Estimation of Peripheral Nerve Conduction Velocity Distributions from a Short Segment of Nerve

by

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ABSTRACT

A complete and accurate description of the conduction properties of the entire population of nerve fibers within a peripheral nerve bundle has long been an important objective of clinical neuromuscular electrophysiology. Access to such information would increase the sensitivity and specificity of neuromuscular electrodiagnostics, and provide improved means of assessing nerve development, growth, regeneration, and response to treatment. One approach has been to generate an estimate of the distribution of conduction velocities (CVD) of all fibers by solving the inverse problem in electroneurography. The complexity and the inconvenience of current CVD estimation techniques have restricted their use to the research laboratory. In order to be accepted and utilized in a clinical setting, conduction velocity distribution estimates should be noninvasive and fast, without the need for complex procedures or costly equipment.

A new technique for improving the estimation of conduction velocity distributions over a short segment of nerve is described and its feasibility is investigated. Using a short segment of nerve can significantly simplify the placement of surface electrodes while reducing the time and the discomfort involved in the acquisition of evoked compound action potential signals. The technique involves normalizing nerve-to-electrode transfer functions at a plurality of closely spaced recording sites. Following normalization, differences between compound action potential waveforms can be attributed to the effects of temporal dispersion and more accurate CVD estimates can be calculated from the short nerve segment. The feasibility of the method is evaluated using simulated and experimental compound action potential data. A formal study is performed to assess the performance of the technique in a clinical setting.

Thesis Supervisor: Roger G. Mark
Title: Distinguished Professor in Health Sciences and Technology; Professor of Electrical Engineering
Many people have provided me with both the inspiration to undertake this research project and the motivation to revisit it each day over the past 2½ years. I am very fortunate to have such strong support in both my personal and my professional relationships.

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-Marty
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**Chapter 1. Introduction / Motivation**

“A major goal in clinical neuromuscular electrophysiology is to describe fully the conduction properties of the entire population of nerve fibers comprising a nerve bundle in a human limb. Optimally, it should be possible to do this in the context of a clinical electroneuromyography laboratory: noninvasively, painlessly, quickly, and without need for complex and costly equipment. Such capability would make electrodiagnosis of neuromuscular disorders more sensitive and specific, and would offer a tool for studying issues related to nerve growth and development, regeneration, and response to treatment. Unfortunately, current capabilities… are still far from this ultimate goal.”[1]

Leslie J. Dorfman, MD

AAEE Minimonograph #21

The Distribution of Conduction Velocities in Peripheral Nerves: A Review

Muscle & Nerve, January 1984

**Introduction**

Diseases that affect peripheral nerves such as multiple sclerosis, Guillain-Barre syndrome, and diabetic neuropathy can not only be painful and difficult to manage, they can be severely debilitating and exact a huge toll to the patient’s quality of life. The most effective way to reduce the impact of these diseases on nerve function is through very specific diagnostics that can lead to treatment or intervention at the earliest possible stages of peripheral nerve deterioration. Similarly, the development of pharmacological treatments for these diseases requires extensive clinical studies that take advantage of the most sensitive possible methods of assessing the effect of therapy on nerve function. [2-10] There are widely recognized shortcomings to standard nerve assessment methods that make them poorly suited to provide this desired sensitivity and the specificity. Many of these methods are highly subjective. The quantitative methods that are clinically available distill electrophysiological
data down to simple parameters that cannot reveal the function of the entire nerve. There are methods that have been developed, however, that have the potential to specifically identify nerve dysfunction prior to any outward symptoms and to gauge, with high sensitivity, small changes in function that may indicate the progression or the regression of peripheral nerve disease. Such enhancements over standard methods are accomplished by extending the parametric description of nerve function to include information about the many different subpopulations of fibers within the nerve bundle.

These advanced methods have been used in research settings for many years. The quote that begins this chapter, however, reflects the frustration that has been felt by researchers and clinicians alike regarding the difficulties involved in realizing such techniques in a clinical setting. The difficulties felt at the time the passage was written were primarily the requirement of extensive computing power to apply the techniques and the complexity of the systems and procedures for electrophysiological data acquisition. The former of these has largely been solved through dramatic advances in integrated circuit and microcomputing technology. The latter, on the other hand, remains an obstacle even with the current state of noninvasive electrode and signal conditioning hardware. The main impediment to further development of these methods is the belief that they necessarily require nerve activation and recording at more proximal regions of the nerve where electrode sites are sparse, limited in extent, and inefficiently coupled to the nerve tissue. This document reports on research to overcome the difficulties in improving peripheral nerve evaluation and to energize the movement to create a more sensitive and specific tool for clinical nerve assessment by applying advanced diagnostic techniques over only a short segment of the nerve.

**Median Nerve Electroneurography**

Electroneurography is the acquisition, study, and analysis of propagating action potentials in peripheral nerves. When electroneurography is performed noninvasively, electrical currents are presented to the active tissue bringing the nerve fibers to threshold, and compound action potential (CAP) signals are recorded from electrodes applied to the surface of the skin at a different location along the nerve. The physiological state of the nerve can be assessed by measuring specific parameters relating to the propagation of the CAP between the stimulation and recording sites. A CAP signal results from a summation of single fiber action potentials (SFAPs) propagating along the axons of the nerve. As such, a CAP contains information about the number of nerve fibers responding to the stimulus, the shape of the SFAP waveforms that contribute to the CAP, and the speed with which each SFAP propagates along the nerve. Knowledge of these parameters and an indication of
how they change with time can be used to detect the onset of pathologies, to track the deterioration or restoration of peripheral nerve function, or possibly to indicate changes in other physiologic systems within the body. Clinical electroneurographic procedures are known as nerve conduction studies (NCS) and are a common component of an electrodiagnostic examination. The clinical value of nerve conduction studies has been shown repeatedly in the diagnosis and evaluation of peripheral nerve injury and disease.[2-4, 6, 7, 9-13]

The median nerve is superficial enough to allow stimulation or recording only at isolated sites along its path: at the digits, along the ventral portion of the wrist, at the elbow, and at the shoulder. A single CAP can be produced by stimulating and recording between two of these sites in any combination.[9, 14-16] There are anatomical and physical factors, however, that often restrict the options considerably. The sites at the elbow and shoulder are small in extent. To stimulate at these locations generally requires stimulation hardware capable of producing very large current pulses. Locating stimulation sites often involves actively monitoring the intensity of the subsequent thumb twitch and adjusting the electrode position until the twitch is maximized. Once the optimal site is found, a stationary surface electrode can be taped or pasted at that location in order to facilitate more stable data acquisition. This involved procedure will only guarantee efficient median nerve stimulation in the healthiest subjects. Testing overweight subjects tends to require the stimulating electrode to be pressed down into the skin in order to be closer to the nerve itself. All subjects require substantially more current be applied in order to activate the nerve. Stimulus pulses at these locations are often required to be above the subject's pain threshold before activation of all fibers is attained.

Recording from the median nerve at the elbow or the shoulder can present an even larger problem because there is no method of feedback, analogous to the thumb twitch, to indicate that the electrode is optimally placed. A time consuming guess-and-check procedure is used, whereby the electrode is repeatedly removed and replaced, until a CAP is reliably recorded following stimulation at another site along the nerve. This procedure, once again, leads to satisfactory recordings only on healthy subjects who are not substantially overweight and who do not suffer from peripheral nerve disease. Previous researchers have resorted to invasive needle electrodes in order to ensure efficient stimulation and recording at these proximal locations.[15, 17, 18] Short of this extreme step, some success with recording at these proximal sites has been found through vigorous skin preparation. Rubbing the recording site with an abrasive pad until the skin remains red can improve CAP recordings and make locating the optimal stimulation or recording site easier.
The median nerve is a mixed nerve, meaning that it contains both motor and sensory fibers in the same bundle. This must also be taken into account when choosing stimulating and recording sites. Stimulation of motor fibers will activate the set of muscles that the nerve enervates. In the median nerve this leads to the thumb twitch mentioned previously. Motor unit action potentials typically produce a disturbance that is millivolts or larger in amplitude at the surface of the skin and can easily be recorded directly over, or even well removed from the muscle with common amplifying electronics. These electromyographic signals provide a means for assessing the function of motor fibers within the mixed nerve.[7, 14, 19]

Sensory fibers, on the other hand, do not produce an equivalent, easily recorded, response. Assessment of sensory fiber function can only be achieved through analysis of action potentials traveling along the nerve itself. These signals may be more than 1000 times smaller in magnitude than a motor response and therefore require highly specialized and sensitive electronics.[14] Stimulation of mixed nerves activates both motor and sensory fibers. If sensory CAPs are of interest, care must be taken to assure that the much larger motor signal does not interfere in the recording. Motor fibers of the median nerve enervate muscles along the arm and the most distal motor fiber terminations are in the hand. Stimulation or recording from the median nerve at the finger will isolate only sensory fibers and therefore represent the only method of performing purely sensory nerve conduction studies in that nerve. Sensory nerve conduction studies are particularly important because they offer the only quantitative method available to gauge the functional performance of the peripheral sensory system. There are general neuropathies that will leave all motor and mixed nerve results normal while producing deterioration of sensory behavior.[3-6, 9, 12, 13]

The desire to record pure sensory CAPs and the anatomic factors that restrict the number of stimulation and recording sites over the median nerve drastically narrow the choices for clinical procedures. The most reliable method is to stimulate the nerve at the digit and record the subsequent CAP at the wrist.[2, 5, 20, 21] This technique eliminates the need to either stimulate or record from the more proximal sites on the arm where these tasks can be difficult and instead records from the wrist where the path of the nerve is well known as it leaves the carpal tunnel. Although the quality of recordings from sites on the wrist will vary with exact electrode location and with the amount of intervening tissue, placing an electrode over the nerve in this region is a straightforward operation that can generally be accomplished on the first try without requiring a trial-and-error process. Digital stimulation also guarantees the activation of only sensory fibers and therefore avoids the possibility of muscle activation interfering with the sensory recording.[7, 22]
Standard Compound Action Potential Analysis

Conduction Velocity

When sensory nerve conduction studies are performed, standard analysis reduces the CAP to its simplest components, conduction velocity and amplitude. While these parameters are clinically useful, they only provide information about the largest and fastest fiber groups and cannot adequately quantify the activity of the entire nerve bundle.[14, 17, 23] The most common measurement made from CAP recordings is conduction velocity. Conduction velocity (CV) is estimated as the distance between the stimulating and recording electrodes divided by the time required to travel that distance. In practice, the time of travel, or latency, is directly available from a CAP recording but the propagation distance along the nerve must be estimated. Minimum latency is measured from the onset of the stimulus to the onset of the CAP. Often, the initial CAP deflection is difficult to identify so an early peak of the signal is used instead.[16] Since these measurements approximate a minimal latency, with much of the CAP signal trailing behind, they are each a measure of maximum conduction velocity. Typical values for the maximum conduction velocity of a healthy human sensory nerve are between 50 and 70 meters per second.[4, 14-16, 23-25]

Conduction velocity estimates rely directly on accurate measures of the distance of propagation between the point of stimulation and the recording site. Errors in distance measurement will translate to errors in the calculated conduction velocity. Measuring over the surface of the skin is the only way to determine these distances noninvasively.[16, 17, 21, 25] One potential drawback to a digit-to-wrist median nerve recording method is the relatively short distance between the stimulation and recording sites and the consequently large impact of a small absolute error in the measurement of propagation distance. Histological examinations of the path of the median nerve through the hand indicate that it can be circuitous. The true path of the nerve, it is then reasonable to assume, will not be shorter than the noninvasive, straight-line measurement and is likely to be longer.

Amplitude

The other standard analysis parameter estimated from recording compound nerve potentials is amplitude. CAP amplitude is most often defined as the peak-to-peak difference between the largest negative and positive deflections of a CAP.[16] The peak-to-peak amplitude is typically about 10 microvolts when referenced to the potential difference at the skin surface. As with latency, peak-to-peak amplitude is determined by the early CAP components. Action potentials that arrive together at
the beginning of the CAP, corresponding to the fastest subpopulation of fibers, are the only contributors that maintain the coherence necessary to determine amplitude. Amplitude is often used as an indication of the number of fast fibers that respond to a stimulus. This indication is then extrapolated to give a relative measure of the total number of excitable fibers in the nerve.[16] Amplitude measurements are very sensitive, however, to electrode quality, and placement and are highly variable among subjects. Amplitude is therefore difficult to use as a one-time diagnostic. Changes in CAP amplitude over time are generally more robust and can be used to efficiently track longitudinal changes in intra-subject nerve conduction.[2, 4, 10, 14-17, 26-28]

**Advanced Analysis: Conduction Velocity Distributions**

Although conduction velocity and amplitude have proven to be useful parameters for the diagnosis and tracking of neuropathies, they cannot provide an adequate description of the health of the entire nerve because they assess only the behavior of a select group of fibers. Conduction velocity distributions (CVDs), on the other hand, are a measure of the relative activity of all fiber subpopulations that contribute to the compound signal. Conduction velocity distributions can be estimated from CAP recordings given several basic assumptions.[29] They are inherently more informative than simple amplitude and latency. Unlike those standard analysis techniques, which tend to isolate a single fiber population, the CVD also quantifies the activity of slower, smaller amplitude fiber populations. The CVD is further able to distinguish between changes in the excitability of certain populations of fibers that may leave amplitude and latency unaltered. CVD analysis extends the amount of clinical information that can be extracted from the CAP.[1, 29-32]

A considerable amount of research was directed at the development of CVD estimation in the late 1970’s and the early 1980’s.[18, 30, 33-40] Several research groups produced similar methods of calculating CVDs in parallel and these efforts culminated in a symposium drawing participants from many different institutions. The papers presented at that symposium reflect the state of the art in 1980 and were subsequently published together as a proceeding. Since the time of that gathering, research into CVD estimation has been much less prominent. Only a handful of papers have been published that extend the work reported in 1980.[32, 41-46] The seeming lack of interest presumably stemmed from a general feeling that the techniques required to estimate CVDs were too involved to ever be moved from the research lab to a clinical setting. This feeling was likely valid at the time of the symposium given the state of computer, integrated circuit electronics, and noninvasive electrode
technology. Dramatic advances have been made in each of these areas since then, however, warranting another look at the noninvasive estimation of CVD as a potential clinical procedure.

Methods of CVD estimation that are based on the shape of the CAP can be broken into two distinct classes, each with its own advantages and disadvantages. Those requiring only a single CAP recording, 1CAP methods, are the easiest to implement because they minimize the number of stimulation and recording sites. Calculating a CVD from a single CAP amounts to a blind deconvolution and requires that a substantial amount of information about single fiber action potentials be known. The exact shape of the single fiber potentials cannot be recorded using standard methods and therefore must be guessed or estimated.[46-49]

The second class of CVD estimation methods, the 2CAP method, is based on two CAP recordings. Incorporating the additional CAP reduces the amount of a-priori information that is required.[37, 39, 43] The two CAPs can both be stimulated or recorded at the same location. The 2CAP algorithm depends on different amounts of dispersion in the two signals. Two CAPs stimulated at the same site, therefore, need to be recorded at different locations so that the action potentials have traversed different distances. In this case, the 2CAP technique requires that the recording sites are electrically equivalent and that a single fiber action potential will look identical if recorded at the two locations. Similarly, one electrode can be used to recorded two CAPs stimulated at different locations. In this case the requirement of electrical equivalence is automatically met, however, the use of multiple stimulation sites requires that the same fibers be stimulated at both locations.

Regardless of how the CAPs are recorded, 2CAP techniques benefit from large differences in the distances of propagation. The 2CAP analysis is based on several assumptions about the recorded signals. Errors in the CVD solution are inevitable due to the limitations of the assumptions. When there is a considerable difference in the amount that the two signals have dispersed, the relative effect of those limitations will be reduced. Errors will be minimized, therefore, when distances of propagation, and hence dispersion, are maximized. Restricting electrode locations to the digit and wrist also limits the amount of dispersion that recorded CAPs can experience. Any method that takes advantage of those sites where the nerve is most superficial and easiest to find must also address the potential for increased errors due to reduced dispersion.
Clinical Conduction Velocity Distribution Analysis

The 2CAP method, as it has previously been applied to the median nerve, requires the use of at least one of the two more proximal electrode sites, where stimulating and recording CAPs is more difficult.[37, 39, 43] This, more than any other factor, has kept median nerve CVD analysis (and other nerves for analogous reasons) from becoming a common and accepted clinical technique. CVD has had very little use outside of the research laboratory, in fact, where needle electrodes have been used to ensure high quality CAP recordings. Problems associated with CAP recordings at the elbow or shoulder are complicated when the subject being tested has a neuropathy because CAP amplitude can be substantially diminished in these subjects. It is not unusual to be unable to record a CAP response when a disease state has progressed sufficiently. Simply identifying the proper location does not, therefore, solve the problems associated with recording from the proximal median nerve sites. There is the further concern that CAPs may be too small to discern at these locations even with modern electronics. This is particularly true for subjects who are overweight or who have a history of peripheral nerve disease. Unfortunately it is exactly these types of subjects who can often benefit the most from a more sensitive diagnostic procedure such as CVD analysis. One goal of this project is therefore to develop novel methods of median nerve CVD analysis that do not rely on recordings from the elbow or shoulder.

