated nerve fibers is 0.1 msec to 0.3 msec (De Andres and Sala-Blanch 2001).

Also, the ideal frequency for effective and comfortable stimulation is between 1 to 2 Hz (De Andres, Alonso-Inigo et al. 2005). The shorter duration stimulus (≤ 0.1 msec) can trigger the motor components while the longer duration (> 0.1 msec) stimulus can trigger sensory components(Urmey and Grossi 2002), (Kaiser, Niesel et al. 1990).

Thus, in the current study an electric impulse of width 0.3 msec with 1 second inter-pulse period was used to stimulate the rat sciatic nerve. The amplitudes of the electric pulses varied from 1V, 2V and 3V based on the membrane excitability curve (Figure 4.7). There were modifications made in course of the study. These voltages were basically used to trigger the type A (β) fibers within the sciatic nerve.
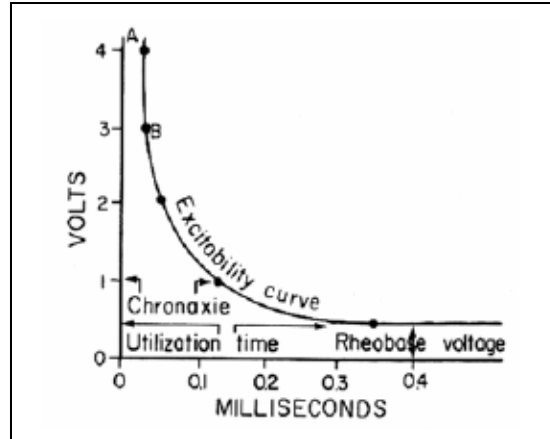
Figure 4.7: Excitability curve of large myelinated fiber (Singh 2006)

A detailed methodology and corresponding results are discussed in following chapters.

CHAPTER 5

SPECIFIC AIM I: ACUTE STUDY I

5.1 SPECIFIC AIMS

Using a rat model, two devices were tested to determine the acute effects on nerve structure and conduction function. The two devices used were Ultrasonic HK 105 blade and Monopolar Electrosurgery (MES). The effect of these devices when applied at distances 1 mm, 2 mm, 3 mm and 4 mm from the sciatic nerve was studied.

5.2 OUTLINE

A total of 75 adult male Sprague Dawley (400-450 gms) rats were used for this study. The test matrix showing the number of proposed experiments is shown below.

Distance of device tip from sciatic nerve	Ultrasonic Blade (HK 105) (Level 5)	Monopolar Electrosurgery (MES) (60W, cut, blend 1)	Sham (no heat)
1 mm	8	8	11
2 mm	8	8	
3 mm	8	8	
4 mm	8	8	

Table 5.1: Test Matrix for Acute Study I

The MES was set at 60 Watt power, cut, Blend 1 which is commonly used in clinical setting to carry out an incision and simultaneously seal the blood vessels during a hip surgery procedure. The ultrasonic blade was set at power level 5 as it is the maximum possible power level this device. This was done so as to maximize the extent of energy exposure by ultrasonic device.

A baseline activity was recorded from the L5 nerve root by stimulating the left sciatic nerve with 1V, 2V and 3V; before Ultrasonic or Monopolar electrosurgery blades were used in close proximity of the nerve. After this recording, 1cc pancuronium bromide, a muscle relaxant (MF Roizen 1978), was injected into the rat so as to stop the muscle twitching during stimulation. Once the muscle twitching was completely ceased, one more baseline recording was taken by stimulating the nerve. Post this recording was completed; either of the surgical devices was used at distances of 1mm, 2mm, 3mm and 4mm from the sciatic nerve. Post application, the sciatic nerve activity was again recorded at specific time points at 2nd minute, 10th minute, 30th minute, 60minute, 120th minute and 180th minute. After completion of all recordings, the rat was euthanized with 1cc Sodium Pentobarbital. No surgical devices were used in Sham experiments. The compound action potentials and conduction velocities of each device with respect to time and distance from sciatic nerve were analyzed.

5.3 METHODOLOGY

5.3.1 Anesthesia

The rats were anesthetized and sedated by an intraperitonial injection of ketamine (40 mg/kg) and xylazine (5 mg/kg) and maintained on ketamine (20 mg/kg) and xylazine

(2.5 mg/kg) before the surgery. The depth of anesthesia was continuously monitored using paw pinch and palpebral reflexes. A maintenance dose of 0.2 cc (50% of initial dose) was injected as and when required.

5.3.2 Pre-recording surgeries

5.3.2.1 L5 nerve root exposure

A midline dorsal longitudinal incision was made over the lumbar spine. The paraspinal muscles were retracted and the L1 to S2 spinous processes was removed. An L2-L6 laminectomy was performed to expose the spinal canal. The dura mater was cut to expose the nerve roots for electrophysiological neural activity recordings. Exposed left L5 dorsal spinal nerve roots were kept intact with their connection to the spinal cord. The nerve roots were immersed in mineral oil maintained at temperature of around 37°C.

5.3.2.2 Tracheotomy

A plastic catheter was inserted and fixed into the trachea as a means to provide mechanical ventilation. The rat was ventilated at breath rate of 90 bpm and a tidal volume of 2.5 ml.

5.3.2.3 Initial Sciatic Nerve Exposure

Using a #10 scalpel a dorsal lateral skin incision between the lateral aspect of knee joint and greater trochanter of femur bone was made to expose muscles and fascia. Conventional scissors were used to detach posterior thigh muscle from the

femur bone. This fascia was located at the posterolateral aspect of the thigh. Forceps were used to retract posterior thigh muscle to expose the sciatic nerve. The sciatic nerve was located posteriorly near midline of the thigh, approximately 10 mm from the cut fascia. After visualizing the sciatic nerve, the gluteus superficialis muscle and biceps femoris muscle (the posterior thigh muscles) that cover the nerve were cut using the designated cutting device. Quantitative data analysis was performed offline.

5.3.3 Device Application

Once the surgeries were completed, the rats were prepared for the neurophysiologic recordings. A set of baseline recordings was carried out before the ultrasonic or MES device application, explained in the following section. Using a micromanipulator (Narishige Inc, East Meadow, NY), the tip of the Ultrasonic Blade or MES device was placed in lateral quadriceps muscle 1, 2, 3 or 4 mm from the sciatic nerve just proximal to the popliteal fossa of the knee joint. The tip of the device was inserted 2 mm into the muscle from the muscle surface. The device was activated for exactly 5 seconds. A schematic representing the application technique is shown in Figure 5.1. The device was activated for 5 seconds and then removed. The ultrasonic blade was activated at level 5 and the MES was activated at 60W, blend 1, in cut mode. Nerve conduction studies were performed pre and post device application at 2, 10, 30, 60, 120 and 180 minutes after the device removal.
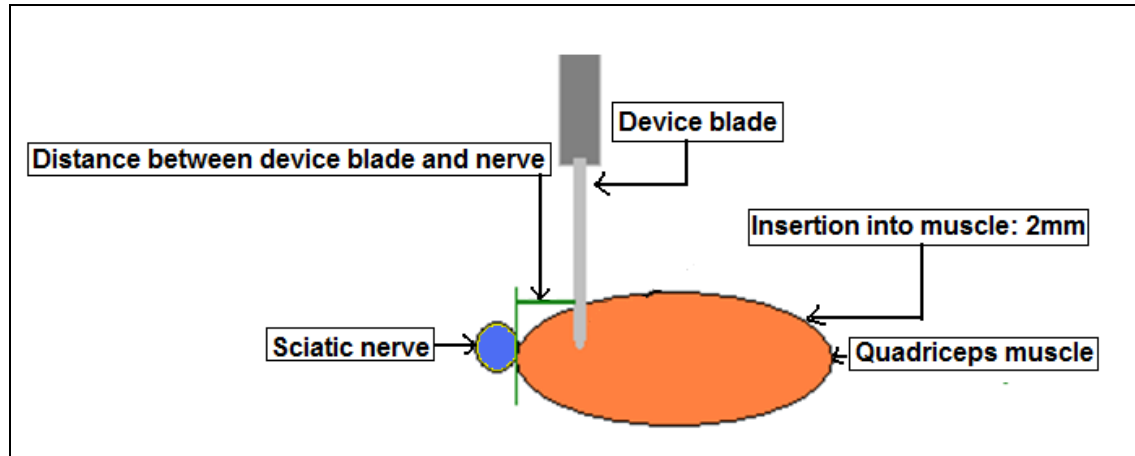
Figure 5.1: Schematic of device application technique

5.3.4 Recording Procedure

5.3.4.1 Neurophysiology Testing

A miniature bipolar stimulating hook electrode (Chen C 2005) made up of platinum wire was placed under the ipsilateral left sciatic nerve. Further, to record the neural activity from the L5 nerve root, two bipolar platinum wire electrodes (RE 1 and RE 2) were placed under the nerve root. The total length of nerve root under which the recording electrodes were placed was approximately 10mm. The recorded data was analyzed from either of the two channels.

A schematic of neurophysiologic recording set up and data acquisition system is shown below:
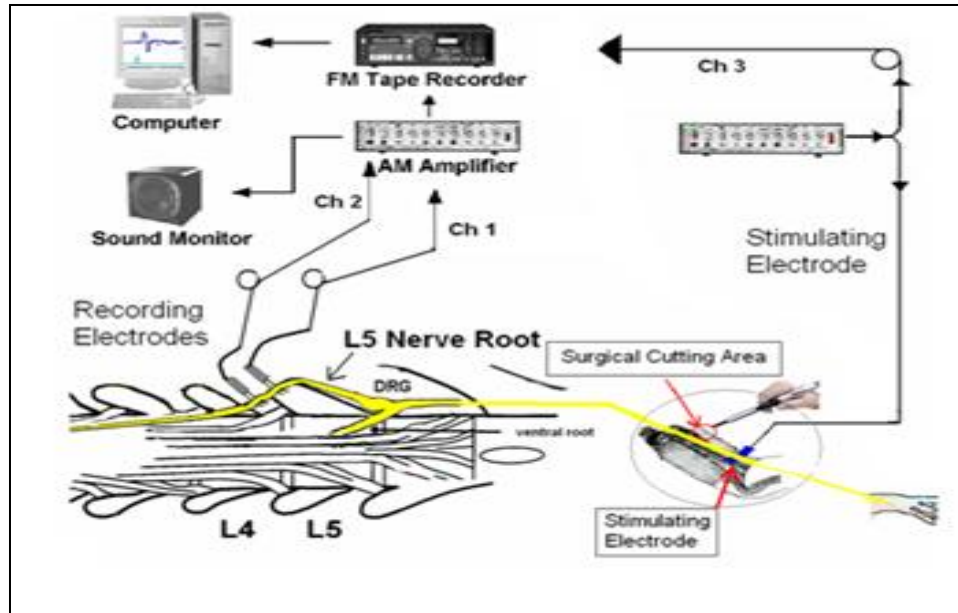
Figure 5.2: Neurophysiologic equipment set up

As shown in the Figure 5.2, the sciatic nerve was stimulated by a pulse signal from the pulse stimulator through the stimulating electrode. The stimulation pulses of amplitude 1V, 2V and 3V were used to activate myelinated axons (Hodgkin and Huxley 1952). The stimulation pulse width was 0.3 msec and the inter-pulse period was maintained as 1 sec (Hodgkin and Huxley 1952). The recording electrodes 1 and 2 (RE 1 and RE 2) were connected to channels 1 and 2 of the A.C. preamplifier (x 1000). Thus, the neural activity was amplified with the amplifier, displayed on an oscilloscope (10-2K mv/div) and recorded on an FM tape recorder (MR-30; TEAC, Montebello, CA). The recordings were simultaneously monitored on a sound monitor. Data was digitized, displayed and recorded real-time on a computer using a window discriminating system (Enhanced Graphics Acquisition and Analysis [EGAA], R.C Electronics, Goleta, CA). Also, output from the pulse stimulator was sent to channel 4 of the EGAA software to display the onset of the stimulation pulse.

Each recording was performed at the designated time points post-device application.

5.3.4.2 Hind Paw Probing

After each electrical stimulation application on sciatic nerve, the hind paw was probed using calibrated Von Frey Hairs (VFH) (Stoelting Inc, Wood Dale, IL) to determine minimal force required for neural response. VFH are calibrated thin nylon filaments which exert specific force when pressed against a surface (Schaeffer, Meyer et al. 2010). The force (in grams) exerted by each VFH was calculated by buckling the VFH on a weighing scale for five consecutive times. An average value of these five consecutive forces for a particular VFH was taken as the final force value exerted by that VFH (Table 5.2). Each VFH starting from VFH 4.56 was used to probe until there was hind paw withdrawal. Thus, minimal force thus required to evoke a sensory response was defined as threshold force.

Figure 5.3: VFH used for paw probing

VON FREY HAIR	FORCE (grams)
4.56	5.2
4.74	6.4
4.93	5.2
5.07	10
5.18	14.6
5.46	24.4
5.88	59.2
6.1	99.6
6.45	104.2
6.65	126.6

Table 5.2: VFH Forces in grams

5.3.5 Data Analysis

The neurophysiologic recordings obtained from the pre and post device application were evaluated for changes in the conduction velocity, and the magnitude of CAP. Complete cessation of CAP indicated a nerve conduction block. The raw data acquired through the EGAA appeared as shown in Figure 5.4. The waveform "A" represents a summation of activity of nerve fibers (type Aβ) from sciatic nerve to the L5 nerve root while waveform "B" represents the stimulating pulse from the stimulator.
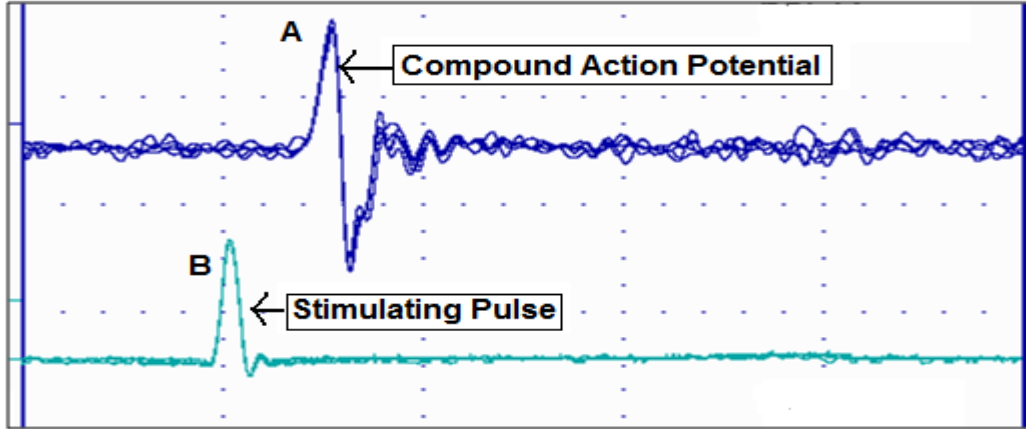
Figure 5.4: Raw data from EGAA **A.** Compound Action Potential recorded from L5 nerve root. **B.** Stimulating pulse from pulse stimulator.

5.3.5.1 Area Under CAP curve (AUC)

Using rectify and integrate functions in EGAA, the raw CAP curve was rectified to calculate the area under the curve (AUC) as shown in Figure 5.5.
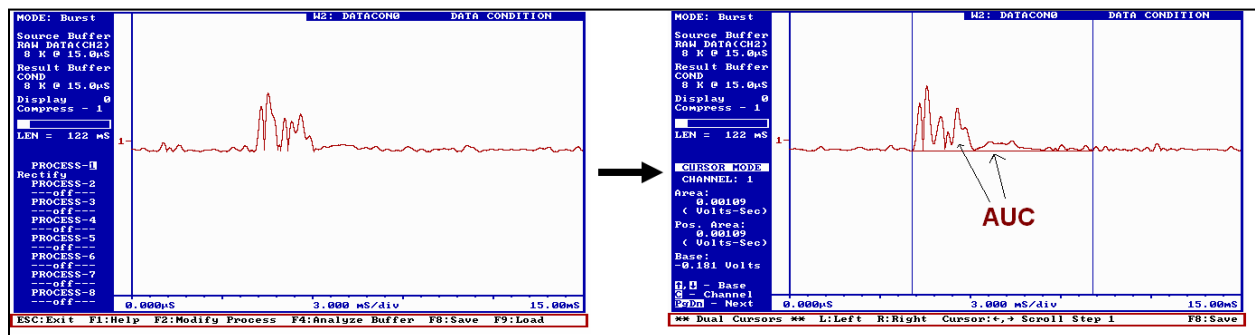
Figure 5.5: Area Under CAP Curve (AUC) using EGAA

The percentage change in AUC values before and after device application was calculated (Eq. 5.1). Thus, a negative percentage change indicated that there was a drop in AUC values as compared to the initial baseline recording and a positive percentage change indicated an increase in AUC values.

$$\text{Percentage Change in AUC} = \frac{(\text{Current AUC value - Before AUC value})}{\text{Before AUC value}} \times 100$$ (Eq 5.1)

5.3.5.2 Conduction Velocity

The conduction velocity was calculated by dividing the distance (D) between the stimulating electrode (S.E.) and recording electrode (R.E) by the latency between the onset of the stimulating pulse and the onset of the CAP (ΔT). This latency was obtained using EGAA system as shown in Figure 5.6. The percentage change in CV was calculated for all designated time points before and after device application. (Eq. 5.2)

$$\text{Percentage Change in CV} = \frac{(\text{Current CV value - Before CV value})}{\text{Before CV value}} \times 100$$ (Eq. 5.2)

A

B
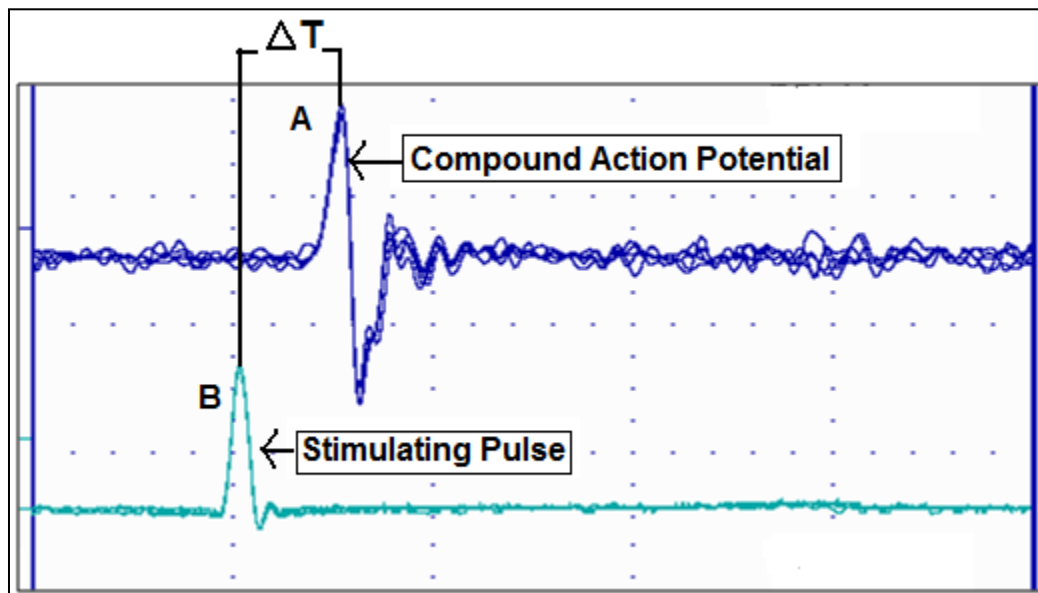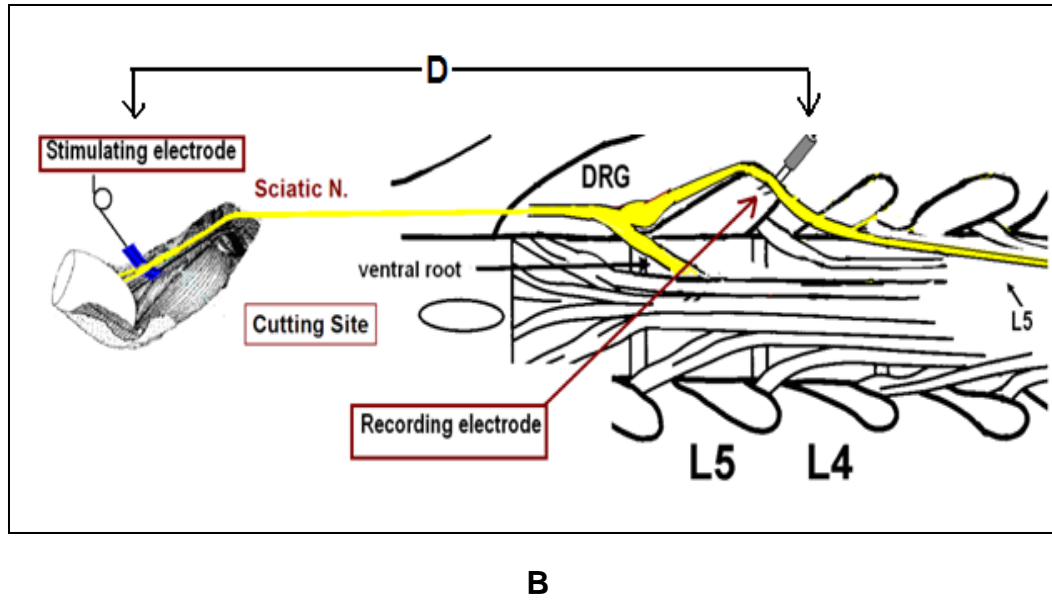
Figure 5.6: Conduction velocity calculation. **A.** Latency between stimulating peak and CAP onset. **B.** Distance between stimulating and recording electrodes.

5.3.5.3 Statistical Analysis

One way ANOVA was performed to determine how the functional changes in AUC and CV were affected by the device application. The comparison of all the post-surgery time points (2 min, 10 min, 30 min, 60 min, 120 min and 180 min) was made with respect to the '0 min' time point at individual time points. One way ANOVA was also performed to determine how the functional changes in AUC and CV were affected by the device application distances (1 mm, 2 mm, 3 mm and 4 mm). The statistical significance level was set at $p < 0.05$. Two Way ANOVA was performed to compare the AUC and CV values for MES and ultrasonic blade (HK 105) at individual distances (1 mm, 2 mm, 3 mm and 4 mm) at all the time points (2 min, 10 min, 30 min, 60 min, 120 min and 180 min). The comparison was made with respect to the '0 min' time point so as to consider the pancuronium bromide effect. All the AUC and CV values considered

for analysis were the values obtained from 3V pulse stimulation in this report. Post Hoc. LSD tests were performed. The statistical analysis was performed, using SPSS (SPSS, Chicago, IL).

5.4 RESULTS

5.4.1 Gross Findings

There were striking differences in the gross effects between monopolar electrosurgery device and ultrasonic blades. As seen in Figure 5.7 A, the use of MES caused burning of tissue, charring, desiccation and a visible black hole on the muscle tissue. Also, during the MES blade application there was high amount of smoke production at the application site along with uncontrolled muscle contraction making it difficult to maintain specific distances from the nerve. On the contrary, in Figure 5.7 B, the ultrasonic blade caused minimal muscle tissue damage with no charring. Further, there was no smoke production and no muscle contraction during application of device.
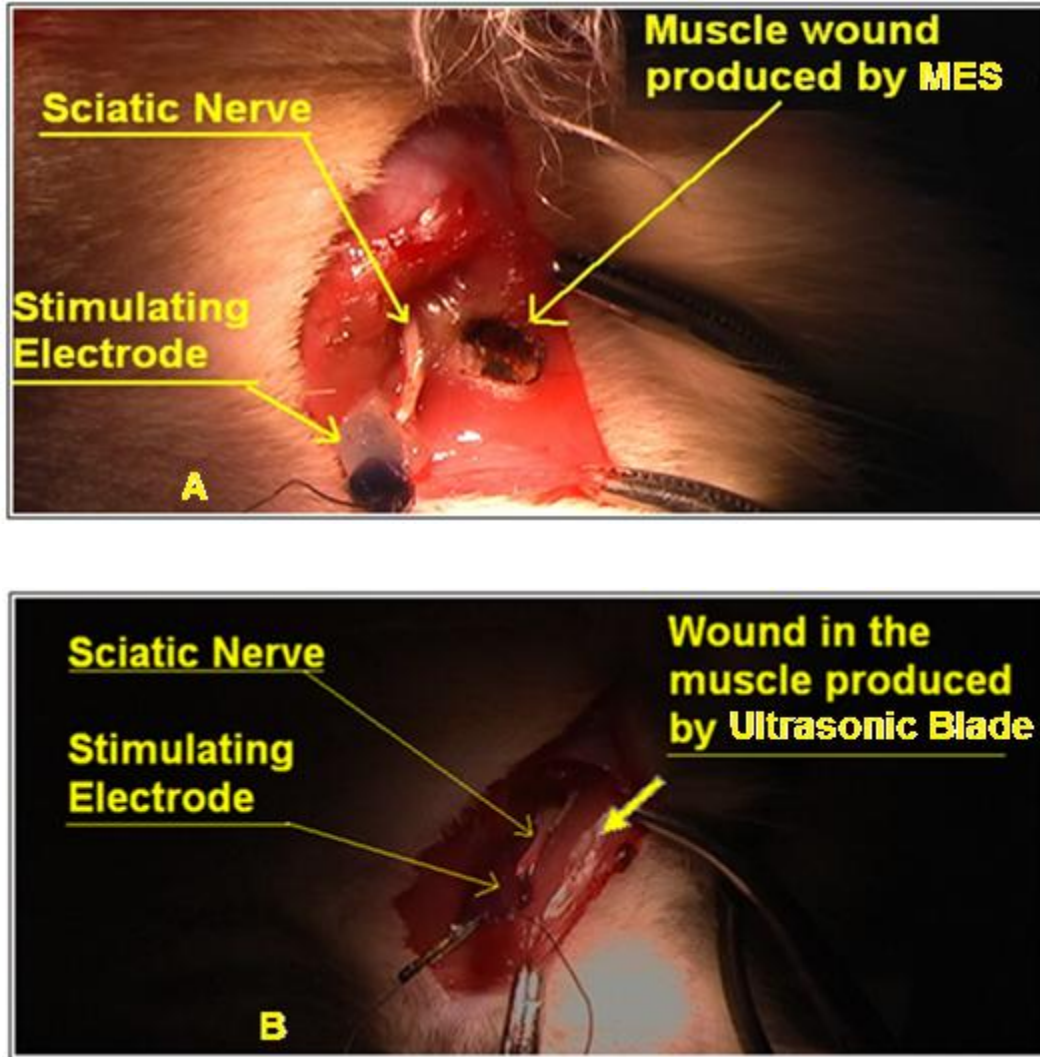
Figure 5.7: Gross effects of devices on muscle.

A. Effect of Monopolar Electrosurgery Blade on the muscle
B. Effect of Ultrasonic Blade on the muscle.

5.4.2 Raw Data

Raw data obtained from EGAA indicated prominent differences between compound action potentials evoked due to two devices. A sample image indicating raw CAP waveforms when MES and ultrasonic blade were used at 1mm distance away from sciatic nerve respect to time for 3V stimulation is shown in Figure 5.8. More raw CAP images with

devices used 2mm, 3mm and 4mm away from sciatic nerve are included in the Appendix A.

A. Ultrasonic Blade   B. MES Device
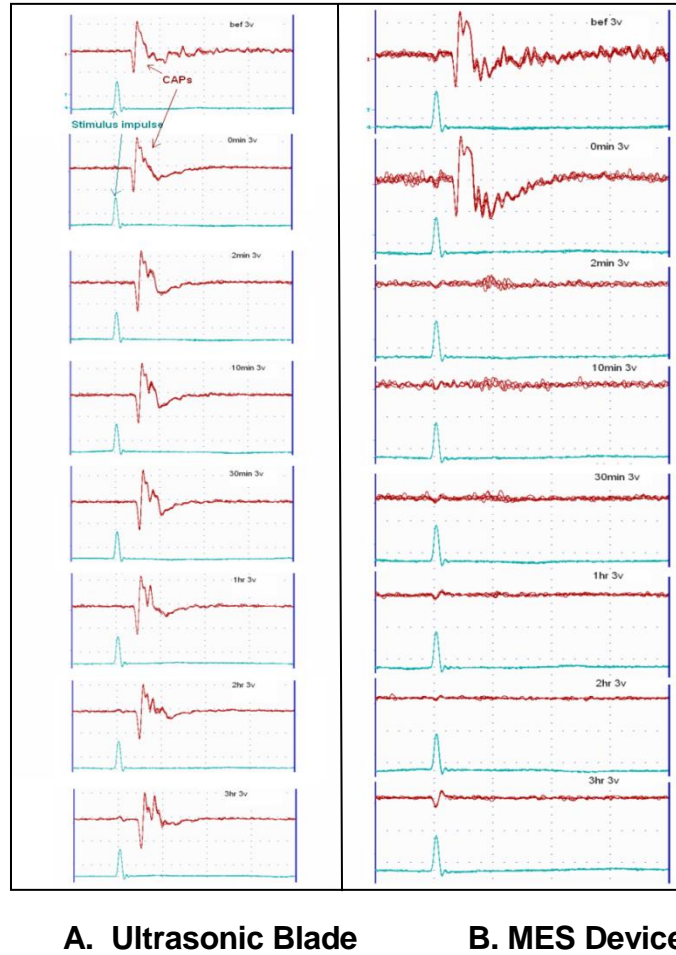
Figure 5.8: Raw Data Comparison between CAP in Ultrasonic blade and MES experiments.

