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Studies in nerve electrophysiology

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Rice University, 1989
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Studies in Nerve Electrophysiology

by

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A Thesis Submitted
in Partial Fulfillment of the
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Master of Science

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Studies in Nerve Electrophysiology

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Abstract

In this thesis, two separate studies in nerve electrophysiology are described. Both of these studies are seen to have clinical relevance in terms of the treatment and diagnosis of peripheral nervous system dysfunction and injury.

The first study, "Electrophysiological and Histological Studies of Laser and Suture Anastomoses in Rat Sciatic Nerves," compares a conventional epineural suture repair technique to a "laser-assist" technique which combines fewer epineural sutures with CO₂ laser welds to seal the epineurium. The results show that the conduction velocity of compound action potentials (CAPs) distal to the anastomotic site was decreased similarly when compared to controls in both groups as seen in the literature. However, the "laser-assist" group exhibited lower stimulation thresholds when compared to the suture group. The suture group exhibited greater branching of regenerating axons proximal to the anastomosis while permitting a number of axons similar to the "laser-assist" group to pass through the anastomosis. The result was greater neuroma formation in suture repaired nerves.

The second study, "Potential Field from an Active Nerve in an Inhomogeneous Anisotropic Volume Conductor--Experimental Verification of the Inverse Problem", a field theoretic technique previously presented in the literature for the recovery of CAPs originating from nerves embedded in a muscular limb containing a bone via field potentials recorded on the surface of the limb is discussed. This technique was tested experimentally in a bullfrog thigh containing a single sciatic nerve source. The recovered CAP waveforms generally matched the shape of CAP waveforms
recorded at the surface of the nerve. However the conduction velocity parameter used in the field theoretic model utilized to recover the CAP waveforms was out of the physiological range of values recorded for this parameter. The errors are thought to result from errors in the measurement technique employed and idealizations inherent in the field theoretic model itself.
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Chapter 1

Introduction

The role of the clinical neurologist, as with all clinicians, is to provide accurate diagnosis of disease followed by the most effective treatment available. This requires a thorough understanding of the principles involved in the disease process as well as the techniques used to diagnose and treat the disease. New and interesting advances in the basic science of neurophysiology now allow the clinician to accomplish things that were not thought possible. In this thesis, two new techniques associated with the diagnosis and treatment of neurophysiological disorders in the peripheral nervous system are explained. These techniques and the background neurophysiology associated with some peripheral nervous system disorders are discussed in terms of experimental studies conducted and observations found in the relevant literature.

1.1 Background

In order to understand abnormal peripheral nervous system physiology, it is necessary to understand normal peripheral nervous system physiology. As diagrammed in Fig. 1.1, the peripheral nerve consists of thousands of nerve cell axons which run from a centrally located cell body by means of an axonal process to an end organ. Sensory nerve cells have their cell bodies located in the dorsal root outside the central nervous system (CNS) ganglia while motor neurons have their cell bodies located inside the spinal cord, part of the CNS. Nerve cell axons are either myelinated or unmyelinated, depending on the relationship of their associated Schwann
Figure 1.1: Peripheral nervous system anatomy [47].

cells. As shown in Fig. 1.2, myelinated axons are tightly wrapped by Schwann cells while unmyelinated axons are simply surrounded by Schwann cells. The axons and their associated Schwann cells are enclosed in a loose connective tissue endoneurium. This unit is called a fascicle. Fascicles are in turn grouped into fascicular bundles by a connective tissue perineurium. The fascicular bundles are held together in the nerve by a dense connective tissue epineurium. Blood vessels course within the structure supplying the necessary nutrients [78].

Motor fibers are myelinated while sensory fibers are both myelinated and unmyelinated. For example, sensory fibers associated with quick reflexes are typically myelinated while those fibers associated with the sensations of pain and tempera-
Figure 1.2: Comparison of myelinated (A) versus unmyelinated (B) axon. Note the number of Schwann cell myelin wraps in the case of the myelinated axon. 1, nucleus and cytoplasm of Schwann cell; 2, axon; 3, microtubule; 4, neurofilament; 5, myelin sheath; 6, mesaxon; 7, node of Ranvier; 8, interdigitating process of Schwann cells at the node of Ranvier; 9, side view of unmyelinated axon; 10, basal lamina [47].
ture are typically unmyelinated. Postganglionic autonomic fibers are also typically unmyelinated.

Nerve cells are designed to transmit information along the nerve. Generally, motor axons transmit information from the spinal cord to muscles, while sensory axons transmit information from the sense organs to the spinal cord. This information is transmitted by means of action potentials. Nerve cells possess thin plasma membranes which separate positive and negative ions such that while in a resting, passive state, the intracellular medium of the nerve cell has a negative potential with respect to the outside of the cell. This passive state is established by the interaction of ion selective and voltage dependent channels and energy dependent ion pumps present in the cell membrane along with large negatively charged macromolecules that cannot pass through the cell membrane. This interaction results in a higher concentration of Na\(^+\) ions in the volume conductor outside the nerve cell than inside, and a higher concentration of K\(^+\) ions inside the nerve cell than outside. For a more detailed explanation of the resting state of the membrane state, see [38,44,66].

When an action potential is initiated (normally at the cell body or sense organ), positive ions enter the nerve cell, thus depolarizing the membrane. If the membrane is depolarized to a certain threshold voltage, channels which are selective for Na\(^+\) ions are opened in a membrane voltage dependent process. As a result, Na\(^+\) rushes into the cell and depolarizes the cell membrane to some peak value. This depolarization spreads via displacement current down the axon, activating adjacent inactive regions. The in-rush of Na\(^+\) ions is short-lived and rapidly inactivated. Regions that have depolarized are subsequently repolarized as the result of a delayed outward flow of K\(^+\) ions (a hyperpolarizing effect) which restores the original membrane potential. A schematic diagram of the currents flowing within the axon and volume conducting fluid during the process of depolarization and repolarization
is shown in Fig. 1.3. The portion of the action potential which is maximally negative is associated with that portion of the membrane which is acting as a current sink for inward Na⁺ current flow through active Na⁺ channels, while the initial positive region is associated with regions of the membrane experiencing an outward flow of forward displacement current and the trailing positive region is associated with the flow of delayed, outwardly directed K⁺ current through K⁺ channels in the repolarizing region of the membrane. The flow of currents in the axon and the surrounding volume conductor is closed-path in nature (solenoidal) [44,48,66]. The potential distribution in the volume conductor on the surface of the nerve is mathematically approximated by the second spatial derivative of the transmembrane potential. Both the transmembrane potential and the approximation to the potential distribution on the surface of the nerve in the volume conductor are shown in Fig. 1.3.

The rate at which the action potential moves along the axon is dependent on the diameter of the nerve cell, temperature and myelination. Larger diameter nerve cells transmit action potentials faster because the axial resistance of the axon is less [43,44,54]. A linear relationship between fiber diameter and conduction velocity was observed as early as 1927 [29]. Since then it has been observed that myelinated fibers follow this linear relationship [41] while unmyelinated fibers relate to the square root of the fiber diameter [36]. Since larger diameter axons are associated with a larger membrane surface area/volume ratio, they exhibit higher thresholds of activation by intracellular stimulating currents [44], action potentials of shorter duration [56], and larger action potential currents resulting in larger field potentials [54]. In contrast to intracellular stimulating currents, larger axons exhibit lower thresholds of activation by extracellular stimulating currents in which the flow of intracellular current in response to electrodes placed far enough apart produces primarily axial
Figure 1.3: Diagram of axonal membrane voltage changes. The flow of current is diagrammed in (A) for the simple case of an unmyelinated axon. The inward flow of current depolarizes the membrane resulting in the change in transmembrane voltage (B). The transmembrane change in voltage results in a change in the potential at the surface of the nerve (C), which is related to the second spatial derivative of the transmembrane voltage. The transmembrane voltage is from synthetic data formed by the sum of three Gaussian distributions [20]. Note the change in scale from transmembrane voltage to spatial potential.
intracellular currents. Since the resistance to axial current scales by the inverse of the square root of the fiber diameter, the resistance for larger fibers will be less and hence stimulated with less current [44]. Action potentials are transmitted faster at higher temperatures because the channels which allow Na⁺ and K⁺ ions to enter open and close faster at higher temperatures. Axonal resistances (both axial and membrane resistances) are also less at higher temperatures, lending to higher action potential conduction velocities. Again, these effects of temperature are based on mathematical simulations which take account of the temperature dependence of the parameters involved [54]. The myelin sheath of myelinated fibers increases the effective resistance of the axonal membrane, forcing displacement currents to activate more distal regions, thereby increasing the conduction velocity of the action potential. This increase in resistance can be seen in Fig. 1.2 by noting that the resistance associated with successive myelin wraps would tend to increase the total resistance by adding in series. In a similar fashion these successive myelin wraps also decrease the total membrane capacitance. This also enhances conduction velocity by decreasing the amount of current necessary to charge the membrane which then becomes available for activation of further nodal regions. In myelinated axons, these activated regions are further apart, being segregated by the myelin sheath to areas called nodes of Ranvier. The ionic channels responsible for the action potential also follow this segregation with the Na⁺ channels clustering heavily in the nodes and the internodes populated mainly by K⁺ channels [77]. The propagation of the action potential along the axon was first described as a “jumping” of the action potential from one active node to the next and thus termed saltatory conduction [42]. The action potential does not really “jump”, but rather adjacent nodes are in relative states of activation or inactivation. The flow of currents and resulting transmembrane potential is diagrammed in Fig. 1.4. Saltatory conduction results
Figure 1.4: Diagram of flow of currents in a myelinated membrane and surrounding volume conductor (A). Note how current flow is spread among several nodes and that several nodes are active. Some current flows through the myelin but this flow is restricted somewhat. The separation of nodes results in the “scalloped” transmembrane potential (B) obtained from computer simulations of myelinated fibers [35].
in the action potential being conducted much more quickly along the axon when compared to unmyelinated fibers [44,48,66].

Propagation of the action potential is affected by the volume conduction of currents outside the axon. Volume conductors of low resistance allow adjacent regions of an active nodes to be easily activated by the flow of solenoidal current. In the case of highly resistive volume conductors, a very small volume or a resistive fluid, the flow of volume conducted currents will be impeded and will thus be expected to slow action potential conduction velocity.

When nerve cell axons are bundled together in a nerve, the Schwann cells and surrounding connective tissue prevent the volume conducted potentials from activating neighboring axons. However, in the case of a highly resistive volume conductor, individual fibers interact with each other, provided they lie sufficiently close to one another. This interaction does not induce action potentials, but serves to facilitate them such that the action potentials in adjacent fibers tend to pull each other along in the direction of propagation [49,50].