Restricting stimulation and recording to the more distal finger and wrist sites might also substantially simplify the clinical protocol for performing CVD analysis. Innovative approaches to the recording of biopotentials have recently been developed with direct applications to electroneurography. These techniques involve the integration of an array of electrodes onto a flexible substrate that is also capable of supporting printed circuits. The use of an integrated electrode array can simplify electrode placement to a single step, eliminating all but one subjective decision about the best electrode site. It can also eliminate questions concerning the distance between two electrode sites that are joined by the substrate. The ability to include printed circuitry allows temperature-sensing circuitry to be incorporated directly into the integrated electrode and kept in contact with the skin during data acquisition. It also potentially provides a means to reduce recording noise by placing preamplifiers very close to the electrodes, or to incorporate signal processing integrated circuitry directly into the electrode array. Array electrodes are not feasible for recording from different sites that are separated by a large distance. Variations in physical anatomy among subjects make it very difficult to develop a single placement electrode that will fit exactly between two recording sites on all subjects. If multiple, closely spaced recording sites were to be used, however, electrodes could be used interchangeably.
among subjects. The regions of the finger and the wrist where the median nerve is superficial enough to allow noninvasive recording are generally long enough to provide space for more than one recording or stimulating location. Techniques that take advantage of the size of these regions to make additional CAP data available can also utilize integrated electrode technology and the benefits in recording ease and accuracy that this new technology provides.
Chapter 2. Theory / Background

Introduction

Surface recorded compound action potential (CAP) waveforms result from a superposition of single fiber action potential (SFAP) signals. The manner in which the SFAPs superimpose and the relationship between the SFAPs produced by different fibers are crucial factors in any solution to the electroneurographic inverse problem. It is also important to understand the influence that corrupting signals, such as noise or artifacts, have on the shape of recorded CAPs and how those shape changes will, in turn, effect the estimation of diagnostic parameters.[4, 14, 29, 50-53]

Single Fiber Action Potential Components

A single fiber action potential (SFAP) traveling along a peripheral nerve will cause a disturbance in the electric potential outside the nerve bundle sheath. This disturbance has been shown to be between 10 and 50 nV and is therefore much too small to record, even with low-noise electronics. The subsequent disturbance on the surface of the skin, after traversing a largely resistive volume, will be even smaller making the noninvasive detection of individual SFAPs impossible. Only concurrent stimulation, electrical or otherwise, of multiple fibers within the nerve will result in a compound action potential (CAP) large enough to be detected on the skin. These evoked CAPs represent a sum of contributions from all stimulated fibers. The shape of a surface recorded CAP signal will therefore be collectively determined by the shape, amplitude, and arrival time of every single-fiber response that passes the recording site.[9, 16, 19, 54]

Peripheral nerve trunks contain tens of thousands of individual nerve axons that range in cross-sectional diameter from 2 to 20 μm.[9, 29] Figure 2.1 shows a cross-section of one fascicle of a peripheral nerve. Larger axons in the micrograph are outlined in black and some are referenced by arrows. Figure 2.1 gives an indication of the variety of both sizes and shapes that occur naturally among the fibers of a nerve bundle. There is a theoretically and experimentally well-documented relationship between fiber diameter and the speed at which an action potential will propagate along the fiber. A velocity of five m/s per um diameter is generally accepted for healthy fibers at core body temperature. The spectrum of fiber sizes in Figure 2.1 thereby leads to a distribution of
propagation velocities. SFAPs that are activated at the same location and time will not reach a distant recording site in unison. As the distance between the stimulating and the recording site increases, the time between the first SFAP to arrive and the last will also increase. This temporal dispersion will lead to a decrease in the amplitude of the surface recorded CAP and an increase in duration as the distance of propagation is lengthened.[21, 28, 29, 55, 56]

Transmembrane action potentials have been modeled very well by the Hodgkin-Huxley equations and are generally mono-phasic. However, the surrounding volume conductor will change the shape of the SFAP that is recorded at the skin. Surface SFAPs are believed to be proportional to the second derivative of the transmembrane voltage and are therefore triphasic with a small initial positive deflection, a large negative deflection, and a trailing positive deflection. This complicated shape leads to overlap and mutual cancellations when time dispersed SFAPs superimpose. The shape is also affected somewhat by the velocity of propagation, and hence the fiber diameter. Faster action potentials will pass by the recording site in less time than slower SFAPs and will therefore be shorter in duration.[16, 29, 53, 57]

The amplitude of a SFAP also varies throughout the fibers of a peripheral nerve. Again, there is a relationship between fiber size and SFAP magnitude. The core conductor model predicts that the transmembrane potential of an isolated in-vitro fiber will increase as the square of the fiber diameter. It is not clear how this variation will be transmitted to the signal at the surface of the skin. Researchers in the past have fit reconstructions of experimental data in order to determine an exponential relationship between fiber diameter and surface SFAP amplitude of the form \( A = v^p \), where \( A \) is the signal amplitude, \( v \) is the SFAP conduction velocity, and \( p \) is a constant. These reconstructions have resulted in the use of values of \( p \) that range between zero and two, with some researchers finding a non-integer exponent to best fit their own data. Since individual surface SFAPs have yet to be recorded it is difficult to determine which relationship is the most accurate. In summed CAPs, the mutual cancellation of multi-phasic SFAPs and the wider dispersion of slower propagating action potentials over the same distance as faster signals may be more dominant effects.[4, 58, 59] The basis for variations in electromagnetic potential and the production of surface CAPs are the currents that flow across the membrane of each fiber during an action potential. A single location on the membrane will have ionic currents flowing outward, inward, and then outward again during the time course of the action potential. In a cell axon these transmembrane currents propagate so that while one region of membrane is seeing the initial outward current of an action potential, the region just ahead of the propagating signal is still at rest.
Similarly, when the second region is experiencing the final outward current, the first region is now behind the propagating signal and is in its recovery phase.[58, 60]

In the body, these currents may be thought of as electromagnetic field sources lying within a large volumetric conductor. The high ionic concentration of the tissues surrounding the nerve and extending to the skin allow each fiber current source to produce an effect on the electric potential at other locations throughout the volume, and on the surface of the volume. It is this effect on the potential that can be recorded with sensitive electronics that are coupled to the volume conductor through surface electrodes. The size and shape of a surface SFAP is largely a function of the geometry, conductivity, and permittivity of the volume conductor, as well as the location and distribution of the transmembrane current sources within that volume.[51-54, 61]

The core conductor model predicts that the transmembrane current density is proportional to the second spatial derivative of the transmembrane potential. This is a useful relationship for producing forward models of the effect of volume conducted SFAPs since transmembrane potentials are closely approximated by the extracellular potential, which is relatively straightforward to measure in-vitro compared to transmembrane currents. Simple bioelectrical models of peripheral nerve bundles are often generated by assuming a perfectly homogeneous, cylindrical volume. Such models predict surface SFAPs that are low-pass filtered versions of the transmembrane current profile. SFAPs propagating at a constant velocity will satisfy the wave equation so that spatial derivatives can be converted to temporal derivatives through a change of variables. The net result is a description of the surface SFAP waveform that is proportional to a low-pass filtered version of the second temporal derivative of the extracellular action potential at the fiber membrane.[52, 53, 58] Eccentricities in the location of the nerve within the volume, deviations from perfect cylindrical geometry, and additional tissue boundaries such as those of skeletal and muscular structures will alter this simple relationship.[29, 52] Calculating the shape of a single fiber action potential at the surface of a volume conductor is, therefore, a most challenging problem.

**Mathematical Formulation**

The derivation of a useful mathematical description of surface-recorded CAP’s is an important aspect of any forward model of such signals and in solving the inverse problem, given a
noninvasive recording.[29, 32, 50-54, 62-65] Generating such a description requires a series of assumptions about the nature of nerve conduction while simplifying the description to a point of usefulness involves several mathematical and physiological approximations. The initial assumptions leading to a very general description are as follows:

1. **The CAP is a linear superposition of individual fiber components.**

This assumption is the most basic and the most important in the forward model. The volume conductor that surrounds the active tissue must display electric linearity. For the distances and types of tissue involved this condition is generally met. 2. **Individual action potentials propagate independently**

This assumption is equivalent to saying that there is no cross talk between fibers within a nerve bundle and that the propagation of an action potential in one fiber is unaffected by the activity, or lack of activity, in all other fibers. Theoretical studies have estimated that extracellular potentials within the bundle are on the order of several hundred microvolts which is too small to evoke additional responses or to affect action potential propagation in-vitro and presumably in-situ as well.[59]

3. **All action potentials due to a single stimulus are activated simultaneously, instantaneously, and at the same location.**

This assumption is equivalent to setting the activation time to zero for all fibers in the bundle regardless of individual morphology or location. In reality, the delay before activation is at most a fraction of a millisecond and its neglect can therefore be justified. This assumption will induce errors in the measure of conduction velocity as determined from individual latencies. These errors are small however, when compared to systemic variability of the measurement technique, and become smaller with increasing distance of propagation. At high stimulus levels there is phenomenon known as the virtual cathode effect in which some action potentials are activated away from the actual site of stimulation. Again, this effect is reduced as the propagation distance is increased. The localization assumption will only err on the side of decreased latency while neglecting activation delay time will tend to result in longer measured latencies. In general, and over reasonable propagation distances, the errors due to these two assumptions will tend to cancel each other out.

4. **The conduction velocity in an individual fiber is constant.**
Another way to state this assumption is that the average conduction velocity, the velocity that results from the ratio of distance to latency, is an accurate measure of the actual conduction velocity in the fiber. This is not strictly true due to variations in fiber diameter along the length of an axon and changes in temperature, determined largely by the proximity of the nerve to the skin surface. Unfortunately, these variations are impossible to predict or to measure noninvasively. This final assumption is common to virtually all nerve conduction studies and will be made in this case without further justification.[29]

These four assumptions allow the surface-recorded CAP to be described in its most general form as a summation of individual SFAP components. That is,

\[ C(t; I) = \sum_{j=1}^{M} \phi_j \left( t - \frac{l}{v_j} ; v_j \right) \]  

(2.1)

where

\[ C(t; I) = \text{the CAP signal as a function of time and distance,} \]

\[ \phi_j (t; v) = \text{the surface SFAP associated with fiber } j, \]

\[ l = \text{the distance of propagation between the stimulating and recording electrodes,} \]

\[ v_j = \text{the propagation velocity associated with fiber } j, \]

and

\[ M = \text{the total number of activated fibers.} \]

This formulation is appropriate to any CAP signal that meets the four assumptions above, regardless of recording method. Using this equation to develop a forward model of a CAP is extremely difficult, however, because it requires a-priori knowledge of the SFAP and CV for each of the tens of thousands of activated nerve fibers. In order to simplify the forward problem, and eventually the inverse solution, researchers in the field have adopted several approximations. The first of these approximations involves the ability to break the fibers of the nerve into distinct and discrete classes. Each fiber class contains all fibers in the nerve bundle that have a distinguishing feature in common. One feature that could potentially be used to define fiber classes is fiber
diameter. Although there is a more or less continuous spectrum of fiber diameters within the nerve, one can easily define fiber diameter classes that are referenced by their central value and include fibers that have a diameter within a given range. For instance, convenient reference values for diameter would be whole numbers that fall in the range from 2 \( \mu m \) to 20 \( \mu m \). Every fiber having a diameter within 0.5 \( \mu m \) of a whole number falls within that class. For the sake of mathematical formulation, every fiber in a given class, or bin, is approximated as having the reference diameter. It is also possible to determine classes by latency or by velocity. Classes based on velocity are equivalent to those defined by diameter because of their linear relationship. Latency is inversely related to fiber diameter, so that equally spaced diameter classes are not directly translatable to equally spaced latencies. Since CAPs are recorded in the time domain, however, it is conceptually easier to continue the mathematical formulation by discussing fiber classes defined by latency.

The continuous SFAP spectrum is approximated as \( N \) fiber classes, distinguished by progressively longer but evenly spaced arrival times. The sampled CAP signal can then be expressed as

\[
C(t_k) = \sum_{j=1}^{N} n_j \phi_j (t_k - \tau_j)
\] (2.2)

where \( C(\cdot) \) is the recorded CAP signal, \( t_k \) is the \( k \)-th sample of continuous time, \( \tau_j \) is the propagation delay (latency) of fiber class \( j \), \( \phi_j \) is the SFAP waveform for fiber class \( j \), and \( n_j \) represents the number of nerve fibers in that class. The latency of each class is a function of the distance of propagation, \( l \), and is given by \( \tau_j = l/v_j \). In this equation, the further approximation has been made that all fibers in a class share the identical surface SFAP waveform. Figure 2.2 is a graphical description of the mathematical formulation to this point. Starting at the left hand side of the diagram, the single fiber action potential for each fiber class is shown. SFAPs are delayed by an amount appropriate to their conduction velocity and the distance of propagation, \( \tau_j \), resulting in a series of shifted SFAPs. The total number of fibers in each class is incorporated as a scaling factor, \( n_j \), before summing all components along with any corrupting signals that may be present. Finally, the compound action potential that is ultimately recorded is shown at the right. Figure 2.3 is a more detailed plot of an actual CAP recording and will be further described in the section of this chapter on nerve conduction studies.
If we continue the formulation by further assuming that SFAPs are the same for all fiber classes, the summation can be written as

\[
\overline{C}(t_k) = \sum_{j=1}^{N} n_j \overline{\phi}_0(t_k - \tau_j)
\]  
(2.3)

where \(\overline{\phi}_0\) is the SFAP waveform common to all fibers. A vector, \(\overline{n} = \{n_1, n_2, \ldots, n_N\}\), whose components are the number of fibers belonging to each of the \(N\) fiber classes is defined. \(\overline{n}\) represents a histogram of SFAP arrival times (latencies). If equally spaced latencies, \(\tau_j\), are used to define the latency histogram, and the spacing between latencies is equal, the summation is simply a convolution of two finite sequences and can be expressed as

\[
\overline{C} = \overline{\phi}_0 \otimes \overline{n}.
\]  
(2.4)

It is convenient to think of the latency histogram, \(\overline{n}\), as a function of the conduction velocity distribution, the ultimate function of interest. Doing so is also useful because the CVD remains the same for multiple CAPs recorded from the same nerve even though the latency distribution changes with propagation distance. We define a CVD, \(\overline{\omega} = \{\omega_1, \omega_2, \ldots, \omega_M\}\), with \(M\) components representing \(M\) discrete fiber velocities. \(\omega_i\) is the number of fibers activated following the stimulation of a nerve at a particular point whose propagation velocity falls within class \(i\). The SFAP arrival time histogram at a recording site a distance \(l\) away can then be calculated as a linear transformation of \(\overline{\omega}\). The distance-dependent latency distribution is then

\[
\overline{n}_l = Q_l \overline{\omega}
\]  
(2.5)

where \(Q_l\) is a sparse matrix with \(M\) columns (one for each velocity class) and \(N\) rows (one for each discrete latency). Each column of \(Q_l\) contains a single, non-zero component whose value is unity. The location of the non-zero matrix entries relates each fiber velocity class (column number) to an appropriate arrival time (row number) for the specific propagation distance, \(l\). The distance dependent latency distribution, \(\overline{n}_l\), can be used in equation 2.4 to specify a CAP that was activated with a certain conduction velocity distribution, \(\overline{\omega}\), and propagated a given distance, \(l\), before being recorded.
The fact that equation 2.4 is a simple convolution reveals another important aspect of this derivation. The SFAP waveform, \( \hat{\phi}_0 \), can be thought of as the impulse response of a linear system. The system, in this case, includes the nerve, the surrounding volume conductor, the electrode, and the recording hardware. Differences in the geometry of the nerve with respect to the electrode or the type, size, and quality of the electrode will affect the impulse response of the system at different recording locations. It is useful to group all of these factors into a single function. We refer to this as the nerve-to-electrode transfer function, or NETF. Although we have assumed that the SFAP waveform is the same for every fiber in the nerve, there is no reason to expect that it will be the same for two different recording sites. The form of the SFAP will be determined by the NETF for each particular recording location. The impulse response of the nerve-electrode system, \( \hat{\phi} \), will change accordingly.