A. 3V CAP due Ultrasonic blade at 1mm away from sciatic nerve over 3hr time period.
B. 3V CAP due to MES at 1mm away from sciatic nerve over 3hr time period.

As seen from Figure 5.8, the magnitude of CAP in case of MES drops over time as compared to the same in ultrasonic blade experiment. In Figure 5.8 B, it is seen that there is a consistent drop in CAP magnitude from 2nd minute after MES device application as compared to the before application magnitude. On the contrary, in Figure 5.8 A, it is seen that there is no major drop in CAP from 2nd minute after using ultrasonic blade. This raw

data was further processed to determine the mean values of CAP, conduction velocities (CV) and percentage changes of CAP and CV as compared to the pre-application recordings.

5.4.3 AUC Analysis

The AUC data obtained from EGAA was analyzed for 1V, 2V, 3V. However, there were no major differences between 1V and 2V data. Also, as compared to 2V, the increase in CAP amplitude was apparent in 3V data. Due to this, 3V was considered to be optimum for analysis.

5.4.3.1 AUC assessment for individual distances

Figure 5.9 shows mean 3V- AUC values for n=8 experiments in Ultrasonic blade, MES and Sham groups. Plots showing 1V and 2V mean AUC changes over time for distances 1mm, 2mm, 3mm and 4mm groups are included in Appendix B. One way ANOVA, Post-hoc: LSD was performed to compare the distance effect with respect to each time point. The p values from the One Way ANOVA: post hoc LSD tests are shown in Table 5.3 (the significant values marked bold). MES showed significantly ($p<0.05$) lower AUC values as compared to the '0 min' time point.

Standard Error of Measurement (SEM) was calculated using Eq. 5.3, and represented as the error bars on each data point.

$$SEM = \frac{\text{Standard Deviation}}{\sqrt{\text{Total Number of samples}}}$$
(Eq. 5.3)

Figure 5.9: 3V Mean AUC change over time for blade-nerve distances 1mm, 2mm, 3mm and 4mm at individual time points. (* indicates One way ANOVA, $p<0.05$; PostHoc LSD)

LSD					
MES		**1 mm**	**2 mm**	**3 mm**	**4 mm**
0 min	Before	0.768	0.923	0.927	0.175
	2min	0.309	**0.002**	**0.013**	0.229
	10min	0.398	**0.001**	**0.002**	0.244
	30min	0.346	**0**	**0**	0.093
	60min	0.288	**0**	**0**	0.111
	120min	0.094	**0.001**	**0**	0.415
	180min	0.142	**0.001**	**0**	0.456

LSD					
US		**1 mm**	**2 mm**	**3 mm**	**4 mm**
0 min	Before	0.59	0.768	0.728	0.294
	2min	0.971	0.781	0.786	0.975
	10min	0.999	0.758	0.928	0.604
	30min	0.939	0.387	0.993	0.695
	60min	0.9	0.578	0.944	0.563
	120min	0.58	0.974	0.802	0.643
	180min	0.556	0.777	0.678	0.861

LSD					
SHAM					
0 min	Before	0.722			
	2min	0.914			
	10min	0.879			
	30min	0.824			
	60min	0.906			
	120min	0.832			
	180min	0.891			

Table 5.3: One Way ANOVA Post-hoc: LSD Analysis for mean AUC values

Figure 5.9 shows that in any group, at any time point, the AUC values in ultrasonic group are higher than in case of MES. AUC values for the ultrasonic blade groups are close to the sham AUC values indicating that ultrasonic blade caused possibly minimal nerve damage. In MES group, there is significant decrease in groups 2 mm and 3 mm at

all the time points after the device application (2 min to 180 min) with respect to '0 min' time point (marked with '*' in the Figure). The ultrasonic blade HK 105 did not have any significant drop in the AUC after the blade application at 1 mm, 2 mm, 3 mm and 4 mm distances from sciatic nerve.

AUC did not decrease overall after US application, while MES caused significant decrease in AUC (Two-way ANOVA, with significance at $p < 0.05$). The P values summarizing these findings are presented in the table 5.4 with the significant values shown in bold. All the data points were compared to the '0 min' time point, thus considering the pancuronium bromide effect. The AUC values were significantly lower on MES application as compared to ultrasonic blade application at a distance of 3 mm from nerve at time points 2 min, 10 min, 30 min, 60 min, 120 min and 180 min after device application.

MES AUC	P value 1mm	P value 2mm	P value 3mm	P value 4mm
before	0.77	0.77	0.93	0.17
2 min	0.31	0.31	**0.01**	0.23
10 min	0.4	0.4	**0**	0.24
30 min	0.35	0.35	**0**	0.09
60 min	0.29	0.29	**0**	0.11
120 min	0.09	0.09	**0**	0.42
180 min	0.14	0.14	**0**	0.46

US AUC	P value 1mm	P value 2mm	P value 3mm	P value 4mm
before	0.59	0.59	0.59	0.29
2 min	0.97	0.97	0.97	0.97
10 min	1	1	1	0.6
30 min	0.94	0.94	0.94	0.69
60 min	0.9	0.9	0.9	0.56
120 min	0.58	0.58	0.58	0.64
180 min	0.56	0.56	0.56	0.86

Table 5.4: Two Way ANOVA Post-hoc: LSD Analysis for mean AUC values

5.4.3.2 Percentage AUC Change

The percentage change in mean AUC values with respect to the 'before surgery' time point was plotted for each device for distances 1 mm, 2 mm, 3 mm and 4 mm. Negative values of percentage values indicate percentage drop in AUC while positive values of percentage indicate increase in AUC.
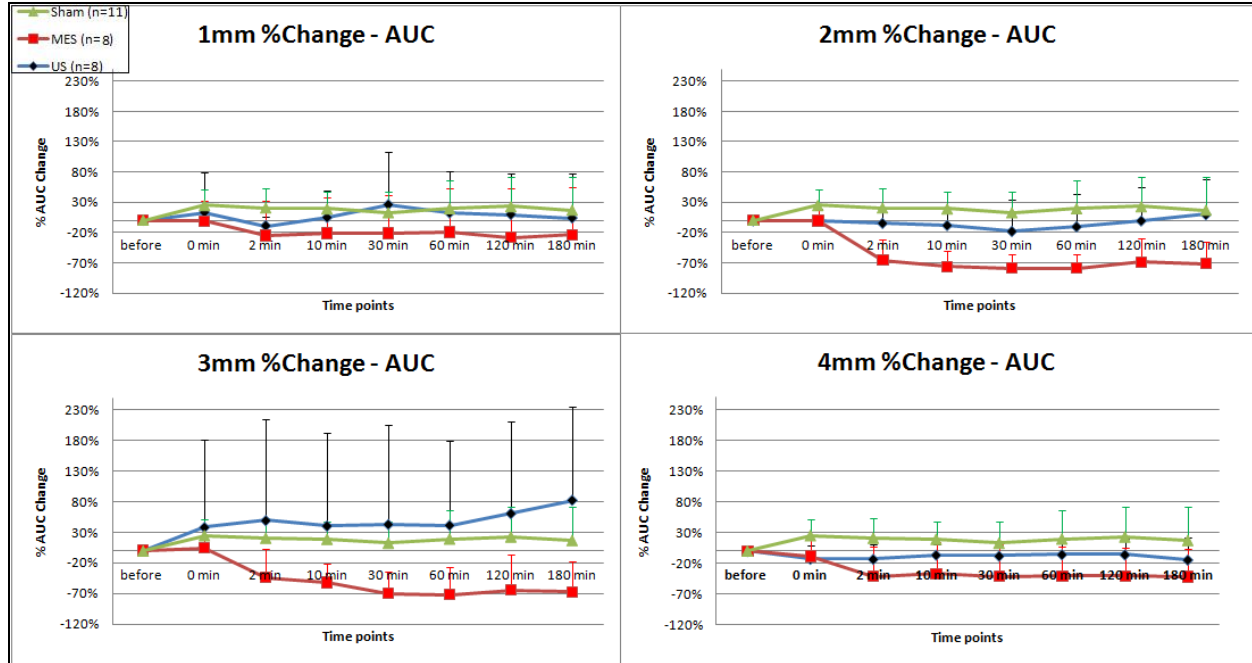
Figure 5.10: Percentage Change in AUC at individual time points for distances 1 mm, 2 mm, 3 mm and 4 mm

In MES group at distances 1 mm, 2 mm, 3 mm and 4 mm all the percentage values were negative, thus, indicating that AUC dropped after application of MES blade. At distances 1 mm and 2 mm there was at least 20 % drop in AUC even by the end of 3 hours. Also, at 2 mm distance, the MES AUC values dropped up to 80 % from 2 minutes after device application till about 1 hour.

In ultrasonic blade group (US), there were positive percentage changes in AUC indicating that there was no drop in AUC. At 2 mm distance, ultrasonic blade caused 20% drop 30 minutes after the blade application. However, after 30 minutes there was a steady increase in AUC.

5.4.3.3 AUC assessment for all distances

As application of MES caused uncontrolled muscle contraction, there was a possibility that the distance of the blade tip from the sciatic nerve was not accurately maintained. Thus, AUC values (3V) were analyzed combining all the distances at the individual time points. One way ANOVA was performed for AUC at all individual time points (Figure 5.11). Post Hoc: LSD was performed comparing all the data at different time points with respect to the "0 minute" time point. Table 5.5 shows the p values obtained from this analysis (the significant values marked bold).
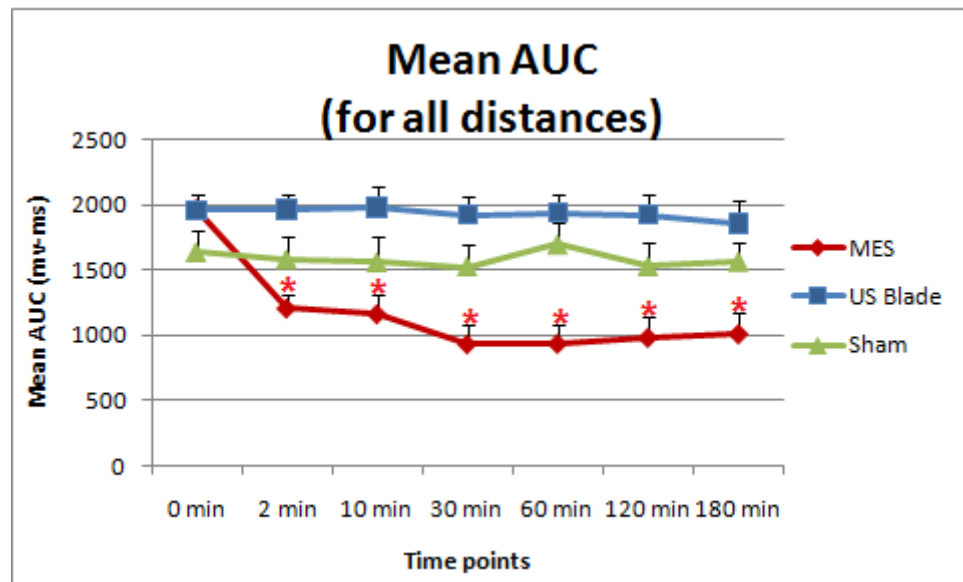
Figure 5.11: Mean AUC (3V) comparison for MES, Ultrasonic blade and Sham over individual time points for all distances (* indicates statistical difference with respect to the '0 min' data point p<0.05)

		LSD MES	LSD US	LSD Sham
0 min	before	0.318	0.469	0.459
	2 min	**0.000**	0.978	0.821
	10 min	**0.000**	0.929	0.752
	30 min	**0.000**	0.868	0.644
	60 min	**0.000**	0.924	0.807
	120 min	**0.000**	0.868	0.659
	180 min	**0.000**	0.666	0.774

Table 5.5: One Way ANOVA Post-hoc: LSD Analysis for mean AUC values

Ultrasonic blade had higher AUC values as compared to MES AUC values all throughout the post device application time points. The AUC values for MES group dropped significantly (p<0.05) at all the time points after completion of MES application as compared to the 0 minute time point. Ultrasonic blade HK 105 did not have any significant drop in AUC at any time point after the device application.

5.4.4 Conduction Velocity (CV) Analysis

Conduction velocity was analyzed for each group of experiments at 1V, 2V and 3V. However, there were no major differences between 1V and 2V conduction velocity values. Also, as compared to 2V, the change in AUC was much more evident in 3V data. Due to this, 3V was considered to be optimum for analysis.

5.4.4.1 CV assessment for individual distances

Figure 5.12 shows mean 3V- AUC values for 8 experiments in Ultrasonic blade, MES and Sham groups. Plots showing 1V and 2V mean CV changes over time for distances 1mm, 2mm, 3mm and 4mm groups are included in Appendix C. One way ANOVA, Post-hoc: LSD was performed to compare the distance effect with respect to

each time point. The p values from the One Way ANOVA: post hoc LSD tests are shown in Table 5.6 (the significant values marked bold). The Standard Error of Measurement (SEM) was calculated using Eq. 5.3, and represented as the error bars on each data point.

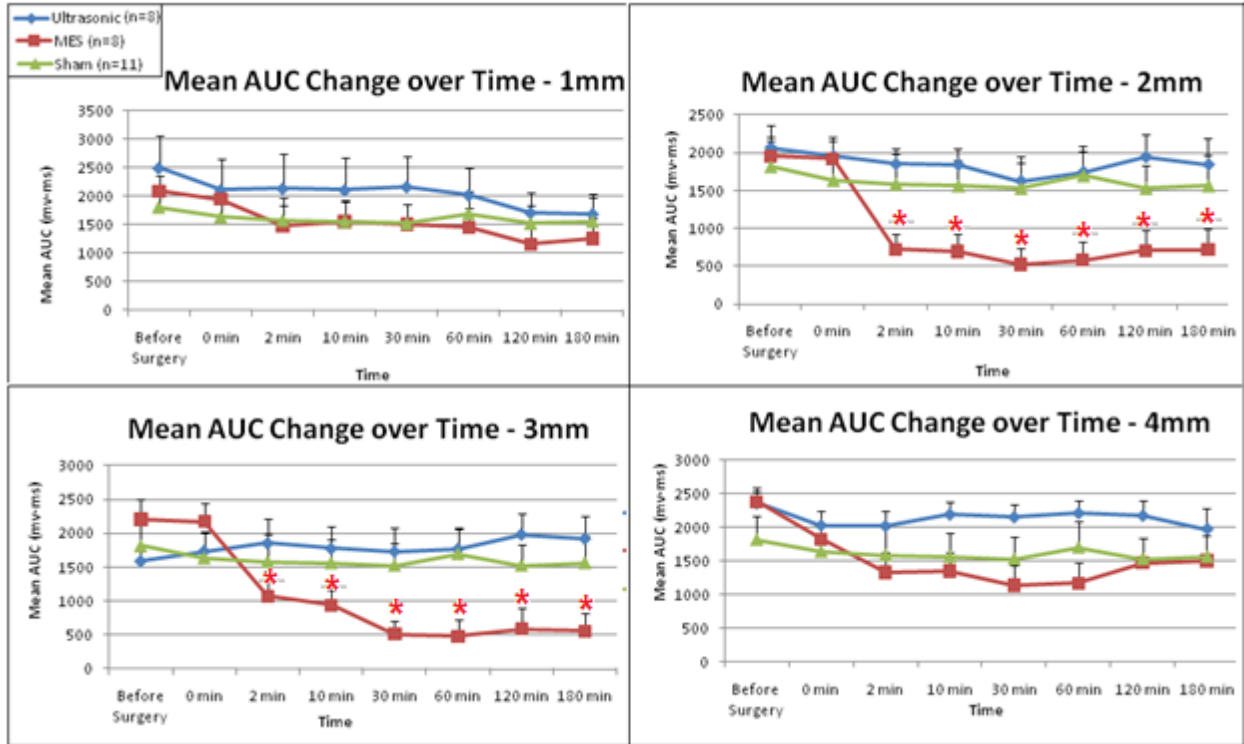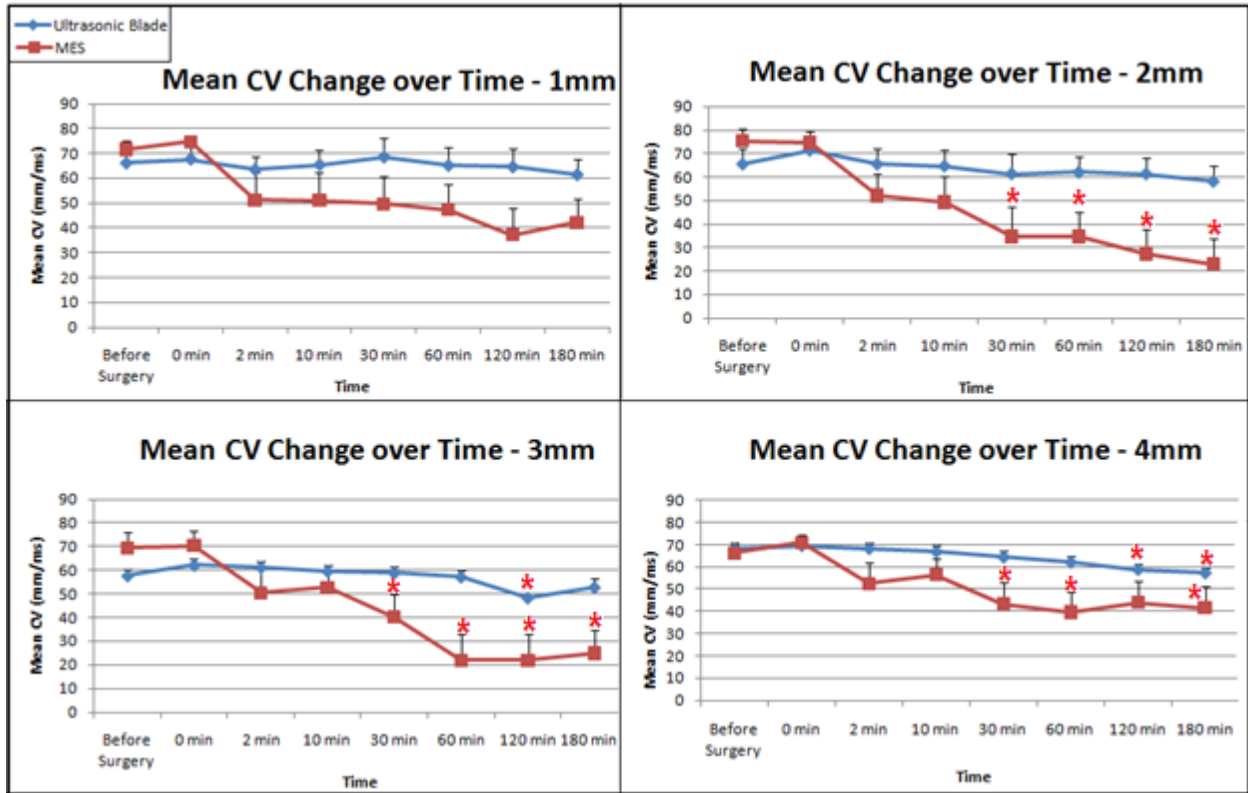**Figure 5.12:** 3V Mean CV change over time for blade-nerve distances 1mm, 2mm, 3mm and 4mm. (* indicates One way ANOVA, p<0.05; PostHoc LSD)

LSD					
MES		**1 mm**	**2 mm**	**3 mm**	**4 mm**
0 min	Before	0.820	0.955	0.940	0.680
	2min	0.131	0.085	0.181	0.230
	10min	0.128	0.065	0.237	0.388
	30min	0.104	**0.003**	**0.041**	**0.048**
	60min	0.072	**0.003**	**0.001**	**0.021**
	120min	0.012	**0.000**	**0.001**	0.066
	180min	0.031	**0.000**	**0.002**	**0.044**

LSD					
US		**1 mm**	**2 mm**	**3 mm**	**4 mm**
0 min	Before	0.864	0.553	0.245	0.710
	2min	0.777	1.000	0.351	0.964
	10min	0.938	0.933	0.610	0.762
	30min	0.798	0.690	0.763	0.376
	60min	0.920	0.724	0.894	0.149
	120min	0.867	0.672	**0.038**	**0.028**
	180min	0.618	0.456	0.213	**0.011**

LSD					
US		**NA**			
0 min	2nd min	0.914			
	10th min	0.879			
	30th min	0.824			
	1 hr	0.906			
	2 hr	0.832			
	3 hr	0.891			
	before	0.722			

Table 5.6: One Way ANOVA Post-hoc: LSD Analysis for mean CV values

Figure 5.12 indicates that after the device application i.e. from 2nd minute recording, mean MES CV values were lower than mean US CV values at all the time points. MES showed significantly ($p < 0.05$) lower CV values as compared to the '0 min' time point at distances 2 mm and 3 mm at all time points after 30 min post device application. Also, following MES application at 4 mm from nerve, there was a significant drop in CV at time points 30 min, 60 min and 180 min post device application. On the

contrary, ultrasonic blade application at 1 mm and 2 mm from the nerve did not cause any significant drop in CV at any time point. However, ultrasonic blade application did show significant drop in CV at 3 mm distance at time point 120 min and at 4 mm distance at time points 120 min and 180 min.

CV values did not decrease overall after US application, while MES caused significant decrease in AUC (Two-way ANOVA, with significance at $p < 0.05$) at certain time points. The P values summarizing these findings are presented in the Table 5.7 with the significant values shown in bold. All the data points were compared to the '0 min' time point, thus considering the pancuronium bromide effect. The CV values were significantly lower on MES application as compared to ultrasonic blade application at 1 mm distance at time points 60 min and 120 min, at 2 mm distance at time points 10 min, 30 min, 60 min and 120 min, at 3 mm distance at time points 10 min, 30 min, 60 min and 120 min and at 4 mm distance at time points 10 min, 30 min and 120 min.

MES CV	P value 1mm	P value 2mm	P value 3mm	P value 4mm
Before	0.13	0.08	0.18	0.23
2 min	0.13	0.06	0.24	0.39
10 min	0.1	**0**	**0.04**	**0.05**
30 min	0.07	**0**	**0**	**0.02**
60 min	**0.01**	**0**	**0**	0.07
120 min	**0.03**	**0**	**0**	**0.04**
180 min	0.82	0.96	0.94	0.68

US CV	P value 1mm	P value 2mm	P value 3mm	P value 4mm
Before	0.86	0.55	0.24	0.05
2min	0.78	1	0.35	0.05
10min	0.94	0.93	0.61	0.05
30min	0.8	0.69	0.76	0.05
60min	0.92	0.72	0.89	0.05
120min	0.87	0.67	**0.04**	0.05
180min	0.62	0.46	0.21	0.05

Table 5.7: Two Way ANOVA Post-hoc: LSD Analysis for mean CV values

5.4.4.2 Percentage CV Change

The percentage change in mean CV values with respect to the 'before surgery' time point was plotted for each device for distances 1 mm, 2 mm, 3 mm and 4 mm. Negative values of percentage values indicate percentage drop in CV while positive values of percentage indicate increase in CV.
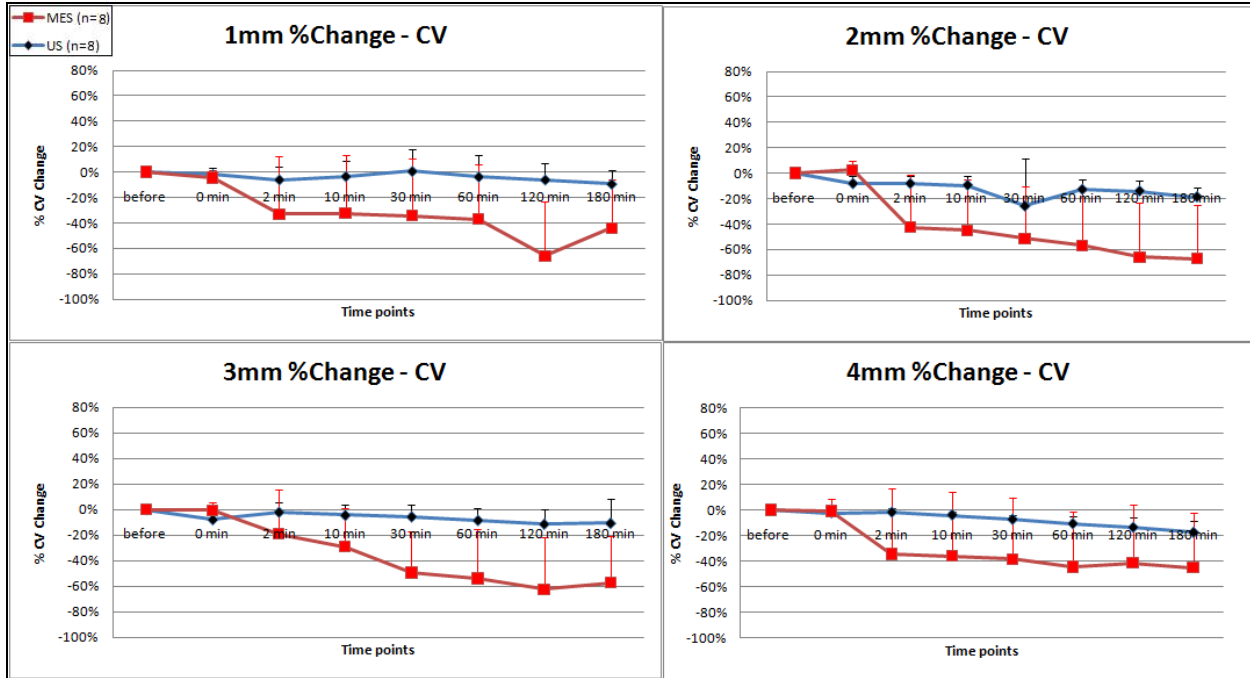
Figure 5.13: Percentage Change in CV at individual time points for distances 1 mm, 2 mm, 3 mm and 4 mm

MES group had negative percentage values for CV change at all distances (1 mm, 2 mm, 3 mm and 4 mm). This indicated that the CV dropped after MES application. At distances 2 mm and 3 mm, there was about 65-70% CV drop in MES group. Even at a distance of 4 mm from the nerve, there was about 45% CV drop 3 hours after device application.

In ultrasonic blade group (US), there was less than 10% drop in CV at distance 1 mm from the nerve. At distances 2 mm, 3 mm and 4 mm, there was less than 20% drop at 3 hours after application. Thus, the maximum percentage drop in CV did not exceed 20%.

5.4.4.2 CV assessment for all distances

As in case of AUC, one way ANOVA was performed on CV for all distance combined (1 mm, 2 mm, 3 mm and 4mm) at the all individual the time points (Figure 5.14). Post Hoc: LSD was performed comparing all the data at different time points with "0 minute" time point. The corresponding p values are shown in Table 5.8.
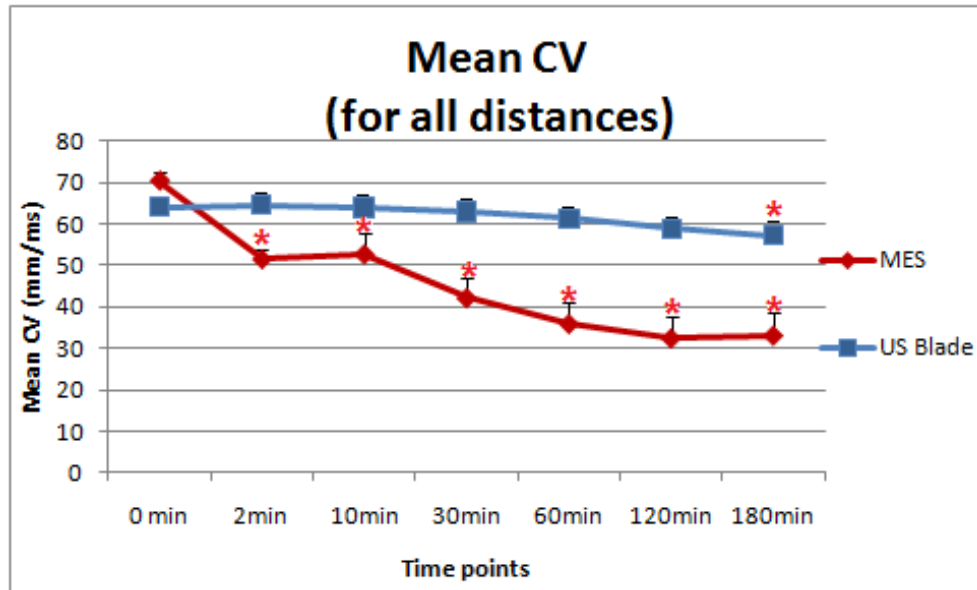
Figure 5.14: Conduction Velocity comparison for devices MES and ultrasonic blade over individual time points for all distances

* indicates statistical difference with respect to the '0 min' data point (p<0.05)

		LSD MES	LSD US	LSD Sham
0 min	before	0.744	0.304	0.459
	2 min	**0.003**	0.872	0.821
	10 min	**0.006**	0.964	0.752
	30 min	**0.000**	0.729	0.644
	60 min	**0.000**	0.389	0.807
	120 min	**0.000**	0.122	0.659
	180 min	**0.000**	**0.038**	0.774

Table 5.8: One Way ANOVA Post-hoc: LSD Analysis for mean CV values

It was seen that ultrasonic blade had higher CV values as compared to MES throughout the post device application time points. The CV values for MES group dropped significantly ($p < 0.05$) at all the time points after the MES application as compared to the 0 minute time point. The ultrasonic blade did not cause any significant drop in CV at time points 2 min, 10 min, 30 min, 60 min and 120 min after the blade application.