When several axons simultaneously transmit action potentials, the field potentials produced by the individual fibers add in the volume conductor surrounding the nerve resulting in the compound action potential (CAP). Such a summation is shown in Fig. 1.5. The magnitude of the different peaks of this summation are dependent on the number and fiber diameter of the active component fibers in the nerve trunk. If the nerve trunk contains nerve fibers of uniform diameter and hence uniform conduction velocity, then the total field potential magnitude reflected in the CAP will remain constant, otherwise the magnitude will decrease as the CAP travels the length of the nerve and faster action potentials speed away from the slower action potentials. This dispersion is diagrammed in Fig. 1.6. With regard to the overall shape of the CAP, the impulse volley in the fastest conducting fibers
Figure 1.5: The summation of several single fiber action potentials (SFAPs) is shown to form a compound action potential (CAP). This summation was formed by assuming a general shape for the SFAPs based on the volume conductor and a conduction velocity distribution calculated from a nerve biopsy [67].

determines the conduction velocity and peak amplitude of the CAP, however the total duration and specific shape of the response is dependent on contributions from slower conducting fibers [34]. Schoonhoven relates this impulse volley in terms of the distribution of fiber conduction velocities, with particularly the faster (larger diameter) fibers responsible for the overall shape of the CAP [67].

1.2 Correlation with PNS Injuries and Disease Processes

As a result of the combination of human nature and technology, peripheral nerve injuries due to grenades, switchblades, and sliding glass doors will always be prevalent
Figure 1.6: The compound action potential (CAP) is seen to disperse as the CAP travels the length of the nerve. In this schematic representation, the nerve is seen at (A) the site of stimulation, (B) 2.5 cm from the site of stimulation and (C) 5 cm from the site of stimulation (data modeled after [67]).
While the incidence of peripheral nervous system disease is not as common as heart disease, its incidence and impact on an individual are such that it cannot be ignored. It is estimated that 40 people in 100,000 will suffer some sort of peripheral neuropathy, while 2 in 100,000 will suffer from Guillane-Bare syndrome [40]. An unknown number of critically ill patients will suffer from a form of neuropathy which is often undetectable in these patients [14]. One in one thousand births will suffer from peripheral nervous system complications associated shoulder dystocia [55].

Peripheral neuropathies can be divided into three groups: neuronopathies, axonopathies, and myelinopathies [75]. Neuronopathies are characterized by cell death of the entire nerve cell, due to death of the cell nucleus, while axonopathies are characterized by death of a distal portion of the axon and myelinopathies are characterized by demyelination. Typically, diseases are often a combination of these to varying degrees. Neuronopathies and axonopathies would be expected to both show a reduction in the amplitude of the CAP. The findings associated with axonopathies would vary postionally, while neuronopathies would be symptomatic along the entire length of the nerve. Myelinopathies demonstrate a slowing or even block of the CAP. These are either focal or symptomatic (ie. localized or extended) over a length of the nerve. The location of some peripheral neuropathies is diagrammed in Fig. 5 [73]. Generally, except for the uncommon diseases, the largest myelinated fibers are the most sensitive to damage while the unmyelinated fibers are the most resistant [79].

Peripheral nerve injuries can be divided into three classes: neurotmesis, axonotmesis, and neuropraxia. Neurotmesis is a cut injury of the nerve, axonotmesis is crush and neuropraxia is compression injury [73]. The crush and cut injuries are often indistinguishable and are differentiated histologically, depending on whether
the basal lamina of the Schwann cell is left continuous [75]. The immediate result of a crush or cut injury is the cessation of CAP transmission due to the destruction and death of the nerve from the injury site to the periphery. Conduction block may also be seen in compression injuries, however nerve damage resulting in death is not typical. The presence of a compression injury may go undetected until conduction block is noticed. Prior to this time the compression injury may present to the clinician as a focal slowing of the CAP due to focal demyelination caused by the compression [75]. Some compression injuries are acute, such as when one temporarily disturbs the circulation to a nerve and the limb “falls asleep”.

As described in Chapter 2, in order for functional return to occur in the case of the crush and cut injuries, the nerve must grow from the injury site back to the original peripheral end organ to which it was attached. In the case of the crush injury, recovery is usually complete, however, recovery is only around 50% in the case of cut injuries [57].

Diagnosis of peripheral nerve injuries and peripheral neuropathies is dependent on clinical testing. With the above discussion in mind, the parameters that are useful are the amplitude, shape and conduction velocity of the CAP. Current technical limitations and misunderstandings often prohibit accuracy in electrophysiological measurements. Such misunderstanding range from lack of technical expertise (saturation of amplifiers evident in recordings [8]) to misinterpretations of the volume conductor and the nature of the first peak in the CAP [3, pg. 268]. The parameters from a recorded CAP are all influenced by the volume conductor surrounding the nerve, ie. the muscle tissue, and the electrodes used to record the CAP. Volume conducted CAP recordings (eg. limb surface potential measurements) can be spatially averaged by large recording electrodes because the currents producing the CAP originate from several sites along the nerve and not just the point of inter-
est directly underneath the electrode and hence the current from several sites will appear simultaneously at the electrodes [67]. This can be circumvented by the use of small needle electrodes which can be placed next to the nerves. However, in addition to not being well-tolerated, recordings with needle electrodes exhibit low repeatability (typically compound action potential (CAP) amplitudes can vary 20-40 % in one patient and subject to subject variations can be twice as much) and as a result are difficult to quantify [13,62]. Additional limiting factors associated with electroneurological examination include the current clinical state of the patient as well as the possible inaccessible location of the nerve of interest due to overlying anatomy.

1.3 Regeneration in Cut Rat Sciatic Nerve Repaired with a Carbon Dioxide Laser

Surgical technique in the repair of peripheral nerve lesions has been for the most part empirical. Since relatively little is known about the neural regeneration process that takes place, the results of surgical repair techniques are judged largely on the basis of patient outcome. Originally, it was thought that it was necessary to realign cut fascicles as exactly as possible so that regenerating axons could find their original paths to the periphery. Now, it is thought that this original path is almost completely destroyed and that all guidance to peripheral end-organs is centrally directed (see review by Wynn Parry [57]). Undoubtedly, alignment must still be important to some degree, however there are other factors that must be considered such as scar and neuroma formation at the site of reanastomosis of cut nerve ends. Scar associated with a foreign body response to suture material is thought to impede regrowth of axons and to play some role in neuroma formation [27,71]. Neuromas are now associated with the excruciatingly painful syndrome of causalgia as well as
with impeding conduction through the anastomotic region [57]. Since it is found clinically that relatively little nervous tissue is necessary for a good to adequate return of function, it is essential that techniques be devised to improve the quality of existing nervous tissue and hence lessen the incidence of neuroma formation.

In Chapter 2 a technique of “laser-assist” reanastomosis of severed nerves is proposed. Results of animal experiments are used to compare this technique with conventional suture methods of nerve repair. As described in the Chapter 2, Sprague Dawley rats were divided into two groups, laser-assist and suture repair. The left sciatic nerve of each animal was cut with a scalpel and the nerve was reanastomosed with one of the techniques. The right nerve was untouched and serves as control for the animal. Approximately 11 months later, both nerves were removed and studied electrophysiologically to measure resulting compound action potentials, thresholds, and conduction velocities. Some of the nerves were then examined in cross section both proximal and distal to the anastomosis to confirm electrophysiological observations. Although the data is not decidedly conclusive, our “laser-assist” repair comparison study demonstrates some interesting aspects of peripheral nervous system regeneration. Specifically, this technique may prove to be useful in the treatment of peripheral nerve injuries due to an apparent reduction in neuroma formation compared with the neuroma formed utilizing the conventional suture method of repair.

1.4 Volume Conduction in Experimental Studies and Its Relation to Clinical Practice

As in most diseases, clinical history and diagnostic tests are the essential elements necessary to establish a diagnosis. In neurological disorders, often it is difficult to obtain useful diagnostic information in the form of electrophysiological recordings.
With an analytical technique derived from electromagnetic field theory, it is possible to account for the effects of the volume conductor associated with overlying anatomy surrounding an active peripheral nerve. Subsequently, the field-theoretic technique can be utilized to predict the form of the compound action potential (CAP) on the surface of the nerve buried within the volume conductor medium based on field potentials recorded elsewhere in the medium (e.g. the limb surface). Normally, neuroelectric examination would require the use of needle electrodes which results in the low repeatability of measurements as discussed above. Since the field-theoretic technique employs limb surface measurements, it does not require needle electrodes, and would consequently be well-tolerated by patients.

The validity of the analytical field-theoretic technique was studied in Chapter 3 in terms of an experimental model, a bullfrog thigh with a single peripheral nerve source (the sciatic nerve) passing through the thigh region. As described in Chapter 3, several traces of limb surface potentials were utilized to estimate the compound action potential (CAP) at the nerve by means of this analytical technique. The estimate obtained was then compared to actual recordings made at the nerve surface by means of an implanted electrode assembly. Despite the obvious simplifications posed by the model, these estimates of CAP waveform and amplitude appear reasonable. At the present time, it appears that this model-based technique is limited in its ability to recover the high frequency components present in the first peak of the CAP. It is thought that this is due to implicit modeling error inherent in the model and a spatial low pass filtering effect of the limb surface electrodes.
1.5 Summary

This thesis studies two problems in nerve electrophysiology. Chapter 2 describes the electrophysiological and histological aspects of nerve regeneration in nerves that have been cut and rejoined with either a conventional suture technique or a combined laser and suture technique. Chapter 3 describes the analytical technique used to estimate nerve surface CAPs from limb surface potentials. The results of studying the application of this technique to a bullfrog thigh are also discussed. The results and conclusions of these studies are discussed in Chapter 4.
Chapter 2

Electrophysiological and Histological Studies of Laser and Suture Anastomoses in Rat Sciatic Nerves

Conventional suture repair of peripheral nerves results in a fibrotic reaction which is detrimental to nerve regeneration [27,72]. As an alternative procedure, a laser can be used, along with a reduced number of sutures, to reanastomose severed peripheral nerves. This technique is referred to as "laser-assisted" repair of nerve and is expected to result in a reduced fibrotic reaction. The right sciatic nerve of Sprague-Dawley rats was surgically cut and reanastomosed either by means of 4 epineurial sutures or 2 epineurial sutures and CO₂ laser welds. After a mean of 347.5 days, both sciatic nerves were excised. Only the properties of myelinated and large diameter unmyelinated axons were considered. The left, unoperated sciatic nerve is normal and serves as a control for the animal. Each nerve was placed in a lucite bath filled with warmed (37°C) or room temperature Kreb's solution; the nerve was stimulated with a brief voltage pulse (0.01 msec duration), and both threshold and supramaximal threshold were measured. Compound action potentials (CAP) were recorded at several spatial sites along each nerve and were used to calculate the conduction velocity as a function of distance along the nerve. Similar to the findings of Bailes et al. [4,5,6], the conduction velocities recorded for suture and laser-assist repaired nerves were not found to be significantly different. However, laser repaired nerves exhibited lower conduction failure rates and stimulation thresholds than nerves with conventional suture repair. If there is a difference in the average nerve fiber diameter of the laser nerves compared to the suture nerves, it was too small to be resolved by the line sampling technique [11] employed due to the small sample size of nerves examined. However, it was apparent that suture nerves exhibit greater branching of myelinated axons proximal to the anastomosis and at the same time permit fewer axons through the anastomosis when compared to laser repaired nerves. This might explain the greater incidence of neuroma formation in sutured nerves, as well as the diminished conduction through the anastomosis in sutured nerves.