**Surface Electrodes**

Noninvasive stimulation and recording of nerves is accomplished with surface electrodes placed on the skin. Modern surface electrodes consist of metal disks or flexible metallic strips that are held to the skin surface with tape or adhesive. The interface between metal and skin includes an intervening layer of electrode paste or electrolytic hydro-gel. The paste or hydro-gel facilitates the translation of electronic to ionic current at the metal-paste interface and greatly reduces the impedance between the electrode and the skin surface. The deceivingly simple system consisting of the skin, hydro-gel, and metallic electrode has been studied extensively but is nonetheless incompletely understood. For the present work it suffices to make a first pass at describing the system and to remain mindful of how the nonlinear electronic behavior may affect CAP recordings.[14-17, 23, 66, 67]

The outer layers of skin are generally thought of as a resistive sheath, insulating the inside volume conductor from the outside world. Applying electrode paste can reduce the resistance of the skin layer by ensuring that the maximum surface area available under the electrode is coupled to the outside system. Increasing electrode size will accomplish this as well and is an effective method of reducing impedance under the ground electrode. Electrodes used to stimulate or record, however, generally rely on depolarizing or recording from a localized region of the nerve so that blindly increasing their size is counterproductive. The skin impedance can be further reduced by a skin preparation procedure that includes mildly abrading the skin underneath the electrode with an abrasive pad or pumice and thorough cleansing with an alcohol preparation pad. Skin impedance
is also affected by physiologic processes such as the opening and closing of sweat glands, and by extrinsic factors such as the density and frequency of the applied current.[15, 16, 66, 68]

The electro-chemical reaction at the interface of the metal surface and the hydro-gel is more complicated and contributes the majority of the nonlinear behavior. Metal atoms dissociate into the gel forming a charge separation layer. This layer is responsible for the half-cell potential, a DC offset characteristic of the materials used, and for establishing capacitances that are frequency- or current density-dependent. Any current traversing the electrode has to bridge the separation gap and undergo a conversion from electronic to ionic current or vice-versa. On top of these complicated effects is the deterioration of both the metal and gel electrode components over time and following exposure to air. The result is a nonlinear system with a variable DC offset.[66, 68]

It is important to note that many factors affecting skin impedance are outside of a researcher’s control and dynamic enough to vary during a single recording session. The minimal preparation procedure for stimulation, recording, or grounding electrodes should therefore consist of an abrasive scrub sufficient to redden the skin, a thorough cleansing with alcohol, and liberal use of electrode paste or gel. These procedures will reduce corrupting noise and artifacts and increase signal amplitude as much as noninvasive studies will allow.

Under low current-density conditions such as those experienced during recording of a CAP the electrical properties of the skin-electrode interface are dominated by the resistive components of the skin. In this case a parallel combination of a linear resistor and a capacitor serves as an adequate model of the electrode’s behavior. When current densities increase, the nonlinear properties of the metal-gel interface begin to dominate and must be taken into account in any model. These nonlinear effects have been modeled as a second order current-voltage relationship of the form \( I = aV + bV^2 \). In this model, \( a \) and \( b \) are constants to be determined empirically.[66, 68]

**Hardware**

The recording of a peripheral CAP has long been a biomedical instrumentation challenge. As recording systems have improved over the years, researchers’ knowledge of neurophysiology and clinicians’ diagnostic abilities have also grown. CAP recording requires two, largely independent hardware components. First, the stimulating circuit must be able to deliver large voltages to the stimulating electrodes in a manner that allows complete control of stimulus onset and duration.
Second, the recording circuitry must combine high input impedance and high-gain analog signal conditioning components with accurate analog to digital conversion while maintaining low-noise conditions. These two elements must be electrically isolated from each other and yet they have to communicate in order to coordinate the stimulation and recording processes. Development and improvement of stimulation and recording hardware is a large part of any project that includes the noninvasive measurement of peripheral nerve signals.\[15, 66, 69-75\]

**Stimulation Hardware**

There are two main types of electrical stimulation that are used to evoke peripheral CAPs, constant-voltage and constant-current. As their names imply, each technique involves the maintenance of either a known voltage or a known current between the two stimulation electrodes. Constant-voltage stimulators are characterized by their low output impedance. The amount of current between stimulating electrodes required to maintain the constant voltage is a function of the intervening impedance. Constant-current stimulators, on the other hand, have a high output-impedance and the voltage difference applied to the electrodes is continually adjusted to maintain a desired current flow. Regardless of the type of stimulation, the stimulation hardware needs to remain electrically isolated from the recording circuitry to avoid direct coupling of the stimulus pulse and isolated from the laboratory ground to avoid short circuits that are potentially dangerous to the subject. Isolation can be accomplished through transformer- or optical-coupling of the stimulation circuitry. No amount of isolation can completely eliminate capacitive coupling between the stimulation and recording hardware, mainly through the lead cables. This coupling can be minimized by using short leads and twisting the leads around each other.\[60, 66, 68, 75, 76\]

**Recording Hardware**

The small magnitude of noninvasive CAP signals mandates very-high-gain analog electronics in order to record them efficiently. Gains greater than 10,000 are required and gains on the order of 100,000 are not uncommon. Besides large gains, large input impedance and a high common mode rejection ratio are required. The input impedance must be much greater than the skin-electrode interface impedance (typically tens of kilo-ohms) to avoid distortions in the recordings. High common mode rejection is required for reducing transient and noise signals at the recording site. Of particular importance is the reduction of 60 Hz interference that would otherwise overwhelm the CAP signals.\[14, 66, 71\] These characteristics are generally accomplished through the use of commercial instrumentation amplifiers. In its simplest embodiment, an instrumentation amplifier
is equivalent to three operational amplifiers. Each differential input is buffered by its own operational amplifier and the outputs of those two amps are fed into the third operational amplifier. Modern instrumentation amplifier integrated circuits commonly have input impedances on the order of $10^{12}$, common mode rejection of 100 dB, and a variable gain that is controlled by an external resistor.

Recording of CAPs further requires effective band pass filtering. The high pass cutoff should be high enough to attenuate the slowly changing DC offset generated by the half-cell potential of the electrodes. Unlike electromyographic responses, the CAP has very little energy near 60 Hz so it is advantageous to use a high pass cutoff above 60 Hz in order to attenuate some of the ambient 60 Hz interference. The low pass filter is needed to reduce the wideband, resistive noise produced at the electrodes but its cutoff should not be below 5 kHz in order to preserve the bulk of the energy in the CAP signals themselves. Distortion in these analog filters can cause CAP components to be shifted in time and care should be taken to keep distortion low in the frequency regions of greatest interest.

**Corrupting Signals**

**Broadband Noise**

As mentioned previously, noise and artifacts are ever-present issues in the field of peripheral nerve recording and analysis. The major contributors to unwanted interference are thermal noise from the recording electrodes, 60 Hz interference from other lab equipment, and artifacts of the evoking stimulus. The problem of thermal (Johnson) noise is impossible to escape. Modern recording hardware often reaches the physical limits of the electrodes and the electronic components in this respect. While lower noise components are always being developed, they cannot be relied upon to solve the problem. Analog filtering helps to improve the signal to noise ratio but cannot be applied over the frequency band that contains the CAP. This leaves ensemble averaging as the only choice for further improving the ratio of CAP signal to inherent Johnson noise. Ensemble averaging relies on a lack of correlation (randomness) in the noise components. The signal to noise ratio will improve as the square root of the number of averaged signals. Studies in which thousands of individual CAPs are averaged to yield each CAP record are common. With today's recording hardware these numbers can be reduced but ensembles containing more than one hundred CAPs are normal. The averaging of multiple evoked signals makes several assumptions about the ergodicity, or periodic stationarity, of the responses over the recording session. There are almost
certainly rapidly changing factors, such as the instantaneous excitability of the nerve, and slowly changing effects, such as the opening of sweat glands under the electrodes or corrosion of the electrode itself, that will violate these assumptions. Ensemble averaged CAP signals contain errors arising from slight differences in the individual contributing CAPs and will consequently be spread in the time domain. Hence, there is a tradeoff between the amount of wideband noise and the accuracy of the CAP representation. Some residual noise is apparent in the CAP recording of Figure 2.3, which is an average of 128 individual time records. Based on the size of the ensemble, the signal-to-noise ratio of each record was more than 11 times lower.

**Stimulus Artifact**

Ensemble averaging will not attenuate signals, such as the stimulus artifact, that are correlated with recording time. These artifacts, along with the CAP signal itself, will persevere and even become more discernable as the amount of averaging is increased. There are several methods for both reducing (a-priori) and removing (a-posteriori) stimulation artifacts. They all stem from a sufficient understanding of the sources that contribute to the artifact.[68, 74, 76-78]

The CAP of Figure 2.3 illustrates a typical stimulus artifact. The largest portions of the artifact are caused by true voltage differences between the recording electrodes due to current flowing through the limb. The current of the stimulation pulse itself causes a large spike at the recording site of the same time course as the stimulus. The discharge of the capacitive components of the stimulating electrodes then causes a voltage gradient that follows the stimulus but falls off rapidly. The duration and amplitude of the electrode discharge component is dependent upon the type of stimulation, constant-voltage or constant-current, because of differences in the output impedance of the stimulator. True voltage differences at the recording site due to stimulation currents will fall off as the recording site moves further and further from the stimulation site. Positioning the recording electrodes to lie on an equi-potential line with respect to the stimulating electrodes can also reduce these elements of the stimulus artifact. The location of equi-potential lines on the surface of the skin is highly dependent upon the geometry and electrical properties of the body. Complicated and ever-changing shapes such as those of the hand and forearm make it impossible to predict where equi-potential lines may lie during stimulation. Some researchers have attempted to influence this component stimulus artifact by selectively moving the anode stimulation site until the artifact is minimized. These technique has proven effective but requires some freedom in anode placement and electrodes that can easily be shifted on the skin.
Another cause of stimulus artifact is the conversion of common mode signals. Differences in the impedance of the two differential recording electrodes will cause voltages that are common-mode on the skin to be amplified. A common mode signal, $V_{CM}$, will be converted as

$$V_{CCM} = \frac{Z_1 - Z_2}{Z_1} V_{CM}$$

(2.6)

where $Z_1$ and $Z_2$ are the impedances of the recording electrodes and $Z_I$ is the input impedance of the recording amplifier. The difference in recording electrode impedance may be on the order of 10 kΩ. This difference, along with the very high input impedance of modern instrumentation amplifiers is usually enough to make this artifact component negligible.

No matter how well isolated the stimulator is from the laboratory, there will be stray capacitance to ground. These capacitances largely arise from coupling of the subject’s body, which is connected to the stimulator, to the surrounding environment. Currents, called escape currents or displacement currents, will flow to charge the stray capacitances during the stimulus pulse.

The final direct cause of stimulus artifact is due to capacitive, or electromagnetic coupling between the stimulating and the recording hardware. The main causes of coupling are unshielded stimulation and recording leads. Shielding to ground increases the stray capacitances and is generally not done. The most effective way to reduce coupling, and the artifacts due to coupling, is to keep stimulation and recording leads as short as possible and as far apart as possible. It has also proven effective to twist stimulation and recording leads (around themselves) in order to eliminate any large loops.

All of the artifacts that are produced through the preceding interactions are susceptible to shaping by the signal conditioning components of the recording hardware. The passage of a stimulus spike through the high pass filter will produce an exponential tail, often lasting several milliseconds. This is particularly dramatic and problematic when the spike is large enough to saturate the input amplifier. Other artifact components will also be altered through filtering but not as severely as the initial spike. The most effective method of reducing spike shaping is by attenuating the recorded impulse as much as possible. Besides the techniques mentioned above, researchers have had some success developing sample-and-hold amplifiers that do not respond, or have a delayed response, during and immediately following the stimulus pulse.
Given the preceding description of the CAP signal and of the corrupting noise and artifacts, each individual recorded signal can be written as the sum of three components. That is

\[ \text{CAP} = C(t) + \text{SA}(t) + \epsilon(t) \]  

(2.7)

where \( C(t) \) is given by equation 2.2, \( \text{SA}(t) \) is the stimulus artifact, and \( \epsilon(t) \) is the broadband noise. If an ensemble of \( P \) pseudo-ergotic signals are averaged, the result is

\[ \text{CAP} = \frac{1}{P} \sum_{j=1}^{P} C_j(t) + \frac{1}{P} \sum_{j=1}^{P} \text{SA}_j(t) + \frac{\epsilon(t)}{\sqrt{P}} . \]  

(2.8)

In this equation the average of the uncorrelated, random noise signals has been written as a stochastic signal itself, \( \epsilon(t) \), scaled by the square root of the ensemble size. If the ensemble is acquired in a relatively short period of time so that physiologic and motion-induced changes during the course of acquisition can be neglected, the averages of individual CAPs and individual artifacts can be simplified to:

\[ \text{CAP} = C'(t) + \text{SA}'(t) + \frac{\epsilon(t)}{\sqrt{P}} . \]  

(2.9)

Since the CAP waveform and the stimulus artifact are highly correlated between stimuli, the average of an ensemble of these signals will yield an estimate of the original. Any differences between the original signal and the averaged signal are due to slight changes that occur over the time of acquisition and that affect waveform shape. Shape differences that affect a single element of the ensemble will be tempered by the size of the ensemble but will alter the average. These small alterations are the reason for the primes in equation 2.9. For the purposes of most studies these differences are neglected and the CAP and stimulus artifact estimates are assumed to be accurate.

**Removal of Corrupting Signals**

While eliminating the causes of the stimulus artifact through improved skin preparation (thoughtful electrode placement and the use of proper electrode technology is the preferred solution) not every component can be removed completely. Several signal processing methods have therefore been developed to remove or reduce artifacts retrospectively. Since stimulus artifact components and the CAP signals themselves generally contain energy in the same bandwidth, frequency domain
methods cannot be used effectively. Most techniques therefore involve subtracting an estimate of the stimulus artifact from the corrupted recording in the time domain. Several different methods of estimating a pure stimulus artifact have been developed.[55, 68-70, 72, 74-80]

The most common way to estimate a stimulus artifact is through sub-threshold stimulation. Stimuli with an amplitude below the level needed to activate the nerve tissue are applied and the response at the recording electrodes is measured. The sub-threshold stimulus artifact is then scaled up to the same amplitude as the artifact of interest and subtracted. The main problem with this approach is that broadband noise in the artifact estimate is also scaled and subtracted from the signal, dramatically reducing the signal to noise ratio and abrogating the work done to reduce recording noise. The other major problem with this technique is that it assumes system linearity, that an increase in stimulus amplitude will lead to a proportional increase in artifact. In reality, the nonlinear aspects of the skin-electrode interface and the recording hardware violate this assumption. This is especially true when the artifact is large enough to saturate the system so that recovery from saturation becomes an issue.[68, 74, 76]

Due to these shortcomings of sub-threshold stimulation, methods have been developed to estimate the artifact with full amplitude stimuli so that dramatic scaling is not required. One such method utilizes an additional set of recording electrodes placed away from the nerve but at a similar distance from, and with a similar orientation to the stimulating site. The stimulus artifact recorded off the nerve is expected to be of approximately the same shape and amplitude as the corrupting artifact recorded over the nerve. Unfortunately, the complicated shape of the limb and the corresponding equi-potential lines on the skin surface make it very difficult to choose two recording sites that yield equivalent stimulus artifacts. It is further difficult, particularly when recording from upper extremity nerves to move the off-nerve electrodes to a position where they will not record any nerve activity. The off-nerve signal often contains a small nerve response and subtracting this signal from the over-nerve recording will, while removing some of the stimulus artifact, complicate the resulting CAP waveform. An analogous method that uses two stimulation sites, one over the nerve and one away from the nerve, has also been attempted. Unfortunately the same limitations exist.[68]

Another technique involves stimulation during the nerve’s absolute refractory period. If two stimuli are presented to the tissue close enough together in time, second pulse will not elicit an action potential because the nerve will still be in its recovery phase. The resulting recorded signal will contain two stimulus artifacts but only a single CAP response. If a standard (single stimulus)
CAP recording is subtracted from the double-pulse signal, the result is a time-shifted estimate of the pure stimulus artifact. This estimate can be shifted back in time and subsequently subtracted from the original to leave only the CAP itself. This method avoids problems associated with multiple electrode sites because only one stimulation site and one recording site are needed. Problems with system nonlinearities and system saturation, however, are amplified. The absolute refractory period of the nerve lasts only one to two milliseconds so that the second stimulus pulse needs to be presented while the artifact from the first pulse is still quite large. This will complicate nonlinearities and increase the possibility of saturating the system amplifiers.[68]

Other researchers have avoided the drawbacks of the previous methods and opted instead to fit the stimulus artifact to a known, parameterized function. This regression method is generally applied by determining the parameters that produce the best fit over a region of the signal that does not include the CAP waveform, usually the region between the stimulus pulse and the CAP. The fitting function is then extended over the entire recording and subtracted. This method can be effective, particularly when the stimulus artifact is minimal in the region of the CAP. Problems arise because no parameterized function will be able to match the complicated artifact exactly no matter what order function is used.[18]

**Nerve Conduction Studies**

Artificial activation and recording of compound action potentials has been performed for many years as a manner of assessing peripheral nerve health.[3, 14-17, 23, 66, 81] Electrical current stimulation is the most common method of activating compound nerve signals and is used exclusively in this work. Noninvasive electrical stimulation of nerve tissue consists of depolarizing the transmembrane potential by injecting current to the region of skin over the nerve. The optimal stimulus for studying nerve propagation will activate every fiber in the nerve bundle. Such a stimulus is called ‘supramaximal’ and can be realized by injecting sufficient current to raise all fibers above threshold. The model stimulus will also evoke an action potential in every fiber at the exact same time and place. This ideal is more difficult to accomplish but can be approached by using a small, localized stimulation electrode and applying a supramaximal current over the shortest time possible. Minimizing stimulus length will also help to reduce artifacts in the CAP recording. For these reasons it is desirable to use the shortest possible stimulus pulse that will remain supramaximal given the maximum available current of the stimulation hardware. The typical current stimulus lasts for less than 0.2 ms and requires as much as 50 mA of current.
The location of most efficient action potential initiation is under the cathodic stimulating electrode. Action potentials will propagate in both directions from this site. Action potentials that propagate towards and under the stimulus anode will pass through a hyperpolarized region of tissue. The transient hyper-polarization may affect the propagating signals and lead to indefinable propagation anomalies. To avoid these problems, the stimulus cathode should be closer to any recording sites than the anode.[60]

Noninvasive peripheral nerve recording is accomplished by placing electrodes over the nerve, on the surface of the skin. The more superficial the nerve is at the recording site, the larger the amplitude of the recorded signal. The small signals being recorded require a differential recording scheme with high gain and a large common mode rejection ratio. Instrumentation amplifiers are used to accomplish this. Differential recordings can be monopolar, with an active electrode directly over the nerve and an indifferent electrode placed away from the nerve activity of interest, or bipolar, with both active and indifferent electrodes directly over the nerve but separated by some distance. Monopolar recordings approximate the potential displacement at a point above the nerve and are therefore triphasic waveforms. Bipolar recordings represent the difference between two such potentials and have a more complicated, multi-phasic shape. Bipolar recordings are further complicated due to a direct dependence on the separation of the two recording electrodes. A separation of 2 to 4 cm is common but signal amplitude and signal shape will both vary widely as this distance changes. [17, 28, 67] A typical monopolar action potential waveform is shown in Figure 2.3. The signal has one large, center peak that is flanked by two, lower-amplitude peaks with the opposite polarity. The central peak represents a negative shift in potential at the site of the active recording electrode with respect to a remote, indifferent electrode. Although negative, standard convention is to graph this peak as an upward deflection. The two positive peaks are, therefore, plotted downward. This convention is used throughout this document without being explicitly indicated.