5.4.5 Hind Paw Probing Analysis

It was seen that in a normal nerve, VFH 5.18 or VFH 5.46 activated the sensory receptors. In cases in which AUC drop, as compared to baseline value, was less than 50%; these VFH forces acted as threshold values. Also, when the AUC drop was greater than 50% the VFH threshold values were around 6.10 VFH. In nerves with 100% AUC drop i.e. a complete cessation of CAP, there was no observable activation of sensory receptors, even with the highest VFH, i.e. 6.65. This indicated significant sensory nerve damage and sensory nerve dysfunction and thus did not respond to any higher force. In nerve injured rats, VFH 6.10 or higher is required to provoke withdrawal from pain

(Chen, Cavanaugh et al. 1997). It was seen that MES experiments caused more cases of hind paw numbness as compared to the ultrasonic blade experiments, irrespective of the distances from the sciatic nerve.

Overall, it was found that MES application at 1 mm distance from the nerve did not show any significant changes in AUC or CV values. The possible reason for this could be the uncontrolled muscle contraction during MES application, Due to the muscle contraction; the device blade moved and could not be precisely positioned all throughout the 5 seconds exposure. As a result, the blade could have possibly touched the nerve or moved away from the nerve. It was seen that in some cases of MES application, there was no CAP generation after device was applied while in some cases there was only a mild drop in the amplitude of CAP after device was applied. This variation would indicate that when the blade tip was moved towards or touched the nerve, as a result of uncontrolled muscle contraction, there was a complete cessation of nerve activity. On the contrary, when the blade tip was pulled away from the nerve, again as a result of muscle contraction, the CAP was not majorly affected. Thus, the 1 mm data obtained after MES application might not have shown the true effects of this device. This limitation was overcome in Acute Study II where in the procedure similar to actual clinical application was developed.

In summary, application of the ultrasonic device at distances 1 mm, 2 mm, 3 mm and 4 mm from rat sciatic nerve resulted in much lower chance of nerve dysfunction as compared to the MES device. Also, MES produces much more rapid and severe nerve dysfunction as compared to the ultrasonic blade.

CHAPTER 6

SPECIFIC AIM II: ACUTE STUDY II

6.1 SPECIFIC AIMS

RF based devices Monopolar Electrosurgery (MES) and LigaSure, and ultrasonic shears Harmonic Focus and Harmonic Ace were tested to surgically expose the rat sciatic nerve. Also, the acute effects of these devices were monitored on the sciatic nerve conduction function.

6.2 OUTLINE

A total of 22 sciatic nerves from 22 adult male Sprague Dawley rats (400-450 g) were used in this acute *in vivo* study as per the test matrix as shown in Table 6.1 to establish the methodology of assessing compound action potential (CAP) changes. Approximately 20 mm length of sciatic nerve was exposed with the surgical device.

Devices	Settings	No. of Rats
Manual Scissors		4
MES	**(60 Watts, Blend 1)**	4
Harmonic FOCUS	**(Level 5)**	4
Harmonic ACE	**(Level 5)**	4
LigaSure	**(Mode 2)**	6

Table 6.1: Test Matrix for Acute Study II

As in previous study, the MES was set at 60 Watt power, cut, Blend 1 which is commonly used in clinical setting to carry out an incision and simultaneously seal the blood vessels. The ultrasonic shears (Harmonic FOCUS and Harmonic ACE) were set at power level 5 to maximize the extent of energy exposure by these devices. LigaSure was set at Mode 2 which is a standard mode used in the clinical setting.

A set of neurophysiologic recordings were taken following a similar technique as described in study 1. The neural activity was recorded from the left L5 nerve root following an electrical stimulation of left sciatic nerve by 1V, 3V and 5V stimulations. A baseline recording was taken. After the initial set of recordings for each voltage stimulation, the sciatic nerve was exposed to any of the five surgical devices. Considering the completion of device application time point as the 0th minute, the post-device application recordings were taken at time points 2nd minute, 10th minute, 30th minute, 60minute, 120th minute and 180th minute from the actual exposure. No pancuronium bromide injection was given to the rat. Thus, there was only one set of recordings performed before the device application, unlike study 1. Once the recordings were completed, the rat was sacrificed using intra-cardiac injection of Sodium Pentobarbital (1cc).

As this was mainly a developmental study, the focus was to develop an appropriate application technique for each surgical device so as to mimic the clinical procedures. A total of 5 expired rats were used to develop and practice the use of these devices before using live anesthetized rats.

6.3 METHODOLOGY

6.3.1 Anesthesia

The rats were anesthetized and sedated by an intraperitonial injection of ketamine (40 mg/kg) and xylazine (5 mg/kg) and maintained on ketamine (20 mg/kg) and xylazine (2.5 mg/kg) as needed throughout the experiment. The depth of anesthesia was continuously monitored using paw pinch and palpebral reflexes.

6.3.2 Pre-recording surgeries

The surgical procedures developed and described here were not a part of Master's thesis project and were developed by others. These procedures are included for sake of completeness of describing the study.

6.3.2.1 L5 Nerve Root Exposure

A L2-L6 laminectomy was performed to expose the spinal nerves. The procedure was similar to that in case of study 1. Also, the nerve roots were maintained in the mineral oil at 37°C.

6.3.2.2 Tracheotomy

Tracheotomy was performed in only those rats where there was a need of artificial ventilation. This was decided during the laminectomy and depending upon if the rat's respiratory rate was normal, a plastic catheter was inserted into trachea. The artificial ventilator had the breath rate of 90 bpm and a tidal volume of 2.5 ml.

6.3.2.3 Sciatic Nerve Exposure

A #10 scalpel was used to make a dorsal lateral skin incision between the lateral aspect of knee joint and greater trochanter of femur bone to expose muscles and fascia. Conventional scissors were used to detach posterior thigh muscle from the femur bone. This fascia was located at the posterolateral aspect of the thigh. Forceps were used to retract posterior thigh muscle to expose the sciatic nerve.

6.3.3 Device Application

The sciatic nerve was located posteriorly near midline of the thigh, approximately 10 mm from the cut fascia. After visualizing the sciatic nerve, the posterior thigh muscles (the gluteus superficialis muscle and biceps femoris muscle) that cover the nerve were cut using the designated cutting device. The tip of the device was applied to the muscles adjacent to the sciatic nerve approximately 1mm away from the nerve. The device was used on both sides of the nerve. The intensity of power as well as the device activation time was recorded. Quantitative data analysis was performed offline. The device settings are showed in Table 6.2.

Devices	Power Applied
Manual Shears	NA
ACE	(Default), Level 5
FOCUS	(Default), Level 5
MES	60 watt, Cut, Blend 1
LigaSure	(Default), Mode 2

Table 6.2: Device Settings

The procedure developed and followed for each of five surgical devices can be explained as follows,

6.3.3.1 Manual Scissors (n = 4)

Manual scissors were used to punch a 2 mm deep hole vertically in the caudofemoralis muscle (cf) (refer to Figure 6.1). A blunt dissection using manual scissors was made approximately 1mm away from the sciatic nerve and about 4mm deep under the muscle. The muscle was then cut with the length of incision being 10 mm. Thus, the total length of cut on each side of sciatic nerve was 10 mm. The same procedure was repeated on the other side of nerve. Thus, there was a 10 mm long incision on each side of nerve.
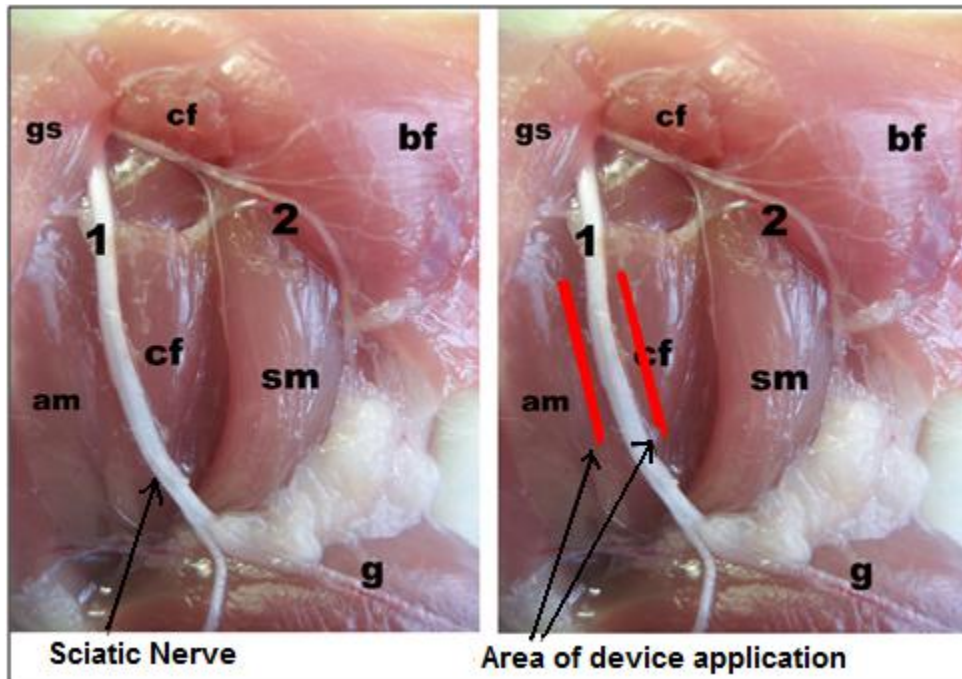
Figure 6.1: Area of device application around sciatic nerve (Rupp, Dornseifer et al. 2007)

6.3.3.2 Harmonic FOCUS (n = 4)

Post the sciatic nerve exposure by cutting and flipping over the gluteus superficialis (gs) muscle and biceps femoris (bf) muscle, FOCUS cutting device was used to punch a hole vertically in the caudofemoralis muscle (cf) by activating the device for 1-2 seconds, with power setting at 5. The hole was approximately 1-2 mm away from the sciatic nerve with the depth of around 5 mm with no bleeding. The muscle was then clamped in jaw of FOCUS. Once the muscle tissue was well positioned in the jaw, FOCUS was activated with tension applied in the upward direction from the muscle so that the device jaws separated from muscle immediately when muscle was cut. This prevented excessive burning of the tissue. This cut produced a 5 mm long wound along the sciatic nerve without bleeding. In a similar fashion another 5mm length of muscle was clamped and cut by FOCUS jaw. These steps are shown in Figure 6.2.

Thus, the total length of cut on one side of sciatic nerve was 10 mm. Exactly the same procedure was repeated on the left side of sciatic nerve with a 10 mm long cut on the adductor magnus muscle (am). Figure 6.1 explains the exact location of device application.
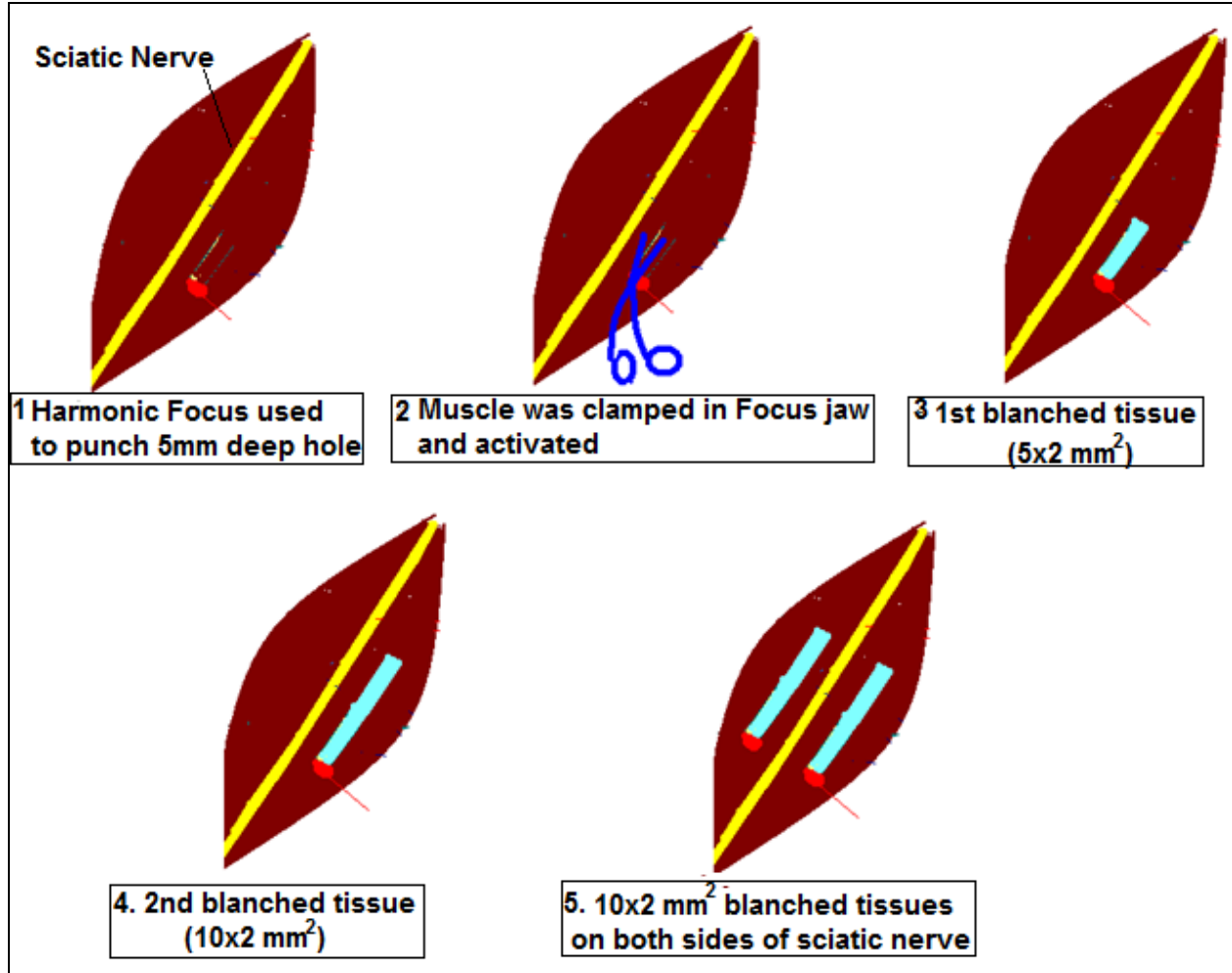
Figure 6.2: Steps in application of Harmonic FOCUS

6.3.3.3 Harmonic ACE device (n = 4)

ACE application was similar to that of FOCUS (Figure 6.2). After the sciatic nerve was exposed by cutting and flipping over the gluteus superficialis muscle and biceps femoris muscle (refer Figure 6.1), ACE cutting device was used to punch a hole vertically in the caudofemoralis muscle (cf) by activating the device for 1-2 seconds, with power set at 5. The 5 mm deep hole was made at a distance of 1-2 mm form the sciatic nerve. Then muscle was then clamped in ACE jaw and the device was activated. An upward tension, as in case of FOCUS, was applied on the muscle. This produced a

5 mm long incision along the sciatic nerve. In a similar fashion another 5 mm length of muscle, in the direction parallel to the nerve, was clamped in the ACE jaw. The muscle was cut in similar way.

Thus, 10 mm long cut was produced on the right side of nerve. The same procedure was repeated on the left side of nerve, producing a 10 mm long cut on adductor muscle (am).

6.3.3.4 MES application (n = 4)

Monopolar electrosurgery cutting blade was used to cut muscle at the same location as mentioned above. The power of MES was set at 60 Watt and mode blend 1. This application also produced an incision 10 mm in length at 1-2 mm away from the sciatic nerve and about 4 mm in depth on each side of sciatic nerve. Muscles on both sides of the sciatic nerve were cut as in case of FOCUS and ACE.

6.3.3.5 LigaSure application (n = 6)

As the LigaSure application technique was developed, three different types of application methods were tried. Each type of application was defined by a protocol number and performed on two rats each. The basic differences in the techniques were based on number of device activations, area of application and exact length of muscle tissue coagulated. A detailed explanation of each protocol is explained below.

- Protocol 1 (n = 2)

Two rats were tested using this protocol. The basic mechanism of the device activation was similar to that ACE and FOCUS. During each application, manual

scissors was used to puncture hole into the muscle adjacent to the sciatic nerve in a similar way as with other devices. Further, the LigaSure jaw was used to clamp approximately 5 mm length of the muscle tissue (cf) (Figure 6.1). The device was activated with the auto-setting Mode 2. This led to 5x2 mm^2 area of blanched tissue. Thus, 1st application (activation 1) caused a total 10 mm^2 of blanched tissue. A similar application procedure (activation 2) was applied to another 5 mm length of muscle along the direction of 1st blanched tissue, parallel to the sciatic nerve. These two activations caused two sections of blanched tissue as shown in Figure 6.3 A. In the same manner, two more activations, activation 3 and activation 4 were carried out parallel to the previous two blanched tissues (Figure 6.3 B).

Thus, all four activations, each producing blanched tissue region of area 5x2 mm^2, were carried out one side of sciatic nerve. The same set of activations was performed on the other side of the nerve as shown in Figure 6.3 B. In protocol 1, there were a total of 8 activations around sciatic nerve.

A.
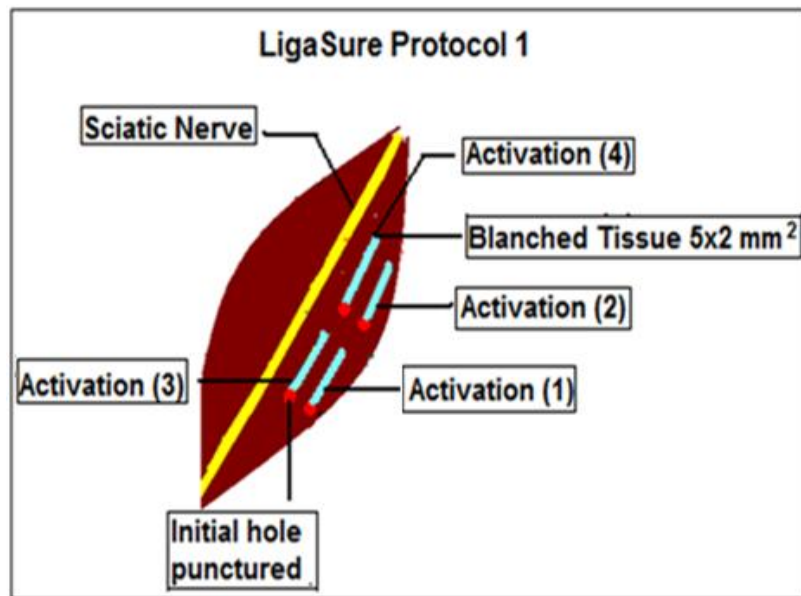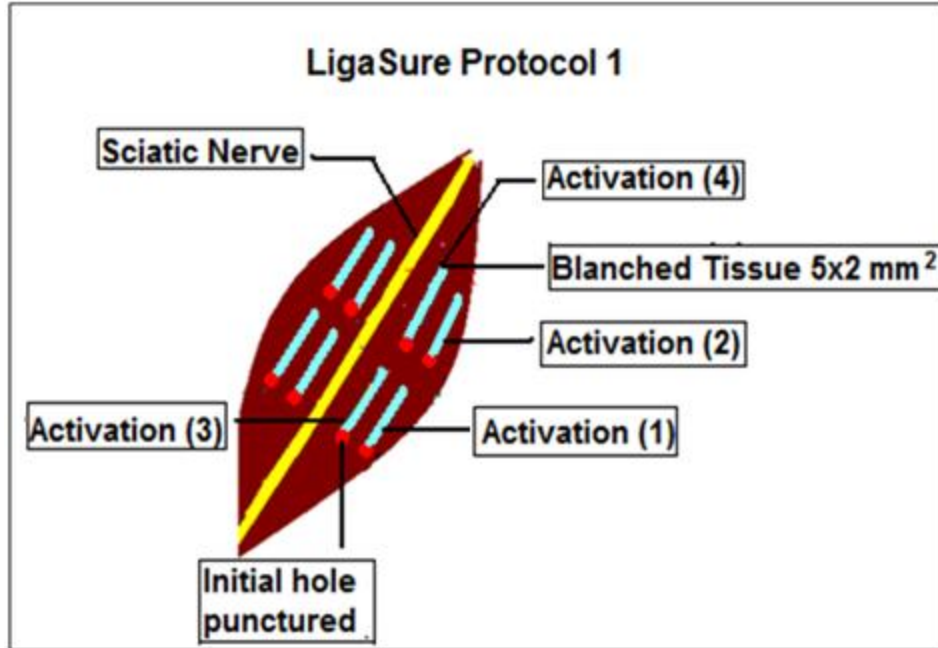
Figure 6.3: Steps in LigaSure application (Protocol 1)
A. First 4 activations on one side of sciatic nerve.
B. Total 8 activations on both sides of sciatic nerve.

- Protocol 2 (n = 2)

In protocol 2, there were only two activations performed on each side of the sciatic nerve. Thus, there were a total of 4 activations on both sides. The application technique during each activation was similar to that of protocol 1 with some minor modifications. In this case, during each activation, a 10 mm length of muscle tissue was clamped. Thus, after activation 1, the blanched tissue area produced was 2x10 mm^2. The 2nd activation was performed parallel to the 1st application of blanched tissue as shown in Figure 6.4 A. Thus, on each side of the nerve, there were two regions of blanched tissue with total area of 2x2x10 mm^2. The same applications were performed on the other side of the nerve as shown in Figure 6.4 B. Thus, protocol 2 had a total of 4

activations on both sides of the sciatic nerve. Once all activations were completed, manual scissors were used to cut the coagulated tissue.
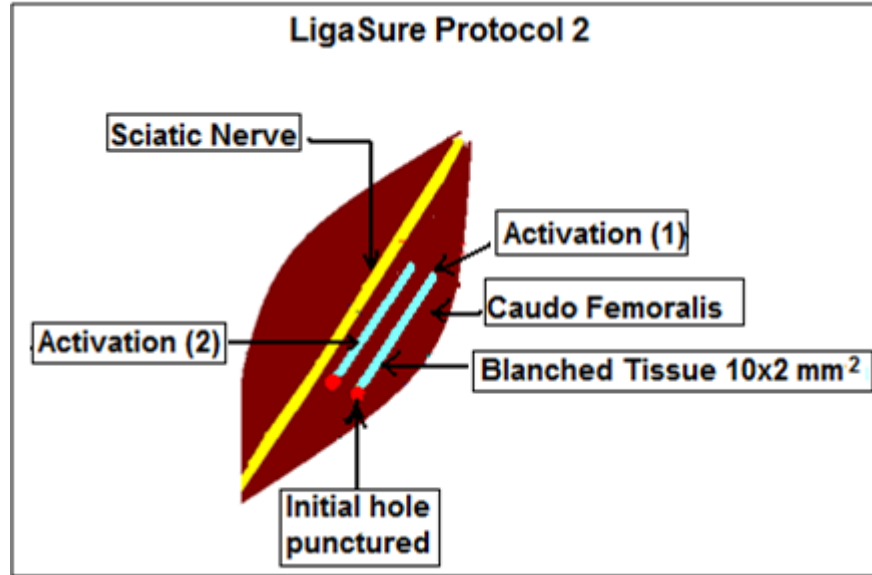
A.
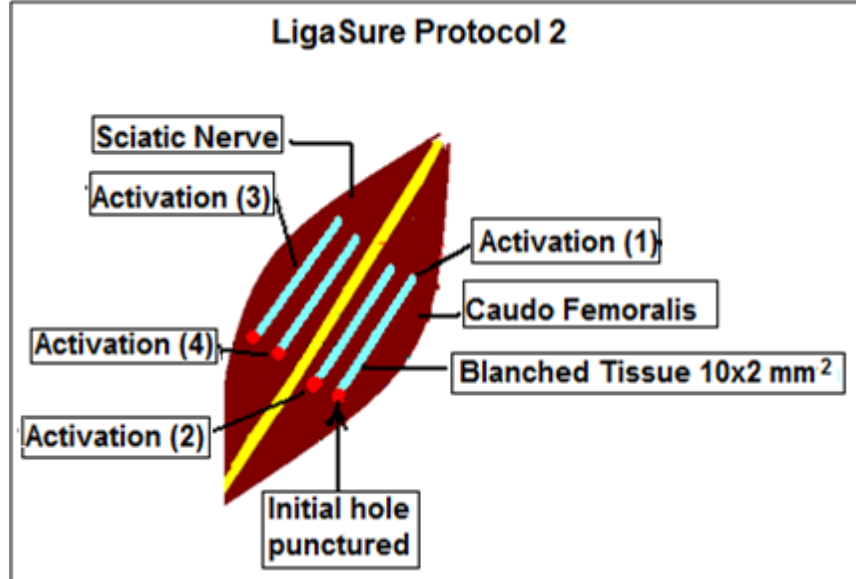
B.
Figure 6.4: Steps in LigaSure application (Protocol 2)
A. 2 activations on one side of nerve
B. Total 4 activations on both sides of nerve

- Protocol 3 (n = 2)

Initially, manual scissors were inserted into the muscle (approximately 4 mm in depth) and the muscle was blunt dissected to produce a 10 mm long cavity under the muscle along the sciatic nerve. LigaSure device was then inserted into the cavity and 10 mm length of muscle was clamped.   LigaSure was activated, producing approximately 10 x 2 mm^2 region of blanched tissue. The manual scissors were then used to cut the blanched muscle tissue along the midline of the blanched tissue. The same procedure was repeated on the other side of the sciatic nerve (Figure 6.5). Thus, there were a total two activations, one on each side of the nerve.
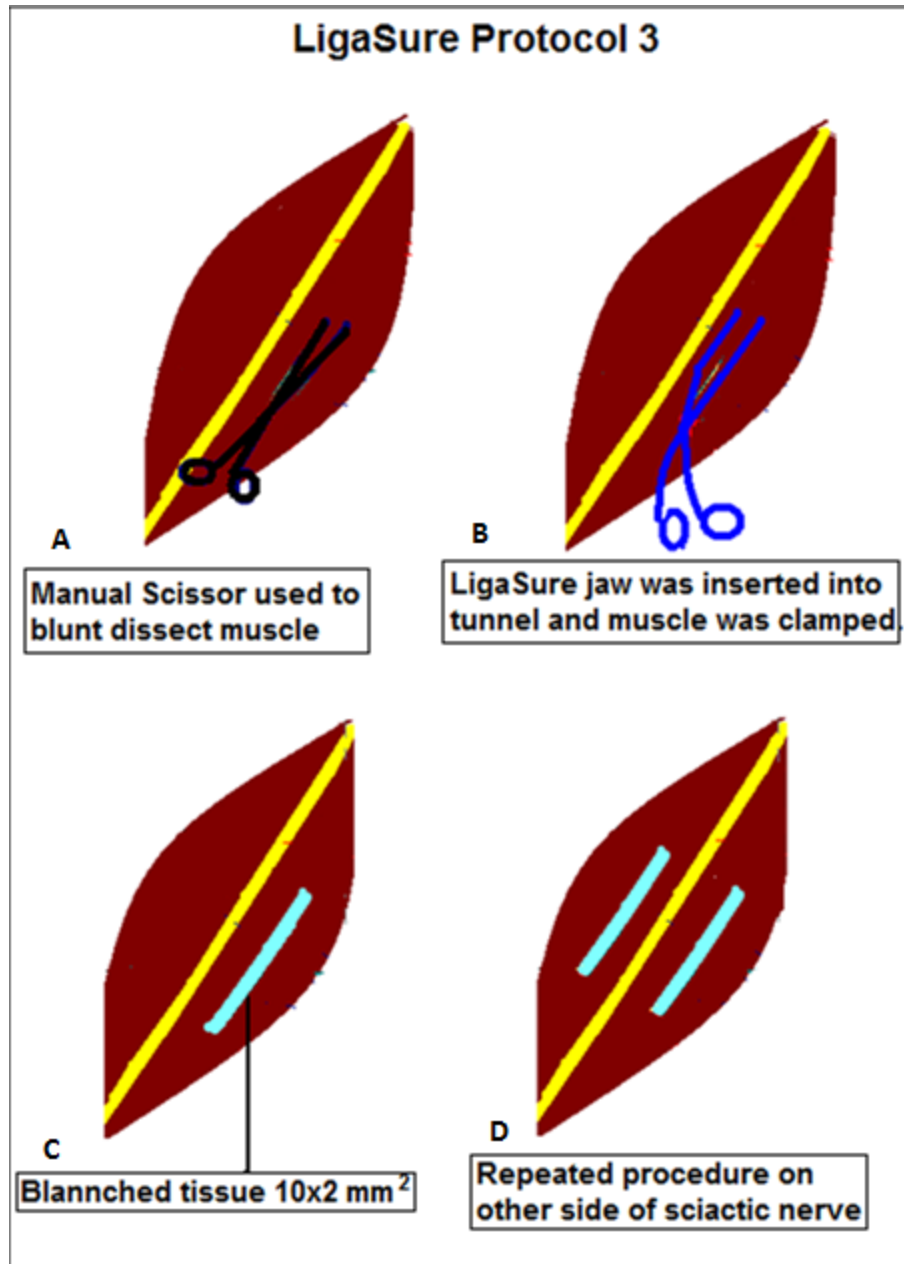
Figure 6.5: Steps in LigaSure application (Protocol 3)

6.3.4 Recording Procedure

6.3.4.1 Neurophysiology Testing

The neurophysiologic settings were exactly similar to that in Study 1. A miniature bipolar stimulating hook electrode (Chen C 2005) made up of platinum wire was placed under the ipsilateral left sciatic nerve. Further, to record the neural activity from the L5 nerve root, two bipolar platinum wire electrodes (RE 1 and RE 2) were placed under the nerve root (Figure 5.2). The sciatic nerve was stimulated by a pulse signal of 1V, 3V and 5V through the stimulating electrode.