2.1 Introduction

Several reviews exist of conventional repair techniques for severed peripheral nerve [53,69,71,80]. Each suture technique demonstrates the supposed necessity of re-
aligning the fascicles in order for any return of function to occur. The preferred method of conventional repair involves mobilization of the nerve to overcome any gap created between lacerated nerve stumps, followed by resection and epineurial suturing of the proximal and distal stumps [51,71]. However, conventional suture repair results in a fibrotic reaction which impedes the growth of regenerating axons past the suture line [27,72]. A careful review of these and other techniques to reduce this effect are reviewed by Fields and Ellisman [26]. Laser techniques which have been attempted include sheathing by a coagulum of red blood cells formed by argon lasing [1,2], and similar to what is described here, CO₂ laser welds of the epineurial sheath [4,5,6,27,28]. Each is designed to attempt to maximize the number of axons regenerating past the line of reanastomosis.

Our studies and others [27,28] have indicated that CO₂ laser welds alone do not provide adequate tensile strength to be practical. In order to augment this, we have combined procedures such that nerves were repaired with half of the usual number of epineurial sutures and laser welds of the epineurial sheath. In this so-called “laser-assist” technique the sutures will serve to augment tensile strength and provide some alignment while the laser welds will also provide some alignment as well as, more importantly, a seal of the epineurium. It is thought that the alignment achieved by laser welds might be superior to sutures in that a coagulated sheath of the epineurial connective tissue will prevent regenerating axons from straying beyond their original course. As a result, neuroma formation should also be less.

Electrophysiological “wellness” of a nerve is based on how easily the nerve fibers composing the nerve are stimulated as well as how fast these fibers then conduct the resulting compound action potential (CAP). In this study, only myelinated fibers were considered. As described in Chapter 1 the shape of the CAP can provide qualitative information on the number of fibers present and perhaps some infor-
mation related to pathologies associated with conduction, such as demyelination. Basal stimulation thresholds are related to nerve diameter, with larger fibers having smaller threshold values. As discussed in Chapter 1, conduction velocity is also related to fiber diameter [68]: myelinated fibers relate linearly to fiber diameter while unmyelinated fibers relate in a square root fashion. Conclusions based on electrophysiological measurements could then be supported by histological measurements regarding fiber diameter.

When a nerve is severed, each nerve fiber within the nerve dies from that point to the periphery in a process known as Wallerian degeneration. The proximal segment of the axon recedes into the proximal nerve stump and swells. The distal myelin sheath of Schwann cells collapses so that the original tube in which the nerve traveled is lost. In the poorly understood process of regrowth, the regenerating fibers begin to branch and grow distally. This growth is thought to be directed by either a locally directed search for the original tube which may exist as a connective tissue framework or by some sort of centrally directed mechanism in which the fiber is “pre-programmed” where to grow [57]. As a result of branching, more fibers may be observed in the region immediately proximal to the anastomosis and within the anastomosis itself. Some of these branches continue towards the periphery, while some die back and some persist in the anastomotic zone resulting in neuroma formation. Eventually Schwann cells reassociate with the regenerating fibers and form a new myelin coat unlike the original. The entire process is quite variable, depending on the prevailing conditions [45,46].

Temperature has been noted to have an unusual effect on demyelinated nerve, such that conduction block is observed both experimentally and clinically in multiple sclerosis and as well as in mathematical simulations of axonal conduction at higher temperatures [64]. Several experimental studies have noted the dependence
of normal CAP conduction velocity on temperature, for example, either a linear [41] or an exponential [36] dependence. Attempts at explaining the nature of these temperature effects have been presented in mathematical models. These mathematical simulations have also demonstrated a linear [41] or exponential dependence [54] on temperature. Simulations of demyelination disease conducted by Schaup and Davis [64] show a linear dependence on temperature with the slope decreasing as demyelination increases. The discrepancy seen in these modeling studies between linear and exponential dependence of conduction velocity on temperature originates from the particular parameters chosen to exhibit temperature dependence. Moore et al. [54] note the sensitivity of conduction velocity to axoplasmic resistance and ionic channel rate constants which are allowed to change in their simulations with temperature. They also note that conduction velocity is sensitive to myelin capacitance, however they do not allow this capacitance to change with temperature. With the foregoing in mind, we felt that more insight concerning regeneration in conventional suture repair or laser-assist repair of severed nerves could be obtained by recording all measurements at both room temperature and elevated temperatures close to body temperatures.

2.2 Methods and Materials

Sprague-Dawley rats were anesthetized with an anesthetic cocktail (ketamine hydrochloride, 42.8 mg/mL, xylazine, 8.6 mg/mL, acepromazine, 1.4 mg/mL; 0.5-0.7 mL/kg body weight i.m.) before each procedure. The right sciatic nerve was cut with a surgical knife and reanastomosed under magnification using either four epineurial sutures (n = 20) or two stay sutures and a CO₂ laser weld of the epineurium (n = 18). Nylon suture material (10-0) was used. The laser employed was a Xanar model XA–20 articulator CO₂ surgical laser with a pinhole attenuator
to reduce normal output of 20 W to 1 W. For laser welds, 0.05 sec exposure times of approximately 100 mW with approximately one minute handling time was typical. The spot size was 0.2 mm in diameter.

Approximately one year later (mean of 347.5 days), both nerves were excised with care taken to remove as much nerve above and below the anastomosis as possible. Each nerve was placed in a lucite bath containing a linear array of 16 silver wire electrodes and filled with warmed (36.6±0.17°C average) or room temperature (25.5±0.14°C average) Kreb’s solution (124 mM NaCl, 5 mM KCl, 1.2 mM K₂HPO₄, 1.3 mM MgSO₄, 26 mM NaHCO₃, 2.4 mM CaCl₂, 20 mM dextrose oxygenated with 100% O₂ [12]). The proximal end of the nerve was lifted up out of the bath and stimulated at a rate of 7-8 Hz with a brief pulse (0.01 msec) and simultaneous recordings were obtained from several sites along the nerve with respect to a large reference electrode placed at the end of the bath as diagrammed in Figure 2.1. Recordings were amplified 20,000 times, filtered (15 Hz to 10 KHz passband), digitized (ISC-16, RC Electronics) at a rate of 1 MHz (62.5 KHz per recording electrode) and averaged (128 times) to obtain a better signal-to-noise ratio. Base threshold and supramaximal threshold were obtained for the particular spatial site lying immediately distal to the anastomosis. Recordings were obtained at a stimulus intensity corresponding to supramaximal threshold. All recordings were obtained within a grounded Faraday cage with stimulus isolation.

The conduction velocity at each site was calculated as the time for a CAP peak to move from the most proximal electrode to subsequent electrodes divided into the distance traveled. This method would not naturally show sudden changes but rather an “averaged” conduction velocity. Each conduction velocity measurement was divided by the averaged conduction velocity obtained from the control nerve in that animal to obtain a conduction velocity index for several spatial sites along the
Figure 2.1: Experimental apparatus used to study electrophysiology of normal and repaired rat sciatic nerve in vitro.

nerve. Since the center of the anastomosis was not located at the same electrode for each nerve, the data was shifted so that the center of the anastomosis fell at the 10 mm mark (n.b. the eight mm mark is halfway along the linear array of 16 recording electrodes which are separated by 2 mm). The conduction velocity at each site was then averaged among each group, laser repairs at 26° C, laser repairs at 37° C, suture repairs at 26° C, and suture repairs at 37° C. To compare the accuracy of the method, the conduction velocity at each site along the normal nerves was divided by the average conduction velocity obtained by that method to obtain another conduction velocity index for several spatial sites along the normal nerve.
Two normal nerves, four suture repaired nerves, and three "laser-assist" repaired nerves were fixed by immersion, embedded in plastic, sectioned into first cross sections and then into longitudinal sections with a microtome into semithin sections (0.5 to 1 μm), and stained with hematoxylin and eosin. Three viewing fields of the cross sections at 400x were digitized and two perpendicular lines were drawn across each field. The outer diameter of all axons (myelinated and any discernible unmyelinated axons) touching the two lines was measured. The technique of line sampling analysis [11] extrapolates this one-dimensional region into a two-dimensional region containing exactly 2000 axons according to

\[ N_i = n_i \cdot (X - d_i)/(d_i + 2 \cdot R) \]

where \( n_i \) counted axons of diameter \( d_i \) in the one-dimensional region of length \( Y \) are extrapolated to \( N_i \) axons of the same diameter in a two-dimensional region of area \( X \cdot Y \). A parameter \( R \) was used to minimize errors associated with diameter measurement inaccuracy and was given a value of 0.56 μm. Given the \( n_i \) and the desired total count of 2000 axons, the data can be written as a system of equations in which the \( N_i \) were solved for by Gaussian elimination. The line length \( Y \) was the total line length in all three viewing fields.

Since only one cross section for each nerve was considered, the data would need to be corrected to account for the deviations from true fiber diameter due to the irregular nodal and perinodal regions as well as the waxing and waning in fiber diameter associated with the internodal region; these deviations are observed to occur as an axon is followed through several cross sections. This correction was suggested by BeMent and Olson and assumes that this deviation is characterized by a Gaussian random variable [9] with a mean value of the associated bin diameter and a standard deviation linearly dependent on fiber diameter. Since the data needed to approximate this relation was not available, a constant standard deviation of 0.5 μm
was used here. In matrix form \( N = A \cdot N' \), where \( N \) is the vector of extrapolated line sampling values, \( A \) is a matrix of probabilities characterized by the random variable, and \( N' \) is the vector of corrected line sampling values which was solved for by Gaussian elimination. When compared to data in which this correction was not taken into account, this correction was not thought to add any more information to the data and was not included in the regular analysis.

The cross sections were again viewed at 100x and the images were digitized in order to measure the total nerve cross sectional area. The extrapolated number of fibers in a cross section is then 2000 times the measured area divided by extrapolated viewing field area.

The longitudinal sections were viewed at 80x to determine the approximate distance of the cross sections from the anastomosis. After approximately 11 months post-operative, this site was not readily discernable. The point was taken to be where any residual suture material was observed or where the neuroma was largest.

### 2.3 Results

#### 2.3.1 Electrophysiology

The voltages required to elicit observed conduction are presented in Table 2.1. Suture repaired nerves exhibited statistically significant larger basal thresholds than the laser-assist repaired nerves and the normal nerves, as shown in Table 2.1. Thresholds for laser-assist repaired nerves were not substantially different from normal nerves. The same observations are made in Table 2.2 which presents the voltages required to elicit observed supramaximal thresholds. These trends were observed at both temperatures.