The importance of nerve propagation studies to clinical neuroscience lies in their diagnostic capabilities. Typically, either the amplitude or the latency (time of propagation) or both are determined from CAP waveforms and used clinically. The amplitude of the CAP gives an indication of the total number of fibers responding to the stimulus pulse.[9] When the stimuli are strong enough to be supramaximal, the total number of responding fibers correlates with the total number of excitable fibers in the tissue. The amplitude can therefore be compared to known normal values or longitudinal data from the same subject to indicate an overall loss of fiber activity. Amplitude is generally measured from the peak of the initial positive peak to the
maximum value of the negative peak. This peak-to-peak measurement is not the only method, however, and other techniques such as zero-to-peak or overall maximum to minimum have been used clinically. [17] What is most important in the comparison of amplitude data is to ensure that the same method was used throughout. The peak-to-peak amplitude of the CAP in Figure 2.3 is indicated and measures approximately 13 μV.

Latency is used to determine the speed at which the CAP propagates along the nerve. Latency determination differs in different laboratories and for different types (monopolar or bipolar) of signals. Generally, latency is determined by the arrival time of either 1) the onset of the leading positive phase, 2) the peak of the leading positive phase, 3) the zero crossing between the leading positive and large negative phase, or 4) the peak of the negative phase. Each of these points reflects early arriving SFAP components and such measurements are often called minimum latency measures.[23] The negative phase latency of the CAP in Figure 2.3 is also illustrated and measures approximately 3.5 ms.

Since latency is inherently dependent on the distance of propagation, it is difficult to compare latency numbers measured at different times or with different techniques. In order to make such comparisons valid, latency must either be measured over a standard propagation distance or converted into a conduction velocity (CV). Standard propagation distances may be determined either by anatomic landmarks, e.g. median nerve propagation between the first interphalangeal joint of the middle finger and the anticubita fossa, or by measurements made over the surface of the skin, e.g. from the first interphalangeal joint of the middle finger to a point on the ventral wrist that is 14 cm away. When standardization is not possible, or when comparing data based on different standards, CV is used as a measure that is independent of propagation distance. CV is calculated as the propagation distance divided by the latency and is generally referenced in meters per second. Reduced CV in a peripheral nerve, when compared to normal values or to prior measurements in the same subject, indicate deterioration in nerve health that may accompany various neuropathies. Both axonal neuropathies, affecting the membranes ability to generate action potentials, and demyelinating neuropathies, affecting the nerve’s resistive sheath covering, will lead to lengthened latencies and hence reduced CV.[14, 15]

The Inverse Problem in Electroneurography

It has long been a goal of clinical neurology to develop an effective manner of estimating the distribution of conduction velocities in peripheral nerve without having to resort to destructive
measures. Measurement of CVD has been shown to be a more sensitive indicator of peripheral neuropathy and has the potential to be a more specific endpoint for clinical studies. Certain disease states may lead to slowed conduction velocity in some fibers while leaving the majority of fibers unaffected. A simple measurement of maximum conduction velocity will not yield diagnostic information in this case. A more thorough parametric description of conduction within the nerve bundle is required. CVD analysis and the inverse problem offers this type of description.[1, 12, 18, 30-33, 35-46, 48, 82-86]

Given the assumptions of the forward problem that allow the CAP to be written as a convolution, Equation 2.4, the inverse problem can be approached in a number of different ways. Methods typically differ in the amount of a-priori knowledge about SFAP waveforms that is assumed and the number of CAP signals that are simultaneously recorded. The most common methods fall into two main categories: single recording (1CAP) techniques and double recording (2CAP) methods. Single recording methods require one CAP recording and considerable knowledge of the SFAP waveforms for all fibers. Double recording methods require two CAPs representing two different propagation distances along the same nerve and an understanding of how SFAPs vary from fiber to fiber.

**Single CAP (1CAP) Formulation of the Inverse Problem**

If the form of the SFAP is known or can be approximated, then a least squares or maximum likelihood estimation algorithm can be used in a 1CAP method to directly calculate the CVD. There are several options for determining the form of the SFAP. The most straight forward is an estimate based on volume conductor theory. Very simple estimates of SFAP waveforms, including piecewise linear, have been used in past research. There are also methods of estimating SFAPs based on CAP measurements. Very weak stimuli have been used in an attempt to excite only a small subset of fibers which have a narrow CV distribution.[18, 29, 38, 44, 46, 83]

To formulate the inverse problem, Equation 2.4 can be expressed in discretized form as

\[
\bar{C} = \Phi_0 \tilde{n}_i \tag{2.10}
\]

where \( \bar{C} \) is a vector representing the recorded CAP waveform, \( \Phi_0 \) is a matrix with elements:

\[
\Phi_{0(i,j)} = \phi_0 \left( t_i - \tau_j; \frac{t}{\tau_j} \right) \tag{2.11}
\]
that are shifted versions of the basis SFAP, $\bar{\phi}_0$, and $\bar{n}_i$ is a latency distribution appropriate to the distance of propagation and defined in Equation 2.5.

If the SFAPs are known, can be guessed, measured, or calculated then the matrix $\Phi_0$ can be specified and the remaining task is to calculate $\bar{n}_i$ from a known $\Phi_0$ and measured $\bar{C}$. The least-squares solution to this equation is given by:

$$\bar{n}_i = \left[\Phi_0^T \Phi_0\right]^{-1} \Phi_0^T \bar{C}$$

(2.12)

The columns of $\Phi_0$ are linearly independent because they are SFAP time series with different latencies. This independence guarantees that the matrix $\Phi_0$ has full rank, that $\Phi_0^T \Phi_0$ is invertible and that a least-squares solution exists. This does not guarantee that the inversion of $\Phi_0^T \Phi_0$ is something that can be performed in a straightforward manner. This inversion is further complicated when the SFAP waveforms are estimated from noisy measurements. Gaussian elimination techniques have largely been employed to solve this equation.

**Double CAP (2CAP) Formulation of the Inverse Problem**

The solution outlined above is based on a single CAP recording. Inherent in this 1CAP formulation is the assumption that the SFAP components can be accurately determined. Errors in the SFAP waveforms will lead to errors and inaccuracies in the estimated CVD. To overcome these assumptions, researchers have developed CVD estimation techniques that incorporate information from two CAP recordings made from the same nerve that have different propagation distances. A 2CAP-recording scheme will allow the CVD to be estimated without the direct calculation of SFAP waveforms. 2CAP algorithms are based on two independent recordings of the same evoked compound signal made at two different recording sites. These algorithms rely on the assumption that both CAPs are due to the same CVD and the same SFAP waveforms. Only the distance of propagation, and hence the distribution of SFAP latencies, is considered to change. This does not simply translate to a shift of the CAP signal in time. The CAP changes in duration and shape due to larger temporal dispersion of the signal at longer propagation distances.[32, 37, 39, 42, 43]

Two CAPs recorded from the same nerve following the same stimulus will share the same CVD. Different distances of propagation, however, will lead to differences in the arrival time (or latency)
distribution of the two signals. If two CAPs, $C_1$ and $C_2$, are recorded from two different locations, 1 and 2, following a single stimulus, then equations 2.4 and 2.5 can be rewritten to apply to each signal. That is

$$C_1 = \phi_0 \otimes \bar{n}_1 \quad \text{and} \quad \bar{n}_1 = Q_1 \bar{\omega} \quad (2.13)$$

for the first CAP, and

$$C_2 = \phi_0 \otimes \bar{n}_2 \quad \text{and} \quad \bar{n}_2 = Q_2 \bar{\omega} \quad (2.14)$$

for the second. Where the SFAP waveform, $\phi_0$, is common to both recording electrodes, and the transformation matrices, $Q_1$ and $Q_2$, are referenced by their respective propagation distances, $l_1$ and $l_2$. This formulation can be extended to any number of compound signals recorded at different locations along the nerve.

If the first equations in 2.13 and 2.14 are post-convolved with $n_2$ and $n_1$ respectively the result is:

$$S_{n_2} = 0 \quad n_1 \quad n_2 \quad (2.15)$$

and

$$C_2 = C_2 \otimes \bar{n}_2 \otimes \bar{n}_1 \quad (2.16)$$

The right hand sides of these equations are identical since $\bar{n}_1 \otimes \bar{n}_2 = \bar{n}_2 \otimes \bar{n}_1$. Therefore, the left hand sides must also be equal so that:

$$C_1 \otimes \bar{n}_2 = C_2 \otimes \bar{n}_1 \quad (2.17)$$

Utilizing the definition of $\bar{n}$ in Equation 2.5, this can be written:

$$C_1 \otimes Q_2 \bar{\omega} = C_2 \otimes Q_1 \bar{\omega} \quad (2.18)$$

Finally, if we define a matrix, $C$, that has columns which are delayed versions of the recorded signal, $C$, then the last equation can be written as:
\[ C_1 Q_2 \bar{\omega} = C_2 Q_1 \bar{\omega} \quad (2.19) \]

implying that,

\[ \left[ C_1 Q_2 - C_2 Q_1 \right] \bar{\omega} = 0 \quad (2.20) \]

This is a homogeneous, linear matrix equation describing the CVD of a nerve bundle with respect to two independent recordings of a compound action potential and the relationships between the single fiber contributions of different fiber classes. This equation can be solved by iterative algorithms, such as steepest descent, that minimize the left hand side while varying the CVD term, \( \bar{\omega} \). [37]
Peripheral Nerve Fascicle Cross-Section

Figure 2.1
Components of a Surface Recorded Compound Nerve Action Potential

Figure 2.2
Typical Compound Action Potential Signal

Figure 2.3

- Stimulus Artifact
- Latency
- Amplitude
- DC Offset

Signal Amplitude (µV)

Time From Stimulation (ms)
Chapter 3. Preliminary Investigations

Introduction

The first experimental stage of this project was undertaken to gain familiarity with noninvasive CAP recording equipment and methodology, to define and refine algorithms for the estimation of CVDs, and to better understand the effect of dispersion on CAPs recorded at different locations. Identifying a robust method of data collection is of primary importance for any study of bioelectric phenomena. The size and location of stimulating and recording electrodes were altered and types and timing of the stimuli were varied in an attempt to consistently generate quality CAP signals. Considerable time was spent attempting to estimate SFAPs from various recording sites, a necessary first step if CVDs are to be estimated from a single CAP recording. It has been hypothesized that estimates of an SFAP can be recorded directly by applying unique stimulation and recording schemes. Attempts were made to duplicate other researcher’s SFAP estimation results using these techniques and new methods were proposed and investigated. Algorithms were written in MATLAB to allow the estimation of CVD, both from single CAPs and from pairs of CAPs recorded at different sites. The performance and behavior of these algorithms was assessed.

Methods

Hardware

The original hardware configuration consisted of a commercial, constant-current stimulator, an analog signal conditioning circuit, and a PCMCIA data acquisition card. All of these components were connected and controlled by a laptop computer running virtual instrumentation software.

The stimulator, World Precision Instruments model A365, is capable of delivering monopolar, constant-current stimulus pulses up to 10 mA in amplitude with over 100 V compliance limit. Stimulus amplitude is controlled by an adjustment knob allowing a 0.1% resolution with three different maxima, 0.1 mA, 1 mA, 10 mA. The stimulator output timing and duration is controlled by a TTL logic pulse input. While the input is high, the stimulator will push the desired current between stimulating electrodes regardless of load resistance but limited by the 100 V compliance limit. Rise and fall time of the stimulus is very fast and output is either on or off, producing square-wave pulses.
The input stage of the single recording channel consists of a Burr-Brown FET input instrumentation amplifier, INA111. The instrumentation amplifier has $10^{12}$ Ω input impedance, 106 dB common mode rejection ratio, and a variable, resistor-set gain. The INA also has very low offset voltage and voltage drift. These characteristics have made the INA111 an excellent choice of input stage throughout this work. The rest of the analog circuit consists of an integrated band pass filter chip with first order low- and high-pass filters and additional, resistor-set gain. Cutoffs of the filters are set at approximately 200 Hz and 5 kHz to capture the majority of signal energy in the CAP while rejecting 60 Hz interference at the low end and broadband noise at higher frequencies. The analog signal conditioning board has a fixed gain of approximately 80,000 in the pass band.

The data acquisition card, National Instruments DAQCard 1200, has 8 single-ended or 4 differential input channels, 2 analog voltage output channels, and 3, 8-bit digital outputs. The DAQCard also has a high input impedance and variable input gain. The DAQCard is connected to both the analog conditioning output and to the stimulator input. The output of the DAQCard and the A/D and sampling of the input are controlled by a National Instruments software package, Labwindows CVI, which generates virtual instrumentation devices based on a C code compiler. The CVI software allows the user to alter the sampling rate, DAQCard gain, and the number and timing of stimuli.

**Recording Methods**

The single-channel hardware used during this portion of the project did not allow recording of multiple CAPs in response to the same stimulus. In order to compare multiple CAPs an assumption of stationarity must be made. It must be assumed that the same fibers will be stimulated by the same stimulus applied to the same location regardless of how many stimuli have been applied previously and how much time has past since the first stimulus. For CVD estimation, it is also an important assumption that the properties of propagation within each of the fibers remain constant over time. Requiring supramaximal stimuli helps to guarantee that the same fibers are activated from the start of the experiment to the end since supramaximal stimuli activate all excitable fibers in the nerve. The propagation properties of the nerve, however, are very much out of the experimenter's control. The nerve itself is a vastly complicated system that undergoes changes that occur on several time scales. Each activating stimulus will induce a recovery cycle in a fiber that lasts as much as several hundred milliseconds. During this recovery cycle, the fiber's activation threshold and the propagation velocity of activated action potentials are both affected. Since it is part of a much larger physiologic system, the nerve is also continuously undergoing changes that occur due to more global variations. One example is changing temperature within the subject body at the location of the peripheral nerve.
Another possible source of variation are changing glucose levels within the interstitial fluid surrounding the nerve. These systemic changes might take place over a time scale of minutes. The best way to ensure that the stationary assumption is a good one is to perform the experiment over as short amount of total time as possible to guard against systemic changes while leaving enough time between stimuli for the nerve to fully recover. A period greater that 0.5 seconds will generally allow enough time for complete tissue recovery. The total time of testing is then determined by how many individual stimuli need to be presented.