A set of baseline recordings were carried out before the device application. This set of recordings was named as 'Before Surgery'. After this, one of the 5 surgical devices was used at a distance of 1-2 mm from the left sciatic nerve. The very end of the surgical procedure was counted as 0th minute. A set of recordings were taken at 2, 10, 30, 60, 120 and 180 minutes after completion of device application.

6.3.5 Data Analysis

The raw data acquired from EGAA was processed using rectify and integrate functions to get area under CAP (AUC) and conduction velocity (CV) values. These values were evaluated to assess the changes in nerve function and also monitor complete nerve conduction block, if any. The before and after device application recordings were studied so as to check any changes in these parameters indicating effect of these devices on the nerve.

6.3.5.1 Area under the CAP (AUC)

This analysis was done by calculating the area under the rectified CAP curve obtained from the recording electrodes, using EGAA system (Figure 5.4). The percentage change in the AUC before and after use of the proposed devices was determined. The before recording was considered as 0% and all the percentage changes of the following values were calculated (Eq. 6.1). This was used to calculate the decrease in the area (if any) of the evoked CAP as a result of device application. Thus, a negative percentage change indicated that there was a drop in AUC values as compared to the initial baseline recording and a positive percentage change indicated an increase in AUC values.

$$\text{Percentage Change in AUC} = \frac{(\text{Current AUC value} - \text{Before AUC value})}{\text{Before AUC value}} \times 100$$ ..(Eq. 6.1)

6.5.3.2 Conduction velocity (CV)

CV was calculated by dividing the distance (D) between the stimulating and recording electrodes by the latency between the onset of stimulus pulse and the onset of CAP (ΔT, difference of CAP onset time). The latency between the evoked potentials was determined using the EGAA system (Figure 6B, Study 1). The change in CV was analyzed before and 2, 10, 30, 60, 120 and 180 minutes after surgical procedure. The percentage change in CV was calculated for all designated time points before and after device application. (Eq. 6.2)

$$\text{Percentage Change in CV} = \frac{(\text{Current CV value} - \text{Before CV value})}{\text{Before CV value}} \times 100$$ ..(Eq. 6.2)

6.4 RESULTS

6.4.1 Gross Findings

As seen in study 1, MES device application caused extensive tissue damage. During application, there was severe muscle contraction and smoke production which posed great difficulty to the surgeon using the device. Also, there was a higher chance of the MES blade touching the nerve tissue which can cause complete cessation of nerve function. It also caused charring, desiccation and left a burn wound on the muscle. On the contrary, application of Harmonic FOCUS and ACE produced minimal thermal injury with no charring or smoke production. Moreover, there was no muscle contraction during application thus making it relatively easy to use. Similarly, the application of LigaSure produced minimal thermal muscle tissue damage. There was no charring of tissue or smoke production. However, there was very slight muscle contraction but not strong enough to make the application uncontrollable.

6.4.2 Time of activation comparison

The time of activation of each device was calculated for every experiment. A mean for all the experiments in specific device group was taken and compared with other devices. Figure 6.6 shows a comparison plot of total activation durations of all devices.
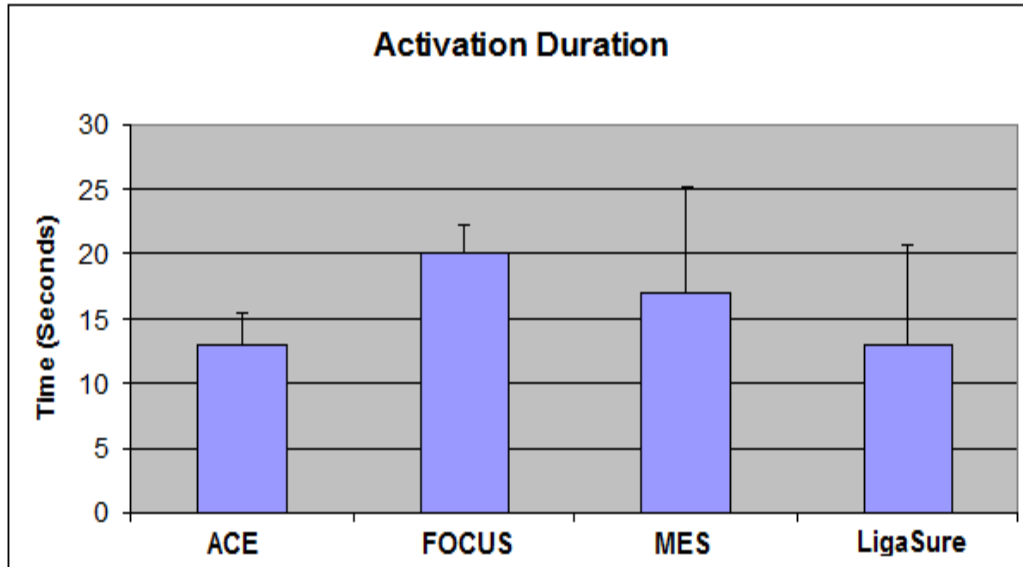
Figure 6.6: Mean of activation duration comparison between all surgical devices

In case of LigaSure experiments, each of the three protocols had different activation duration of the device. This was mainly because the number of activations differed in each protocol.
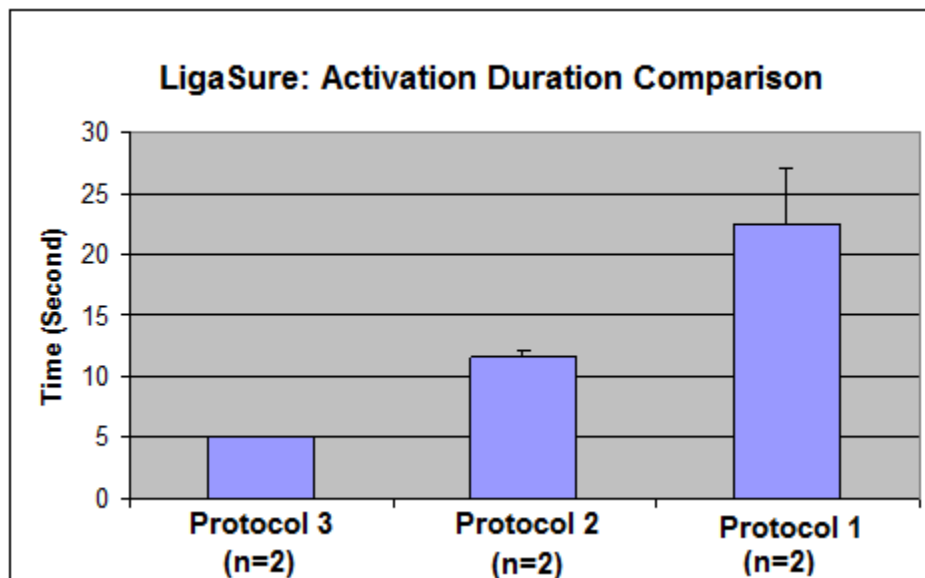
Figure 6.7: LigaSure activation duration comparison between three protocols

6.4.3 Raw Data

The raw CAP waveforms acquired by EGAA indicated that MES caused consistent drop in CAP through 3 hours. Also, there was no recovery seen by the end of experiment. The other groups showed slight dips in CAP after device application with some recovery towards end. This data was further analyzed in detail. Figure 6.8 shows sample plots of 3V stimulation CAP for all the devices.

A   B   C
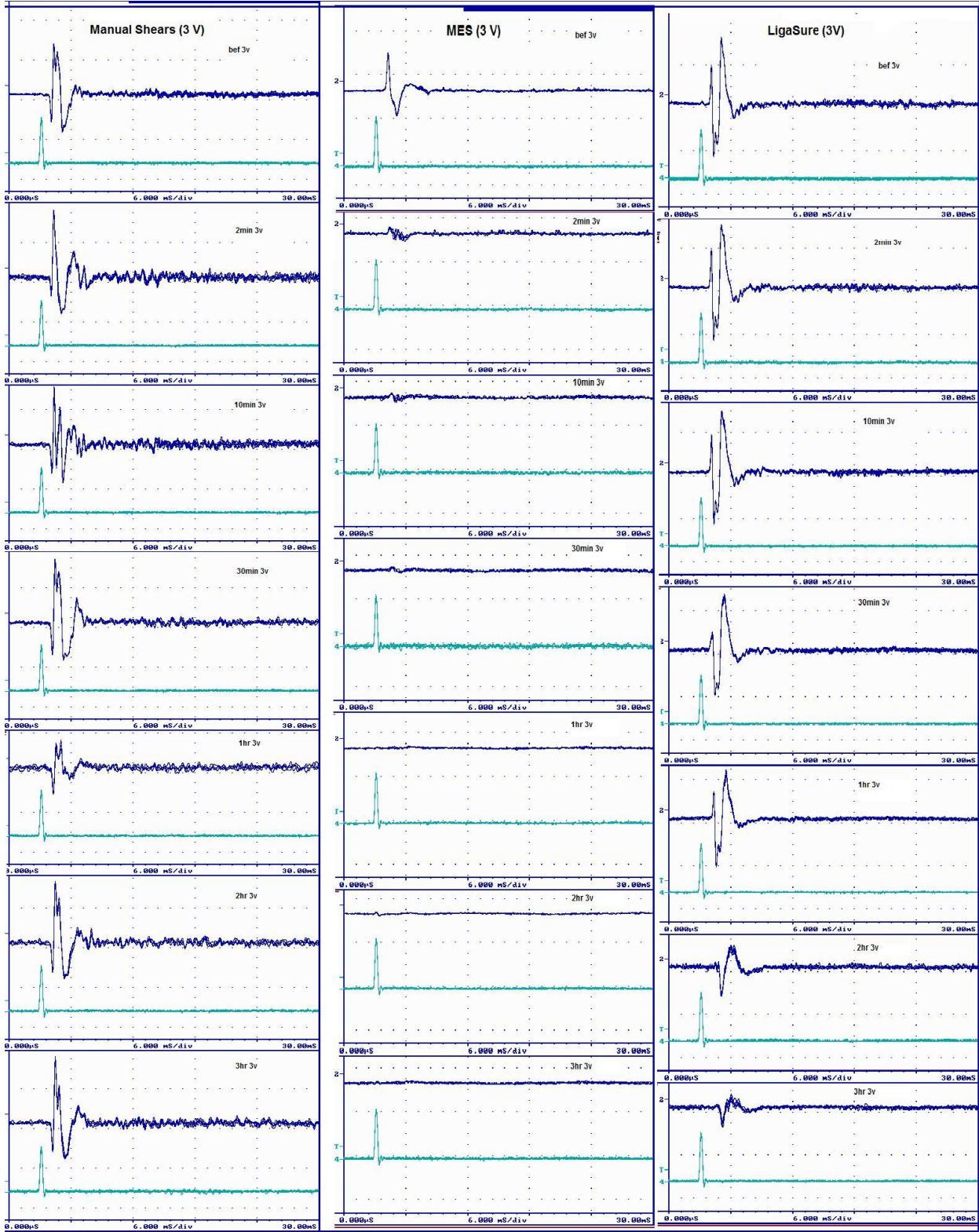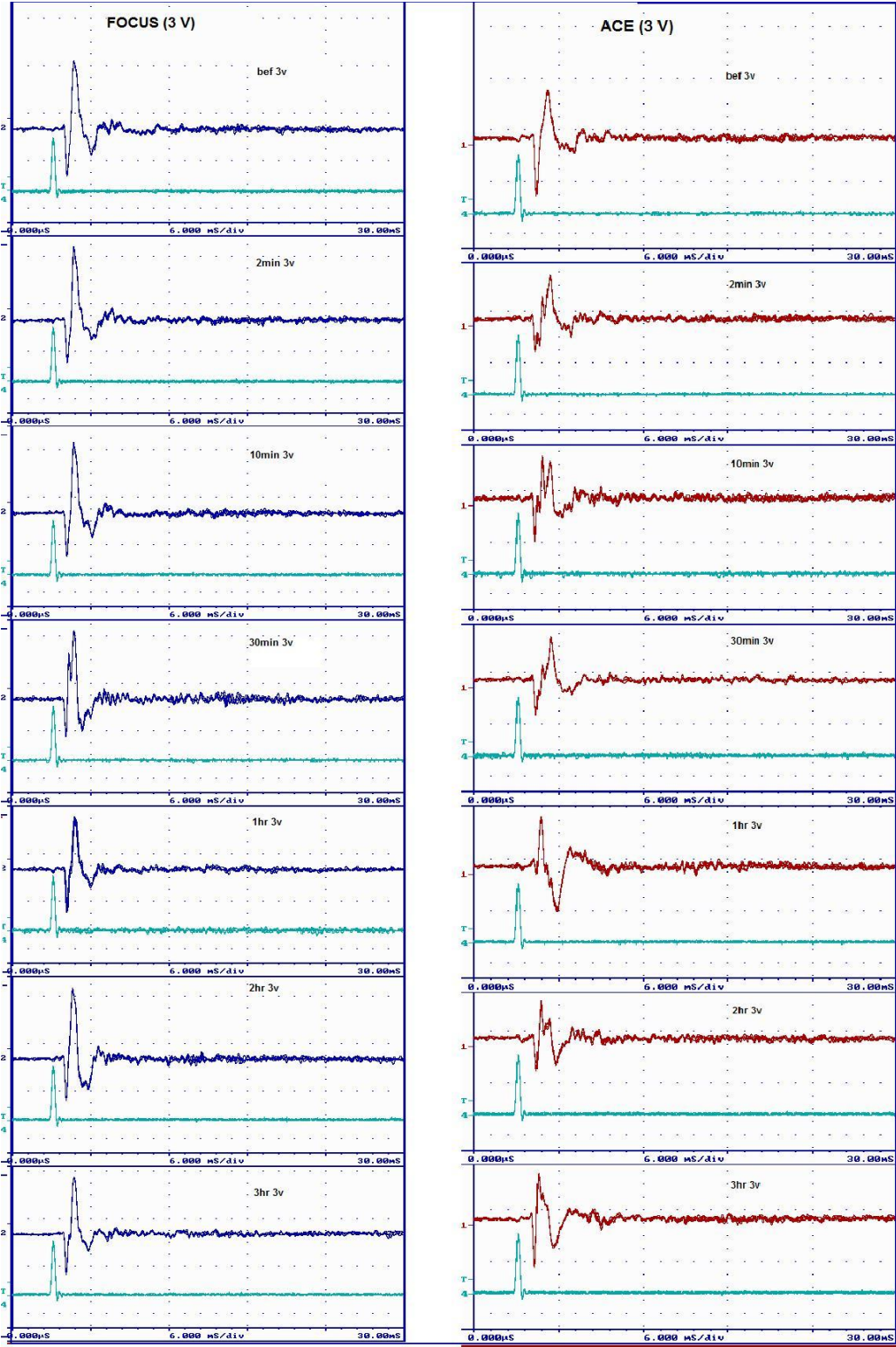
Figure 6.8: Raw 3V CAP Change over time for each group

A: Manual Shears, **B:** MES, **C:** LigaSure **D:** Focus, **E:** ACE

Figure 6.8 shows that MES caused a large drop in CAP after device application. Also, as seen in Figure 6.8 B, by the end of 3 hours there was no CAP evoked indicating that there was a nerve conduction block. In all other plots, there is very slight decrease in CAP. These CAP plots were processed using EGAA to get area under CAP curve (AUC) and the conduction velocity (CV) values. In each case, a percentage change with respect to the before application (baseline) recordings was studied.

6.4.4 AUC Analysis

The AUC data obtained from EGAA was analyzed for 1V, 3V and 5V. After a detailed review, it was seen that there were no major differences between 3V and 5V data. Moreover, the changes in AUC were much more apparent in 3V data. As a result, 3V data was considered to be the most optimum. A mean of all AUC values was calculated and compared between different groups (Figure 6.9) at individual time points. Standard Error of Measurement (SEM) was plotted as error bars for each data point.
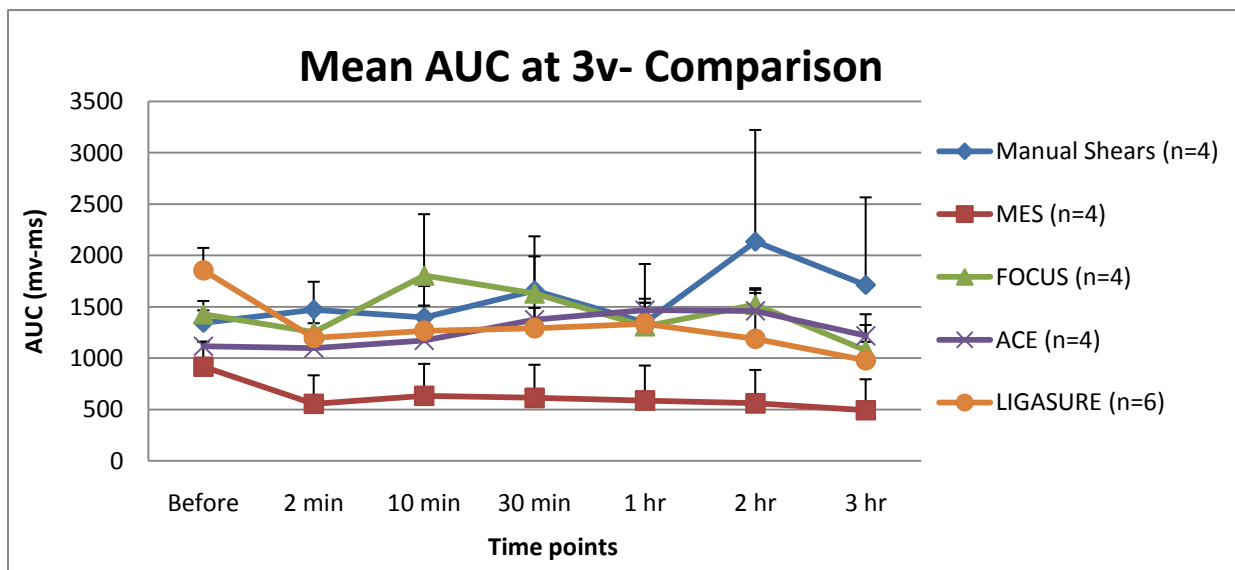
Figure 6.9: Mean AUC (3V) values comparison at individual time points

It was seen that the AUC values in MES group are consistently lower as compared to all the groups. LigaSure group shows a dip in the AUC after device application. On the contrary, FOCUS and ACE showed relatively lower drops after the device was applied. Also, percentage changes in AUC with respect to 'before' recordings i.e. pre-surgery recordings based on Eq. 6.1 (Figure 6.10).
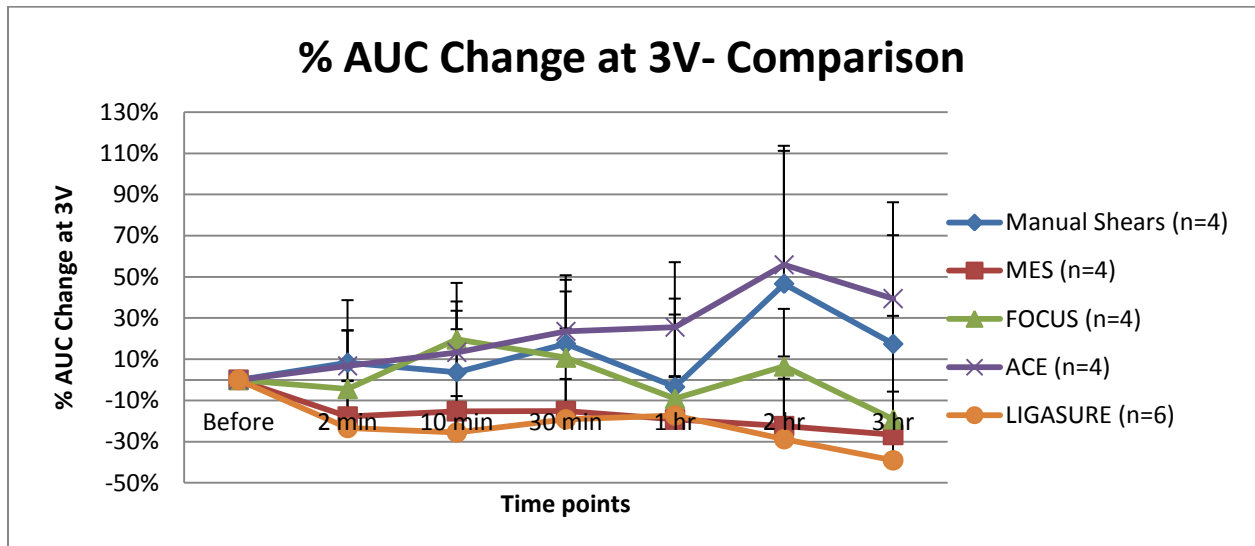
Figure 6.10: Percentage AUC Changes with respect to time

The MES group showed about 30% drop in AUC as compared to the baseline. LigaSure group showed about 10-30% drop after device application. After 2 hours, LigaSure group showed up to 40% drop in CV. Harmonic FOCUS showed less than 10% just after the application but eventually showed about 25% increase in CV. By the end of 3 hours, there was a drop of 25% in FOCUS group. Manual Shears and Harmonic ACE showed positive percentage changes through all the time points, indicating an increase in CV values post device application.

6.4.5 CV Analysis

The mean of CV values was calculated for all the stimulation voltages 1V, 3V and 5V. There were no major differences between 3V and 5V data. Moreover, the changes in AUC were much more apparent in 3V data. Hence, as in case of AUC, mean of 3V CV values were plotted with respect to time and compared for different groups (Figure 6.11).
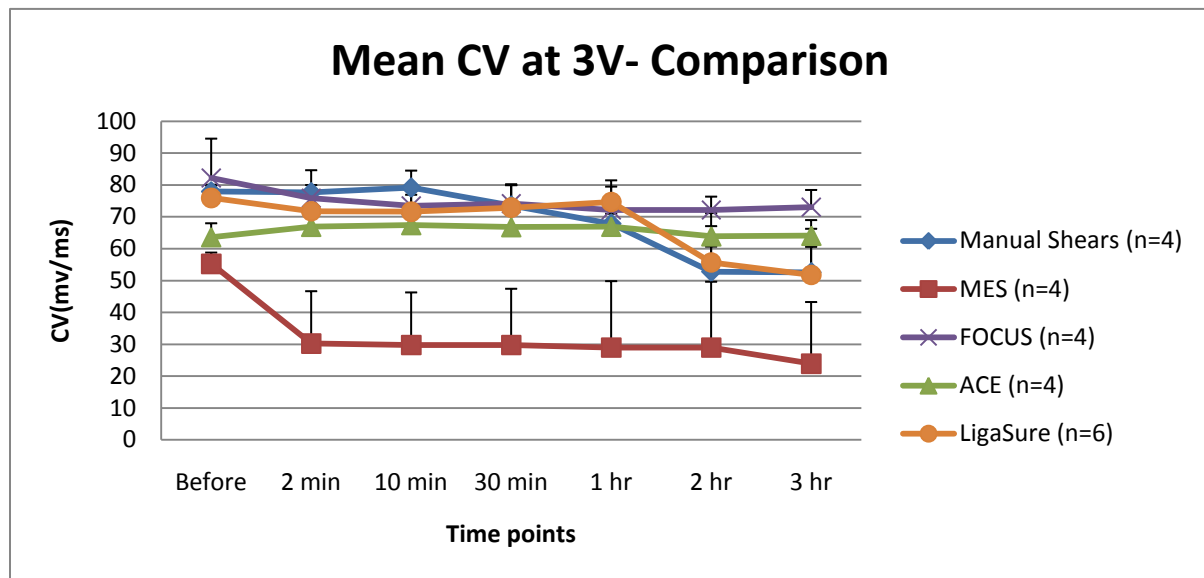
Figure 6.11: Mean CV values with respect to time

Figure 6.11 shows that the FOCUS and ACE have stable CV values throughout. Also, by the end of 3 hours there is recovery in ACE CV. LigaSure group shows a drop in CV after 1 hour of device application. Manual Shears showed a drop 10 min after surgery. Percentage changes in CV were plotted (Figure 6.12).
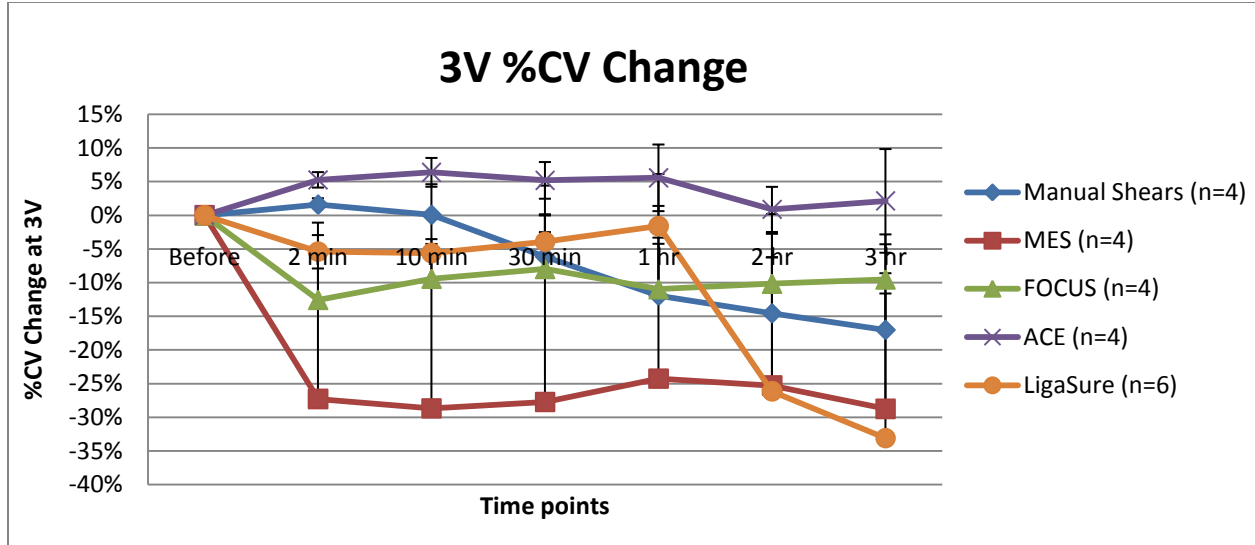
Figure 6.12: Percentage CV change with respect to time

The 'before' recording in each group was considered as 0% and all values were compared with respect to this recording (Eq. 6.2). Figure 6.12 shows that, in MES group, immediately after the device application there was almost 30% drop in CV. There was no recovery by the end of experiment. LigaSure device showed about 5% drop after device application with an eventual drop of 30-35% after 1 hour post surgery. Manual shears showed a consistent drop in CV throughout 3 hours with a decrease of around 15-20% by the end of experiment. Harmonic FOCUS showed a decrease of 5-10% through all the time points. However, ACE showed positive % change values from 2nd minute to the 180th minute after using the device. Thus, ACE showed an increase in CV as compared to the baseline values.

6.4.6 Comparison between LigaSure application protocols

The mean AUC and CV values at individual time points were compared between protocols 1, 2 and 3 for application of LigaSure device.

6.4.6.1 AUC Analysis

The mean of all 3V AUC values for each LigaSure experiment was calculated. These values were plotted with respect to time points (Figure 6.13).
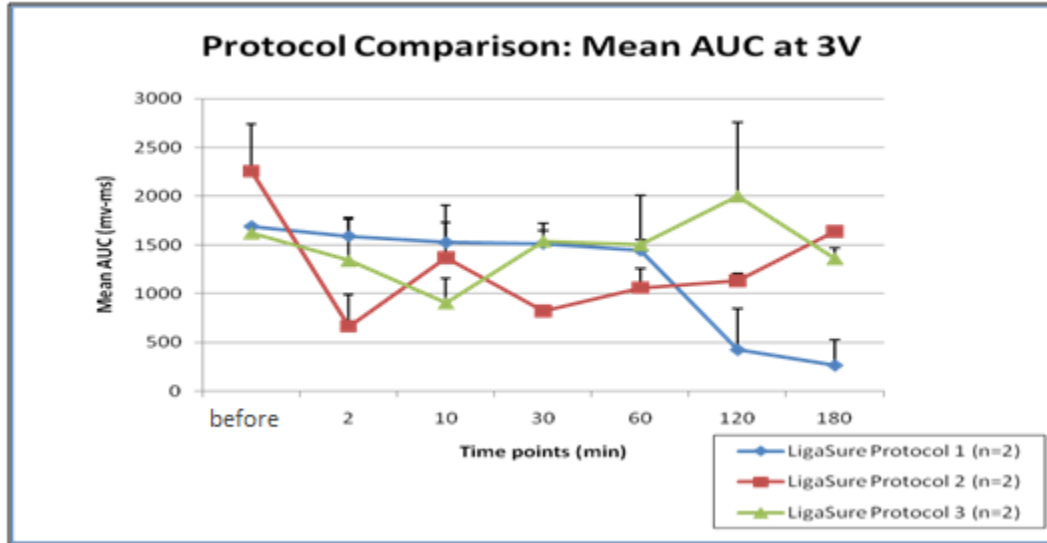
Figure 6.13: Mean AUC (3V) comparison between LigaSure protocols

Protocol 1 showed a decrease in AUC values 2 hours after surgery. Protocol 3 appeared to have a drop in AUC after device application which recovered after 30 min. Protocol 2 also caused a decrease in the AUC values indicating nerve dysfunction. These changes in AUC by same device were mainly because of the difference in the number of activations of LigaSure in each protocol.