Typical CAP waveforms are depicted in Figures 2.2 and 2.3. A diminishment in CAP amplitude is observed in the traces as the recording electrode approaches
Figure 2.2: Typical CAP waveform traces from an animal with laser-assist repair. Each trace was obtained from one of 16 electrodes in the lucite chamber. (a) Normal nerve at 26°C. Note the strong positive potential on the last electrode. (b) Normal nerve at 37°C. (c) Laser-assist repaired nerve at 26°C. Note the conduction delay in waveforms. (d) Laser-assist repaired nerve at 37°C.
Figure 2.3: Typical CAP waveform traces from an animal with suture repair. Each trace was obtained from one of 16 electrodes in the lucite chamber. (a) Normal nerve at 26°C. (b) Normal nerve at 37°C. (c) Suture repaired nerve at 26°C. Note the conduction delay in waveforms. (d) Suture repaired nerve at 37°C.
Table 2.1: Basal Stimulation Threshold in Volts

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Normal</th>
<th>Laser-Assist</th>
<th>Suture</th>
</tr>
</thead>
<tbody>
<tr>
<td>26°C</td>
<td>1.71±0.14</td>
<td>1.99±0.19</td>
<td>4.51±0.81†</td>
</tr>
<tr>
<td></td>
<td>(n=16)</td>
<td>(n=8)</td>
<td>(n=9)</td>
</tr>
<tr>
<td>37°C</td>
<td>1.90±0.39</td>
<td>1.52±0.13</td>
<td>3.21±0.70†</td>
</tr>
<tr>
<td></td>
<td>(n=14)</td>
<td>(n=8)</td>
<td>(n=7)</td>
</tr>
</tbody>
</table>

† Statistical Difference with Normal Controls (Student’s t-test: P ≤ 0.05)

Table 2.2: Supramaximal Stimulation Threshold in Volts

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Normal</th>
<th>Laser-Assist</th>
<th>Suture</th>
</tr>
</thead>
<tbody>
<tr>
<td>26°C</td>
<td>8.30±2.20</td>
<td>4.23±0.43</td>
<td>18.08±5.41†</td>
</tr>
<tr>
<td></td>
<td>(n=16)</td>
<td>(n=8)</td>
<td>(n=9)</td>
</tr>
<tr>
<td>37°C</td>
<td>5.80±1.62</td>
<td>3.60±0.54</td>
<td>11.30±3.15†</td>
</tr>
<tr>
<td></td>
<td>(n=14)</td>
<td>(n=8)</td>
<td>(n=7)</td>
</tr>
</tbody>
</table>

† Statistical Difference with Normal Controls (Student’s t-test: P ≤ 0.05)

the distal end of the nerve. This same phenomena was observed by Lorente de No in his experiments on excised frog sciatic nerve [52]. The waveforms obtained at higher temperature all show the expected reduction in peak CAP amplitude and narrowing of the base of the peaks. Waveforms obtained from repaired nerves show an observed delay in the peaks occurring at the approximate site of the anastomosis. In general there was little observed difference between the overall shape of CAP waveforms obtained from laser-assist and suture repaired nerves, however suture repaired nerves sometimes exhibited the overshoot noted in Figure 2.3.

Figure 2.4 shows the average conduction velocity and standard error versus recording electrode position determined for all normal nerves at two temperatures. One will note that conduction velocity is relatively constant along the length of the normal nerve and that the standard error in this measurement is relatively small.

Table 2.3 presents average conduction velocities measured. An average was
Figure 2.4: Standard error associated with the calculation of conduction velocity (see Methods) for normal nerve. Y-axis is conduction velocity index; x-axis is recording electrode position along the nerve. The velocity at 0.0 mm represents the velocity of the CAP waveform peak in traveling from electrode 1 to electrode 2. The velocity at 2.0 mm represents the velocity of the CAP waveform peak in traveling from electrode 1 to electrode 3 and so on. Data is presented as average ± standard error (N ≥ 23) at two temperature, (a) 26°C and (b) 37°C. Average conduction velocity for normal nerve at 26°C was 47.11±0.52 m/sec while at 37°C was 82.58±0.91 m/sec.
Table 2.3: Conduction Velocity of Repaired Nerves in meters/sec

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Normal</th>
<th>Laser-Assist</th>
<th>Suture</th>
</tr>
</thead>
<tbody>
<tr>
<td>26° C</td>
<td>47.11±0.52</td>
<td>22.69±0.64†</td>
<td>22.09±0.57†</td>
</tr>
<tr>
<td></td>
<td>(n=409)</td>
<td>(n=89)</td>
<td>(n=88)</td>
</tr>
<tr>
<td>37° C</td>
<td>82.58±0.91</td>
<td>43.82±1.074†</td>
<td>44.40±1.08†</td>
</tr>
<tr>
<td></td>
<td>(n=406)</td>
<td>(n=93)</td>
<td>(n=82)</td>
</tr>
</tbody>
</table>

† Statistical Difference with Normal Controls (Student’s t-test: P≤0.05)

obtained from all of the spatial sites for the normal nerves and from the the spatial sites corresponding to 12 mm from the distal end of the repaired nerves. Our calculated values for conduction velocity are somewhat higher than those reported by Fields and Ellisman [26] for suture repaired rat sciatic nerve, however their results were obtained in a moist chamber and not in a volume conductor and therefore would be expected to exhibit lower conduction velocities. When the conduction velocity index is considered, the repaired nerves at 37° C exhibited approximately a 45% decrease in the conduction velocity index (Figs. 2.5a, 2.6a). The repaired nerves at 26° C, however, show approximately a 55% decrease in the conduction velocity index. This is compared to the 40% decrease reported by Fields and Ellisman. This decrease was deemed significant (by the student’s t-test with a 95% confidence interval) and can be viewed in Figures 2.5b and 2.6b where the augmentation at higher temperatures is observed. The large error associated with the conduction velocity index proximal to the anastomotic site might be attributed to the variability of damage associated with the initial cutting of the nerves.

Table 2.4 shows the peak CAP voltages measured under supramaximal stimulation at the electrode immediately distal to the anastomosis site. Both the laser-assist and the suture repaired nerves exhibited significantly lower peak voltages than the normal nerves at an equivalent electrode. As noted by Fields and Ellisman [26],
Figure 2.5: Variation in conduction velocity index calculated for laser-assist repaired nerves. Y-axis is conduction velocity index, x-axis is recording electrode position along the nerve. The arrow indicates the center of the anastomosis. Data presented as average ± standard error. (a) Laser repair at 26°C $N \geq 9$.(b) Laser repair at 37°C $N \geq 10$. 
Figure 2.6: Variation in conduction velocity index calculated for suture repaired nerves. Y-axis is conduction velocity index, x-axis is recording electrode position along the nerve. The arrow indicates the center of the anastomosis. Data presented as average ± standard error. (a) Suture repair at 26°C \(N \geq 10\). (b) Suture repair at 37°C \(N \geq 10\).
Table 2.4: Peak Voltage Immediately Distal to the Repair Site in mVolts

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Normal</th>
<th>Laser-Assist</th>
<th>Suture</th>
</tr>
</thead>
<tbody>
<tr>
<td>26° C</td>
<td>0.133±0.013</td>
<td>0.0367±0.0083†</td>
<td>0.0314±0.0079†</td>
</tr>
<tr>
<td></td>
<td>(n=16)</td>
<td>(n=8)</td>
<td>(n=9)</td>
</tr>
<tr>
<td>37° C</td>
<td>0.105±0.014</td>
<td>0.0246±0.0069†</td>
<td>0.0123±0.0037†</td>
</tr>
<tr>
<td></td>
<td>(n=16)</td>
<td>(n=8)</td>
<td>(n=7)</td>
</tr>
</tbody>
</table>

† Statistical Difference with Normal Controls (Student's t-test: P≤0.05)

These lower values suggest incomplete numbers of regenerated fibers, subnormal fiber size, and less uniform utilization time in the repaired nerves distal to the anastomotic site. The lower numbers and decreased fiber size were confirmed histologically.

The overall performance of the nerves is presented in Table 2.5. Laser-assist repaired and suture repaired nerves respond differently to temperature in borderline cases of conduction, as diagrammed. Specifically, one laser-assist repaired nerve which exhibited slight conduction at 37° C failed completely to conduct at 26° C while conversely three suture repaired nerves exhibited slight conduction at 26° C and failed completely to conduct at 37° C. Complete failures and the total number

Table 2.5: Nerve Overall Performance

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Laser-Assist</th>
<th>Suture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Failed Conduction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>at 26° C</td>
<td>0</td>
<td>1/18 (5.5%)</td>
<td>0</td>
</tr>
<tr>
<td>Failed Conduction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>at 37° C</td>
<td>0</td>
<td>0</td>
<td>3/20 (15%)</td>
</tr>
<tr>
<td>Complete Failure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1/18 (5.5%)</td>
<td>2/20 (10%)</td>
</tr>
<tr>
<td>Total</td>
<td>0</td>
<td>2/18 (11.0%)</td>
<td>5/20 (25%)</td>
</tr>
</tbody>
</table>

Average Healing Time 347.5±2.4 days
of failed conductions show that suture repairs failed to conduct approximately twice as often as laser-assist repairs. No normal nerves failed to conduct.

2.3.2 Histology

Comparison of the fiber diameter histograms obtained by the line-sampling method for normal nerves to those found in the literature for myelinated fibers in rat sciatic nerve [65] demonstrates that the method supplies reasonable results, even at such low magnification and with fewer samples. The observed average fiber diameter is larger for our data which is reasonable since our animals were almost a year old versus the data shown in [65] for 4 week old animals. The calculated standard deviation also is larger which is to be expected since the larger unmyelinated fibers were included. Our method also predicted a total number of axons present in normal nerve that generally agreed with that found by Schmalbruch [65]. These observations are tabulated in Table 2.6 and shown in Figure 2.7(a-b).