Recording CAPs in different locations with a single-channel system requires applying recording and ensemble averaging responses at one site, and then moving the recording leads to another set of electrodes and repeating the process. The number of stimuli applied for each recording site (the ensemble) has a direct relationship to the noise level in the averaged response. The total number of required stimuli is therefore a function of the noise characteristics of the recording hardware and the ambient noise.

The hardware system described above was sufficient for these preliminary studies. Several drawbacks of the system were, however, identified. The primary drawback was that this system had only a single recording channel. Both 1CAP and 2CAP CVD estimation can be made more efficient with the use of multiple recording channels. In 1CAP studies, multiple channels can be used to record more than one CAP from the same electrode following each stimulus. The total number of stimuli necessary to generate an ensemble can thereby be reduced by a factor equal to the number of channels. The benefits of this method are fewer total stimuli, easing patient discomfort, and less testing time, improving the assumption that members of the ensemble are samples of a stationary system.

Another drawback of the preliminary hardware system is the inflexibility and low current limits of the commercial stimulator. The stimulator’s maximum constant current output is 10 mA. While this is adequate to elicit supramaximal responses in superficial peripheral nerve in most cases, the duration of the pulses often needs to be lengthened in order to guarantee that all fibers are being activated. Longer stimulus pulse durations result in more substantial and troublesome stimulus artifacts and elevate the possibility that the fibers within the nerve are activated over a finite time rather than at the same instant as the forward model assumes. Constant current stimuli should be kept as short as possible, less than 200 μs, in order to reduce artifacts and ensure that all fibers are activated within a small amount of time. This can only be accomplished, and supramaximal conditions maintained, if the current is increased, generally above 10 mA.
Discussion

Stimulus Artifact

One goal of this project phase was a better understanding of the causes of stimulus artifacts in upper extremity nerve conduction studies and how the presence of a stimulus artifact will affect parameter estimation solutions. It was determined that the complete elimination of stimulus artifacts by changing experimental techniques and the total removal of stimulus artifacts following CAP recording are both difficult to achieve. It seems, however, that carefully applying a combination of stimulus artifact reduction and removal techniques can effectively lead to adequate estimates of artifact-free signals.

Stimulus Artifact Reduction

There is significant evidence that changing the location of stimulation and/or recording sites can help to reduce the amplitude and duration of the stimulus artifact. It has been shown for instance that rotating the anodal stimulating electrode around the cathode can be used to find an anode location that minimizes the stimulus artifact. Changing the spatial relationship between the anode and cathode changes the location and orientation of equi-potential lines in the vicinity of the recording electrodes. When a single equi-potential line passes through both the active and the indifferent recording electrodes, the differential stimulus artifact will be minimized. This is unfortunately true only in the most ideal conditions, including point recording electrodes and an absence of motion. Electrodes of finite and varying size will rarely be at the exact same potential so that artifacts can only be minimized to a certain degree. Also, different sets of recording electrodes, such as one has when performing 2CAP CVD, will not lie close to their minima at the same time. Different orientations of the stimulating anode with respect to the cathode may be necessary for different recording sites.

In many cases, and in particular for the studies at hand, moving the electrodes is not an option. If stimuli are presented to the finger for instance, two ring electrodes are used. The cathode needs to be connected to the electrode closest to the recording site and anode further away. The only degree of freedom for moving the electrodes with respect to each other is their distance of separation along the finger. Changes in the separation of ring electrodes has been shown to affect the amplitude of signals recorded by those electrodes but seems to have little influence over the stimulus artifact produced at recording electrodes away from the finger.
Two factors that do affect distant stimulus artifacts and that can be easily changed are the orientation of the limb and the separation of the stimulation and recording leads. Changing the orientation of the limb will alter the shape of the volume conductor through which the artifact must travel. Changing the shape of the conductor also changes the location of equi-potential surfaces within the conductor produced by the stimulus pulse. Unfortunately, the complicated shape of the arm and hand make the location of these equi-potential surfaces difficult to determine and the prediction of their location impossible. The only feasible way to minimize the stimulus artifact through changes in the volume conductor orientation is through an educated guess-and-check routine. For signals recorded at the wrist following digital stimulation with ring electrodes, it has been demonstrated that simply rotating the hand can be an effective method of reducing stimulus artifact. It is often the case that artifacts are worse when the hand is in a palm-up position and can be reduced by rotating the hand so it is palm-down or with the palm at a 90° angle to the table.

Electrode lead position also affects the production of stimulus artifact due to mutual inductance and/or capacitive coupling between the stimulating and recording leads. Inductive interference can best be reduced by ensuring that there are no loops in the closed circuit path of the stimulating current that can generate magnetic fields during the stimulus and that there are no loops in the path of the recording circuit that will lead to an induced voltage. In order to eliminate loops in the electrode leads, individual anode and cathode pairs are twisted around each other to the extent possible. Wire leads from the active and indifferent inputs of a recording channel are also twisted. Capacitive coupling can be reduced by keeping the stimulating and recording leads as short as possible. Both capacitive and inductive effects will be further reduced by spatially separating the stimulating leads from the recording leads and from all recording hardware.

**Stimulus Artifact Removal**

Stimulus artifacts cannot be completely eliminated from CAP signals by the preceding methods. Techniques must be developed to remove the artifact after recording. Researchers have previously applied stimulus artifact removal techniques that rely on estimating the stimulus artifact contribution and subtracting the estimate from the recorded signal. The quality of the stimulus artifact estimate, then, determines how well the method performs.

During the preliminary stage of this research, three simple methods of stimulus artifact removal were utilized. The first algorithm that was developed followed the work of Kovaks.[18] Artifacts were removed by fitting each ensemble averaged artifact to a function of the form $A(1 - \gamma t)\exp(-\gamma t)$,
where $t$ is an independent variable representing discrete time and $A$ and $\gamma$ are to be determined. The fit was performed on a region of the data immediately prior to the CAP signal and optimized by minimizing the mean square error in that region. The function was then extrapolated over the entire signal and subtracted from the data. This method effectively removed stimulus artifacts that infected the CAP but did not account for the large deflections during, and immediately following the stimulus itself. The first 1-2 ms of each recording was zeroed to remove these deflections.

Another, similar method was to fit the artifact to a linear function just before and/or just after the CAP. The line was then extrapolated over the entire time range and subtracted. This technique assumes that the exponential tails of the stimulus artifact in the region of the CAP can be approximated by a linear function. Once again initial deflections, as well as the artifact before and after the CAP need to be zeroed.

Finally, stimulus artifact can be removed without the need for a regression step at all. If the beginning and the end of the CAP are known, and if the signal passes close to zero at those points, the entire time series prior to and following the CAP can be zeroed without altering CAP shape. This method of stimulus artifact removal assumes that the exponential artifact tails have almost died out entirely prior to the CAP. This method is distinguished from the linear regression approach even though the latter will reduce to the former if the circumstances are right. The distinction comes because this method is more easily automated because the onset and termination of the CAP are constrained to lie on or close to the zero voltage axis. This approach required much smaller artifacts in order to yield results that were at all realistic. Only when data was seen to reach a steady baseline around zero well before CAP onset was this method employed. Otherwise the previous two techniques were utilized.

**Conduction Velocity Distribution Algorithms**

MATLAB programs were written to implement CVD estimation algorithms. Single CAP and 2CAP algorithms used by past researchers have been investigated. Variations of these techniques have been applied to specific recording situations. A frequency domain technique has also been implemented.

In its simplest form the mathematical expression for a CAP waveform, Equation 2.4, can be expressed as a matrix, whose columns are SFAP waveforms for each fiber class, operating on a vector representing the distribution of SFAP arrival times. That is,

$$\vec{C} = \Phi \vec{n}$$  \hspace{1cm} (3.1)
where $\overline{C}$ is the recorded CAP signal, $\Phi$ is a matrix containing the SFAP waveforms, and $\overline{n}$ is the distribution of SFAP latencies. The inverse, 1CAP problem is how to obtain an estimate of $\overline{n}$ from the measured $\overline{C}$ and known $\Phi$. Given a clean CAP recording and perfect estimates of the SFAP waveforms, this equation could be solved exactly using Gaussian elimination methods. Due to the corrupting noise and artifacts, however, and the inability to directly record individual SFAPs a matrix inversion problem cannot be solved exactly, and approximate solutions must be generated. Least squares estimates are commonly used. The non-negativity of the entries in an arrival time distribution has been explicitly included by some researchers by using an iterative, non-negative least squares algorithm. Others have used the non-negative the requirement to recursively update their SFAP waveforms in a search for the correct solution.

**Algorithms Involving a Single CAP**

Single CAP algorithms solve the inverse problem by fitting single fiber action potentials with known latency and shape to the recorded CAP signal. Since any real conduction velocity distribution will be positive, a variation of the least squares solution, non-negative least squares (nnls), is often used. The professional version of Matlab contains a nnls function that relies on the algorithm of Lawson and Hanson. This is the same algorithm that has been utilized in previous CVD studies.[46, 55] The 1CAP CVD algorithm that has been developed in this lab requires a SFAP waveform and CAP data both sampled at the same frequency. It then utilizes Matlab’s nnls function to estimate a CVD.

The first step in applying a 1CAP algorithm is to define or determine the form of the single fiber action potential waveforms of every fiber in the nerve bundle. There is presently no way to record SFAP waveforms noninvasively and it is not feasible to define individual SFAPs for each of the tens of thousands of fibers in a nerve. Most research methods rely on substantial assumptions to be made concerning the relationship between SFAPs of various fibers. The most restrictive, and the most common, of these assumptions is that SFAPs do not vary with fiber conduction velocity and that all fibers in the nerve will have an identical contribution to the CAP. Less restrictive variations on this assumption that allow for changes in amplitude and/or duration while retaining the same wave shape have also been applied. The evidence to support a specific power relationship between amplitude, duration, and velocity comes from extrapolations of in-vitro and theoretical studies or from empirical analysis of in-vivo data.[9, 29, 38, 45, 47] Controversies concerning the exact manner in which amplitude and duration may vary with velocity and the fact that these relationships are generally included at the end of an algorithm as a somewhat artificial scaling factor, often make it more feasible to use a single SFAP in the 1CAP solution.
The 1CAP solution is defined by and dependent upon the SFAP waveforms that are used in the calculation. The challenge then becomes determining a method for SFAP estimation. Several possible methods that have been used in the past and at least one new method of SFAP estimation were explored for this work.

*A-priori SFAP Approximation*

When the first CVDs were estimated noninvasively the electronics were substantially less sophisticated than they are now. The recording of large, compound signals was enough of a challenge at that time and recording of SFAPs was not even considered. Researchers at that time used predefined triangle shaped waveforms. These SFAPs, although known to be incorrect, were capable of producing very reasonable estimates of the CVD. These researchers had the advantage of dealing with mono-phasic CAP data recorded from excised nerve. SFAPs were therefore purely additive without phase cancellation. This not only simplifies the 1CAP solution, but also allows for the fact that CVDs are, by their nature as a distribution, positive. Least squares estimates can then be implemented with linearly independent SFAPs without the need for algorithms that ensure non-negativity.

Contento et. al. (1983) investigated the dependence of the 1CAP CVD solution on the choice of a-priori SFAP [47]. These researchers found that CVD estimates are strongly influenced by the choice of SFAP waveform. They suggested that, for inter-subject studies, the method of generating an a-priori SFAP may be less important than choosing a recording site where single fiber signals will be most similar between subjects. This suggestion indicates the these researchers were more interested in comparing latency distributions between subjects and perhaps tracking changes in latency distributions in disease states rather than generating accurate estimates of the distribution of conduction velocities or of fiber size. It also indicates a resignation on their part to the fact that noninvasive CVD estimates are inherently imprecise and that consistent experimental methods may be just as important as the estimation method when applying the technique to multiple subjects.

The complicated nature of the CAP waveforms recorded for this study, and particularly of the bipolar CAPs used for this preliminary phase, makes a-priori determination of SFAP waveforms difficult to implement in CVD estimation. Mutual cancellation occurs among the SFAPs often leading to under determined CVD estimation problems. The use of piecewise linear, triphasic SFAP estimates has nonetheless been attempted. The deconvolution of multi-phasic CAPs requires more complicated algorithms in order to ensure that the resulting CVD is non-negative. These algorithms dramatically
increase the time required to obtain a CVD solution and often make convergence impossible. These methods have therefore been rejected as infeasible for clinical applications of CVD estimation.

**Minimal Stimulus SFAP Estimation**

One method of determining SFAP wave shape from recorded data is through a minimal stimulus technique. The amount of current injected during stimulation is reduced to the point at which only a small number of fibers are activated. The assumption is that the activation threshold is different for every fiber and that fiber diameter is the main factor in determining threshold. Small stimuli will therefore discriminate among fibers based primarily upon fiber size rather than fiber position. If this assumption is accurate, the resulting CAP signal is produced by a coherent subpopulation of fibers with similar diameters and, hence, conduction velocities. Since the conduction velocity of all contributing fibers is similar, dispersion among SFAPs will be minimal and all action potentials will reach the recording site in unison. The minimal stimulus CAP signal will therefore approximate a scaled SFAP and it can be used as an SFAP estimate. This technique is advantageous since SFAPs are estimated using the same stimulation and recording sites as CAPs that are being studied. If the assumptions above hold, the minimal-stimulus SFAP estimate should be an accurate representation of how an SFAP recorded at that site would appear and not simply a realistic possibility.

One observation that substantiates the belief that the minimal stimulus CAP represents a coherent subpopulation of fibers is a consistent difference in shape and time of occurrence between the minimal stimulus and the supramaximal CAPs. If the minimal stimulus activated the same spectrum of fibers as a supramaximal stimulus, one would expect the minimal stimulus CAP to be simply a scaled version of the CAP generated by larger stimuli. The signals would have the same overall shape and duration. However, with very few exceptions, minimal stimulus CAPs have onsets that occur after the onset of the supramaximal CAP and have shorter durations. This would indicate that the fibers being excited by minimal stimuli do represent a sub population with conduction velocities slower than the maximal conduction velocity within the nerve bundle and that the amount of dispersion in the minimal stimulus CAP is reduced.

Figure 3.1 demonstrates the use of the minimal stimulus technique in estimating SFAPs. The largest trace is a supramaximal CAP waveform. The supramaximal CAP is overlaid on a minimal stimulus CAP, plotted in gray, produced by stimulating at 15% of supramaximal. The minimal stimulus CAP is shown again at the bottom of the figure after scaling by a factor of five in order to better show its shape. The data of Figure 3.1 indicate that a slower (smaller) population of fibers is excited and that
dispersion is diminished. In fact, it is possible that in even smaller subpopulation of fibers is activated by lower amplitude stimuli but that their response is too small to be recorded.

**Paired Pulse SFAP Estimation**

Another possible method of SFAP estimation involves stimulation with pairs of pulses so that the second stimulus arrives during some of the fibers’ relative refractory period, a late phase of the recovery cycle. A fiber that is in its refractory period will require a larger stimulus in order to fire again. If the first stimulus pulse was just large enough to generate an action potential, a second stimulus of the same size will not be able to activate the fiber until the relative refractory period has ended, generally 10 to 20 ms from the first stimulus. If an entire nerve bundle is excited by pairs of pulses, they can be timed so that the majority of the fibers have recovered enough to fire again. A minority of fibers, however, will not be activated by the second stimulus. If there are no other interactions, the difference between the CAP produced by the first stimulus and the CAP generated by the second will represent the contribution of those fibers that did not fire. If it can be assumed that those fibers that remain refractory and are not activated by the second stimuli belong to a class that share a similarity in size, then the difference between the signals may represent an estimate of the SFAP due to a fiber of that class. Once again, this technique relies on the ability of the experimental method to discriminate among fibers based primarily on fiber size irrespective of fiber position or any other physiologic or anatomic parameters. A second inherent assumption is that no factors other than differences in recovery affect changes in the second CAP.

**Differential Stimulus SFAP Estimation**

The final method of SFAP estimation investigated, the differential stimulus method, was motivated by the experience with minimal stimulus SFAP estimation. Sub-maximal stimuli, which do not activate all fibers in the bundle, were used. If the amplitude of a sub-maximal stimulus is increased slightly, additional fibers will be recruited. If those additional fibers represent a coherent subpopulation of similar size, the difference between the CAPs activated by the two stimuli will represent an SFAP estimate for that subpopulation. One advantage of this method may be that multiple SFAP estimates can be made by continuing to systematically increase the stimulus amplitude.

Estimation of the SFAP waveforms using differential stimuli is inherently different from the other methods mentioned above because allows multiple SFAPs to be produced. If each SFAP, by virtue of its arrival time, can be assigned to a specific conduction velocity class, then this method may
eliminate the need to assume that all SFAPs for all fiber classes are identical. This technique also has advantage over the paired pulse method is stimuli are presented using the same paradigm for SFAP estimation as for CAP production.