6.4.6.2 CV Analysis

The mean of 3V CV values for each LigaSure protocol were calculated and plotted with respect to time (Figure 6.14).
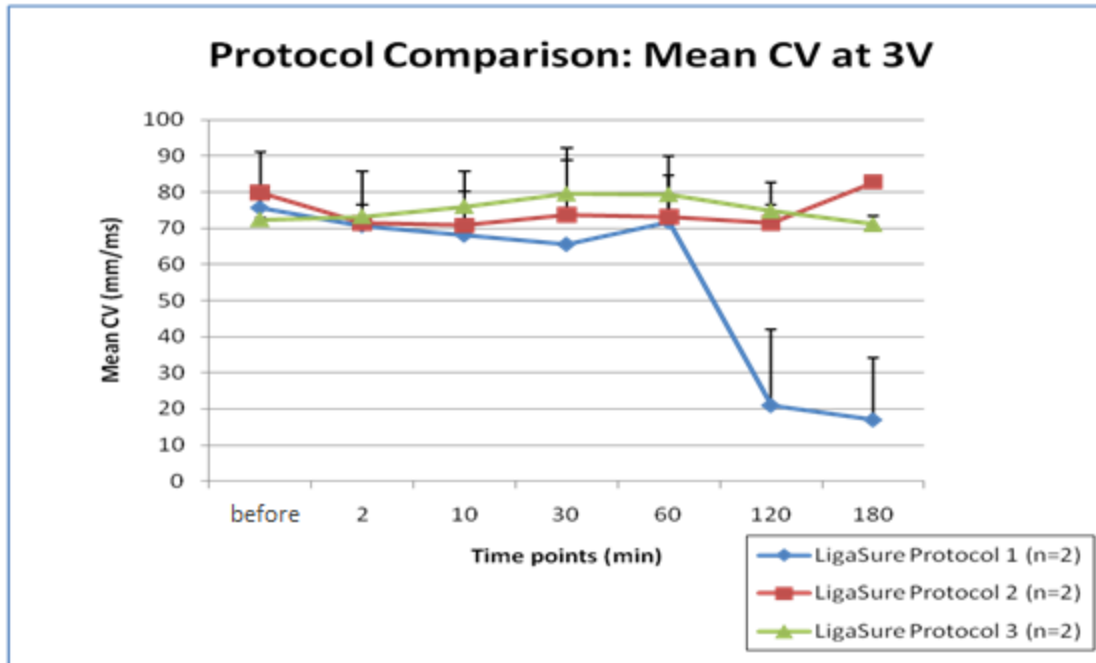
Figure 6.14: Mean CV (3V) comparison between LigaSure protocols

The preliminary outcomes showed that Protocol 1 could cause decrease of CV indicative of nerve dysfunction. Also, protocol 1, with total of 8 activations, showed a decrease after 1 hour of application. Protocol 2 with 4 activations showed slight decrease in CV post application but there was a recovery after 2 hours. On the contrary, protocol 3 with 2 activations showed no decrease in the CV values.

Due to small sample size, a statistical analysis was not performed. This is a preliminary study to assess sciatic nerve function after use of surgical cutting devices using both ultrasonic energy and radiofrequency energy. The preliminary findings show a safer outcome when US devices are used as compared to RF devices. However, in the current study the sample size per group is relatively small. A larger sample size is required to further evaluate these devices.

CHAPTER 7

SPECIFIC AIM III: SUBACUTE STUDY

7.1 SPECIFIC AIMS

The surgical devices Monopolar Electrosurgery (MES), LigaSure, Harmonic Focus, Harmonic ACE, and manual shears were used based on the technique developed in Study 2 to surgically expose the sciatic nerve. The sub-acute effects of these devices on the nerve conduction function and behavioral changes were analyzed for a period of 3 or 7 days after the device application.

7.2 OUTLINE OF STUDY

Twenty-two male Sprague Dawley rats (400-450 grams) were used to perform these in-vivo studies. This was a sub-acute study where in the rats were survived either for 3 days or for 7 days after the surgical devices were applied to the muscles around left sciatic nerve (Table 7.1). The rats were used based on the test matrix shown below.

	Manual Shears (MS)	MES RF (60 W)	Harmonic Focus (Level 5)	Harmonic Ace (Level 5)	LigaSure RF (Mode 2)
3 Days Survival	3	2	2	3	2
7 Days Survival	1	3	2	2	2
Total	4	5	4	5	4

Table 7.1: Test matrix for Subacute Study with all devices used and the survival

period of rats

In total, each experiment performed lasted for a period of 9 or 13 days. This

included 5 days of recordings pre-surgery and 3 or 7 days of recordings post surgery.

There were three main sections in this study;

- Surgery Using Sterile Techniques (Device Application)

- Behavioral Study

- Neurophysiology

On the first day of study, i.e. 5 days before surgery (Day -5), the left and right

hind paws of the rat were probed with Von Frey Hairs. This probing continued at

alternate days i.e. Day -5, Day -3, Day -1 (pre-surgery) and Day +1, Day +3, Day +5

and Day +7 (post-surgery), considering Day 0 as the day of sciatic nerve exposure

surgery. On Day 0, the surgical devices manual shears, MES, LigaSure, Harmonic

FOCUS or Harmonic ACE were used around the left sciatic nerve. On Day +3 or Day

+7, designated rats were used for neurophysiology studies. On these days, the nerve was stimulated by electrical pulses of 1V, 3V and 5V. The corresponding compound action potential was recorded from the L5 spinal nerve. After the neurophysiology tests were completed, the rats were sacrificed using intra-cardiac injection of sodium pentobarbital (1 cc). Table 7.2 summarizes the time points for each individual study.

Day	-5	-3	-1	0	1	3	5	7
Behavioral study	yes	yes	yes		yes	yes	yes	yes
Device application				yes				
Neurophysiologic assessment						yes		yes

Table 7.2: Time points for individual studies

7.3 SURGERY (DEVICE APPLICATION)

A length of sciatic nerve was exposed in sterile fashion using each of the surgical devices. The surgical procedure was carried out using standard aseptic procedures.. About 10mm long incision was made parallel to the length of sciatic nerve using the surgical devices. A distance of 1-2mm was maintained from the sciatic nerve.

7.3.1 Surgical procedures

7.3.1.1 Anesthesia

The rats were anesthetized and sedated by an intraperitoneal injection of ketamine and xylazine as in Study 2. The depth of anesthesia was continuously monitored using paw pinch and palpebral reflexes and maintenance dose was given if required.

7.3.1.2 Sciatic nerve exposure

The sciatic nerve exposure was basically performed in the same way as in Study 2. The tip of the blade was applied to the muscles adjacent to the sciatic nerve approximately 1-2 mm away from the nerve. The intensity of power from the devices as well as the device activation time was recorded. Quantitative data analysis was performed offline.

7.3.2 Device Application Procedure

The device application techniques were performed in accordance with procedures developed in the specific aim 2. The device power and intensity settings were as shown in Table 6.1. Each device blade was applied at a distance of approximately 1mm from the left sciatic nerve. The length of the cut was 10 mm on each side of the nerve (Figure 6.1).

7.3.2.1 Manual Shears

Manual shears were used to punch a 4 mm deep hole at the same location as mentioned above, 1-2 mm from the sciatic nerve. A blunt dissection was carried out beneath the muscle followed by a 10 mm long incision on the muscle using the manual shears. The same procedure was repeated on the other side of sciatic nerve.

7.3.2.2 Harmonic FOCUS and ACE Application

The device application procedure for Harmonic Focus and ACE were exactly same. After the sciatic nerve was exposed by cutting and flipping over the gluteus superficialis muscle and biceps femoris muscle, either of these cutting devices were

used to punch a hole vertically in the caudofemoralis muscle (cf) by activating the device for 1-2 seconds, with power set at 5. The hole was 5 mm deep at a distance of 1-2 mm form the sciatic nerve. The muscle was then clamped in jaw of device followed by activation to complete the cut. This cutting produced a 5 mm long wound along the sciatic nerve without bleeding. Another 5 mm cut in continuation with the first cut parallel to the nerve. This produced an incision of a total length 10 mm on one side of the nerve. Same procedure was repeated on the other side of nerve.

7.3.2.3 MES Application

MES blade was used to cut muscle at the same location as mentioned above, 1-2 mm from the sciatic nerve and about 4 mm in depth. The power was set at 60 watt, blend 1 level. Thus, 10 mm long incision was produced on either side of the sciatic nerve.

7.3.2.4 LigaSure Application

The application techniques for LigaSure were based on Protocol 3 developed in specific aim 2. Manual scissors were inserted into the muscle (4 mm in depth) and the muscle was blunt dissected to produce a 10 mm long cavity under the muscle along the sciatic nerve. LigaSure was inserted into the cavity and 10 mm length of muscle was clamped. LigaSure was activated, producing approximate $10 \times 2 \text{ mm}^2$ region of blanched tissue. Then the manual scissors were used to cut the blanched muscle tissue along the middle line of the blanched tissue (Figure 6.5). The same procedure was repeated on the other side of the sciatic nerve.

Once the device application was completed, the muscle and skin were then closed in sterile manner using sterile sutures (Ethicon Endo-Surgery Inc). These rats were then survived for either 3 days or 7 days.

7.3.3 Results

The time of device activation was measured to determine the total time of energy exposure on the muscles around the nerve.

7.3.3.1 Time of Device Activation

The time of activation required for complete exposure was averaged for all the rats in one group (Figure 7.1).
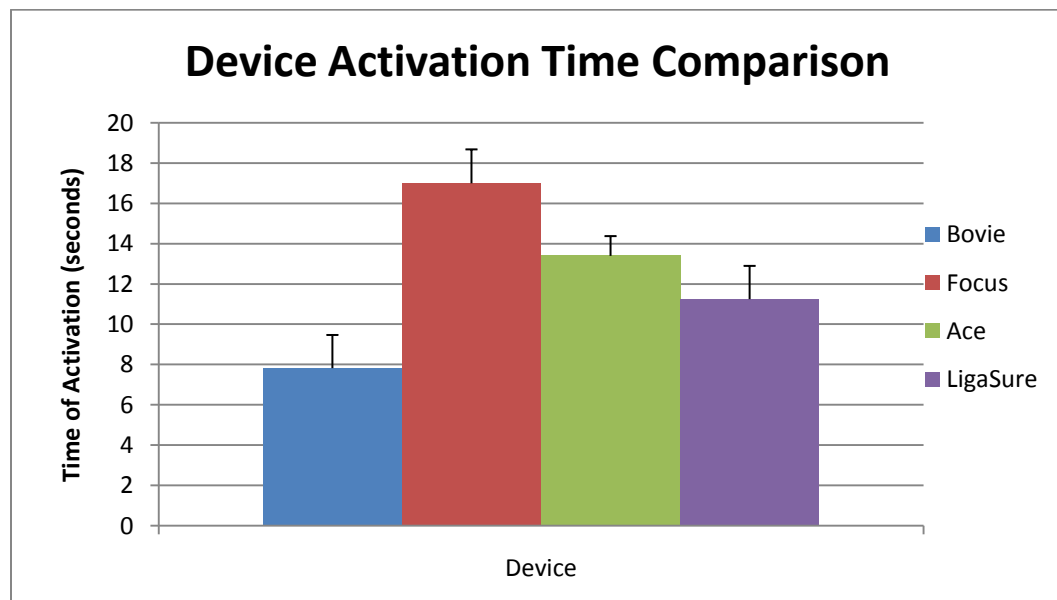
Figure 7.1: Average time of device activation

Harmonic Focus showed highest time of activation to complete the procedure. Harmonic ACE required relatively less time of activation followed by LigaSure. The MES required the least time of activation to complete the entire procedure.

7.4 BEHAVIORAL STUDY

This study was performed to assess the hind paw sensory function in terms of behavioral changes. These changes were investigated by hind paw withdrawal when a series of forces were exerted on the plantar surface of the paw.

7.4.1 Methods

7.4.1.1 Experimental Set up

The rats were placed on a wire mesh platform so that the hind paws can be viewed from the bottom so as to perform probing. Two rats were probed at once. An acrylic barrier was placed over each rat. The barrier separated the rats as well as restricted their mobility. The Von Frey Hair (VFH) filaments were used to probe the hind paw surface to exert varying forces on the paw (Figure 7.2). The VFH filaments used were VFH 4.31, VFH 4.56, VFH 4.74, VFH 4.93, VFH 5.07, VFH 5.18, VFH 5.46, VFH 5.88, VFH 6.1, VFH 6.45, and VFH 6.65. These filaments exerted corresponding forces (grams) when pressed against the paw surface (Table 5.2).

Figure 7.2: Behavioral study experimental set-up
A: Rat placed on wire mesh platform
B: Von Frey Hair probed on hind paw

7.4.1.2 Probing Procedure

The rats were initially acclimated for about 5 minutes each with the investigator as well as the wire mesh and acrylic box so as to reduce the anxiety. Once the rats settled down, the probing procedure was started.

During probing session, the rats were probed on the plantar surface of each hind paw alternately. One VFH was used for maximum 5 times with an interval of 4-5 sec maintained between each probing. Starting from the lowest calibrated VFH (VFH 4.31), the hind paws were probed by each VFH. If there was a withdrawal on probing, it was considered as a positive response (count 1) while no withdrawal was considered as no response (count 0). The basic aim of this probing procedure was to determine following parameters.

- Threshold Force

Threshold force was defined as that VFH force which causes at least 80% of positive responses i.e. at least 4 positive responses out of 5 probings. It was calculated by taking average of five repeated stimuli which produced paw withdrawal. In order to achieve this, each VFH was first probed twice. If within these two probings if there were no positive responses, then that VFH was discarded and a higher VFH filament was tested. This was done as two 'no responses' abandoned any possibility of obtaining 80% positive responses. Thus, whenever there was an instance of two 'no responses', the corresponding VFH filament was discarded and a filament with higher force was tested. Finally, the VFH force that evoked 80% or more positive responses was considered as the threshold force. A decreased threshold force might be an indication of neuropathic pain and allodynia whereas an increased threshold force might indicate loss of sensation.

- Withdrawal Frequency

VFH 4.56, VFH 4.93 and VFH 5.18 were probed for 5 times irrespective of positive or no responses. These exceptions were made to analyze the 'Withdrawal Frequency' (Schaeffer, Meyer et al. 2010). The frequency of withdrawal (% of occurrence) for each of these specific VFH was counted in terms of percentage. For example, if there are 3 positive responses out of total 5 probings, the withdrawal frequency was counted as 60%. The values of these frequencies were further compared and analyzed before and after device applications. The withdrawal frequency was counted to track the sensitivity of the hind paw.

7.4.2 Data Analysis

7.4.2.1 Threshold Analysis

A mean of all the threshold forces for Day -5, Day-3 and Day -1 (all before surgery data) in each device group were combined. This data was normalized to 100 %. All the post-surgery threshold values were compared with respect to this combined pre-surgery data by using Eq. 7.1.

$$\% \text{ Threshold Value for post surgery data point} = \frac{\text{Actual post-surgery Threshold Force (grams)}}{\text{Actual pre-surgery Threshold Force (grams)}} \times 100$$ ..(Eq. 7.1)

7.4.2.2 Frequency Analysis

A mean of all frequency values for Day -5, Day -3 and Day -1 (all before surgery data) in each device group were combined. These were the true values of frequency (%). The post-surgery frequency values were then compared to this combined pre-surgery data.

7.4.3. Results

The threshold and frequency values for group of devices were compared with respective pre-surgery values. The standard error for all the data points was plotted as error bars.

7.4.3.1 Threshold Analysis

- Exception

For the threshold values calculation, there were two exceptions within the MES group. Out of the 5 rats in this group, no threshold values could be determined for two rats. Even with the highest VFH filament (VFH 6.65 with corresponding force 126.6 grams), there was no paw withdrawal i.e. not a single positive response occurred at Day +1, Day +3, Day +5 and Day +7. This result indicates hind paw numbness or loss of motor function post device application. However, threshold values were calculated in the remaining three rats. For fair analysis, the MES group was divided into two sub groups: one including rats without threshold (no TH; n = 2) and other including rats with threshold (with TH; n = 3).

- Group Comparison

Figure 7.3 shows the threshold comparison for left paw (vertical bars) and right paw (inverted bars) for each device. Day +1, Day +3, Day +5 and Day +7 percentage threshold values were compared with the normalized pre-surgery data.
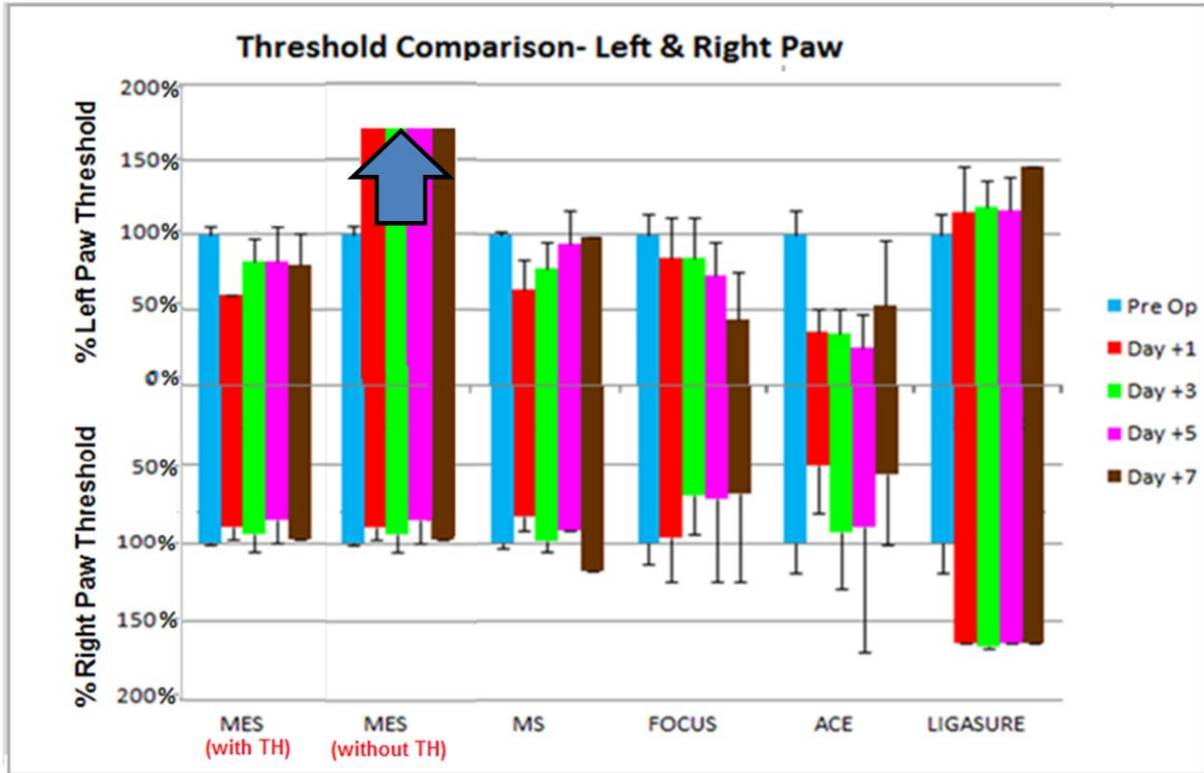
Figure 7.3: Threshold comparison for left and right hind paws for each device

The MES (with TH) showed a drop in the left paw threshold at Day +1. The right paw, however, showed no major changes with respect to the pre-surgery data. The MES (without TH) group is shown by increasing vertical bars (indicated by upward arrow), as threshold was higher than highest value of VFH force (126.6 grams) for the left paw.

The manual shears group showed a slight drop in the threshold values after the surgery. For left paw, at Day +1 there was a drop in threshold with a slight recovery by the end of Day +7. The right paw did not show major changes after the surgery.

In the Harmonic Focus, there was a continuous drop in threshold values of left hind paw post device application. Also, there was a drop in the threshold values of the right paw. There was recovery by the end of 7 days.

Harmonic ACE showed a considerable drop in the threshold values of the left hind paw at all days after surgery. The right paw threshold values also showed a decreasing trend. Like Focus, there was no recovery even by the 7th day.

LigaSure device showed a steady increase in threshold values of the left paw. At Day +7, the threshold was higher than values at Day +1, Day +3 and Day +5. The right paw threshold values increased at Day +1 and remained constant throughout.

7.4.3.2 Frequency Analysis

The frequency analysis was carried out for the frequency values for VFH 4.93 (5.2 grams) and VFH 5.18 (14.6 grams). The frequency values for VFH 4.56 were not analyzed as these values were 0% for most rats (except two rats). The true frequency values for left paw were plotted as vertical bars and those for right paw were plotted as inverted bars (Figure 7.4 and 7.5).

- 4.93 VFH

The frequency values for VFH 4.93 for all days post surgery were compared with the combined pre-surgery data.
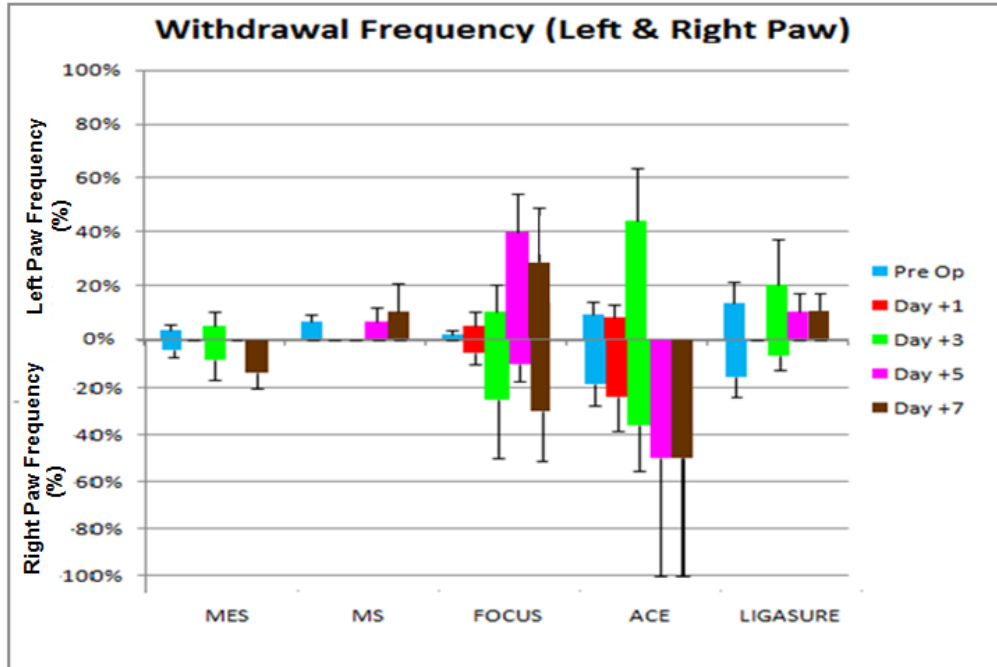
Figure 7.4: VFH 4.93 withdrawal frequency comparison for left and right hind paws

The MES and Manual shears showed no major changes in the withdrawal frequency after the surgery. Harmonic Focus showed an increase in withdrawal frequency for the left paw. The right paw frequency values in this group also increased after surgery but the increase was relatively smaller than that of the left paw. Harmonic ACE showed an increase in frequency of left paw at day +1 and +3. The right paw frequencies showed a steady increase after ACE application. LigaSure showed increased frequencies in the left paw after device application. Unlike the left paw, the right paw frequencies dropped after surgery and remained 0% at days +5 and +7.

- 5.18 VFH

The frequency values for VFH 5.18 for all days post surgery were compared with the combined pre-surgery data.
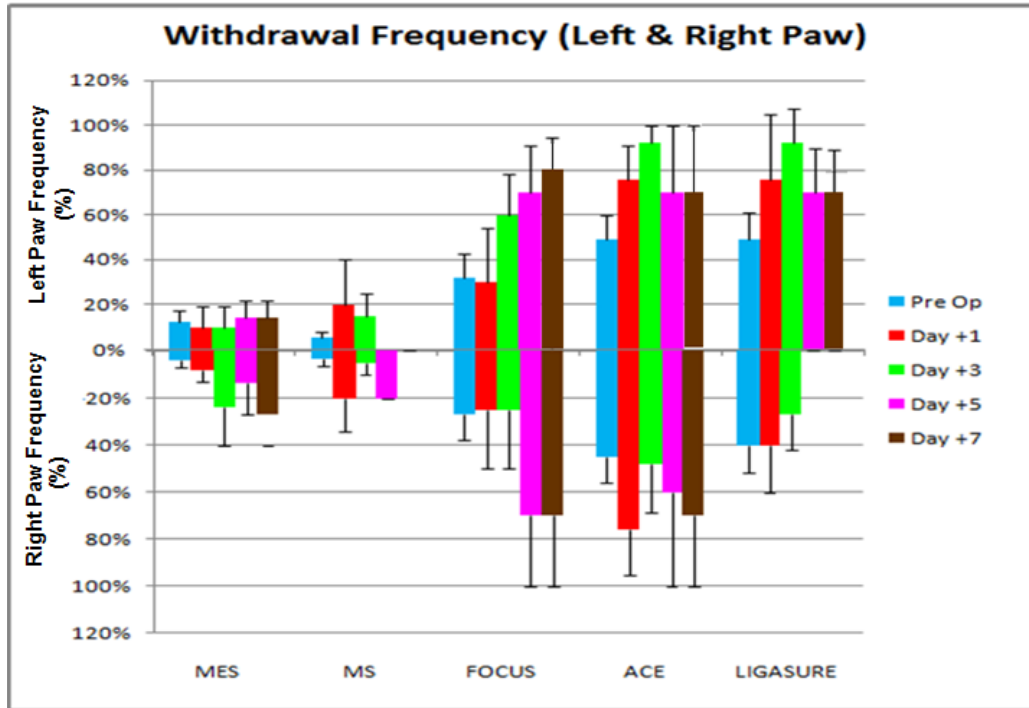
Figure 7.5: VFH 5.18 withdrawal frequency comparison for left and right hind paws

The VFH 5.18 withdrawal frequencies showed similar trends as VFH 4.93. The MES and manual shears groups showed no major changes between before and after surgery. Harmonic Focus and ACE showed a considerable increase in the left paw frequency. Also, in both these groups, the right paw frequency also increased after device application. As in case of VFH 4.93, the LigaSure group showed increasing frequency trend with VFH 5.18 for the left paw. The right paw frequencies dropped at Day +1 and Day +3 and eventually became 0 % at days +5 and +7.

7.5 NEUROPHYISOLOGY

The neurophysiology recordings were taken on days +3 or +7. On this particular day, after the behavioral study, the rat's sutured sciatic nerve was exposed. The nerve

was then stimulated by applying voltages of 1V, 3V and 5V. The corresponding CAP was recorded from the L5 spinal nerve.

7.5.1 Methods

7.5.1.1 Anesthesia

The rats were anesthetized and sedated by an intraperitonial injection of ketamine and xylazine as in Acute Study II. The depth of anesthesia was continuously monitored using paw pinch and palpebral reflexes. Also, a maintenance dose of 0.2 cc was injected, if required.

7.5.1.2 Pre-recording surgeries

- L5 nerve root exposure

A laminectomy was performed to expose the left L5 nerve root so as to record the compound action potential. Exposed left L5 dorsal spinal nerve roots were kept intact. The nerve roots were immersed in the mineral oil maintained at temperature of around 37°C.

- Tracheotomy

Tracheotomy was performed in a similar way as in previous studies. The artificial ventilator was set with the breath rate of 90 bpm and a tidal volume of 2.5 ml.

7.5.1.3 Recording Procedure

As in case of previous studies, the nerve was stimulated by a pulse stimulator and the corresponding neural activity was recorded by recording electrodes (Figure 5.2, Acute Study I). The total length of nerve root under which the recording electrodes were placed was approximately 10 mm. A set of baseline recordings was taken stimulating the sciatic nerve by 1V, 3V and 5V.   After this, 1cc of an intra-peritoneal injection of pancuronium bromide was given to the rat. This was done to cease the muscle twitching occurring due to nerve stimulation. Once the muscle was completely relaxed and there was no muscle twitching, a set of recordings was taken. Thus, the baseline recording was the one 'with no pancuronium bromide' effect and the second recording was the one 'with pancuronium bromide' effect.

7.5.2 Data Analysis

Neurophysiological recordings obtained were evaluated for changes in the conduction velocity (CV), occurrence of conduction block and the area under CAP curve. These parameters were compared with the pre-device application (control) data for 1V and 3V obtained in Study 1 (n = 73). To compare the 5V data in this study, the control 5V data from the Study 2 (n = 24) was used.

7.5.3 Results

Two parameters Area Under the Curve (AUC) of CAP and conduction velocity (CV) were analyzed. Both these parameters were calculated based on the same technique as described in Study 1 and Study 2. However, there were two groups of data

points studied: one with no effect of pancuronium bromide and one with the effect of pancuronium bromide. The 3V data was considered as optimum for comparing the effects of all devices.