Table 2.7 lists the results of the line sampling analysis. Since the sample was so small, only general trends with regard to diameters can be considered. The normal nerves have the highest average diameter. The average diameter for laser repaired nerves proximal to the anastomosis appear slightly larger than similar suture repaired nerves. The average diameter of nerve fibers distal to the anastomosis in

<table>
<thead>
<tr>
<th></th>
<th>Mean(μm)</th>
<th>Standard Deviation(μm)</th>
<th>Average Number of Axons Present in Cross Section</th>
</tr>
</thead>
<tbody>
<tr>
<td>Literature[65]</td>
<td>7.09</td>
<td>2.57</td>
<td>7288</td>
</tr>
<tr>
<td>Line-Sampling</td>
<td>8.80</td>
<td>2.69</td>
<td>8762</td>
</tr>
</tbody>
</table>
Figure 2.7: Comparison of literature rat sciatic nerve fiber histogram for normal nerve with that obtained by line-sampling of a normal nerve. Axes are fraction versus diameter in microns. (a) Nerve fiber diameter histogram reconstructed after Schmalbruch [65]; $\bar{x} = 7.09, \sigma = 2.57$. (b) Nerve fiber diameter histogram obtained by line-sampling of a normal nerve; $\bar{x} = 8.80, \sigma = 2.69$. 
Figure 2.8: Typical fiber diameter histograms. Axes are fraction versus diameter in microns. (a) Normal nerve, $\bar{x} = 8.80, \sigma = 2.69$. (b) Laser-assist repair, proximal to anastomosis, $\bar{x} = 8.08, \sigma = 3.17$. (c) Suture repair, proximal to anastomosis, $\bar{x} = 7.48, \sigma = 2.19$. (d) Laser-assist repair, distal to anastomosis, $\bar{x} = 6.82, \sigma = 2.50$. (e) Suture repair, distal to anastomosis, $\bar{x} = 7.36, \sigma = 2.37$. 
Table 2.7: Nerve Fiber Diameter Line-Sampling Results: Myelinated and Unmyelinated Fibers Visible at 400x

<table>
<thead>
<tr>
<th></th>
<th>Mean(μm)</th>
<th>Average Number of Axons Present in Cross Section</th>
<th>Distance from Anastomosis (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (n=3)</td>
<td>9.34</td>
<td>8238</td>
<td></td>
</tr>
<tr>
<td>Laser Prox. (n=3)</td>
<td>8.37</td>
<td>8601</td>
<td>3.20</td>
</tr>
<tr>
<td>Suture Prox. (n=4)</td>
<td>7.25</td>
<td>10599</td>
<td>3.53</td>
</tr>
<tr>
<td>Laser Dist. (n=3)</td>
<td>7.13</td>
<td>6871</td>
<td>3.15</td>
</tr>
<tr>
<td>Suture Dist. (n=3)</td>
<td>6.92</td>
<td>7699</td>
<td>2.30</td>
</tr>
</tbody>
</table>

both cases is similar. Typical nerve fiber histograms are presented in Figure 2.8(a-b). The standard error associated with each bar on the histograms from a sample of size \( n = 2000 \) in Figure 2.7(b) and Figure 2.8(a-e) is given by [11] as:

\[
S.E.(p) = \sqrt{p \cdot q / n}
\]

where \( p \) is the proportion of the population with a certain diameter and \( q = 1 - p \).

The average number of axons present in cross section shows that laser repaired nerves exhibited in proximal cross sections a number of axons similar to normal nerves, while suture repaired nerves in cross section exhibited a much larger fiber count. The reduction in fibers crossing the anastomosis is thus observed to be greater for the suture repaired nerves.

2.4 Discussion

In this study, we have attempted to discern differences that may exist between conventional epineurial suture repair of cut nerves and a laser-assisted repair technique. With regard to the techniques employed, we can only discuss the effects of the su-
tures or laser in the myelinated fibers, which were those observed histologically and electrophysiologically, i.e. no information on small unmyelinated fibers which outnumber myelinated fibers in normal nerves almost 2 to 1 [65] was obtained. In favor of the laser repair, the suture repaired nerves were noted to exhibit conduction failure in more cases than the laser-assist repaired nerves. However since the sample size is small, this statistic is of unknown significance. The laser-assist repairs were also noted to exhibit lower basal thresholds than the suture repaired nerves. However, with regard to conduction velocity, no difference was observed between the two techniques. This suggests that both groups are undergoing a similar regenerating process in which both are subjected to the same remyelination conditions. Waxman [15,74,76] notes that regenerating nerves exhibit shorter internodal distances and hence slower conduction velocities. Both of these possibilities are attributed to the regenerating and remodeling myelin coat. Interestingly, both repair groups showed less slowing through the anastomosis at temperatures closer to body temperature. Hence, it should be emphasized that regenerating nerves are not remyelinated in a simple way. Specifically, regenerating nerves undergo a process of myelin remodeling such that normal internodes do not reappear even 11 months post-injury [37]. In this remodeling process, the myelin sheath internodes grow and retract in a continuous process of remyelination and demyelination. Waxman [74] remarks (with the observations of Hirano and Dembitzer [39] in mind) that “subtle pathologies such as those which loosen the paranodal junction to expose the internodal axon to the extracellular space may shunt the entire internode and consequently interfere with conduction even if the sheath itself is maintained.” Waxman and Brill [76] note that the impedance mismatch of a demyelinated region can result in a complete conduction block. Such subtle pathologies combined with this impedance mismatch may be present in sutured nerves which could then produce the increased
thresholds observed. It is thought that the impedance mismatch can be overcome by shorter internodes proximal to the demyelinated region. Shorter internodes are observed in the remodelling process of regeneration [37] and may serve this purpose. Such short internodes would result in a lowering of CAP conduction velocity [74].

The results of the line-sampling analysis point to a lower average fiber diameter for both laser and suture repaired nerves distal to the anastomosis. This is also consistent with the lower conduction velocities measured in this region. Since the laser repaired nerves exhibited lower thresholds than the suture repaired nerves, one would expect a larger diameter for the laser repaired nerves, however a large difference was not observed. This poorer conduction through the anastomosis could be attributed to the greater neuroma that is assumed to exist in the suture repaired nerves since it was observed that a greater number of axons entered the region while a number similar to the laser repair case exited the anastomotic region as seen in Table 2.7. The fact that more branching was observed in proximal segments in the suture repair case is interesting and cannot be explained. This greater degree of branching is also reflected in the lower average diameter observed in proximal suture cross sections when compared to proximal laser-assist cross sections. The greater degree of branching could be due to the influences of a greater foreign body response since more sutures are present. Jenq and Coggeshall [45,46] have found that moving the distal segment further from the proximal segment in experiments using silicone tubes as conduits results in less branching. With this in mind, one could postulate the presence of chemical and cellular factors present in the degenerating distal segment that affect branching.
Chapter 3

Potential Field from an Active Nerve in an Inhomogeneous Anisotropic Volume Conductor—Experimental Verification of the Inverse Problem

Since in the electrodiagnosis of peripheral nerve disorders clinical information is usually obtained noninvasively, a solution to the inverse problem may be of considerable interest to the clinical neurologist. A fast, computationally efficient method has been proposed for cylindrical geometries applicable to the lower limb [81]. However, the experimental validation of this model remains to be demonstrated. To do this, large bullfrogs were sacrificed, the skin on the lower limb was removed, and the sciatic nerve was freed from the muscle and supportive connective tissue as it travels from the lower vertebral column to the knee. The objective was to study the field potentials from the single sciatic nerve source in a passive, anisotropic limb medium containing skeletal muscle and the femur bone. The nerve was stimulated with a brief voltage pulse and the compound action potential (CAP) is simultaneously recorded, amplified, and digitized at several sites on the surface of the limb. Several recordings were averaged to obtain an improved signal-to-noise ratio. An additional set of recordings was obtained on the nerve surface itself using a thin silver wire multiple electrode assembly which was located at the sciatic nerve. Nominal conductances and geometric information obtained from a magnetic resonance (MR) imaging scan of the limb in cross section were used. The potential at the surface of the nerve was predicted using an inverse digital filter obtained from a solution to the forward problem with a centric-bone [81]. This potential is then compared to that measured at the surface of the nerve.

3.1 Introduction

As previously discussed, estimation of the potential on the surface of an active nerve source from measurements obtained on the surface of a limb could provide the clinical neurologist with information for a more complete diagnosis in the treatment of peripheral nervous system disease. In this estimation problem, the information lost due to the "low-pass filter" behavior of the limb could be regained to show the
higher frequency characteristics of the normal compound action potential (CAP) source, as well as those unusual higher frequency characteristics associated with some nervous system pathologies. This problem is known as the "inverse problem" in electrophysiology, whereas prediction of the potential on the surface of the limb using a nerve CAP is known as the "forward problem." The forward problem is, understandably, theoretical and not applicable to clinical measurements, however its formulation is fundamental to an understanding of the more clinically relevant inverse problem.

The forward problem for a nerve in a cylindrically shaped, infinite medium has been approached using several different methods [7,18,19,20,21,32,33,52,63,70]. The formulation used here is a computationally efficient analytical technique presented by Wilson et al. [81,82] for a finite, cylindrically symmetrical medium representing a limb that contains a centered bone and an off-center single nerve trunk located at some reference angle $\epsilon$ as diagrammed in Figure 3.1. Each of the concentric cylinders represents a specific conducting medium, either marrow, bone, muscle or surrounding media. For each medium, the transverse specific conductivity ($\sigma_T$) is not necessarily equal to the specific longitudinal conductivity ($\sigma_L$). In this case, that particular medium is characterized by an anisotropy ratio ($\sigma_L/\sigma_T$). These conductivities are assumed to be constant and with no capacitive component [60]. The boundaries between adjacent media are considered to be membraneous, possessing both resistive and capacitive properties.

The solution of the forward (and resulting inverse) problem results from a solution to Laplace's equation under quasistatic conditions [59] with the limb centered in cylindrical coordinates. A single active nerve source is located within the limb at a specified radius from the center of the bone. (This constraint of a single nerve source leads to a unique inverse solution.) Due to the linear nature of Laplace's
Figure 3.1: Diagrammatic representation of frog thigh. In a bone-centered cylindrical coordinate system, the nerve is located at a distance $R$, and angle of $\epsilon$ from the reference angle $\theta = 0$.

equation, the technique may be extended to include additional sources provided that they lie parallel to the sciatic nerve and their geometry within the limb can be specified. With this idealized description, Laplace's equation is solved analytically as a two-dimensional Fourier transform. The forward solution can be viewed as an equivalent Fourier domain operation in which a two-dimensional filter (known as the forward filter) operates on nerve source potentials (CAPS) to predict electric fields throughout the limb.

Since the forward filter is uniquely specified, it can be inverted to obtain an inverse filter. As a result, the inverse solution can also be viewed as an equivalent Fourier domain operation in which the two-dimensional inverse filter operates
on field potentials at some distance from the nerve to predict source potentials originating from the nerve. A schematic representation of the forward and inverse techniques is shown in Fig. 3.2. The studies of Wilson et al. [81,82] utilized simulated compound action potential data. The goal of this study was to validate the model using limb surface CAP data and to compare the predicted nerve source CAPs to measured nerve source CAPs.

3.2 Mathematical and Computational Aspects

Following the development in Wilson et al. [82], the potential $\Phi(\rho, \theta, z)$ at any point in the thigh is described by Laplace's equation in cylindrical coordinates:

$$\nabla \cdot (-\sigma \nabla \Phi(\rho, \theta, z)) = 0$$

where the specific conductivity $\sigma$ was assumed to be real, and conditions of quasistationarity prevail, so that field propagation effects and medium inductivity can be neglected [59]. Although CAPs vary with time, the nerve was assumed to be uniform and the CAP wavefront was assumed to travel with uniform conduction velocity. Under these conditions, the field distribution at any given time instant is a spatially translated version of that at any other instant. The spatial extent of the current density field from the active region of the nerve source is also assumed to be very small relative to the length of the nerve. Therefore the nerve and limb may be treated as though they are of infinite length. Time dependence may therefore be converted to spatial dependence by scaling time points by a measured conduction velocity, even though the resulting spatial extent of the CAP waveforms may be longer than the physical thigh. Thus the time dependent CAP waveforms measured at several points around the thigh perimeter may be represented as a two dimensional (angular x longitudinal) spatial electric field [82].
Figure 3.2: Schematic diagram of forward and inverse problems. Forward filter $V(n, m)$ operates on internal limb potentials $\Phi_i(\theta, z)$ to produce external limb potentials $\Phi_e(\theta, z)$. Likewise, the inverted forward filter $V^{-1}(n, m)$ operates on external limb potentials to estimate internal limb potentials corresponding to the location of the nerve source. Waveforms are synthetic data from Wilson et al. [81].