Figures 3.2 and 3.3 demonstrate the use of the differential stimulus method for SFAP estimate. Figure 3.2 shows a pair of sub-maximally stimulated CAP waveforms and their difference. The top trace is a CAP that was stimulated with a 8.8 mA pulse of 200 μs duration. The middle trace, plotted in gray, was recorded from the same nerve at the same recording session but with a stimulus pulse that was only 8.2 mA in magnitude. The mathematical difference between those two CAPs represents the additional fibers activated by the increase in stimulus amplitude. This difference is shown as the bottom trace of the figure. This technique can be applied repeatedly in order to generate a series of SFAP estimates. Figure 3.3 shows three such SFAP estimates plotted on the same time scale.

Although each of the above techniques for estimating SFAP waveforms has some theoretical basis and some technical feasibility, the resulting SFAPs do not represent the true single fiber waveform as it would be recorded at surface of the skin. The a-priori methods utilizing piecewise linear or computer simulated SFAPs can never take into account differences and recording site geometry or recording hardware as they change from subject to subject. Methods that estimate SFAPs from CAP recordings, whether through minimal stimulus or other techniques, do take recording site geometry and other subject specific parameters into account but will invariably result in noisy estimates and estimates that are corrupted by stimulus artifact. There is some merit to the idea of choosing a recording site for every subject that will minimize SFAP variation so that a single a-priori SFAP estimate can be applied for every test. To do so, however, cannot be thought of as CVD estimation but rather as the estimation of a series of parameters describing the temporal characteristics of the CAP waveform. If the intention is only to generate a multi-parameter description of CAP waveforms and compare them across multiple subjects, it may be that principal component or higher order statistical methods would do a better job.

ICAP CVD estimates using measured SFAPs are further complicated by the computational effort required. Minimal stimulus SFAPs, paired pulse SFAPs, and differential stimulus SFAPs all have lower signal to noise and lower signal to stimulus artifact ratios. The design of deterministic algorithms to identify, isolate, and cleanup these estimates is therefore very difficult. These preliminary studies have resorted to the use of interactive algorithms whereby the operator selects the onset and termination of the SFAP signal. While interactive algorithms help to identify small amplitude signals, they are highly time intensive and introduce subjective decisions to the process.
Algorithms Involving Two CAPs

The problems identified with 1CAP CVD estimation have led to the development of 2CAP methods. 2CAP algorithms are based on two independent CAP recordings with different distances between the stimulation and recording sites. In exchange for more difficult recording methodologies, 2CAP methods do not require the single fiber contributions to be estimated. 2CAP algorithms rely on the assumption that both CAPs result from activation of an identical CVD and that the SFAP waveforms contributing to the two recordings are the same. They further require that the differences in SFAP shape among different fibers can be accounted for by convolving an unknown SFAP basis waveform with known shaping functions. Only the distance of propagation, and hence the distribution of SFAP latencies, is considered to change between the two CAP recordings. This does not simply translate to a shift of the CAP signal in time. The CAP changes in duration and shape due to larger temporal dispersion of the signal at longer propagation distances. Since 2CAP algorithms look at the dispersion between the two signals, larger differences in the propagation distance will lead to more accurate estimates of CVD.

Barker's Approach

Barker et al. (1979) have developed a 2CAP technique that is implemented much like a 1CAP method [39, 40]. It is included in this section because the SFAP estimate is generated from a very short distance recording of a CAP. As the distance of propagation decreases so does dispersion of the SFAP constituents. If it were possible to record CAP from a site just adjacent to the stimulation site there would be no dispersion and the SFAPs would overlap completely. Provided the prior assumptions still hold and the SFAPs are similar for all fibers in the nerve, then such a CAP would be simply be a scaled version of the SFAP waveform.

Unfortunately, it is impossible to record CAPs within the immediate proximity the stimulation site because large stimulus artifacts will overwhelm the input amplifier of the analog recording system. Barker's approach is to record CAPs as close to the stimulation site as possible while maintaining sufficient signal to artifact ratio in the region of the CAP signal. A CAP recorded in this manner will have very short distance over which to disperse and will therefore remains a reasonable estimate of the SFAP. Once this SFAP estimate is generated, least squares or non-negative least squares algorithms can be applied to estimate the CVD.

Barker's technique is included in this section because it requires a minimum number of electrode sites, one for stimulating and two for recording or two stimulation sites and a single recording site. The
former approach is generally not used because the use of two separate recording sites does not guarantee that the SFAP estimate is appropriate to apply to the CAP recorded elsewhere. The goal is to record an SFAP estimate that is representative of the recording site at which the CAP is recorded and this can only be accomplished if the SFAP estimate and the CAP are recorded from the same site. For this reason, two stimulation sites are generally used, one located close to recording site and one further way. Although the use of a single recording site eliminates differences in the SFAP due to recording site geometry, the use of two separate stimulation sites has drawbacks of its own. Termination and/or branching of the fibers between two stimulation sites will result in different populations of fibers being activated at the two sites.

**Cummin’s Method**

Around the same time as the work of Barker, Cummins et al. (1979) developed their own 2CAP method based on a solution to Equation 2.20.[37, 38] The main advantage of this technique is that the CAP recorded over a short distance is not assumed to be an estimate of the SFAP. Rather, the two CAPs are considered to be equivalent compound signals, due to identical CVDs activated at the same location. Cummins solved Equation 2.20 by minimizing the left-hand side using an iterative steepest descent method. The nonnegativity requirement was included explicitly as a condition of the minimization.

Once again the issue of whether to use multiple stimulation or multiples recording sites comes into play. Cummins chose multiple recording sites in order to ensure that the fibers activated in each CAP recording are identical. This technique lends itself to that approach and in general it is easier to find a location for CAP recording that is to isolate a site that will allow supramaximal electrical stimulation. Differences between the SFAP waveforms at the two recording sites were not accounted for in this work. It was proposed that the longer difference in the distance of propagation would minimize the effect of differences in recording site geometry.

**Frequency Domain Approach**

An approach that solves the inverse problem in electroneurography in the frequency domain has also been proposed and implemented.[43] It has been shown that this technique results in an estimated solution to equation 2.20, just as the previous method did.[29] The frequency domain solution involves recursive estimation of unevenly spaced samples of the discrete time Fourier transform (DTFT) of the CVD. Since the method is recursive, it requires a known starting point. Initial values of the sampled DTFT must therefore be assumed. The number of initial values that need to be assumed
is a function of the difference in the distance of propagation of the two CAP signals. The minimum number of parameters that need to be generated a-priori is one. This occurs when the longer distance of propagation is at least twice the first. If the difference in propagation distance is reduced, as this project hopes to accomplish, that number is increased so that more and more of the sampled DTFT is due to ambiguous or assumed information and the accuracy of the method declines rapidly.

**Conclusions and Key Research Questions**

The preliminary work discussed in this chapter has laid a foundation of understanding for the remainder of the project. A hardware system was developed for the electrical activation and recording of CAPs. Signals recorded with this hardware were used to develop digital signal processing algorithms for noise and stimulus artifact removal. Methods for estimating single fiber action potentials from the compound signals, to facilitate 1CAP CVD calculations, were developed and explored. Multiple compound action potential signals, having been recorded and processed, were applied to three different 2CAP CVD algorithms to yield a better understanding of the strengths and weaknesses of each. A 2CAP method was selected as the most appropriate and promising to apply to the problem at hand. The choice of algorithm, as well as other insights gained in this preliminary investigation, resulted in the development of the following three key research questions.

1. *Can noninvasive methods be used to empirically estimate the transfer function between a peripheral nerve and a surface electrode and/or the relative transfer function between a pair of surface electrodes?*

2. *Can information about the nerve-to-electrode transfer function be incorporated into an algorithm designed to solve the inverse problem in electroneurography and thereby improve the estimate of the conduction velocity distribution of a peripheral nerve?*

3. *Can peripheral nerve CVD be accurately estimated from data noninvasively recorded over a short segment of human peripheral nerve where temporal dispersion is limited, by incorporating nerve-to-electrode transfer function information into the CVD algorithm?*

The remaining chapters of this document describe research undertaken to answer these three questions.
Figure 3.1
Figure 3.2
Figure 3.3
Chapter 4. Simulations

Introduction

The preceding preliminary work made it clear that additional insight into the manner in which dispersion affects noninvasively recorded CAPs, and a better intuitive understanding of the limitations of the two CAP CVD algorithm were necessary. It was determined that the most straightforward way to gain these insights would be to produce a detailed mathematical model describing the propagation and recording of peripheral compound action potentials. An appropriate model would duplicate the effects of dispersion that are seen in experimentally recorded data, allow for the inclusion of corrupting noise and artifact, and provide a means for testing the efficacy of the inverse problem solution.

This portion of the project focused on development of a simulation environment in which realistic CAP signals could be generated. This tool was used to quantify the relationships between CAPs recorded at different locations, to evaluate the performance of stimulus artifact removal and CVD estimation techniques, and to investigate the effect of artifact and noise on the CVD estimation result. In order to meet these goals, the assumptions regarding CAP production and recording, discussed in Chapter 2, were presumed to hold. The task was then to generate simulated CAP signals that fit those assumptions.

Methods

The Calculation of a Simulated CAP

The compound nature of a noninvasively recorded action potential is modeled by summing the contributions of many individual fibers. If the contributing fibers are separable into $N$ discrete velocity classes, then a basis matrix of single fiber action potentials can be constructed. The basis matrix, $M$, has $N$ columns. The data in each column represents the single fiber contribution appropriate to that class. Each row of $M$ represents an equally spaced sample of time. The shape of the SFAP in each column is appropriate to a particular velocity action potential that is recorded by a particular electrode. Each SFAP is also delayed an amount specified by its conduction velocity and the distance that it has traveled from the
site of stimulation. Even though each column of the basis matrix is referenced by a velocity, the column vectors themselves are signals represented as a function of time.

We can further define a $N \times 1$ column vector, $\overrightarrow{w}$, whose entries are the number of fibers in each velocity class. $\overrightarrow{w}$ is the conduction velocity distribution (CVD) of the nerve bundle. A compound action potential signal, $\overrightarrow{C}$, can then be calculated as a linear combination of single fiber waveforms weighted by the value of the CVD for that fiber class, or

$$\overrightarrow{C} = M\overrightarrow{w}.$$  

In this formulation, $M$ contains all information about the geometry of the recording site, the position of the nerve with respect to the electrode, the distance of propagation, and the form of the single fiber action potentials for each velocity class. $\overrightarrow{w}$ simply dictates the number of fibers from each class that contribute to the compound signal. While the $M$ matrix will be different at different recording sites, any CAP signal that is recorded along the nerve due to the same stimulus will share the same CVD.

Further assumptions can be made to simplify this formulation. Many authors assign identical single fiber action potentials to every velocity class or allow the single fiber waveforms to vary only in amplitude. Scaling the amplitude by the square of the velocity, for instance, is common.[29, 37, 38, 40] Once the amplitude dependence is established, it can be factored into $\overrightarrow{w}$ as an appropriate scaling of each entry of the distribution. With this simplification, the columns of $M$ become shifted and possibly scaled copies of each other. In this way only one, single fiber action potential must be estimated or approximated to determine $M$. Once this waveform is known, the velocity of that fiber class and the distance of propagation determine the delay for each column.

The above formulation is useful because it explicitly includes the CVD, $\overrightarrow{w}$. It is convenient and informative, however, to develop this model in the temporal domain so that it directly matches experimentally recorded data. In the time domain the components of $\overrightarrow{w}$ must be transformed from a distribution of velocities to a distribution of arrival times, or latencies. Since the velocity classes are predetermined and the propagation distance is known, this transformation involves calculating the latency of each fiber class and shifting each component of $\overrightarrow{w}$ to the corresponding location in a new vector defined over a range of latencies. The latency distribution, $\overrightarrow{\tau}$, consists of a series of scaled impulses spread out over
a suitable range of time. This transformation can be represented as a linear operator, \( G \), that is uniquely determined by the range of fiber velocities, the range of arrival latencies, and the distance of propagation to the recording site so that

\[
\tau_i = G_i \{ \bar{w} \},
\]

where \( l \) is the distance of propagation, \( G_i \) is the operator appropriate for a distance \( l \), and \( \tau \) is the latency distribution appropriate to \( l \).

If we continue to assume that all single fiber action potentials recorded at one location have the same waveform, then that waveform can be thought of as the impulse response of a linear, time-invariant system whose input is a latency distribution. The compound action potential recorded at electrode \( j \) after propagating a distance \( l \) can then be calculated as

\[
C_{ij} = G_i \{ w \} \ast h_j = \tau_i \ast h_j
\]

where \( \bar{h}_j \) is the single fiber action potential waveform common to all fiber classes and specific to one electrode.

The calculation of a simulated CAP is then performed in the following steps:

1. A conduction velocity distribution, \( \bar{w} \), is defined over a range of velocities appropriate to the fibers that can contribute to a noninvasively recorded CAP.

2. A scaling factor is applied to the CVD to account for variations in the amplitude of the single fiber action potential components derived from different sized fibers.

3. The CVD is acted upon by a linear operator, \( G \), that transforms it from a distribution defined over a range of velocities to a distribution defined over a range of latencies, \( \tau \). \( G \) is completely defined by specifying the velocity range of the CVD, the temporal range of the latency distribution, and the distance of propagation.

4. A time series representing a typical single fiber action potential waveform, \( \bar{h} \), is generated (see next section).
5. Finally, the CAP, \( \overline{C} \), is generated as a convolution of the latency distribution, \( \overline{\tau} \), and the single fiber waveform, \( \overline{h} \).

The overall procedure is outlined in Figure 4.1. The top panel shows a single fiber action potential estimate that is to be attributed to every fiber in the simulated CAP. The distribution of velocities within the nerve, \( \overline{w} \), is plotted in the second panel. Knowing \( \overline{w} \) and knowing the distance of propagation allows the CVD to be converted into a latency distribution appropriate to that distance. The third panel of Figure 4.1 shows the distribution of arrival times (latencies) that stems from the CVD, \( \overline{w} \), being activated and propagating 14 cm along the nerve. Since the arrival time distribution for a particular recording site and the SFAP waveform for all fibers in the nerve are known, the simulated CAP can be calculated by convolving those two signals with each other. The result is shown in the bottom panel of Figure 4.1.

It is interesting to note the similarities between the shape of the SFAP and the CAP that results from it. You can also begin to notice one of the shortcomings of this simulation method. SFAPs from neighboring bins of the CVD that are associated with the slower fibers of the nerve disperse much more with respect to each other than neighboring-bin SFAPs in the faster end of the spectrum. Eventually, slower SFAPs will disperse so much that their arrivals begin to appear as discrete events rather than a continuum. This is evident at the end of the CAP signal in Figure 4.1 where individual SFAPs can be seen. This problem is associated with the spacing of the velocity bins in the CVD. To reduce the effect, CVDs have been defined with closely spaced bins and down-sampled when necessary to compare with CVD estimates. The steps described above can be repeated for different CVDs, different SFAPs, or both in order to study the effect that each of these components has on the CAP.

**Calculation of Simulated SFAP's**

A quasi-static, infinite homogeneous volume conductor model has been adopted in order to generate simulated single fiber action potentials for this study. A cylindrically symmetric geometry is used so that the relationship of the nerve to the field point can be represented as a function, \( D(\cdot) \), of a single spatial variable, \( x \). This provides for a straightforward interpretation of \( D(x) \) as the path of the nerve below a horizontal surface containing the field point. The transmembrane potential is assumed to be constant over the entire membrane while the tissue is at rest. During action potential propagation the transmembrane
potential is assumed to vary in two step functions, the first represents a depolarization event and the second a tissue repolarization. These two events travel together along the nerve separated by a constant distance, $2\delta$.

This model allows the depolarization and repolarization events to be approximated as two, oppositely oriented dipole surfaces traveling along the nerve as depicted in Figure 4.2. These dipole surfaces span a cross-section of the nerve fiber perpendicular to its cylindrical axis. The potential at the field point at any time can then be calculated as a superposition of the far-field effect of the two dipole surfaces. The contribution of each can be shown to be proportional to the total dipole moment across the surface and to the solid angle that the surface subtends at the field point, $\Omega$.

Since the dipole moment is assumed to be constant across the surface, the total dipole moment reduces to $\pm m$, where the sign differentiates between a depolarizing and a repolarizing event. The solid angle term for each surface is a function of the location of the surface, the cross-sectional area of the surface, and the instantaneous direction that it is traveling with respect to the field point. For a field point located at $x = 0$, the solid angle subtended by a nerve fiber cross-section is given by

$$
\Omega = \frac{Ax}{[x^2 + D^2]^{3/2}} \cos(\theta)
$$

where $A$ is the cross-sectional area and $\theta$ is the angle that the unit vector $d\hat{A}$ makes with the position axis, $\hat{x}$. The general anatomic path of the nerve is parallel to the recording surface, the dive angle of the fiber, $\theta$, is expected to be small. For this reason, and because $\theta$ will generally be common to both dipole surfaces, we further simplify by assuming $\cos(\theta) = \text{constant} \equiv 1$. In this way, the total contribution to the potential at the field point can be written as

$$
V_p = Km(\Omega_1 - \Omega_2) = KmA \left\{ \frac{(x + \delta)}{[\delta^2 + D^2(x + \delta)]^{3/2}} - \frac{(x - \delta)}{[\delta^2 + D^2(x - \delta)]^{3/2}} \right\}
$$
where \( x \) is the horizontal distance from the field point to a point half way between the dipole surfaces, \( K \) is a constant, and all other variables are as previously defined.