7.5.3.1 Without Pancuronium Bromide

This group of data consisted of the baseline recording data compared with the control data from the Study 1 i.e. with no pancuronium bromide effect.

- AUC Analysis

The mean AUC values for 3V stimulation were calculated for Day +3 and Day +7 for each group of devices. These values were compared with the mean of all the control AUC values (n =73) (Figure 7.6).
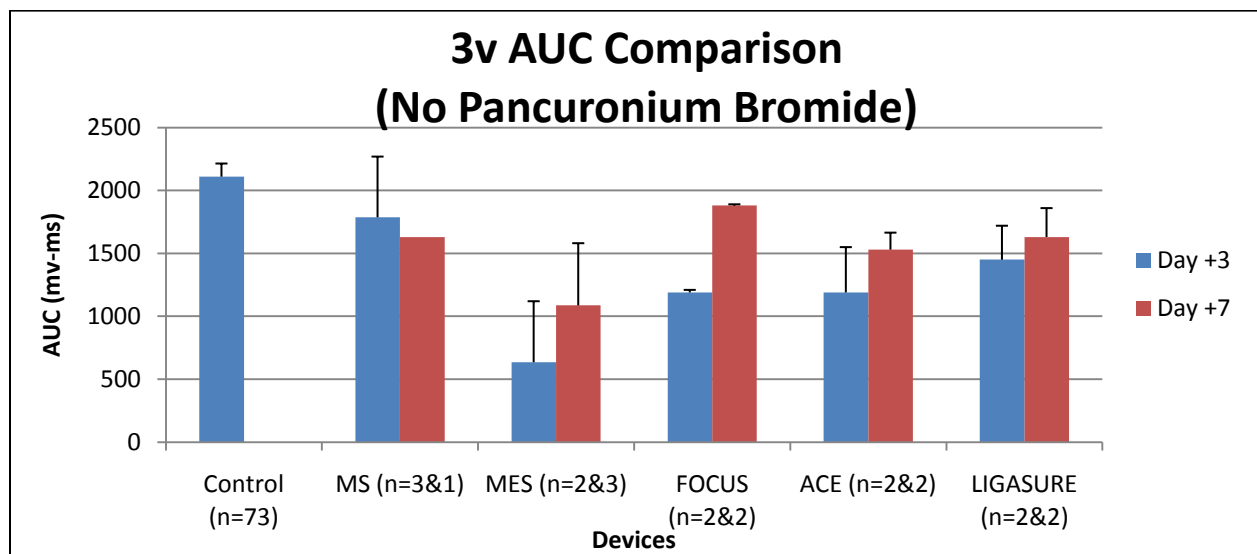
Figure 7.6: AUC comparison for 3V stimulation without pancuronium bromide effect

The MS group AUC values on Day +3 and Day +7 were very close to the control AUC values. The MES group showed a very low AUC values as compared to the control group on Day +3 but showed a slight recovery on Day +7. Harmonic Focus produced lower AUC values on Day +3 but recovered greatly on Day +7. Harmonic ACE had similar trend as Focus with relatively lesser recovery on Day +7. LigaSure produced AUC values higher than Focus and ACE on Day +3. On Day +7, the Focus AUC values were higher than the LigaSure AUC values. The AUC values for all the groups other than Manual Shears showed an increasing trend from Day +3 to Day +7 indicating a recovery by end of 7 days. Statistical differences were not calculated due to small sample size.

- **CV Analysis**

The mean values for all the CV values at 3V were calculated for days +3 and +7. These values were compared with the mean of AUC values from the Study 1 (n = 73).
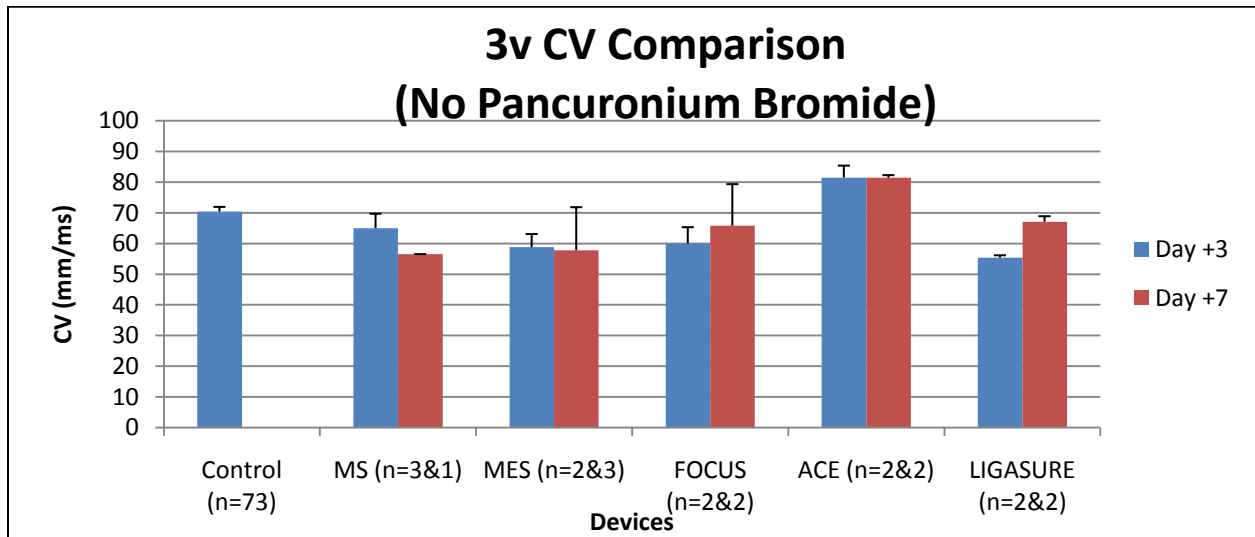
Figure 7.7: CV comparison for 3V stimulation without pancuronium bromide effect

The CV values did not show major differences as compared to the control values. Manual shears, MES, Focus and LigaSure almost had same values of CV on days +3. There was a slight increase in the CV values on Day +7 in Focus and LigaSure groups. Harmonic ACE had CV higher than the control values on days +3 as well as +7.

7.5.3.2 With Pancuronium Bromide

This group of data consisted of the recordings after pancuronium bromide was injected and there was no muscle twitching on stimulation. This data was compared to the 'after pancuronium' control data (0 minute recording) from the Study 1 i.e. with pancuronium bromide effect.

- AUC Analysis

The mean AUC values for each group were plotted for Day +3 and +7 (Figure 7.8).
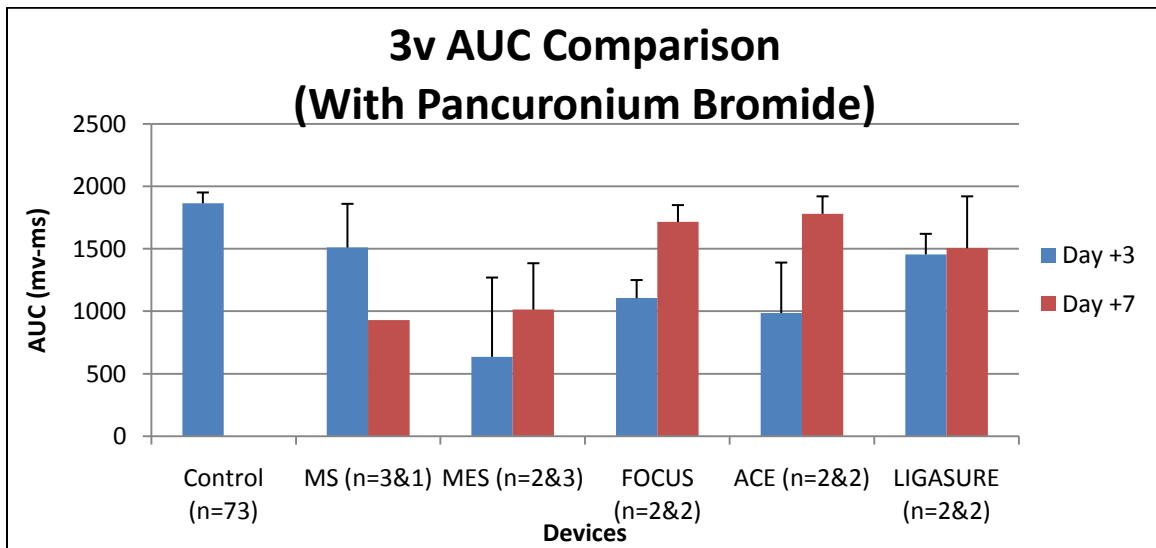
Figure 7.8: AUC comparison for 3V stimulation with pancuronium bromide effect

The AUC values in MS group on days +3 and +7 were lesser as compared to the control values with more decrease on Day +7. The MES group showed the least AUC values in all the groups on Day +3. Harmonic Focus and ACE had reduced AUC on Day +3 but eventually there was an increase by Day +7. The Day +7 AUC values for these two groups were almost same as the control values. The LigaSure group CV values remained almost same on Day +3 and Day +7. Like in 'no pancuronium bromide' AUC analysis, an increasing trend of AUC values in groups MES, Focus, ACE and LigaSure was seen.

- **CV Analysis**

The mean CV values for 3V stimulation were calculated for each device group.
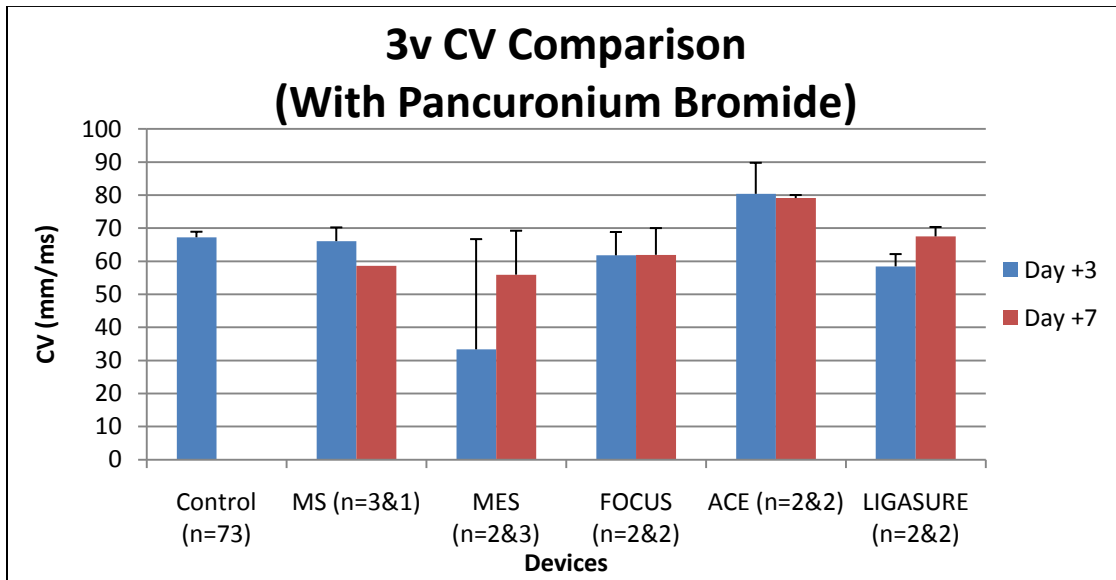
Figure 7.9: CV comparison for 3V stimulation with pancuronium bromide effect

The CV values for Manual shears, Harmonic Focus and LigaSure on days +3 and +7 were not much different from the control values. The Harmonic ACE had higher

values of CV on days +3 and +7. This CV analysis was consistent with the one with 'no pancuronium bromide' group.

The results obtained from 'no pancuronium' group and 'with pancuronium' group did not show major differences indicating that there was no major effect of this drug on the compound action potentials produced on stimulation.

7.6 SUMMARY

The behavioral study and the neurophysiology results together indicated that MES can be produce most harmful effects as compared to all other surgical devices. Two out of five rats showed signs of numbness immediately after using the MES blade. There was clenching of the left hind paw as well as limping while walking. This might be indicative of severe nerve injury which caused a total cessation of CAP in these rats. Also, there was no sign of recovery by the end of 7 days.

The manual shears showed drop in CAP amplitudes by Day +7 thus indicating some delayed nerve dysfunction. This could be caused as a result of delayed inflammation as the incision made with the manual shears was not treated by any haemostatic procedure. However, no major behavioral changes were observed in this group.

Harmonic Focus and ACE both produced mild damage of nerve function by Day +3 but eventually showed a recovery trend by end of 7 days. These devices induced increased sensitivity of the left as well as right paws after the device applications.

LigaSure caused increased sensitivity of the left hind paw after the device application. In some cases, the rats appeared stressed at Day +1 but eventually calmed down. It produced lower amplitudes of CAP indicating some damaged caused to the nerve.

In this study, a small sample size is the major limitation especially in behavioral studies where the sensitivity of the hind paws varied significantly for different rats. Increased sample size is required to statistically compare and conclude on the effects of these devices.

CHAPTER 8

DISCUSSION

The development of ultrasonic devices have lead to increased efficiency in laparoscopic dissections (Emam Tarek 2003). The ultrasonically activated scalpel significantly reduces the operational time and blood loss as compared with standard techniques using surgical diathermy (Marcin KOS 2007). The ultrasonic shears have many applications in head and neck surgeries mainly in thyroidectomy, parathyroidectomy, anterior neck dissection, parotidectomy and submandibular gland excisions (Leonard 2008). Harmonic Focus and Harmonic ACE, specifically, reduce the operational time in head and neck procedures and more considerably in thyroid and parotid surgeries by 20-25% as compared to conventional haemostatic techniques (Prgomet, Janjanin et al. 2009). Alternatively, electrosurgery techniques have been extensively employed for a variety of applications since its development in 1927 (McCauley 2003).

A growing number of studies have been undertaken to understand the thermal and biological effects of ultrasonic and electrosurgery cutting and coagulating devices on different types of tissues. As compared to MES, ultrasonic devices have no risk of electric shock to the operator as well to the patient (Amaral 1994). This is mainly because the patient is not a part of the electric circuit and no current thus passes through the patient body.

One of the most prominent differences between the ultrasonic devices and the monopolar electrosurgery (MES) is the gross appearance of the tissue where the device is applied. In the present study, it was seen that the findings were very similar to the following reports in the literature. The MES causes charring and desiccation of the tissue surface (Amaral 1994). The ultrasonic scalpel, on the contrary produces tissue blanching without tissue charring or burning (Hambley, Hebda et al. 1988), (Amaral 1994). The tissue damage caused by ultrasonic devices is more localized and less extensive (Birch, Park et al. 1999), (Hambley, Hebda et al. 1988). Similar results were seen in the present studies: Acute Study I, Acute Study II and Sub-acute Study.

Moreover, during MES application, there is smoke production at surgical site which hinders the visualization making the procedure time consuming. This might also necessitate frequent removal of smoke during the procedure which can be inconvenient (Corbitt 1991; Voyles CR 1991). Other than affecting visualization, the smoke produced is highly toxic (Hensman C 1998). The ultrasonic device applications do not produce smoke at the surgery site (Emam Tarek 2003). During the MES application, the resulting muscle contraction can be troublesome while performing the surgery. This can cause difficulty in achieving precision during the procedure. This factor is well avoided in case of ultrasonic scalpels (Carlander, Johansson et al. 2005). This was very clearly evident in the current study; where in the application of MES could not be precisely maintained due to uncontrolled contraction of muscles adjacent to the sciatic nerve. Also, in the Acute Study I, the muscle contraction caused a great difficulty while applying the MES blade at a distance of 1 mm from nerve without touching the nerve. In contrast, the ultrasonic blade could be easily applied at 1 mm from the nerve causing no

significant muscle contraction. Moreover, the incisions produced by electrosurgical techniques show slower wound healing period as compared to that of ultrasonic techniques (Hambley, Hebda et al. 1988).

The basic mechanism of vessel coagulating in both electrosurgery and ultrasonic techniques is by constriction of the vessel and coaptation by denatured protein coagulum (Koch, Friedrich et al. 2003), (Amaral 1994). However, in case of MES, coagulation is caused by heating of tissue and vaporization of tissue fluids. In ultrasonic devices vessel sealing is caused by application of mechanical energy to break the hydrogen bonds (Amaral 1994).

The causative injury factors in MES technique may include thermal and electric energy. In case of ultrasonic techniques, the causative factors may include thermal and mechanical energy; however, the thermal effects are less than with MES devices. The thermal effects of both these techniques have been compared in many studies. The MES procedures cause a rapid rise in temperature of tissue up to more than 300°C which can lead to carbonization, desiccation and charring of the tissue (Kinoshita, Kanehira et al. 1999). Such high temperatures can cause harmful effects on the vital structures like nerves within close proximity (Birch, Park et al. 1999). In the Acute Study I, it was seen that MES caused a severe decrement to the nerve conduction function when used in close proximity of the sciatic nerve. It was also seen that, there was significant drop in area under the curve (AUC) of CAP 2 mm and 3 mm distances from the sciatic nerve at specific time points. Moreover, from Acute Study I, it was evident that combining all the distances (i.e. 1 mm, 2 mm, 3 mm and 4 mm) together, there was significant drop in AUC as well as conduction velocity (CV) at all the time points after

the MES device application. Moreover, this energy travels collaterally as well as vertically affecting the surrounding tissues. In order to acquire the desired effects, the temperature induced by electrosurgery may go as high as 400 °C as compared to ultrasonically activated scalpel which induces around 150 °C (Feil 2002). In our current study the temperatures near the devices were not measured.

There is limited amount of heat produced by ultrasonically activated devices at the surgery site thus causing smaller area of thermal injury (Amaral 1994). The maximum heat generation occurs at the vibrating tips of the active blade and surrounding area where this heat can get diffused (Emam Tarek 2003). Thus, the temperature elevation at the surgery site is greatly dependent on the distance from the active blade. A study considering the possible safe distance to prevent the thermal damage to the surrounding tissues suggested that 3mm distance provided a safety margin. It suggested that the temperature elevation was less than 6°C at about 2mm distance from the blade application. Also, it reported that there was a temperature elevation of about 30 °C at a distance 1 mm from the ultrasonically active blade (Koch, Friedrich et al. 2003). A study determining the tissue temperatures at different angles and distances from the point of application of an ultrasonically activated scalpel reported that there was approximately 40-45 °C temperature at a 90° angle (collateral) and 4 mm distance from the application site (Katz, Danai et al. 2010). In the current studies, the temperature at the site of application was not measured. However, in the Acute Study I, it was seen that at 3 mm distance from the sciatic nerve, there was no observed drop in the magnitude of compound action potential after applying the ultrasonic blade thus indicating no injury to the nerve. It was also seen that there was no significant drop

in AUC or CV at any time point after applying the ultrasonic blade at 1 mm, 2 mm, 3 mm or 4 mm distance from the sciatic nerve.

A study based on determining the safety of ultrasonic dissection suggested that the power level 5 in an ultrasonic device can cause significant proximal and collateral injury up to 10 mm from the point of application. It indicated that the power level 3 is an ideal setting for applications around the vital structures. Moreover, it suggested that the time duration for each activation should not exceed 5 seconds (Emam Tarek 2003). In the current studies, power level 5 was used as a standard setting for all ultrasonic devices. It was seen that even when used at power level 5, no significant damage was caused to the nerve conduction function by ultrasonic devices. In Acute Study I, the activation time of the ultrasonic blade was 5 seconds. In Acute Study I and Sub-acute Study, the total activation time for the ultrasonic shears varied from 13 seconds to 20 seconds to complete the surgical procedures.

A study by Carlandar et. al. compared the rat sciatic nerve injury caused by ultrasonically activated scalpel and electrosurgery. In this study, the nerve dysfunction was quantified by analyzing the electromyographic potential (EMG) potential evoked as a result of a 2 Hz square wave and 0.5 ms stimulation pulses (current level 0.5-1 mA). The EMG potential was compared before and after the device applications. The device application was carried out by two types of surgical procedures. The first type was the one where in muscle and fascia were cut perpendicular to the nerve by ultrasonic shear device and bipolar electrosurgery device. In the second type, a 5 mm long longitudinal incision was made in close proximity of the nerve (approximately 1 mm) by ultrasonic shear, MES and bipolar electrosurgery. Carlander et. al. reported that EMG

measurements indicated significantly more damaged nerves after longitudinal MES application as compared to that with ultrasonic shears. Also, longitudinal incision by MES application caused extensive morphological injury as compared to ultrasonic shears. This coincided with the Acute Study I and Sub-acute Study where in a similar longitudinal incision performed by MES caused extensive nerve conduction injury as compared to the ultrasonic devices. It was suggested that extended time of activation may affect the extent of nerve injury. The ultrasonic shears make an incision at a relatively slower speed as compared to MES, thus increasing the exposure time (Carlander, Johansson et al. 2005).

In the present studies, the impairment of the nerve function was quantified by means of changes in the compound action potential (CAP). CAP represents a summation of neural activity of activated nerve fibers within a particular nerve. The range of conduction velocities obtained in these studies was about 40 to 70 m/sec indicating that the neural activity was mainly recorded by the stimulation of Aβ fibers. Thus, a drop in CAP would indicate a decreased number of fibers getting activated by stimulation while a drop in CV would indicate a reduced conduction velocity of nerve fibers within the nerve following stimulation. A reduction amplitude or velocity in the CAP may be a result of nerve damage caused by exposure of nerve fibers to the external energy sources of the devices.

When the surgical devices were used near the sciatic nerve, the resultant energy exposure affected the activation of nerve fibers and hence the CAP. Each device produces different intensities of energy exposure. Thus, a change in the CAP could be used as a measure to quantify the effect of each surgical device on the nerve function.

The ultrasonic blade HK 105 showed no significant nerve dysfunction even at a distance of 1 mm from the nerve with the AUC drop not exceeding 20% as compared to the baseline recording AUC. The nerve conduction data indicates that the ultrasonic blade appears to be much safer than MES in avoiding nerve injury. MES caused significant loss of nerve function when applied in close proximity to the sciatic nerve. This severe nerve injury could result in numbness, motor weakness, reduced jerk reflex, paresthesias, acute or chronic neuropathic pain.

Different safe distances from the nerve tissue have been reported in different studies (Emam Tarek 2003; Koch, Friedrich et al. 2003; Katz, Danai et al. 2010). The possible reasons for these variations could be the differences in the time of application of the device as well as the angle of the tissue from the device tip. The ultrasonic vibration transmitted through the active blade passes through the body tissue in specific directions. Thus, there are different levels of heat production within the tissue area near the application site.

A study by Katz et. al. determined the heat spread patterns and the subsequent tissue temperatures around the point of UltraCision device tip application in ten rats. The device was applied for 10 seconds at power level 3/5 and the tissue temperatures were measured at distances 4 mm, 8 mm, 12 mm and 16 mm from the device in a circumferential manner by means of thermocouples. Thus, an angle of 0° would indicate the part behind the device blade while an angle of 180° would indicate the part right in front of the device blade (Figure 8.1). Also, thermal mapping of the device tip was performed by the use of infrared camera. Additionally, the same device was applied at varying distances from the sciatic nerve. The rats were sacrificed after 1 week survival

period and the nerves were obtained for histological assessment. In this study, it was reported that average absolute temperatures at 4 mm from the device were 52.7± 13.2°C exactly below the device (angle of 180°) with the peak temperatures recorded below the device (180°) were recorded as high as 80°C. Also, the average absolute temperatures at 4 mm adjacent to the device tip (angle of 90°) recorded were 40 °C to 45 °C. It was seen that the mean tissue temperatures were relatively higher at angles higher than 90° from the device tip as compared to the lower angles i.e. increased tissue temperatures were found exactly below the device tip as compared to the adjacent (collateral) tissue areas. Further, potentially neurodestructive temperatures and morphologic axonal damage were reported within a distance of 4 mm to 8 mm from the application site when applied for 10 seconds (Katz, Danai et al. 2010).
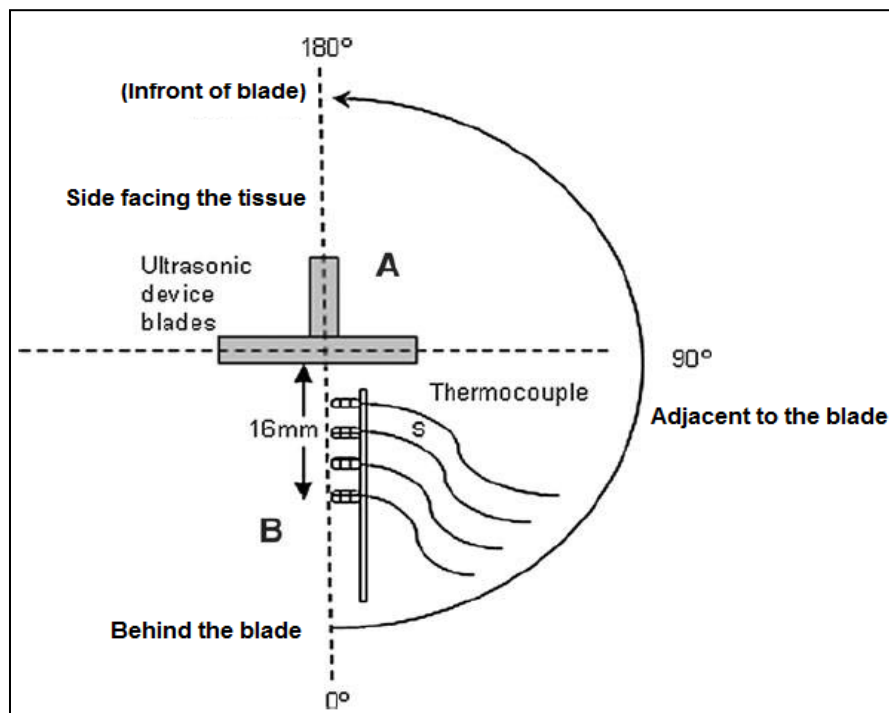

Figure 8.1: Experimental set up showing the ultrasonic blade, thermocouples and angles around the blade. Modified from (Katz, Danai et al. 2010)

The study by Koch et. al. determined the temperature elevations in the tissue during the application of the harmonic scalpel UltraCision to different tissues (lung parenchyma, tongue, parotid gland) in a pig model. In this study, the tissue temperatures were recorded exactly below the device tip (at an angle of 180° from the device tip, Figure 8.1) by means thermocouples of specific designs. Power level 5 was used in lung surgery by dissection hook HS2 with the flat side of the blade while power level 3 was used to perform the surgery of the tongue and the parotid gland with the blunt side of the HS2. The HS2 dissection hook was applied for a period of 15 seconds to cut the different tissues with an incision 2 cm long and 4 mm deep. The temperature sensors when positioned at about 2 mm below the surface at different distances from the cut, recorded temperature elevation of more than 40 °C at 1 mm distance from the device tip. From this study it was concluded that thermal damage did not exceed a distance of 3 mm distance from the device blade in lung tissue, musculature of the tongue and tissue of parotid gland. Thus, it was suggested that at least 3 mm distance would be justified as a conservative limit for safe distance of application (Koch, Friedrich et al. 2003).

A study by Emam et. al. determined the safety of the ultrasonic dissections in pigs. Harmonic scalpel UltraCision was used in random fashion to mobilize the cardia and fundus, bile duct, hepatic arter, portal vein, aorta from the inferior vena cava, renal vessels, colon and ureters. The dissections were carried out at power levels 3, 4 and 5 and time of activation was recorded. Thermal mapping of the tissues during dissection was performed by means of an infrared camera. The animals were sacrificed at the end of experiment and tissue sample was harvested for quantitative histology. It was seen

that with power level 5, the peak temperatures at a distance of 10 mm away from the device was 91.4 °C with 5 seconds exposure, 134.6°C with 10 seconds exposure and 144.8°C with 15 seconds exposure. It was reported that at power level 5, the heat generation was substantial at the vibrating tip as well as considerable zone of tissue around device tip with the temperatures exceeding about 60 °C over a distance of 25 mm from the application site. Also, it was reported that power level 5 could cause significant collateral injury to important structures within 10 mm of the dissection site. Moreover, it was seen that the tissue damage caused by the ultrasonic devices was not macroscopically visible wherein the tissues appeared normal even after extensive histologic injury. In conclusion, it was suggested that power level 3 would be an ideal setting as compared to power level 5. Also, it was reported that the risk of damage would be reduced if the device activation time did not exceed 5 seconds at once .

In the Acute Study I, the application of HK 105 blade (at power level 5) approximately at an angle of 90° from the sciatic nerve (i.e. collaterally) with the constant activation time of 5 seconds produced no significant effect on the nerve function at distances of 1 mm, 2 mm, 3 mm to 4 mm from the nerve.

The basic differences within these studies are the device, the application techniques, time of exposure and the animals. These varied parameters can have varied effects on the body tissues. Moreover, even the different angles from the device tip can have varying effects on the tissue temperatures and subsequent tissue damage. When an ultrasonic scalpel is activated the mechanical energy from the ultrasonic device is transmitted to the tissue with blade tip pointing to just below the blade tip. . Thus, it would be safer to apply these devices parallel to the nerve. In Acute Study I, the

HK 105 blade was applied with a precisely controlled application distance and time which might not hold true during actual clinical procedures. For this purpose, Acute Study II and Subacute Study were developed where the surgical procedures were comparable to the clinical surgical techniques. However, the current studies do involve a limitation wherein the effect of ultrasonic energy on the tissue surface below the device tip is not studied which can result in different heat and energy transfer compared to the adjacent tissue area.