Since the limb medium is modeled as concentric cylinders symmetrically disposed about the bone, a separable product solution to Laplace’s equation can be assumed to exist. This solution is a two dimensional Fourier transform relationship with $F_{SO}(n, k)$ representing the spatial two dimensional Fourier transform of the nerve source potential while $W_{R,r}(n, k)$ is regarded as a two dimensional filter which represents the combined effects of all thigh tissues on the source potentials. In this notation, $n$ is the angular spatial frequency and $k$ is the longitudinal spatial frequency. If the nerve is located at the reference angle, then $\epsilon = 0$ and this factor becomes unity.
\[
\Phi(\rho, \theta, z) = \sum_{n=-\infty}^{\infty} \sum_{k=-\infty}^{\infty} W_{R,r}(n, k) \cdot F_{SO}(n, k) e^{-j k z} e^{-j n(\theta-\epsilon)} = 2D - \text{IDFT}[W_{R,r}(n, k) \cdot F_{SO}(n, k) \cdot e^{j n \epsilon}]
\]

The filter \( W_{R,r}(n, k) \) reflects the consequences of confining an isolated nerve in the finite medium of the thigh (which enhances the nerve potentials) and the volume conductor filtering effects of the thigh medium between source and thigh source, which results in attenuated and spatially low-pass filtered CAPs measured on the limb surface. The general solution can be decomposed into a product of two components where \( W_{R,R}(n, k) \) is responsible only for the complex confining effects and \( V_{R,r}(n, k) \) (Fig. 3.3) is responsible only for the passive attenuating effects of volume conduction [81]. Only \( V_{R,r}(n, k) \) is inverted to solve the inverse problem, because only the enclosed CAP at the outer surface of the nerve is desired.

Two dimensional Fourier transformed thigh surface CAPs \( G(n, k) \) are multiplied by \( V_{R,R}^{-1}(n, k) \) (obtained from the forward filter designed for a nerve of diameter \( d \) located at a distance \( R_s \) and angle \( \epsilon \) in the central coordinate system of Fig. 3.1.) to obtain an estimate of potential corresponding to a radius concentric with the outer surface of the nerve \( (R_s + d, \theta, z) \) that is:

\[
\Phi(R_s + d, \theta, z) = 2D - \text{IDFT}[V_{R,R}^{-1}(n, k) \cdot G(n, k) \cdot e^{j n \epsilon}].
\]

The potential corresponding to the \( \theta \) position of the nerve, is taken to be the inverse problem approximation to the CAP.

For computational efficiency, the infinite summations are truncated above and below and appropriately discretized in \( k \), so that the 2DIFT can be evaluated by means of a fast Fourier transform algorithm.
Figure 3.3: Typical forward and inverse filters. (a) Filter responsible for the complex confining effects of the limb, $W_{RR}(n, k)$. (b) Filter responsible for the passive attenuating effects of volume conduction of potentials due to the limb region between the nerve and limb surface, $V_{RR}(n, k)$. This filter is inverted in the solution of the inverse problem.
As observed in Fig. 3.3b, $V_{R,s}(n, k)$ is strongly low pass in both spatial frequencies, and as a result its inverse $V_{R,s}^{-1}(n, k)$ will be very sensitive to the high frequency components of the noise present in $G(n, k)$. In order to prevent this sensitivity, $V_{R,s}^{-1}(n, k)$ is windowed in the frequency domain by the elliptic filter function $H(n, k)$ shown in Fig. 3.4a. The spatial domain impulse response of this window is shown in Fig. 3.4b to demonstrate the Gibb's phenomenon ripple that will be associated with it due to the slope discontinuity. This effects of the slope discontinuity will be greater in the inverted filter due to the sharper slope discontinuity, as seen in Figure 3.13. These effects could perhaps be alleviated by the use of an additional smoothing window (i.e. a Hamming window). The size of this window is adjusted to allow only frequencies pertaining to the CAP to pass.

An inherent periodicity of $L = 2\pi/\Delta k$ is introduced into the longitudinal spatial frequency by discretization, however the physical periodicity of $2\pi$ of the $\theta$ dimension cannot be changed by altering the spacing or number of samples. This can be done by decimating the angular frequency domain by some factor of the samples. Specifically, if every $p$th angular frequency domain sample is retained, including the zeroth, then the $\theta$ dimension associated with the filter is reduced $p$-fold. Effectively this is $\theta$-dimension windowing, just as truncation in the $z$-domain is $z$-domain windowing. This $\theta$-domain windowing introduces a periodicity of $2\pi/p$ which then requires that transformed CAP data be limited to a $2\pi/p$ $\theta$-dimension interval in order to prevent aliasing [81].

The modeled limb assumes that the muscle medium is uniformly symmetric in the $\theta$-dimension. As a result, field potentials would be expected to be filtered symmetrically, that is, the fall-off in signal power for electrodes further from the nerve would be the same on either side of the nerve. If this is not the case for
Figure 3.4: Inverse filter frequency window (a) and impulse response (b).
the recorded limb field potentials, then the data could be mirrored in $\theta$ about the location corresponding to induce this symmetry.

In regions where noise and CAP signal power overlap, a weighting function known as a posteriori Weiner filtering can be applied [17,23,24]. This weighting function is obtained from estimates of noise and CAP signal power. Signal power is estimated by the power spectrum of each signal. If $S_{ss}(n, k)$ and $S_{ss}(n, k)$ represent CAP and noise signal power respectively, then the $(n, k)$th weight is

$$U(n, k) = \frac{S_{ss}(n, k)}{S_{ss}(n, k) + S_{ss}(n, k)}.$$

As seen in Figure 3.14, the noise power was relatively low.

Combining all of these techniques, frequency domain windows, $\theta$ domain windows, and noise weighting function, the estimated nerve potential is then:

$$\Phi(R_d, d, \theta, z)$$

$$= 2D - IDFT[H(pn, k) \cdot U(pn, k) \cdot V^{-1}_{R_d}(pn, k) \cdot G(pn, k) \cdot e^{int}].$$

Methods and Materials

Electrophysiology

Large (6"-7") bull frogs were sacrificed by cervical dislocation ("pithing"), the skin of the lower limb was removed and the sciatic nerve was freed from surrounding muscle and supportive connective tissue as it travels from the vertebral column to the knee. The path of the nerve was then traced to the knee with care taken to cut and strip from the limb all nerve branches. The nerve was then allowed to settle into its approximately original place and the surrounding muscle tissue that was
disturbed was sewn back in place with 4-0 prolene suture material. To prevent the nerve and muscle tissue from drying out, the tissue was frequently irrigated with a frog Ringer solution (111mM NaCl, 2mM KCl, 1.5mM CaCl₂, 2mM NaHCO₃, 0.1mM NaH₂PO₄, 11mM glucose). The nerve was stimulated on the central end as it leaves the cord with brief (0.01 msec) voltage pulses of approximately 2 volts amplitude and surface potentials were simultaneously recorded at 16 sites on a cylindrical array of 80 electrodes (4 latitudes separated by 0.5cm of 20 electrodes each) encased in a lucite cylinder applied to the thigh surface with respect to a reference electrode placed at the knee. The nerve was not stimulated supramaximally, but at the stimulation voltage perceived to result in the strongest CAP signal with the lowest stimulus artifact. Limb surface electrodes were stainless steel pin heads with a lead off surface of approximately 0.5 mm². Potentials were also recorded at the nerve surface with an array of four silver electrodes with lead off surfaces of 0.05-0.1mm² approximately positioned next to the nerve. Recordings were amplified 20,000 times, filtered (15 Hz to 10 KHz passband, first-order rolloff), digitized (ISC-16, RC Electronics) at a rate of 1 MHz (62.5 KHz per recording electrode) and averaged (128 times) to obtain a better signal-to-noise ratio. All recordings were obtained within a grounded Faraday cage with stimulus isolation (Fig. 3.5). The geometry of the limb was obtained through measurement of a dissected cross section or by magnetic resonance imaging of the limb in a similar lucite cylinder. Prior to imaging, limbs were preserved in a formalin solution. Conduction velocity of the recorded CAPs was calculated for both nerve surface and limb surface traces by computing the time for the second (negative) CAP peak to travel a known distance.
Figure 3.5: Apparatus used in obtaining field potentials from the frog limb and CAPs from frog nerve embedded in the frog thigh.

3.2.1 Magnetic Resonance Imaging

Spin echo (TR/TE 4000/38 msec) proton magnetic resonance (MR) images were acquired on a Bruker Med Spec 24/40 system operating at 2.4 Tesla. A 5 cm imaging coil, 256 gradient steps and 2 averages were used for all acquisitions. A pilot scan taken along the longitudinal axis of the leg was used to position four 3 mm slices along the length of the leg (slice separation: 7-10 mm). Cross sectional images were viewed from the knee. Marrow diameter, bone diameter, distance
of bone from center of the limb, distance of nerve from center of the limb, and angle between a line extending from the center of the limb to the nerve and a line extending from the same point to the center of the marrow were measured on digitized MR images of four cross sections for each limb. The distance from the center of the bone to the nerve was calculated by the law of cosines. For each limb, each geometric measurement from the cross sections was fitted by a third order polynomial with a least squares fit to an equation relating that parameter with distance from the hip. The right limbs were “mirrored” so that the equations would describe an average left limb. The equations were used to calculate geometric parameters used in the filter formulation because MR image cross section scans did not coincide with cross sections from which electrophysiological measurements were obtained. The coefficients for each parameter from all the legs were averaged to obtain an equation describing how that parameter changes with distance from the hip in an average thigh.

Data Processing

Each of the 20 raw data CAP measurements from limb surface electrodes corresponding to a single latitude was adjusted to approximately zero baseline by subtracting the average of the last 2 mSec of data which was reasonably flat from all data points. Each trace was then smoothed over the last 2 mSec and first 0.25 mSec of data by an exponential time window in order to remove any noise associated with the baseline signal. The 20 limb surface traces were then decimated by a factor of 4 with respect to time and interpolated with respect to \( \theta \) to 32 limb traces by means of a clamped cubic spine algorithm [16]. Consequently, the modified data set was in the form of 32 x 128 array. When necessary, the modified data set could be mirrored about the nerve to achieve the necessary \( \theta \)-dimension symmetry inherent in
the model. The discrete Fourier transform (DFT) of the modified data set was then computed by means of a Duhamel-Holman split radix formulation of the fast Fourier transform [25]. This transformed array could then be treated to remove noise by the \textit{a posteriori} Weiner filter weighting coefficients (see below). The forward filter function (calculated using the geometric and electric parameters and conduction velocity of the limb surface CAP as input) was inverted and windowed by an elliptic function filter. The inverse filter and the transformed data could be decimated in the angular spatial frequency to achieve $\theta$ windowing. The inverse discrete Fourier transform (computed by means of an inverse fast Fourier transform) of the product of the inverse filter function and the transformed data yielded the potential in the muscle medium at the nerve radius. The potential at the $\theta$ point corresponding to the nerve was taken as the estimate of the nerve surface potential.