A MATLAB function has been written to estimate the extracellular potential at a field point due to propagation of an action potential along a single fiber whose distance below a horizontal plane containing the field point is allowed to vary. The function requires the following user-defined quantities:

\[
[x] = \text{The range and resolution with which to evaluate the above expression.}
\]

\( x_0 = \text{The location of the field point, defined on the range, } [x]. \)

\( d(x_0) = \text{The depth of the fiber as it passes directly underneath the recording electrode.} \)

\( s = \text{The slope (dive angle) of the fiber with respect to the recording ‘surface’ (although this angle is assumed to be zero in the calculation of solid angle above, it will affect the computed SFAP because it defines the nerve path, } D(x), \text{ in the region under the electrode and, therefore, the distance from the field point to the dipole current sources before and after they pass directly under the electrode).} \)

\( 2\delta = \text{The spatial separation of the depolarization and repolarization events.} \)

The function requires that all of the above inputs to be measured in centimeters and normalizes \( KmA \) to a value of 1.

Varying these parameters results in changes to the size and shape of the SFAP. Figure 4.3 depicts the effect of different nerve paths on the SFAP waveform. The depth of the nerve beneath each of four recording electrodes and the angle of the nerve path with respect to the recording surface are both varied to simulate a complicated nerve course. The values of these parameters that were used are listed next to each of the SFAP waveforms. The path of the nerve as it traverses the volume conductor is represented conceptually. Amplitude and duration of the SFAP are both affected by the depth of the nerve fiber below the surface whereas the shape of the signal is a function of fiber angle.

This procedure computes the field point potential as a function of action potential position, \( x \). In order to accurately simulate data recorded in-situ, this potential must be converted to
the time domain. The transformation is accomplished by incorporating the propagation velocity of the action potential to relate the position of the action potential to the time it passed the point \( x = 0 \). This transformation requires an interpolation step in order for the resulting data to be equally spaced in time. Matlab's linear interpolation function has been used for this purpose. This transformation also introduces velocity dependence to the form of the single fiber action potential. Signals due to action potentials traveling at different velocities will have similar shapes and amplitudes but will vary in duration, with faster propagation leading to shorter temporal duration. Alternatively, a SFAP can be calculated for a single velocity and then applied to every velocity class. This allows the same procedure to be used while maintaining the assumption that all fibers produce the same shape SFAP.

**The Addition of Stimulus Artifacts**

It has also been useful to simulate stimulus artifacts and incorporate them into the CAP data. The determination of a functional representation of stimulus artifacts is a difficult task. The shape, duration, and amplitude of artifacts recorded experimentally varies dramatically from subject to subject and from test to test. In order to get a general idea of how a corrupting artifact will affect the estimation of CVD, however, artifacts have been approximated as a summation of multiple decaying exponentials. To add realism, the exponentials are generated with a random component. This allows calculation of exponentials for which a range of amplitudes and a range of time constants can be predetermined but will differ slightly from simulation to simulation.

For the simulations presented in this chapter, stimulus artifacts are simulated as the sum of two exponentials, each of known amplitude but with a random time constant. The two exponential signals have time constants less than 0.1 ms and less than 3 ms respectively. The influence of the artifact on the CAP signal, and hence on the CVD estimation, is varied by changing the amplitude of the exponentials.

While the addition of stimulus artifacts to the CAP signals is useful in studying the dependence of the CVD algorithm on clean recordings, their subsequent removal can be used to investigate the sensitivity of the algorithm to signal errors resulting from imperfectly removed artifacts. In order to look at CVD algorithm behavior after artifact removal, an automated linear regression script has been used. The script identifies key landmarks in the CAP signal including CAP onset, location of both positive peaks, location of the large
negative peak, and termination. A linear function that connects the onset point and the termination point is then extended to the entire signal and subtracted. The resulting waveform is zeroed, both before the onset and after the termination, to yield the final CAP signal.

Results / Discussion

Simulated CAPs

The procedure outlined above has been implemented to simulate CAP waveforms recorded at different locations, after different distances of propagation, and with different distributions of conduction velocities. In order to examine each of these effects, a standard CVD and SFAP were chosen. The CVD, shown in Figure 4.4, is generated as the sum of two Gaussian distributions. One of the distributions is centered at 50 m/s with a standard deviation of 4 m/s, while the other is approximately 1/3 the amplitude, centered at 40 m/s with a standard deviation of 12 m/s. These two functions are summed and multiplied by a factor proportional to the square of velocity in order to generate the final CVD. The CVD measures the relative percentage of fibers in each velocity class and, therefore, must sum to 100. The shape of the CVD shown in Figure 4.4 was chosen to reflect experimentally determined CVDs in healthy human nerves and referenced in the literature.

SFAPs are determined with the fiber model described in the previous section. The depth of the nerve is set to 0.5 cm, the slope or dive angle of the nerve is 0.02, and the separation of the two dipole components is 0.5 cm. The combination of these parameters leads to the SFAP shown at the top of Figure 4.3. This same SFAP was used for the simulation studies described in the remainder of this chapter. The other SFAPs shown in Figure 4.3 are examples of the differences that can come about from alterations in the geometry of the nerve and electrode system. SFAPs similar to these are used in the next chapter in order to more closely investigate how these differences affect the estimated CVD.

Once the CVD and SFAP are generated, CAPs can be simulated at different propagation distances by transforming the CVD to an arrival time distribution and convolving with the SFAP. The arrival time distribution depends on the range of velocities over which the CVD is defined and the distance of propagation. Figure 4.5 demonstrates the effect of different propagation distances on both the arrival time distributions and on the resulting CAPs.
Arrival time distributions for propagation distances of 14 and 28 cm are shown in the top panel. These two distributions share a similar shape but one is a dispersed version of the other. The same is true for the CAPs shown in the bottom panel. These CAPs were generated by convolving the same SFAP waveform with each of the arrival time distributions plotted above. As with the distributions, added dispersion results in reduced amplitude and increased duration of the second CAP signal.

When calculating an arrival time distribution from a CVD, each velocity bin will translate to a specific arrival time bin. When the velocity bins are very wide and the time resolution is fine, neighboring velocity bins may be separated by several points in the temporal range. This creates the un-physiological effect of impulses in the arrival time distribution that are surrounded by times where no SFAPs are arriving. This is particularly true for the slowest velocities in the distribution and for long distances of propagation where the amount of dispersion is large. In order to reduce this problem, the resolution of the CVD is kept much higher in the simulations than in solutions to the inverse problem. When the width of the velocity bins is very small, smoother, more physiological arrival time distributions are produced, even at longer distances of propagation. To compare to the inverse solution, the simulated CVD can be interpolated to have the same number and spacing of velocity bins.

In order to study the effects of dispersion on propagating CAPs it is useful to generate a series of simulated signals that share the same CVD but have different propagation distances, and hence different arrival time distributions. Once such a data set is produced, changes in CAP parameters with propagation distance can be quantified. Figure 4.6 shows four CAPs from a series of more than 100 that were simulated with propagation distances between 10 and 40 cm. It is clear from the figure that dispersion has a major effect from the CAP recorded at 10 cm to any of the other three. The amplitude of the CAPs decrease with distance while the duration increases. There also appears to be a nearly equal difference in arrival time between neighboring CAPs in the figure that have equal differences in propagation distance.

**Time Shifts**

Since this project is concerned with the propagation and temporal dispersion of compound action potentials, the computer simulation has been used to quantify the changes to standard nerve conduction parameters. The most common of these, and the one that is easiest to distinguish from the time series data of Figure 4.6, is the time shift that occurs to the CAP
onset as propagation distance increases. This is the latency measurement used clinically. It is often measured to the beginning of the initial positive peak, to the positive peak itself, or to the negative peak.

Figure 4.7 is a plot of the latency of simulated CAP signals as a function of propagation distance. Latencies determined to each of the locations on the CAP waveform mentioned above are plotted separately for a range of distances. Three CAP signals, from distances of 10, 25, and 40 cm, are overlaid at the appropriate locations in order to illustrate the dispersive process. The least-mean square linear fit for each of the latency measures is also plotted. The onset latency is by far the cleanest measurement. As the point of interest moves further back on the waveform the certainty of its measurement decreases due to the increased dispersion among slower SFAPs. The slope of the onset latency represents the conduction velocity of the fastest fibers in the simulated nerve. The slopes of the other latency measures roughly track the conduction velocity of other SFAP components. The four latency measures other than onset are not guaranteed to track a single SFAP because the SFAP that arrives concurrently with the negative peak, for instance, is not necessarily the same as the CAP disperses.

**Dispersion**

As a CAP propagates, the individual action potentials spread out over a larger and larger range of time. At a very short distance the SFAPs will theoretically overlap exactly as they pass a detection site, resulting in a compound signal that is close to the same width as the SFAP. Places along the nerve that are further from the point of stimulation will see a volley of single fiber signals. After traversing a meter, SFAPs of only the myelinated fibers in healthy human tissue would be spread out over more than 20 ms. As the finite total energy in the compound signal spreads out in time, the amplitude of the CAP must also decrease. These two measures, the duration and the magnitude of a propagating CAP, indicate the amount of temporal dispersion that the CAP has undergone. The duration, along with latency, is a direct approximation of conduction velocities of the fastest and slowest recordable fibers in the nerve. CAP amplitude at a known distance gives some indication of the total number of fibers firing while the changes in amplitude with increased distance reflect the amount of dispersion.
Duration

CAP duration as a function of propagation distance is plotted in Figure 4.8 for the simulated CAP dataset. Duration is difficult to determine in an automated fashion for this data due to quantization errors and the ultimate discrete nature of the SFAP arrival time. Duration, however, like latency changes approximately linearly with distance. The least-mean square linear fit to the duration data is also plotted. The fit intercepts the duration axis at 1.2 ms which corresponds approximately to the duration of the SFAP used in the simulations. This relationship is expected because an undispersed CAP will be a scaled version of the SFAP itself. Added propagation will change the duration of the CAP by approximately 0.021 ms per cm of additional distance.

CAP amplitude with respect to distance of propagation has also been determined and the natural logarithm of that data is shown in Figure 4.9. The two individual plots correspond to amplitude measured from zero to the negative peak of the CAP (peak amplitude) and from the initial positive peak to the negative peak (peak-to-peak amplitude). While these two measures differ in values, they share the same relationship with respect to distance. The slope of the linear fit of the logarithmic data indicates that the amplitude falls off approximately as distance raised to the -0.075 power. The deviation of the data from the linear fit indicates that there are other, higher order dependencies as well.

Conduction Velocity Distributions

Another reason for producing computer simulations of CAPs at different distances was to test the performance of an algorithm written to calculate a two CAP solution to the inverse problem. The performance of the CVD algorithm on clean simulated data is shown in figure 4.10. The smooth plot punctuated by open circles is a down-sampled version of the CVD used in the simulation. The stair-step plot is the CVD estimated from two CAPs simulated at distances of 14 and 35 cm. Errors in the estimate are due to quantization errors in the data, the discrete nature of the velocity distributions and the temporal data, and the finite length of all vectors used in the forward and inverse problems. This plot indicates the best performance that can be expected from the algorithm because the simulated CAPs had no added noise or artifacts and distances were known exactly.

The simulation allows the effects of additive noise and differences in SFAP shape on the CVD estimate to be quantified.
Error Vs. Added Noise or Artifacts

In order to simulate the effect of additive broadband noise of the CAP waveform and on the two CAP CVD estimate, white noise data was digitally filtered and added to simulated CAPs. The frequency response of the low pass filter is close to that of the analog recording hardware. The amplitude of the white noise was increased to simulate a decreased signal to noise ratio, measured as a comparison of peak voltages. The effect of noise on the accuracy of the CVD algorithm will indicate what signal to noise ratio will be acceptable in experimental studies.

Figure 4.11 illustrates the result of adding broadband noise to the simulated CAPs on CVD estimates. The left-hand panel of this figure shows the same CAP with different levels of additive noise. The signal-to-noise ratio of each plot is indicated. The CAPs that are plotted, along with another CAP with the same amplitude noise added, were used to estimate CVDs. The results of these estimations are shown in the right-hand panel. The smooth plots are the CVD used in the forward simulation. The stair step plots in each case represent the CVD estimated from the noisy data. Deterioration of the CVD estimate is evident as the signal-to-noise ratio decreases. Even the center example, where the signal-to-noise ratio is 20, renders the estimated CVD unrecognizable. A plot of root-mean-square error versus added noise is shown at the bottom of Figure 4.11 for signal-to-noise ratios from 10 to 500. As the signal-to-noise ratio approaches a value of 50, the error in the estimated CVD drops to approximately 1.5. This value is close to the error produced without any added noise. These data indicate that additional improvement in accuracy cannot be achieved by increasing the signal-to-noise beyond 50.

Performance of the CVD algorithm will also be affected by the addition of stimulus artifacts to the CAP signals. Simulated stimulus artifacts should provide a better understanding of the acceptable levels of stimulus artifact in CAP signals used for CVD estimation. Figure 4.12 demonstrates the deterioration of the CVD algorithm as higher amplitude stimulus artifacts are added to the CAP waveforms. For each of the three examples shown, two exponentials with the same amplitude but with different, and variable time constants were added to each CAP. In the top example small exponentials, amplitude approximately 1% of the larger CAP amplitude, were added. Moving down the figure, the amplitude of the exponentials increases to 10% and 25% of the maximal CAP amplitude. While the amplitude of the simulated artifacts is known, the time constants of each exponential has a stochastic component. This
can be seen in the first and last examples in which the artifacts added to each of the two CAPs are much different in shape.

The right-hand panel of Figure 4.12 shows the effect that stimulus artifacts have on the resulting CVD. In each case the actual CVD used in the CAP simulation is plotted as a smooth line and the CVD estimated from the corrupted data at the left is overlaid as a stair-step plot. Deterioration of the CVD result is obvious. The CVD estimated from the middle example, due to a very modest artifact, has already lost most of its resemblance to the actual CVD. It is interesting to note that corrupting stimulus artifacts seem to lead to overestimation in velocity bins at the slower and faster ends of the range of interest. For the bottom example, in fact, many of the bins where the true CVD is the highest were estimated to have very few or no fibers contributing in the CVD estimate.

### Stimulus Artifact Removal

An example of the artifact removal procedure is shown in Figure 4.13. The top panel is a plot of a pair of simulated CAPs with different propagation distances and with corrupting stimulus artifact signals added. Key landmarks on the CAPs are indicated. The onset, the top of the negative peak, and the termination as determined by the artifact removal script are indicated with small stars. Open circles represent the two positive peaks. A linear regression connecting onset and termination of each CAP is plotted as a dotted line. The lower panel of Figure 4.13 shows the same two CAPs after subtraction of the linear regression and zeroing of the signal before and after the CAP. The artifact removed CAPs are shown in a solid line while the actual, pre-artifact CAPs are overlaid as dotted lines. Slight errors due to fitting of an exponential function with a linear regression are apparent in the region of the CAP itself.

### The Effect on CVD

Just as adding stimulus artifacts to the CAPs leads to deterioration of the CVD result, effectively removing those artifacts should improve the estimation accuracy. Differences in the estimated CVD for uncorrupted data and for corrupted but artifact-removed data will be due to imperfect estimation of the stimulus artifact. In the case of the simulations at hand an exponential artifact is estimated with a linear function so that imperfections are guaranteed. Of interest, is the amount of imperfection that can be tolerated and still yield reasonable CVD estimates.
Figure 4.14 demonstrates improvement of CVD estimation following the removal of the corrupting stimulus artifact by a linear regression technique. The data of Figure 4.13 was used to generate this plot. The smooth line punctuated by filled circles represents the actual CVD used in the forward simulation of the CAPs. The dotted stair-step plot is the CVD estimate made from the artifact corrupted CAP data of the top panel of Figure 4.13. The solid line stair-step plot indicates the CVD estimated from the corrupted data after the artifacts have been removed, the bottom panel CAPs of Figure 4.13. In this example, the slight errors in the CAP waveforms after artifact removal do not translate to large errors in the CVD estimate. In fact, the CVD estimate after artifact removal is just as accurate as the estimate made without corrupting artifacts. This indicates that, for this data, the approximation of the stimulus artifact as a linear function in the region of the CAP is a good one and that the removal of that linear approximation can greatly improve CVD estimation accuracy.