From Acute study II, it was seen that the nerve dysfunction, indicated by drop in AUC, induced by MES was rather rapid i.e. immediately after device application (2 min time point) as compared to the LigaSure device where in the nerve dysfunction occurred at about 2 hours after device application. A possible reason for this would be that application of MES device results instant axonal injury referred as primary axotomy. The primary axotomy involves direct rupture or breaking of the axonal membrane which causes a complete dysfunction of the axon immediately. However, application of the LigaSure device might result in a prolonged axonal injury also referred to as secondary axotomy. The secondary axotomy is a delayed consequence of complex axolemmal or cytoskeletal changes which can further lead to cytoskeletal collapse and impairment of axoplasmic transport (Povlishock and Jenkins 1995). Thus, the intra-axonal changes result in impairment of anterograde transport which might lead to subsequent axonal swelling (Stephen G. Waxman 1995).

Also, the nerve injury induced by the use of these devices may be a result in infiltration of leukocytes into the nerve tissue and their further extravasations into the surrounding tissues. It has been reported that MES exposed sciatic nerves have

increased leukocyte cells as compared to ultrasonic blade exposed nerves (Chen C. 2010) . This elevated infiltration could lead to release of inflammatory cytokines which may contribute to prolonged wound healing and altered nerve conduction. The combination of inflammatory cytokines and altered nerve conduction can lead to sensory and motor deficits which ultimately can contribute to an altered behavioral state. It has been reported that application common inflammatory cytokines like Tumor Necrosis Factor (TNF) and Interleukin-1 β (IL-1β) can have harmful effects on the nerve function measured in terms of neural discharge on probing of tail area in a rat model (Ozaktay, Cavanaugh et al. 2002). Moreover, it was seen that application of cytokine TNF resulted in significant decrease of the neural activity (Ozaktay, Cavanaugh et al. 2002).

Heat energy, one of the major causative factors of the surgical devices, can damage the protein structure and interrupt axoplasmic transport. Also, as protein is negatively charged, local accumulation can cause change in the resting membrane potential leading to hyperpolarization and axon inexcitability. Damage to nerve fibers results in blockage of action potential propagation along the axons, leading to a drop of CAP magnitude and decrease of CV. Clinically, such nerve injury can further result in numbness, motor weakness, reduced jerk reflex, parathesias, and acute or chronic neuropathic pain.

In the Subacute Study, the behavioral changes developed in the sub-acute phase after MES application on two rats indicated possibility of numbness with no signs of recovery. This might indicate that the sciatic nerve was functionally dead. A possible reason for the nerve to be completely damaged could be the uncontrolled muscle

contraction which might have caused the MES blade to touch the nerve during application procedure.

In Acute Study II and Subacute Study, Harmonic Focus indicated mild nerve injury with a recovery trend by seven days. Harmonic ACE enabled more efficient cutting of tissue as compared to Focus which required longer duration of device application increasing a chance of nerve injury. Harmonic Focus and ACE resulted in hypersensitivity of ipsilateral and contralateral hind paws. LigaSure showed mild reduction in the CAP and an increased sensitivity in the left hind paw by the end of seven days when the device was applied on left sciatic nerve. Due to a limited sample size, the results were not statistically analyzed.

To summarize, the ultrasonic blade and shears appear to cause less nerve injury. The bipolar LigaSure vessel sealing device might not produce as severe nerve dysfunction as caused by monopolar electrosurgery. However, a small sample size poses a limitation to show significant differences. More studies need to be performed to compare these devices and conclude strongly on their effects on nerve conduction function.

CHAPTER 9

CONCLUSIONS

The acute and sub-acute effects of the RF based and ultrasonic based surgical devices were analyzed on their application in close proximity of the rat sciatic nerve. The nerve dysfunction was quantified in terms of compound action potential (CAP) and conduction velocity (CV) measurements. The sensory nerve function was assessed by the behavioral changes in the ipsilateral and contralateral hind paws.

This study demonstrates that CAP and CV provide valuable data regarding functional deficit and recovery in nerve trunks subjected to exposure by ultrasonic and RF devices. The drop in CAP indicates that a certain number of nerve fibers within the nerve trunk have been damaged. The CAP changes were quantified in terms of area under the CAP curve (AUC) as compared to the AUC before the device application. The acquired results can be summarized as,

1. Acute CAP and CV Changes

The AUC decreased significantly after application of MES device. It was seen that using MES at a distance of 1 mm from the nerve could cause severe nerve damage. There were instances where in there was no CAP generation indicating that there was no nerve activity. MES caused a significant drop in AUC values at all the time points after the device application at distances 2 mm and 3 mm from the nerve. Also, significant drop in CV values was seen after 30 minutes of device application at 2 mm and 3 mm distances from the nerve.

The LigaSure device showed considerable drop in AUC up to 3 hours of device application. The CV dropped to a large extent after 1 hour of device application.

The ultrasonically activated scalpel HK 105 cutting device caused minimal nerve injury during muscle incision. There was slight drop in CAP after device application at 1 mm from the nerve, indicating mild nerve injury. However, no significant drop in AUC values was seen when the blade was applied at distances 1 mm, 2 mm, 3 mm or 4 mm from the nerve.

Harmonic Focus showed slight drop in AUC and CV indicating mild nerve injury. Harmonic ACE did not show any drop in AUC and CV in the acute phase. Harmonic Focus required more activation time to complete the procedure as compared to Ace. Shorter time of activation may reduce heat generation at the surgery site thus reducing the chance of nerve injury. The manual shears did not show any drop in AUC and CV till 3 hours after surgery.

2. Sub-acute AUC and CV Changes

In case of MES device, the severe nerve injury persisted in the sub-acute phase as well. The AUC value was significantly low which continued up to 3 days after device application. There were instances where in there was no CAP developed indicating a severe injury of the nerve which continued throughout the 3 day survival period. There was a mild increase in AUC seen at the end of 7 day period indicating a slight recovery of nerve function. However, even after 7 days, the AUC was considerably lower than the control values. The change in CV was not as significant as AUC and remained consistent through the 7 days.

LigaSure device might cause mild nerve injury when applied at 1 mm from the nerve. It showed slight drop in AUC at 3 days. By the end of 7 days, there was an increase in AUC indicating recovery of the nerve function. There was a slight drop in CV at 3 days with a gradual increase at 7 days.

The sub-acute effects of ultrasonic blade on the nerve conduction function were involved in this study.

Harmonic Focus and Harmonic ACE showed drop in AUC at 3 days. Both Focus and ACE showed a considerable increase in AUC by the end of 7 day period. There was no major drop seen in CV at 3 or 7 days after the device applications.

The manual shears showed no significant drop in AUC and CV at 3 days. Conversely, there was a drop in AUC as well as CV by the end of 7 day period. Thus, manual shears may indicate some delayed nerve dysfunction. This could be possibly because of local inflammation as no haemostatic procedures were used at the surgery site.

3. Behavioral Changes

MES induced motor weakness after the application. Significant discomfort was observed as the rat continued to limp all through the 7 day survival period. MES indicated hypoalgesia in the left hind paw. In 2 cases, MES caused peripheral numbness indicating extremely severe nerve dysfunction. There was clenching of the left hind paw and inability to use the paw while walking. Also, there was no sign of recovery observed by the end of 7 day survival period.

LigaSure device did not cause peripheral numbness but did show some signs of hypoalgesia in the ipsilateral (left) hind paw after surgery. It caused increased sensitivity of the ipsilateral hind paw as compared to that before application of the device. There was no change in the sensitivity of the contralateral (right) hind paw.

The ultrasonic blade did not cause any effect on the sensitivity of the ipsilateral hind paw till 3 hours after the device application. The sub-acute effects of ultrasonic blade on the sensitivity of hind paw were not a part of this study.

Harmonic Focus and Harmonic ACE did not induce peripheral numbness thus showing that the extent of nerve injury is much more in MES till 7 days after surgery. There was no discomfort or distress observed in the rat behavior from 1 to 7 days after device applications. These devices caused hypersensitivity of the ipsilateral as well as contralateral hind paws after the surgery.

The manuals shears did not induce any behavioral changes all throughout the 7 day survival period after the surgery.

To summarize, the monopolar electrosurgery device causes severe nerve injury when used in close proximity of nerve. The ultrasonic blade causes mild nerve injury when used at 1 mm from the nerve. The ultrasonic shears appear to induce less nerve injury. The bipolar LigaSure vessel sealing device might not produce as severe nerve dysfunction as caused by monopolar electrosurgery. Harmonic Focus and ACE might induce mild nerve dysfunction in the acute phase but eventually indicated recovery in the sub-acute phase. However, a small sample size of shears devices in the Acute Study I and Sub-acute Study poses a limitation to show significant differences. More

studies need to be performed to compare these devices and conclude strongly on their

effects on nerve conduction function.

APPENDIX A

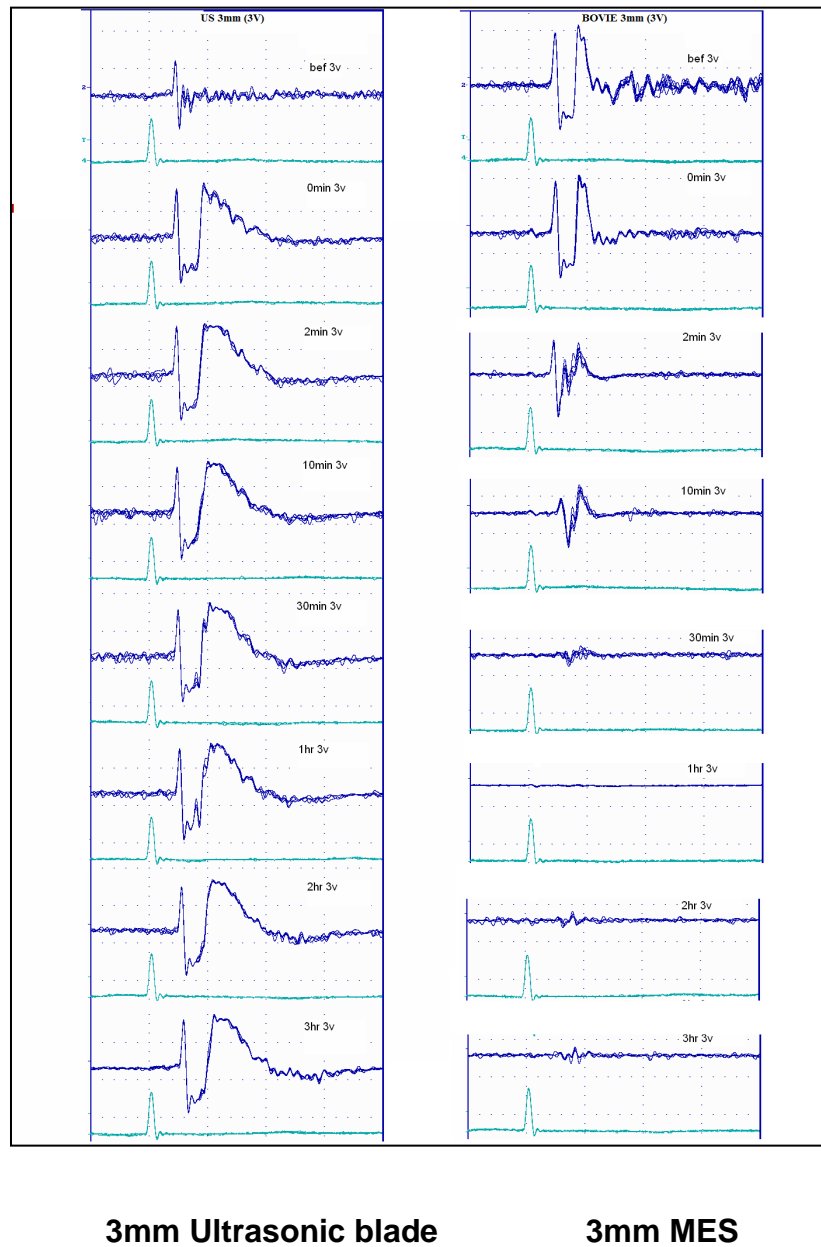**Figure A-1:** Typical changes of CAPs over the time after application of MES and Ultrasonic blade 2 mm from the sciatic nerve. The immediate drop in the magnitude of CAP after MES application recorded at the 2nd minute after application with 100% drop after 1 hour of application (right column). The decrease of AUC did not occur in ultrasonic blade application (left column).
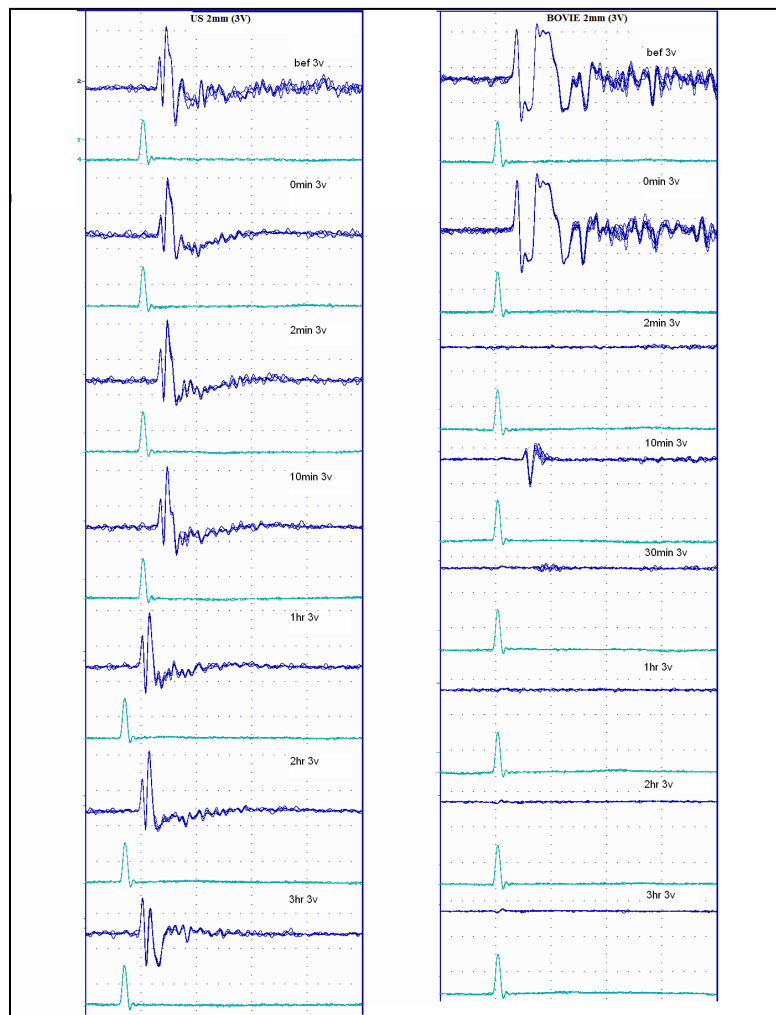
2mm Ultrasonic blade   2mm MES

Figure A-2: Typical changes of CAPs over the time after application of MES or ultrasonic blade 3 mm from the sciatic nerve.   The magnitude of CAP dropped immediately after MES application recorded at the 2nd minute after application (right column). The CAP magnitude decrease did not occur in ultrasonic application (left column).

3mm Ultrasonic blade　　　　**3mm MES**

Figure A-3: Typical changes of CAPs over the time after application of MES or ultrasonic blade 4 mm from the sciatic nerve. There is no clear decrease of AUC in both ultrasonic blade (left column) and MES application (right column).
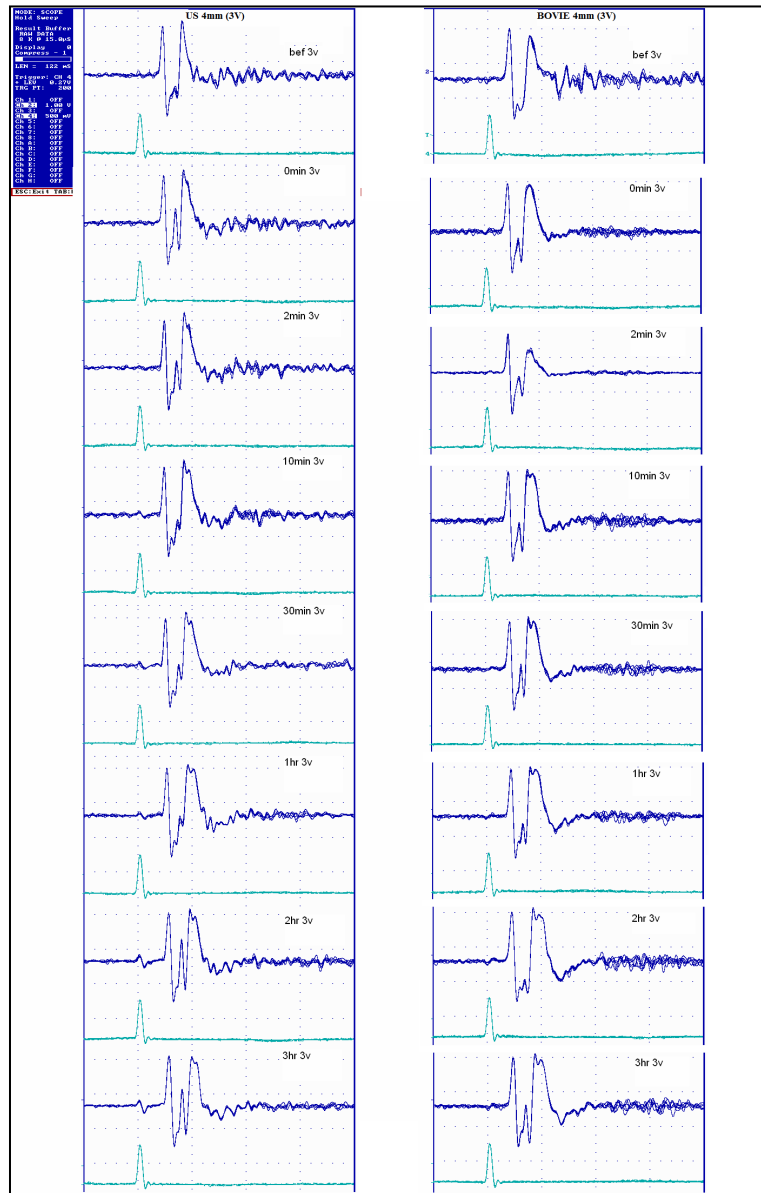
4mm Ultrasonic blade   4mm MES

APPENDIX B

Figure B-1: Application of ultrasonic blade 1 mm from the sciatic nerve resulted in decrease of AUC (One way ANOVA, p>0.05, PostHoc LSD).
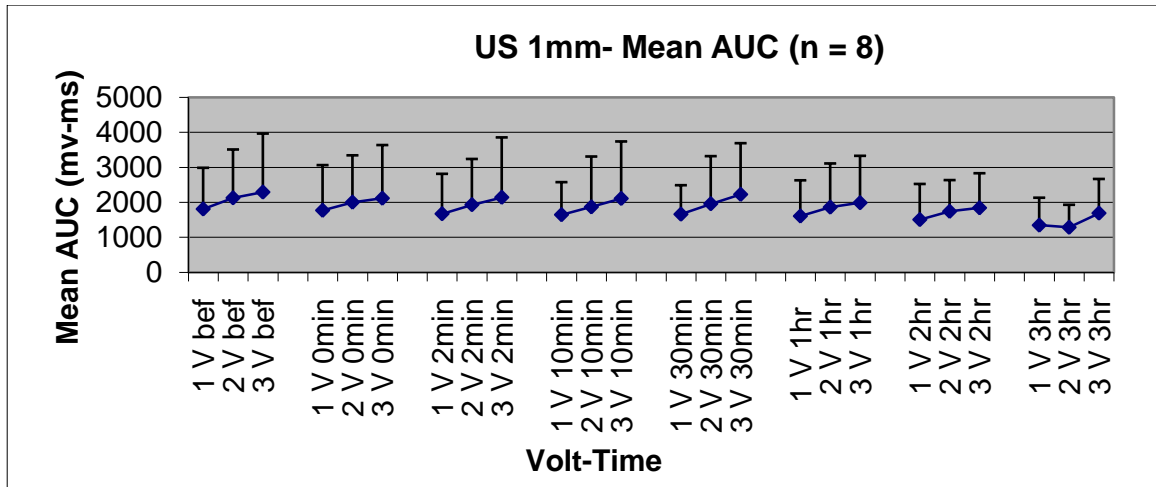
Figure B-2: Application of MES 1 mm from the sciatic nerve caused significant decrease of AUC over time (One way ANOVA, p<0.05; PostHoc LSD).
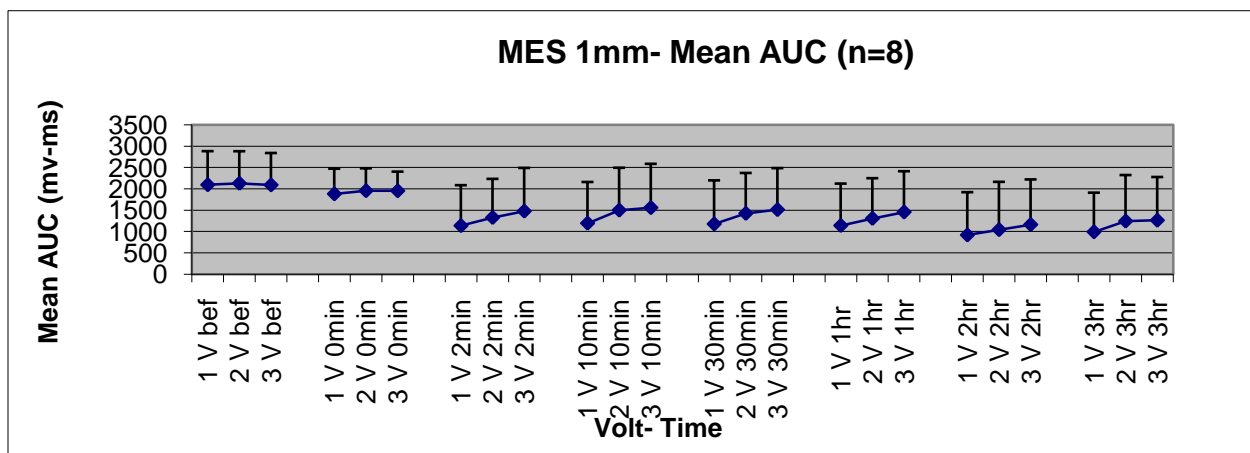
Figure B-3: Application of ultrasonic blade 2 mm from the sciatic nerve did not cause significant decrease of AUC over the time (One way ANOVA, $p > 0.05$, PostHoc LSD).
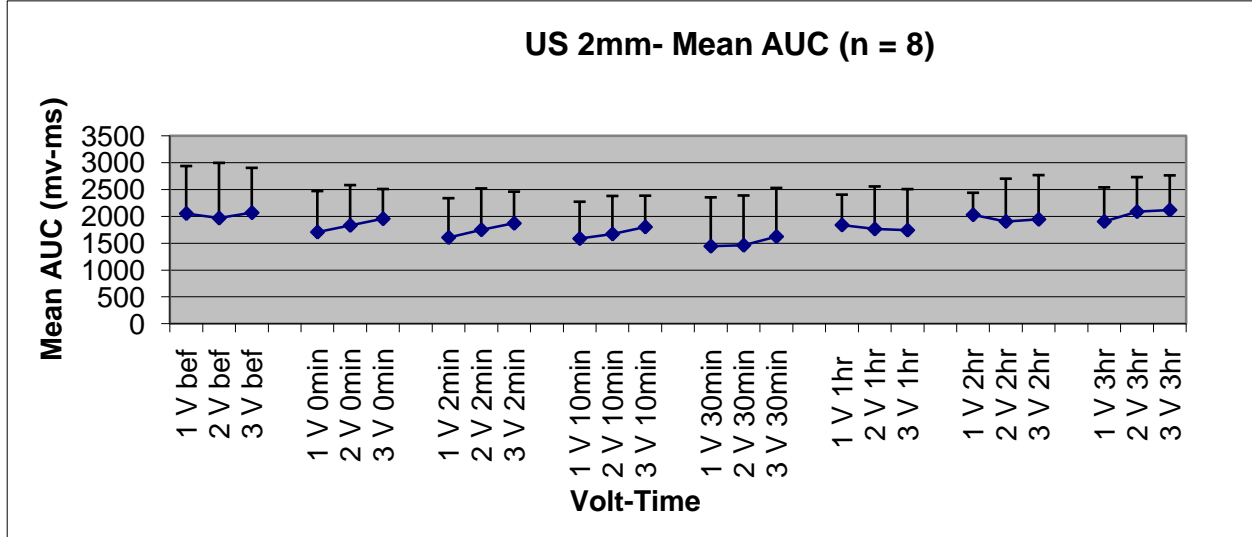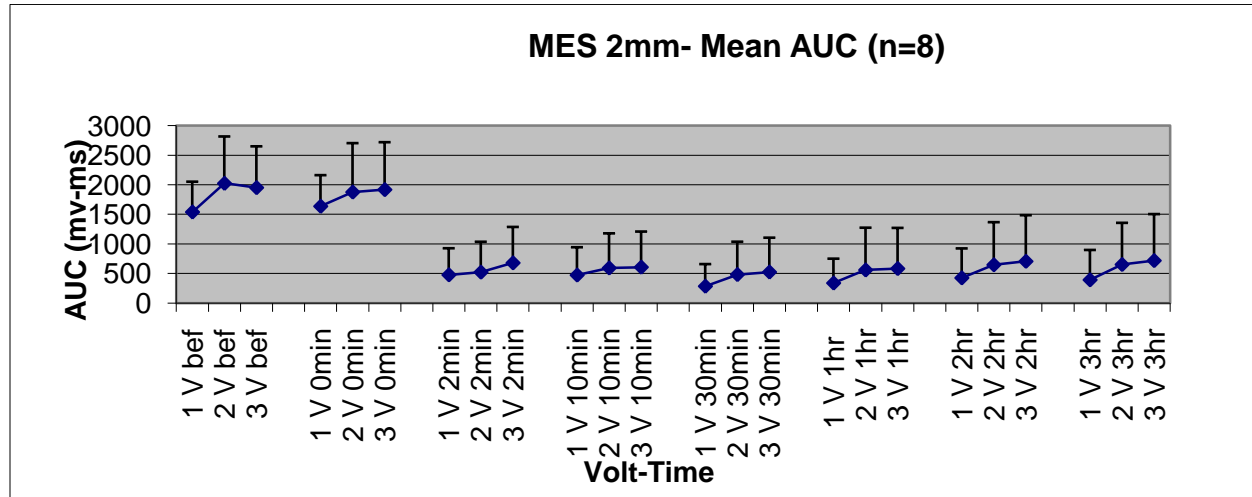

Figure B-4: Application of MES 2 mm from the sciatic nerve caused significant decrease of AUC over the time (One way ANOVA, $p < 0.05$; PostHoc LSD).