The power spectrum of the CAP was calculated by squaring the magnitude of the discrete Fourier transform coefficients obtained by transforming a single nerve surface trace by means of the fast Fourier transform algorithm. Power spectra of a typical limb surface CAPs were obtained in the same fashion. These power spectra are not true power spectral densities of the signal because the signals were not random signals. The power spectral density of the noise was estimated by squaring the magnitude of the discrete Fourier coefficients obtained by transforming an array of noise data that was treated in the same manner as the signal data. The two dimensional spectra of five noise arrays were averaged to obtain the estimated power spectral density. This was not truly the power spectral density because the process was not ergodic [61]. Averaging several power spectral density estimates tended to lower the variance of the estimate.
3.3 Results

3.3.1 Limb Geometry

When viewing the digitized MR images of the frog thigh, the resolution was sufficient to identify the sciatic nerve in most images. A positional offset of areas rich in fat was noticeable, particularly in the marrow of the central bone. This “chemical shift artifact” was due to the resonant frequency difference between water and fat at 2.4 Tesla. The frog thigh in cross section did not appear similar to the ideal cross section as seen in Fig. 3.6. The bone was not in the center of the limb and tended to wax and wane with respect to its distance from the center of the limb. In sections approximately half-way between the hip and knee, the bone was closest to the center. The third order regression equations below were obtained by averaging the coefficients from six frog thighs to obtain equations for an “average” left frog thigh. In these equations, $a$ is the marrow diameter, $b$ is the bone diameter, $r_b$ is the distance from the center of the limb to the center of the bone, $R_a$ is the distance from the center of the nerve to the center of the limb, $\alpha$ is the angle as diagrammed, $R'_a$ is the distance from the center of the nerve to the center of the bone, and $z$ is the distance from hip to knee with the origin at the boundary of the lucite cylinder near the hip:

\[
\begin{align*}
a &= 1.975 - 0.155 \cdot z + 8.19 \times 10^{-3} \cdot z^2 + 1.32 \times 10^{-4} \cdot z^3 \\
b &= 3.162 - 0.151 \cdot z + 5.86 \times 10^{-3} \cdot z^2 - 6.50 \times 10^{-5} \cdot z^3 \\
r_b &= 3.586 + 8.37 \times 10^{-2} \cdot z - 1.32 \times 10^{-2} \cdot z^2 + 2.97 \times 10^{-4} \cdot z^3 \\
R_a &= 7.506 - 0.310 \cdot z + 1.30 \times 10^{-2} \cdot z^2 - 1.92 \times 10^{-4} \cdot z^3 \\
\alpha &= 215.8 + 10.9 \cdot z - 1.11 \cdot z^2 + 2.33 \times 10^{-2} \cdot z^3 \\
R'_a &= 9.533 - 0.190 \cdot z + 4.49 \times 10^{-3} \cdot z^2 - 4.65 \times 10^{-5} \cdot z^3.
\end{align*}
\]
Figure 3.6: Ideal (a) and typical (b) frog thigh cross sections.

Not shown are the equations for each frog thigh which were used for the geometric parameters involved in the calculation of the forward and resulting inverse filters. These equations were substituted with the value of \( r \) corresponding to the latitude of the electrodes which recorded the limb surface traces involved.

The cross sections also showed that the apparent muscle density was not constant. Some frogs showed more fibrous material between muscle groups than other frogs. Typical examples of this from two different frogs are shown in Fig. 3.7. This sort of fibrous difference was also in different cross sections from the same animal.
Figure 3.7: Typical MR cross sections. Note the irregularity in apparent density of muscle groups.

3.3.2 Electrophysiology

The power spectrum of a typical limb surface CAP trace directly over the nerve ($\theta = 0^\circ$) is shown in Fig. 3.8. This is compared to a typical nerve surface CAP power spectrum shown in Fig. 3.9. The spectrum of the nerve CAP is observed to be approximately band limited at 1500 Hz with a tail extending from 1500 Hz to 2500 Hz. The effects of the volume conductor resulted in the spectrum from the limb surface being approximately band limited at 1000 Hz with a slight tail from
Figure 3.8: Power spectrum of typical measured limb surface field potential from electrode directly over nerve.

1000 to 1500 Hz. The goal of the inverse filter was then to restore this lost power, especially at higher frequencies.

When a low-pass filter with a pass-band which could be varied in the frequency domain was passed over a typical nerve surface trace, it was found that the frequencies responsible for most of the signal power in the first peak of the CAP range from 1500 to 2000 Hz. This is demonstrated in Fig. 3.10, which shows the results of several low pass filters passing over the original CAP waveform shown by the dots. Therefore, the gain of the inverse filter in this region was the most critical.

A typical inverse filter prior to inversion is shown in Fig. 3.11. As described, the strong low pass filter characteristics can be seen with regard to both angular and longitudinal frequencies. This filter was inverted and windowed by the elliptic
Figure 3.9: Power spectrum of typical measured nerve surface CAP.

window resulting in Fig. 3.12. The inverse filter was not meant to be "symmetric", even though it is an even length filter. As a result, it is an odd length filter represented in the frequency domain by an even number of points. The gain is observed to increase most with angular frequency. Thus, as one moves further from the nerve in a cross sectional plane, the spatial filtering is greater. A longitudinal frequency cross section of this filter corresponding to dc angular frequency is shown in Fig. 3.13. Here the frequency is shown as Hz, recalling that this is a really a temporal frequency scaled by the assumed constant conduction velocity to result in a spatial frequency.

Most inverse filters were computed with approximately the same conduction velocity and windowed with similar pass-bands as in Fig. 3.13. In the examples
Figure 3.10: Diagramatic representation of frequency content in CAP. The dotted CAP waveform was low pass filtered in the frequency domain by filters with passbands of 3000, 2000, 1000 and 500 Hz.

discussed below, the electrical parameters listed in Table 3.1 remained constant while $R_s$, muscle anisotropy, and CAP conduction velocity were varied to achieve the closest fit possible to the nerve surface CAP, which served as a sort of template. In all examples, the best fits were achieved with values of $R_s$ closer to those of the distance from center of the bone to the nerve ($R'_s$). Conduction velocity was that calculated from the limb surface CAPs.

In all examples, the “half-filter” formulation was used. That is, the data set was reduced by one-half, as described in the section Mathematical and Computational Aspects. In order to induce the assumed $\theta$-dimension symmetry, all data sets were mirrored about the nerve. The half data set that was mirrored was the half data
Figure 3.11: Typical forward filter prior to inversion.

set which resulted in the closest inverse solution estimate to the nerve surface CAP. If mirroring was not performed, the resulting inverse solutions were found to be unacceptable in producing meaningful estimates to the nerve surface CAP. The “Weiner” filtering coefficients were not used because they were not found to make any difference, except when the pass band was increased beyond 2500 Hz. This demonstrated that the signal to noise ratio was very good. A spectrum of the noise is shown in Fig. 3.14.

To illustrate the results of the inverse problem, the data shown in Fig. 3.15 was obtained from the same animal (animal fl107l). Each data set is from a different latitude in the recording cylinder. Since only half of the data was used, this effect of the “hump” present in the middle of the data was minimized, resulting in the two
fits overlayed with the CAP recorded at the nerve surface in Fig. 3.16. It is feasible that the bone itself could act like a secondary source. This hypothesis is supported by the fact that this “hump” appears along those electrodes corresponding to $\theta$ values in which the bone lies between the nerve source and the electrodes. The two fits do not overlay each other in time since they are from different recording cylinder latitudes and would hence, be expected to be delayed in time. These two fits seem to reasonably approximate the second and third CAP peaks but the high frequency content of the first peak is not matched, as seen by the width and slope of the first peak. The conduction velocity of the second peak in the fits was 62.5 m/sec compared to 46.20 m/sec calculated from the nerve surface CAPs. The reasons for
Figure 3.13: Cross section of typical forward filter after inversion and windowing. Section corresponds to $n = 0$.

the error in the shape and conduction velocity of these fits and those in the next example will be discussed below.

The last example demonstrates the best fit achieved. The data sets in Fig. 3.17 are from two different latitudes of the recording cylinder on the same frog (animal fl113l). Again as in the previous example, peaks two and three of the CAP are seen to fit quite well (Fig. 3.18), however, the first peak is again lacking in high frequency content. This is also seen by the excessive width of the first peaks of the fits when compared to the nerve surface CAP as well as a slower slope. These fits do appear better than the previous example, however, in fitting the slope in the transition region between the first and second peak. The conduction velocity of the second peak in the fit CAP waveforms was 104.17 m/sec compared to 58.81 m/sec.
Table 3.1: Parameters Used to Calculate Inverse Filter

<table>
<thead>
<tr>
<th>Conductances</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone Marrow</td>
<td>1.0 S/mm</td>
</tr>
<tr>
<td>Bone (longitudinal)</td>
<td>0.05 S/mm</td>
</tr>
<tr>
<td>Bone (transverse)</td>
<td>0.016 S/mm</td>
</tr>
<tr>
<td>Muscle (transverse)</td>
<td>4.5 S/mm</td>
</tr>
<tr>
<td>Muscle (longitudinal)</td>
<td>1.8-2.25 S/mm</td>
</tr>
<tr>
<td>Air</td>
<td>0.01 S/mm</td>
</tr>
</tbody>
</table>

Specific Membrane Conductances

<table>
<thead>
<tr>
<th>Conductance</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone Marrow-Bone</td>
<td>10 S/mm and 0 nF/mm</td>
</tr>
<tr>
<td>Bone-Muscle</td>
<td>10 S/mm and 0 nF/mm</td>
</tr>
<tr>
<td>Muscle-Air</td>
<td>10 S/mm and 0 nF/mm</td>
</tr>
</tbody>
</table>

in the nerve surface CAPs. The error in the conduction velocity of the fits is much higher here than in the previous example.

The power spectrum of one of these fits is shown in Fig. 3.19. The overall power was increased when compared to Fig. 3.8, however the presence of a tail is seen up to 2000 Hz, however it was still much smaller, perhaps unnoticed, when compared to Fig. 3.9. This was expected, since the first peak which is described by these frequencies, was not reconstructed sufficiently.

To explain the errors associated with the fit nerve CAPs, it is thought that the limb-surface recordings were filtered by the limb surface electrodes themselves. This filtering was both temporal and spatial. When one considers temporal filtering, the electrode-tissue interface can be modeled as described by Geddes [30] and shown in Fig. 3.20. The values of R and C are dependent on current density and frequency. For the current densities involved, the effect of this circuit was perhaps very small, with a pass band outside the area of interest. The low current density was achieved by the high input impedance amplifier used. Spatial filtering was due to the large lead off surface of the electrodes. These electrode, stainless steel pin heads of 0.75-1.00 mm in diameter, effectively subtended an angle of approximately 5°. The
angle between each electrode was supposed to be $360^\circ/32$ or about $11^\circ$ (in the case of spline fit data), as diagrammed in Fig. 3.21. As a result, the electrode took up almost 50% of this region and effectively spatially "smeared" the recorded potentials in much the same way the waveform was temporally "smeared". Since the surface CAPs obtained have been pre-filtered in this manner, it then became very difficult to accurately reconstruct nerve surface CAPs. Since the first CAP peak contains the most high frequency content, it is expected to be affected most, as observed. With this in mind, when the experiment is repeated, point electrodes should be used to minimize this effect.