Figure B-5: Application of ultrasonic blade 3 mm from the sciatic nerve did not cause significant decrease of AUC over time (One way ANOVA, $p > 0.05$, PostHoc LSD).
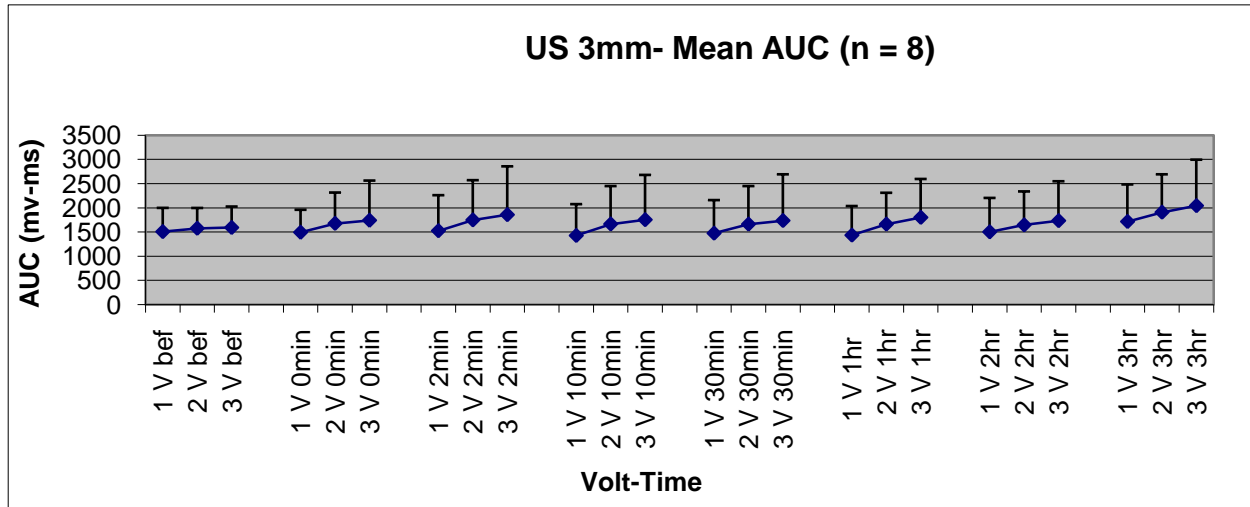

US 3mm- Mean AUC (n = 8)

Figure B-6: Application of MES 3 mm from the sciatic nerve caused significant decrease of AUC over time (One way ANOVA, $p < 0.05$; PostHoc LSD).
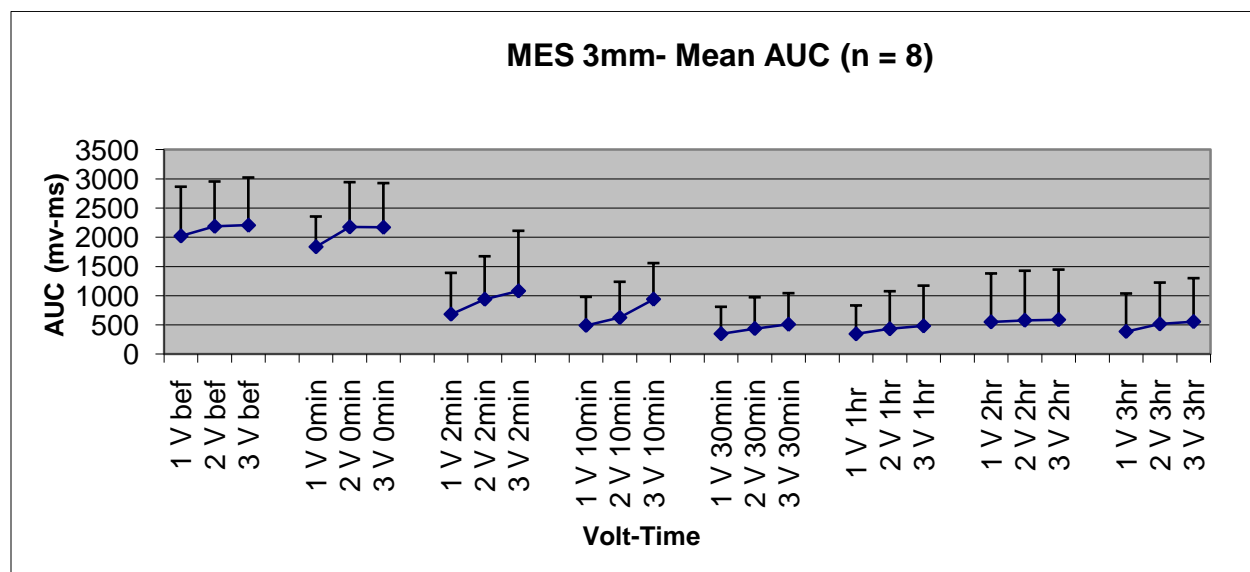

MES 3mm- Mean AUC (n = 8)

Figure B-7: Application of Harmonic 4 mm from the sciatic nerve did not cause significant decrease of AUC over the time (One way ANOVA, p>0.05, PostHoc LSD).
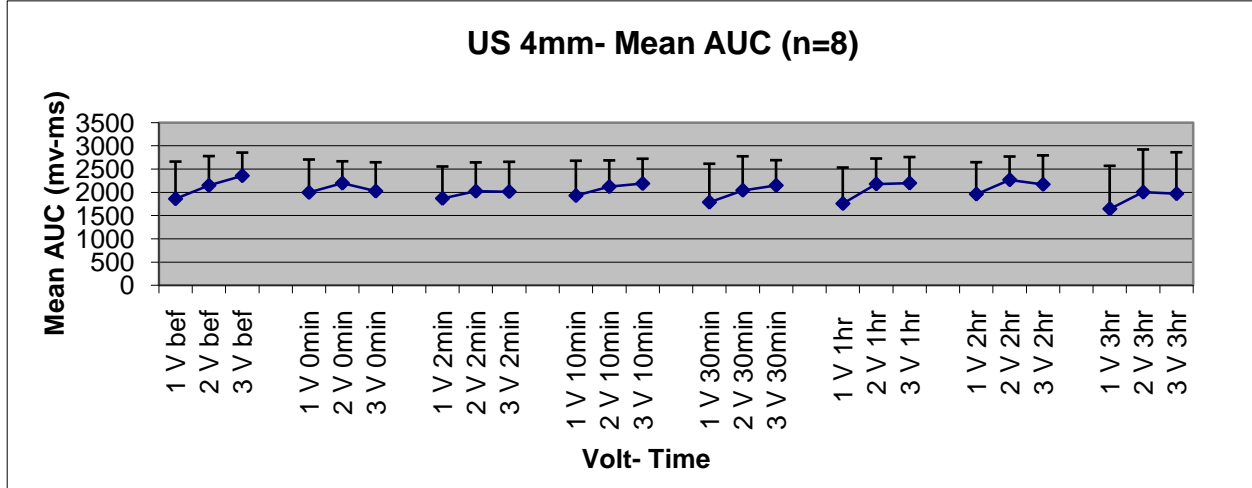

Figure B-8: Application of MES 4 mm from the sciatic nerve still caused significant decrease of AUC over the time (One way ANOVA, p>0.05, PostHoc LSD).
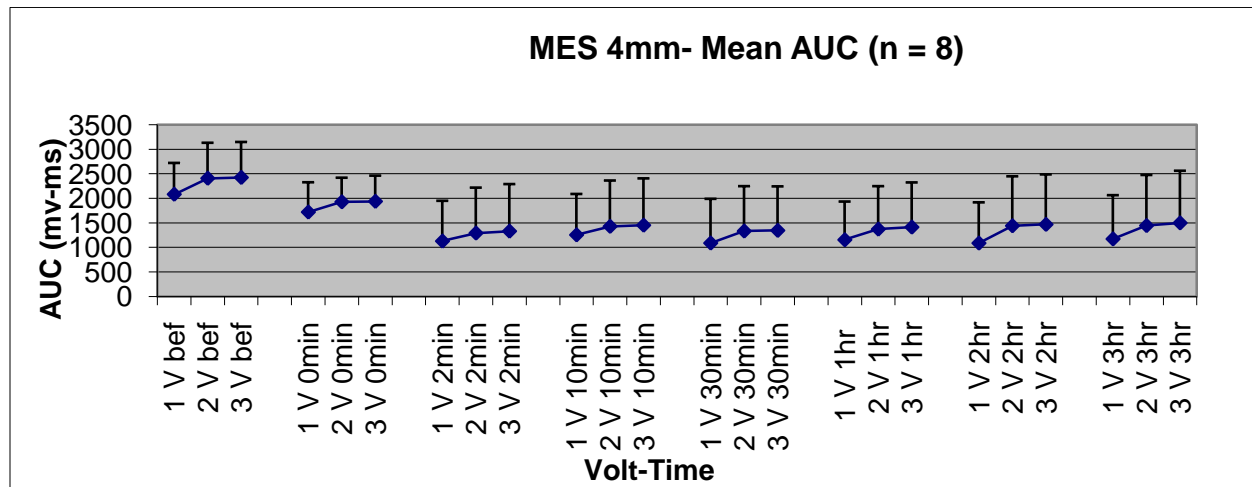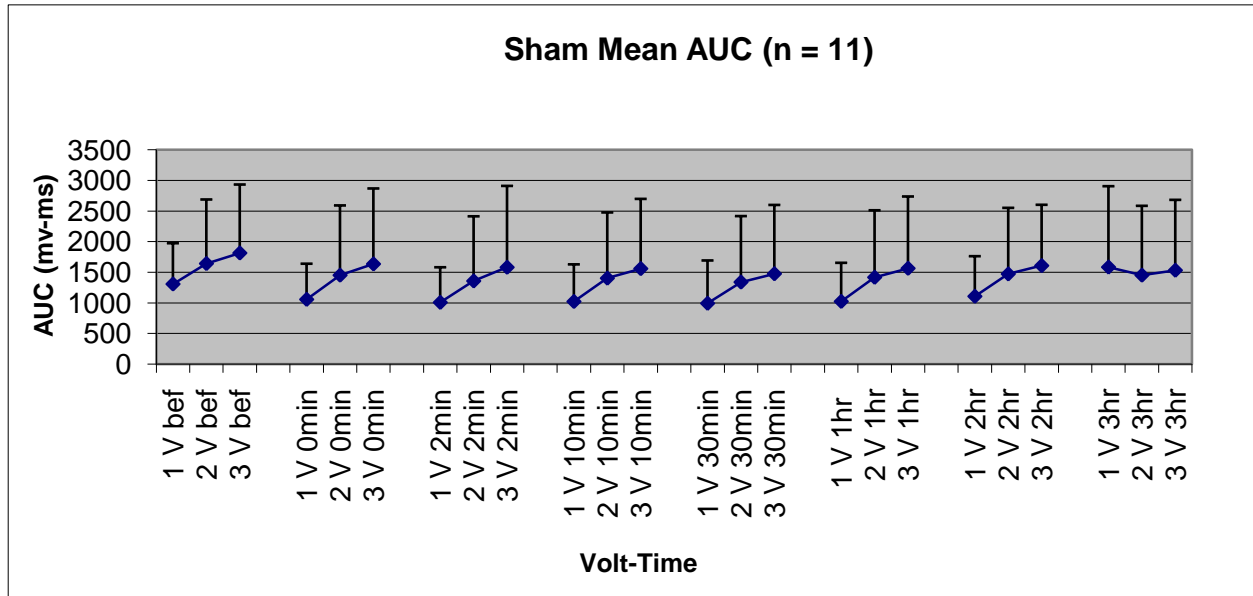
Figure B-8: AUC in sham group did not have significant changes over the time (One way ANOVA, p<0.05; PostHoc LSD).

APPENDIX C

Figure C-1: Application of ultrasonic blade 1 mm from the sciatic nerve did not cause significant decrease of CV over the time (One way ANOVA, $p > 0.05$, PostHoc LSD).

Figure C-2: Application of MES 1 mm from the sciatic nerve caused significant decrease of CV over the time (One way ANOVA, $p < 0.05$; PostHoc LSD).

Figure C-3: Application of ultrasonic blade 2 mm from the sciatic nerve did not cause significant decrease of CV over the time (One way ANOVA, p>0.05, PostHoc LSD).
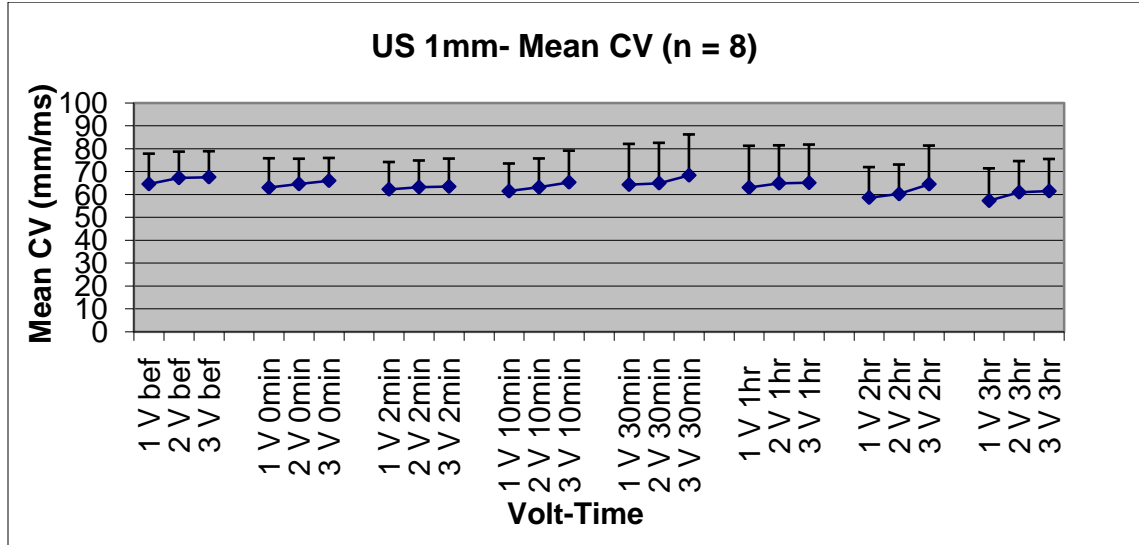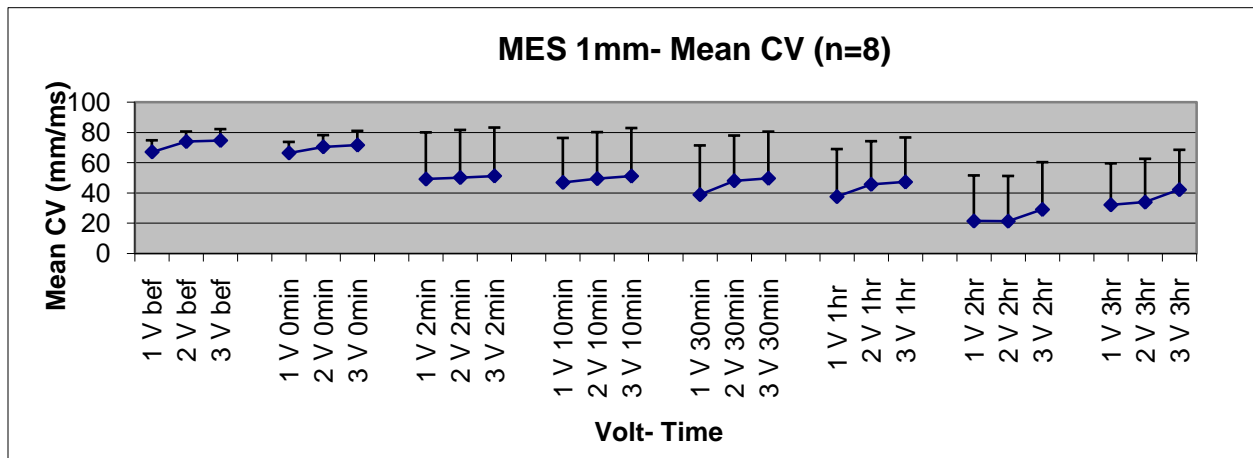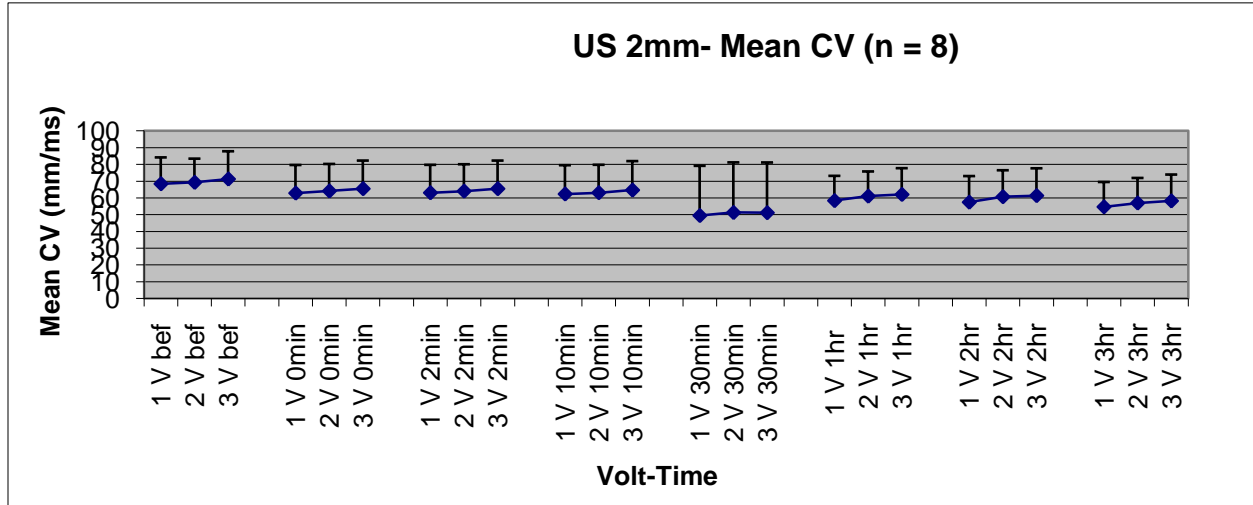

Figure C-4: Application of MES 2 mm from the sciatic nerve caused significant decrease of CV over the time (One way ANOVA, p<0.05; PostHoc LSD).
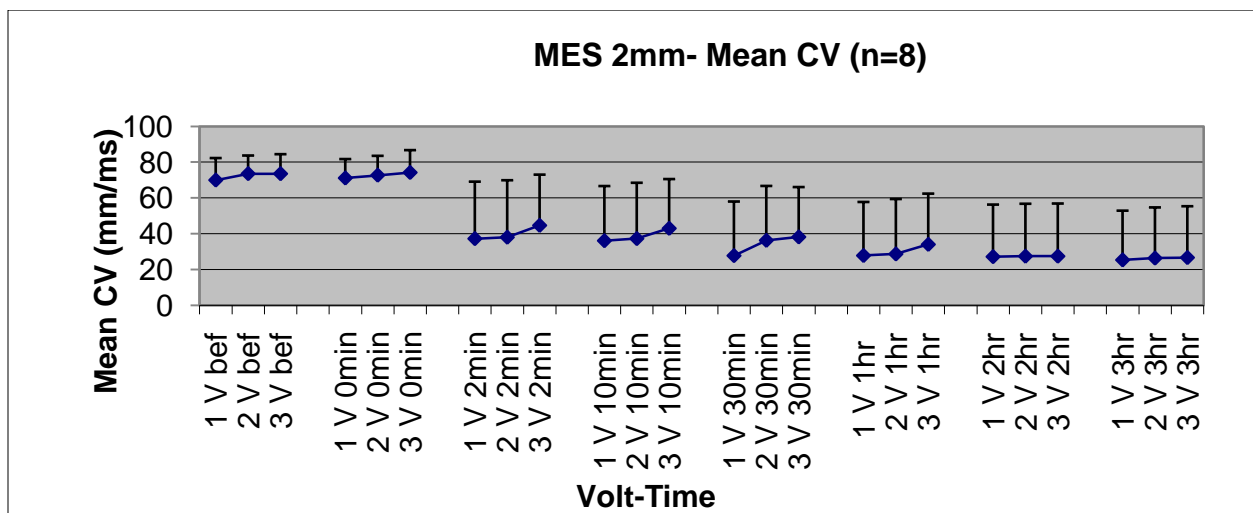
Figure C-5: Application of ultrasonic blade 3 mm from the sciatic nerve may cause decrease of CV over the time (One way ANOVA, p>0.05, PostHoc LSD).

US 3mm- Mean CV (n = 7)

Figure C-6: Application of MES 3 mm from the sciatic nerve caused significant decrease of CV over the time (One way ANOVA, p>0.05, PostHoc LSD).

MES 3mm- Mean CV (n = 8)

Figure C-7: Application of ultrasonic 3 mm from the sciatic nerve may cause decrease of CV over the time (One way ANOVA, p>0.05, PostHoc LSD).

Figure C-8: Application of MES 3 mm from the sciatic nerve did not cause significant decrease of CV over the time (One way ANOVA, p>0.05; PostHoc LSD).

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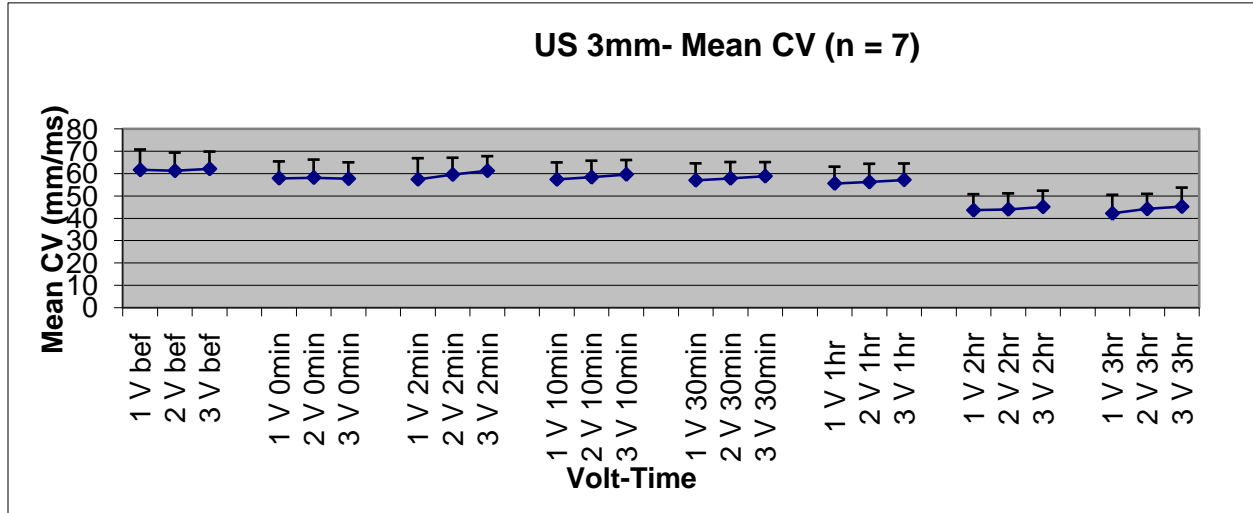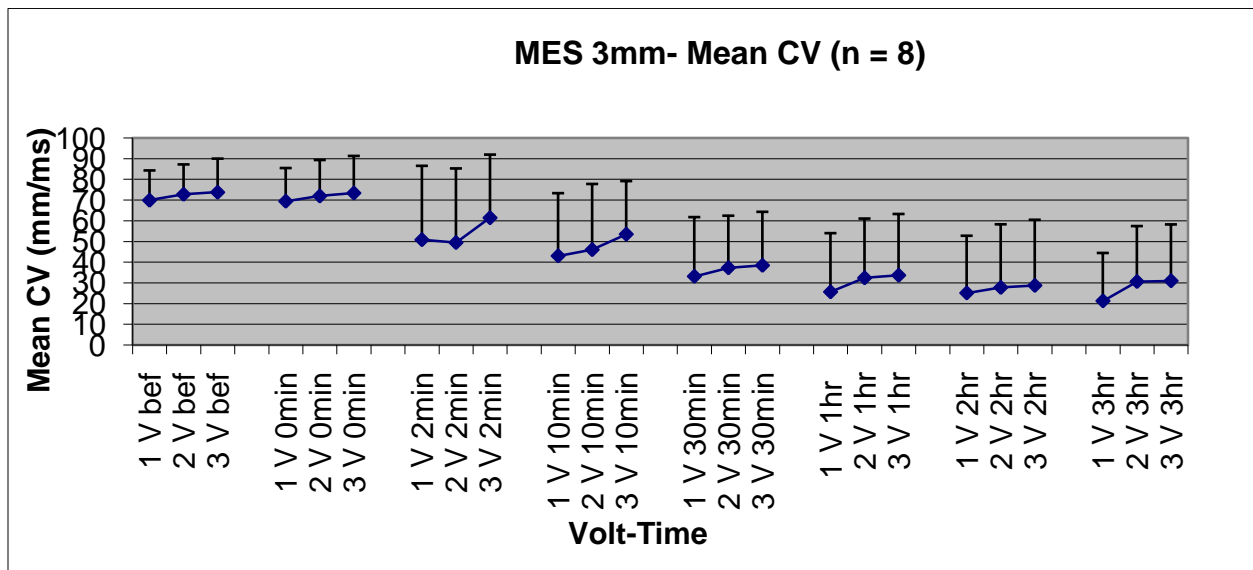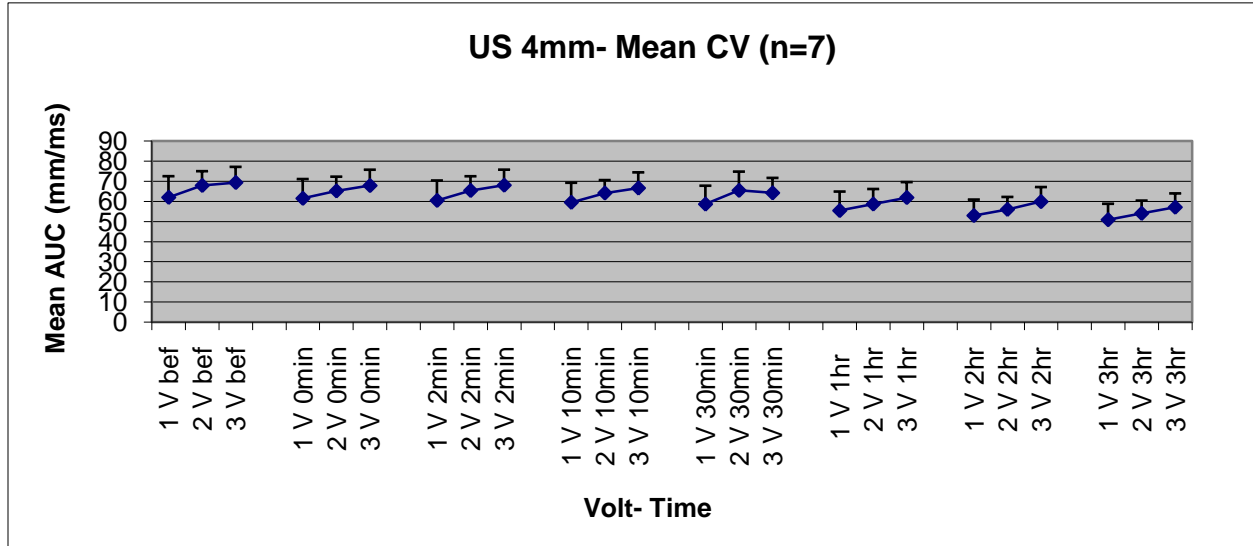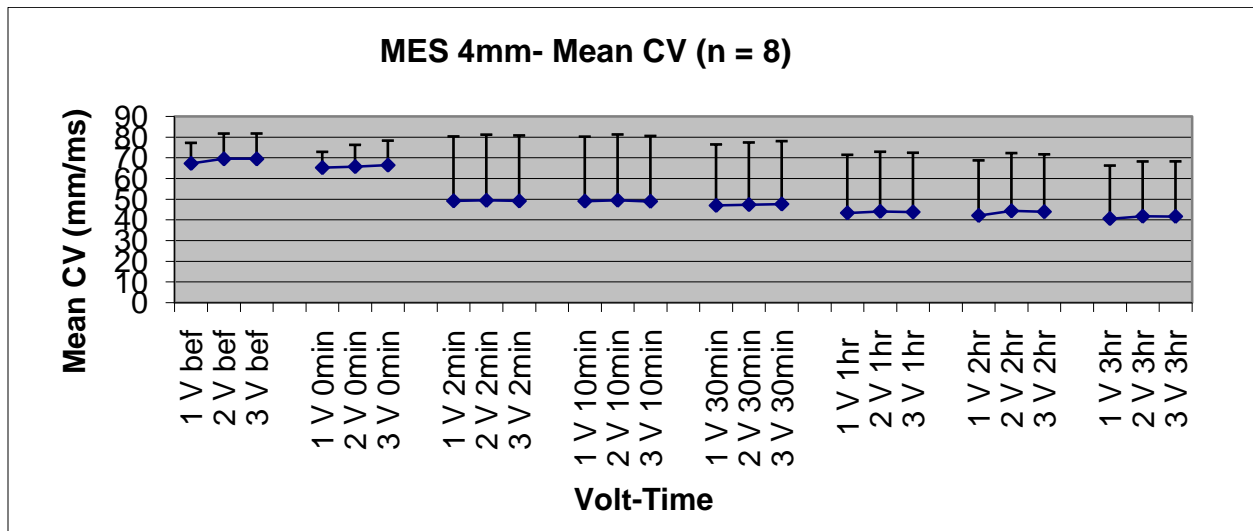Amaral, J. F. (1994). "The experimental development of an ultrasonically activated scalpel for laparoscopic use." Surgical Laparoscopy and Endoscopy **4**(2): 92-99.

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ABSTRACT

THE ACUTE AND SUB-ACUTE EFFECTS OF ELECTROSURGICAL AND ULTRASONIC SURGICAL DEVICES ON THE NEUROPHYSIOLOGIC CHANGES OF SCIATIC NERVE FUNCTION

by

ANUJA VEDPATHAK

December 2010

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Major: Biomedical Engineering

Degree: Master of Science

Iatrogenic blood loss during surgery can have several harmful effects on the patient. The blood loss can be significantly minimized by using a variety of vessel sealing devices that use different types of energy sources to seal the blood vessels or tissue bundles and simultaneously cut and coagulate tissues.

The two types of vessel sealing techniques accepted worldwide are based on electrosurgical and ultrasonic technology. The electrosurgical technique uses high frequency electric current while the ultrasonic technique uses ultrasonic energy to cut and coagulate tissues. These devices have been widely employed in various surgical procedures. However, it is not clear if they can induce nerve injury when applied near the nerve tissue. The safe distance to apply these devices from nerves has not been profoundly investigated.

In the current study, a set of electrosurgical and ultrasonically activated surgical devices were surgically tested around the sciatic nerve in a rat model. Monopolar electrosurgery (MES) device and ultrasonically activated (US) blade were applied on the muscle tissue at specific distances from the sciatic nerve. The consequent change in the nerve conduction function was neurophysiologically assessed and analyzed. Also, developmental studies were performed to determine the effects of electrosurgical and ultrasonic shear devices on the sciatic nerve function in the acute as well as sub-acute phase. The sensory nerve function was assessed by means of behavioral changes.

The current study indicates that the US blade appears to be superior in terms of controlling muscle contraction, providing visibility at the surgical site and safe application around the nerve as compared to the MES device. Overall ultrasonically activated devices appear to be superior to electrosurgery devices.

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<u>CONFERENCE PUBLICATION</u>

Chen C. Y., **<u>Vedpathak A. G.,</u>** Kallakuri S., Malaviya P., Cavanaugh J. M. (2010) "Effect of Ultrasonic Versus Electrosurgery Cutting Devices on Sciatic Nerve Function: A Neurophysiological and Histological Assessment" 56th Annual Proceedings of the Orthopedic Research Society, March 6-9, New Orleans, LA.

<u>AWARD</u>

Graduate Professional Scholarship, Wayne State University   (2009 - 2010)