Coupled to this affect, as noted by Wilson et al. [81], it is crucial to center the nerve underneath the electrode at $\theta$-position 0.0. If this is not done, it is very
Figure 3.15: Measured limb surface field potentials from animal fl1071.
Figure 3.16: Comparison of measured CAP with inverse fits from animal f110711. Solid curves are fits while triangles are measured CAP. Y-axis units are microvolts.

difficult to reconstruct the first peak of the CAP. This is diagrammed in Fig. 3.22. The simulated results presented by Wilson et al. in such cases are similar to the fits obtained in Figs. 18 and 21. In order to minimize this effect, perhaps the area directly over the nerve should be oversampled in order not to miss the point at which the first peak of the limb surface CAP is a maximum.

To illustrate the combination of centering errors and the spatial filtering aspects of the electrodes, a theoretical study using simulated CAP data was conducted similar in principle to that by Schoonhoven to elucidate the spatial filtering effects of needle electrodes [67]. Since the forward and inverse model predicts the potential at punctate points, the potential at several sites across the surface of the electrodes can be calculated by the forward model using as input a nerve surface CAP. To estimate
Figure 3.17: Measured limb surface field potentials from animal fl1131.
Figure 3.18: Comparison of measured CAP with inverse fits from animal f113hl. Solid curves are fits while triangles are measured CAP. Y-axis units are microvolts.

the effects of the electrodes, the potential at several sites was simply averaged. This estimate of spatially filtered field potentials was then used as input to the inverse model to calculate a nerve surface CAP which was compared to the CAP used in the forward model. Schoonhoven suggested in his study that this be done in incremental steps of 0.1 mm across the electrode surface. This was done here for 11 sites across the 1 mm electrode surface for two cases. In the first case, the nerve was ideally centered underneath the electrode and the field potentials corresponding to the center site and 5 sites on either side of the electrode were calculated and averaged. The result of the inverse model applied to this data compared with the nerve surface CAP used in the forward model is shown in Fig. 3.23. The error in reconstructing the first peak is seen to be greater than the error in reconstructing the second
Figure 3.19: Power spectrum of typical inverse fit. Note the lack of signal power in the 1500-2000 Hz range.

and third peaks, as seen in the reconstructions with actual data presented above. However, this error is not that large and was significantly reduced by reducing the size of the electrode by one-half. The original CAP used in the forward model is compared with the reconstructed CAP for this case in Fig. 3.24.

When the nerve was centered on the edge of the electrode and the field potentials calculated by the forward model were averaged across the electrode, the results of the inverse model were worse than when it was centered in the middle of the electrode. These results are shown in Fig. 3.25. This case also demonstrated the effects of mis-centered electrodes. As the nerve is moved further away from the electrode, the results of the inverse estimates are expected to get worse. In the
Figure 3.20: Temporal electrode filter circuit.

experimental data described above, it is supposed that the poor fits of the first peak were then a combination of these two effects.

3.4 Discussion

In this study, the frog thigh was modeled as an infinitely long cylinder with a central bone and a single sciatic nerve source. The muscle medium was given specific conductance and allowed to exhibit a constant anisotropy with regard to these conductances. Experimentally the frog sciatic nerve was stimulated and volume conducted CAPs were recorded at the limb surface. These CAPs were used to predict using the model the source nerve CAP. The predicted nerve CAP was then compared to a measured nerve CAP. As reported [81], the model parameters which
Figure 3.21: Diagram of origins of spatial electrode filtering effects.

most affect the predicted nerve CAP are the location of the nerve in the thigh, anisotropy of the muscle medium and the conduction velocity of the nerve CAP.

The location of the nerve utilized by the inverse model was a value close (usually slightly less than) to the distance from the center of the bone to the nerve as measured by MR imaging. The anisotropy ratio for muscle utilized was typically 2.25-2.5, a value that lies in the low range of values given for this measurement [31]. The CAP conduction velocity utilized was that measured at the surface of the limb (typically 70 m/sec). This value was larger than the correct value measured at the nerve (typically 45 m/sec) apparently due to the spatial and temporal filtering affects of the limb surface electrodes. Since the peak values are no longer representative of the correct time points at which would normally appear, but rather
Figure 3.22: Effects of electrode misalignment on recorded limb field potentials.

averages due to the convolving nature of the spatio-temporal filtering, the peaks have been displaced in time, resulting in the error in conduction velocity calculation. Such an effect can be observed in the simulation shown in Fig 3.25. If a smaller value for the CAP conduction velocity was chosen, the gain of the inverse filter was such that predicted nerve CAPs were too large. To alleviate this effect, the distance of the nerve from the center of bone needed to be increased and/or the anisotropy decreased. Both of these changes begin to move these parameters values out of realistic physiological ranges. These gain increases were not found to alter the shape of the predicted CAPs (ie. width of peaks). Only overall and relative height of the peaks were changed. It must be mentioned that despite the errors,
Figure 3.23: Theoretical effects of large electrodes on solution of inverse problem. In this case the nerve was centered underneath 1mm diameter electrode. Triangles represent original waveform while solid line represents inverse fit after corruption by spatial averaging.

when measured values were used, the predicted nerve CAPs were actually close to measured nerve CAPs.

The errors associated with the spatial electrode filter could be corrected by using different electrodes. However, the errors associated with mis-centering the electrodes may be difficult to correct unless the region directly over the nerve is oversampled to determine the exact location directly over the nerve. This may present a real technical limitation to the application of the inverse model in situations in which the electrode array cannot be easily adjusted to center the electrodes over the nerve source, which was not the case in the experiment protocol used here.
Figure 3.24: Theoretical effects of large electrodes on solution of inverse problem. In this case the nerve was centered underneath 0.5mm diameter electrode. Triangles represent original waveform while solid line represents inverse fit after corruption by spatial averaging.

The usefulness of the model to recover the high frequency information present in the CAP is essential to its utility, however this was not demonstrated. The reasons for this were errors in the measurement technique as described as well as the idealized nature of the model. For example, it was found by MR image studies that the location of the source was not constant along the length of the limb. It was also found that the bone was not in the center of the limb. The irregularity in muscle mass demonstrated by the two cross sections in Fig. 3.6 would be expected to alter the muscle conductivity described by the model. These areas of fibrous material could represent current paths of higher conductivity which could, in effect, short out the volume conductor, resulting in spurious results. However, what is
Figure 3.25: Theoretical effects of large electrodes on solution of inverse problem. In this case the nerve was placed on the edge of a 1mm diameter electrode. Triangles represent original waveform while solid line represents inverse fit after corruption by spatial averaging.

more striking is that the muscle was not one homogenous muscle as represented by the model, but rather several muscles, undoubtedly of different density, resulting in areas of varying conductivity.

With regard to anisotropy, Gielen [31] notes that muscle anisotropy is not a constant, but rather it exhibits frequency dependence depending on distance from the bioelectric source, in this case the nerve. The model does not take this into consideration and treats muscle anisotropy as a constant. If this frequency dependence were real, then the regions where reconstruction of the first CAP peak is important would be affected, 1000-3000 Hz. Gielen’s figures indicate that this frequency dependence is bandlimited from 1-500 kHz. It is of interest to note, however, that
Gielen's technique in obtaining these results has been questioned in the literature [58].

It must be noted that some of the effects of errors may be unique to the frog thigh preparation. For example, in the case of a larger human limb such as the thigh, the effect of electrode size would be much less with regard to spatial filtering. The frog thigh preparation was not readily amenable to adjusting the nerve source directly underneath the electrodes. The results are encouraging in showing that with nominal parameters, cylindrical inverse volume conduction problems can be addressed by the fast and computationally efficient Fourier transform method. It therefore offers a promising approach to a variety of clinically oriented problems.
Chapter 4

Conclusion

In this thesis, a brief explanation of the properties of nerve conduction prefaced the explanation, results and conclusions of two nerve physiology studies. Both of these studies are seen to have clinical relevance in terms of treatment and diagnosis of peripheral nervous system dysfunction and injury. The repair technique described in Chapter 2 discussed the results of experience with application of a “laser-assist” versus traditional suture repair technique to intentionally cut sciatic nerves in rats. In Chapter 3 a potential clinical diagnostic technique for the measurement of compound action potentials (CAPs) from nerves embedded in muscle tissue was described in terms of a field theoretic inverse problem and tested in a bullfrog thigh model.

In Chapter 2 rats were divided into two groups. In one group the right sciatic nerve was cut and reanastomosed by means of a conventional suture repair technique, while in the other group the right nerve was cut and repaired by means of a “laser-assist” technique in which fewer sutures were used to rejoin the nerve. After approximately eleven months, both nerves were removed from the animals in each group and examined electrophysiologically and histologically. The left nerve served as a control for that animal. The “laser-assist” technique of suture repair of cut sciatic nerve in rats was shown to be similar to suture repair with regard to conduction velocity as in previous studies. However, it was shown that suture repair of cut nerves resulted in greater neuroma formation. This greater neuroma formation was explained to possibly be responsible for the higher stimulation thresh-
olds and greater number of conduction failures observed in suture repaired nerves. The utility of the "laser-assist" technique in keeping regenerating axons within the bounds of the nerve, while not necessarily aligning fascicles, supports the theory that alignment is not as important as containment.

This study was part of a large study of "laser-assist" repair that included the evaluation of tensile strength and functional return observed after injury and repair and healing over several months [10]. In this study, it was found that tensile strength is proportional to the number of sutures used in the immediate weeks after healing. However, after 11 months, the tensile strength of "laser-assist" repaired nerve versus suture repaired nerves was not significantly different. The functional return observed was measured by means of the sciatic nerve function index [22]. This index is not a linear measure of functional return and so the interpretation of the results was difficult. However, there appeared to be no difference in functional return when "laser-assist" and suture repaired groups were compared.

Chapter 3 contains a description of a field theoretic technique for the recovery of compound action potentials (CAPs) originating from nerves embedded in a muscular limb via field potentials recorded on the surface of the limb. This technique was tested in a bullfrog thigh model containing a single sciatic nerve source. The recovered CAP waveforms generally matched the shape of CAP waveforms recorded at the surface of the nerve. However, the conduction velocity parameter used in the field theoretic model utilized to recover the CAP waveforms was out of the physiological range of values recorded for this parameter. The conduction velocities measured using these recovered CAP waveforms was also out of the physiological range of values. These errors are thought to result from errors in the measurement technique employed and idealizations inherent in the field theoretic model itself. The errors in measurement technique are thought to have arisen from spatial fil-
tering of recorded field potential waveforms by the large electrode surfaces. The idealizations include the model's shortcomings in describing the asymmetric thigh with a symmetric model.
Bibliography