The same experiment has been extended to a range of stimulus artifact amplitudes to determine how robust the artifact removal technique is for these data. Figure 4.15 shows the results of three individual trials with varying artifact amplitudes. This figure shows corrupted CAP signals in the left-hand panel, artifact-removed CAPs compared to the original CAP simulation in the center, and CVDs estimated before and after artifact removal in the right-hand panel. The top trial is performed with a small (~1% of maximal CAP amplitude) artifact identical to that shown at the top of Figure 4.12. The artifact is not large enough to dramatically reduce CVD estimation performance, as seen in figure 4.12, and the estimate does not change significantly after artifact estimation and removal. The middle trial is performed with larger artifact estimates. The poor performance of the CVD algorithm on the corrupted signals is evident in the right-hand panel. Following artifact removal, however, the CVD estimate returns to very close to the pre-corruption estimate. The same is true for the bottom trial in which the stimulus artifact is quite large (~75% of maximal CAP amplitude). In all three examples, the CVD that is estimated after removal of the simulated stimulus artifact does a good job of duplicating the actual CVD and is as accurate as the estimate based on uncorrupted data.

**Conclusions**

An algorithm has been developed that allows simulated CAP waveforms, which fit the linear model previously discussed, to be produced and studied. The general CAP simulation
procedure requires a user-defined CVD, a distance of propagation from stimulation to recording, and parameters reflecting the geometry of the nerve and the electrode at the site of recording. The CVD and the propagation distance together uniquely define a distribution of arrival times at the recording site. The parametric description of the recording site geometry, and a simple volume conductor model, are used to calculate a SFAP for that recording site. The convolution of the arrival time distribution and the SFAP results in a simulated CAP. Simulating multiple CAPs, corresponding to different distances of propagation and recording sites, allows 2CAP CVD estimation to be tested with knowledge of the actual CVD. This has been useful in examining the effect of corrupted data on the CVD estimation procedure.

The simulations reported in this chapter have improved understanding of CAP production under the forward model and provided insight to the estimation of CVDs from pairs of CAPs with and without corrupting influences. In particular, the effects of dispersion along a nerve bundle have been modeled and quantified. It has been observed that the 2CAP CVD algorithm that has been developed for this work can effectively estimate distributions from clean simulated data that fits the forward model of compound peripheral nerve action potentials. As the CAPs vary from the forward model, through the addition of increasing amounts of corrupting noise or of simulated artifacts, the performance of the estimation algorithm deteriorates.

It has been noted that the accuracy of the algorithm also suffers when less direct errors in the forward model are included. First, as the distance of propagation between the two recorded CAPs decreases the estimated CVD becomes less accurate. Theory would predict that, with the clean simulated data studied, as long as the propagation distances were known the algorithm would perform well regardless of the distance between the recording sites. Quantization errors, however, and approximations made in the simulations lead to poor CVD estimates in this case even without any added noise or artifact. Similarly, while CVDs that were corrupted with small levels of additive noise produced adequate CVDs with large propagation distances, the algorithm performance dramatically decreased as the distances were reduced. It should be expected that experimental data, which contains both quantization errors and additive noise, will be affected similarly to the simulated data in this chapter. This will be particularly important for the remainder of this project as an attempt is made to estimate CVDs from experimental recordings using short inter-electrode distances.
Simulation of a CAP Waveform

Simulated Single Fiber Action Potential

SFAP = h

Time (ms)

Velocity Distribution

CVD = \omega

Velocity (m/s)

Equivalent Arrival Time Distribution

G{\omega} = \tau

Time (ms)

Simulated Compound Action Potential

CAP = C = \tau \cdot h = G{\omega} \cdot h

Time (ms)

Figure 4.1
Figure 4.2
Figure 4.3

Single Fiber Action Potential Simulations

Depth = 0.5 cm

Depth = 0.2 cm

Depth = 0.6 cm

Depth = 0.8 cm

Nerve Bundle

Nerve Slope

Nerve Depth
Figure 4.4
Multiple CAPs Produced by the Same CVD

Latency Distributions for Two Different Propagation Distances

14 cm

28 cm

Resulting Simulated Compound Action Potentials

14 cm

28 cm

Figure 4.5
Simulated CAP Signals Due to Dispersion over Different Distances

Figure 4.6
Latency of Various CAP Landmarks and linear Fits as a Function of Propagation Distance

Figure 4.7
Simulated CAP Duration vs. Propagation Distance

Figure 4.8

Linear Fit:
Duration = 0.021 ms/cm × Distance + 1.2 ms
Figure 4.9

Log of Simulated CAP Amplitude vs. Propagation Distance and LMS Linear Fit

- Log of Peak-to-Peak vs. Propagation Distance
- Log of Negative Peak vs. Propagation Distance

Linear Fit Exponents
Two CAP CVD Estimate Compared to Actual for Simulated CAPs

Mean-square Error = 1.4

Figure 4.10
Simulated CAPs with Added Noise

Resulting CVD Estimates

SNR = 500
SNR = 20
SNR = 10

Time (ms)

0 2 4 6 8 10

RMS Error in CVD Estimate vs. Signal to Noise Ratio

SNR = 500
SNR = 20
SNR = 10

Velocity (m/s)

20 40 60 80

Figure 4.11

Peak Voltage Signal to Noise Ratio

10^1 10^2 10^3

1 2 3 4 5
Simulated CAPs with Added Stimulus Artifacts

Resulting Two CAP CVD Estimate

Figure 4.12
Linear regression Removal of Stimulus Artifact

Figure 4.13
The Effect of Stimulus Artifact Removal on CVD Estimation

![Graph showing the effect of stimulus artifact removal on CVD estimation. The graph compares CVD estimates prior to and after artifact removal, with actual CVD values indicated by solid dots.](image)

Figure 4.14
Simulated CAPs with Added Stimulus Artifacts

CAPs after Removal of Linear Approximation to Stimulus Artifact

CVD Estimates Before and After Stimulus Artifact Removal

Figure 4.15
Motivation

In CAP data recorded from multiple sites along the wrist, waveform differences of a type other than those expected due to dispersion have been observed. The classic model of the CAP as a superposition of similar SFAPs that are dispersing does not account for this behavior. The differences in CAP shape indicate that there are factors other than dispersion, such as recording site geometry, that influence the shape of CAP waveforms at different recording locations along the array. Classically, algorithms that estimate a CVD from multiple CAPs assume all differences in the shape of the signals comes about from additional dispersion between recording sites. Typically, pairs of CAP signals recorded at widely spaced locations are used so that dispersion has a dominant influence over CAP shape differences. When short distance recordings are used dispersion is minimal and geometric differences that would be overshadowed by dispersion in the classic method have a relatively large influence over CAP shape differences. 2CAP algorithms that do not account for differences other than dispersion will be prone to errors in this case.

In order to produce consistent and accurate CVD estimates over a short segment of nerve it is therefore necessary to account for intrinsic recording site differences in the CVD estimation algorithm. Cummins et.al. (1979) discuss a technique in which differences in the SFAPs generated at different locations and by different nerve fiber classes could be incorporated into the minimization routine as part of their 2CAP algorithm.[37] This approach required the SFAP for each fiber class to be written as a convolution of a basis SFAP with some known shaping function. Application of the method therefore required knowledge of the relationship between each SFAP and the basis waveform and explicit, a-priori expressions for all weighting functions. In order to avoid such requirements, the approach described here incorporates intrinsic recording site differences into the forward model and attempts to remove those differences through Fourier analysis methods prior to application of the CVD estimation algorithm. This is accomplished by using electrode arrays at both the stimulating and recording sites and incorporating information from multiple CAP recordings.
Short Segment Electrode Array Recordings

Figure 5.1 illustrates the stimulation and recording methodology used in this portion of the work [15-17, 23]. The stimulating cathode is connected to one of two ring electrodes around the middle finger at the positions labeled $\alpha$ and $\beta$. A four-channel electrode array records monopolar CAP signals from the median nerve along the ventral portion of the wrist with the most distal electrode near the proximal wrist crease. The indifferent input for each differential amplifier consists of a pair of surface electrodes that straddle the active electrode. The common reference, R, for all four recording channels is placed on the palm or on the back of the hand or forearm. The distance between adjacent recording electrodes is constant and is equal to the separation between stimulating sites. This configuration leads to five different distances of propagation, the shortest between stimulation site $\alpha$ and recording electrode 1, the longest from stimulation site $\beta$ to electrode 4.

Compound action potentials are recorded from all four channels following both proximal (cathodic electrode $\alpha$) and distal (cathodic electrode $\beta$) stimulation. The eight resulting CAP signals can be classified by the following three parameters:

1. The site of stimulation.
   
   Whether the stimulation cathode is at site $\alpha$ or $\beta$ will determine the exact distribution of conduction velocities activated by a stimulus pulse.

2. The distance of propagation.

   The distance between the stimulation and recording sites uniquely determines the $Q$ matrix for each CAP and the amount of temporal dispersion of the SFAP constituents. The electrode configuration and spacing of Figure 5.1 results in five different distances of propagation; a, b, c, d, and e.

3. The nerve-to-electrode transfer function (NETF) at the recording electrode.

   This function is largely determined by the geometry of the nerve-electrode system. Each of the four recording electrodes (labeled 1-4) will have a unique NETF whose impulse response is the SFAP waveform for that channel.

Figure 5.1(b) shows a plot of multichannel data recorded from a single subject using the three parameters listed above as subscripts. CAPs evoked by distal and proximal stimulation but recorded
at the same electrode are plotted as an overlapping pair using the same vertical axis. The four pairs of overlapping CAPs correspond to the four recording electrodes of the array. By convention, positive differential signals are plotted as downward deflections.

Among the recordings of Figure 5.1(b) there are pairs, such as $C_{α,β,2}$ and $C_{β,β,1}$, that have the same amount of dispersion but different stimulation sites and NETFs. There are also pairs, $C_{α,α,1}$ and $C_{β,β,1}$, for instance, that were recorded at the same electrode with the same NETF after having dispersed over different distances. Examples of experimentally recorded CAPs exhibiting these relationships are shown in Figure 5.2. The top panel contains two compound signals that have undergone the same amount of dispersion but were recorded at different electrode sites. The shapes of these two CAPs are considerably different. Besides a substantial difference in signal amplitude, latencies measured to the onset and each of the signal peaks are shifted. The most striking difference, however, and the strongest evidence of the effects of individual NETFs is the relative size of the two positive peaks that give the CAP its distinctive shape. The positive peaks of the CAP plotted in black have approximately the same amplitude, giving the CAP a balanced look. The gray waveform, on the other hand, has a second positive peak that is substantially larger than the first. These CAPs can be contrasted with the two waveforms in the bottom panel that are recorded by the same electrode (have equal NETFs) but have dispersed over slightly different distances. The black CAP in this panel is identical to the previous graph. The gray CAP, however, is noticeably different. The positive peaks on this CAP have the same relative amplitude as the other signal recorded at the same electrode. These results are not only evidence that electrode-specific variables (NETFs) determine CAP shape, they also begin to indicate a way in which NETF differences might be removed (normalized) from a set of CAP data.

The waveforms of Figure 5.2 also suggest that there are differences in stimulation sites that must be accounted for. The decrease in amplitude between the two CAPs in the lower panel, which are stimulated at two different sites, seems more dramatic than a further three centimeters of propagation could account for alone. The additional assumption that the fiber distributions activated at points $α$ and $β$ will differ by a simple scaling factor is therefore made. This is quite reasonable when using ring electrodes to stimulate nerves in the finger. Anatomically, any fibers that pass under the proximal electrode will either continue on to also pass under the distal stimulating electrode, or terminate between the electrodes. For supramaximal stimuli, this assumption is equivalent to arguing that the fibers that terminate between points $α$ and $β$ represent a uniform cross-section of the entire
distribution. In this case, the CVDs activated at the stimulation sites will be the same to within a constant multiplier. So, we can then write:

\[ \bar{\omega}_\beta = k \cdot \bar{\omega}_\alpha = k \cdot \bar{\omega} \quad (5.1) \]

where \( \bar{\omega}_\alpha \) and \( \bar{\omega}_\beta \) are the CVDs activated at points \( \alpha \) and \( \beta \) respectively, and \( k \) is a constant of proportionality representing the percentage of fibers passing under \( \alpha \) that are also stimulated at \( \beta \).

Our forward model of CAP propagation and this one additional assumption allow differences in NETF to be removed from each of the recorded signals.

**Normalization Theory**

**Fourier Development**

We have used equations (2.13), (2.14), (5.1) and the eight CAP signals of Figure 5.1(b) to produce Table 1, which indicates the time and frequency domain formulae for each recorded CAP. Capital letters in the third column of Table 1 represent the Fourier transform of their lowercase complements. Using Table 1, we can write:

\[ \frac{F\{C_{\beta,1}\}}{F\{C_{\alpha,2}\}} = k \cdot \frac{N_b \Phi_3}{N_b \Phi_2} = k \cdot \frac{\Phi_3}{\Phi_2}, \quad (5.2) \]

and similarly

\[ \frac{F\{C_{\beta,2}\}}{F\{C_{\alpha,3}\}} = k \cdot \frac{\Phi_4}{\Phi_3}, \quad (5.3) \]

and

\[ \frac{F\{C_{\beta,3}\}}{F\{C_{\alpha,4}\}} = k \cdot \frac{\Phi_4}{\Phi_4} \quad (5.4) \]

where \( F\{\} \) indicates Fourier transform. Excluding the factor \( k \), which takes into account the termination of fibers between the two stimulation sites, the RHS of (5.2) is itself a transfer function that relates the NETF at electrode 2 to the NETF at electrode 1. Using this idea we can define:
The subscript notation is intended to indicate that the transfer function \( \Phi_{2 \rightarrow 1} \) can be used to determine how a signal recorded with the NETF of electrode 2 would have looked if it had instead been recorded with the NETF of electrode 1. This can be illustrated by performing the following multiplication and inverse Fourier transformation:

\[
\Phi_{2 \rightarrow 1} \cdot F\{C_{\beta,c,2}\} = k \cdot N_c \Phi_1,
\]

\[
k \cdot N_c \Phi_1 \leftrightarrow C_{\beta,c,1}.
\]

The right hand side of (5.7) is a time domain signal that approximates a CAP stimulated at the distal cathode, propagated a distance \( c \), and recorded by electrode 1. In this way we have used the transfer function calculated in (5.5) to simulate a “virtual electrode” with the NETF of electrode 1 but at the location of electrode 2.

The right hand side of (5.2) can be multiplied by (5.3) and (5.4) to yield two more transfer functions, \( \Phi_{3 \rightarrow 1} \) and \( \Phi_{4 \rightarrow 1} \). They are calculated as:

\[
\Phi_{3 \rightarrow 1} = \frac{1}{k^2} \frac{F\{C_{\beta,b,1}\} \cdot F\{C_{\beta,c,2}\}}{F\{C_{a,b,2}\} \cdot F\{C_{a,c,3}\}} \]

and

\[
\Phi_{4 \rightarrow 1} = \frac{1}{k^3} \frac{F\{C_{\beta,b,1}\} \cdot F\{C_{\beta,c,2}\} \cdot F\{C_{\beta,d,3}\}}{F\{C_{a,b,2}\} \cdot F\{C_{a,c,3}\} \cdot F\{C_{a,d,4}\}}.
\]

respectively. The three transfer functions can be used, in the manner of (5.6) and (5.7) to create three “virtual electrodes” located at recording electrodes 2, 3, and 4 but with the NETF of electrode 1. Once the NETFs have been normalized, any differences in the CAPs can be attributed to increased temporal dispersion.


Digital Fiber Termination and the k-Factor

A Description of k

The description of CAP waveforms stimulated and recorded at multiple sites and the theory of waveform normalization presented above both rely on the use of the proportionality constant, \( k \). In the electrode array model, \( k \) represents the percentage of fibers that pass under and are activated by the more proximal stimulating electrode, which subsequently pass under and become activated by the distal stimulating electrode. If the stimulus pulses are of large enough amplitude, it is appropriate to assume that all fibers that pass under a stimulating electrode will be activated. In this case, \( k \) represents the number of nerve fibers that terminate in between the two stimulation sites.

In light of the electrode array model, \( k \) can be thought of as a scaling factor representing the only difference, apart from intrinsic and geometric recording site differences, between two CAPs that are stimulated at the two different stimulation sites but propagate the same distance. The comparison of two such CAPs shows that the differences do go beyond a simple scaling. Even when the two signals are plotted over one another with normalized amplitudes differences in the shape of the CAPs are apparent. These intrinsic recording site differences are the focus of the normalization procedure and this chapter. In order to identify and remove these differences they must first be isolated from any other influences. Recording CAPs with the same amount of dispersion but that are recorded at different sites is the first step because it eliminates the effect of dispersion and leaves only geometry and \( k \). The next step is to remove the effect of \( k \) from these CAPs leaving only the differences that are inherent to the two recording sites. Unfortunately, intrinsic recording site differences can also include a simple scaling factor. This scaling factor will have an identical effect as \( k \) on the CAPs. The challenge then becomes to generate an estimate of \( k \) that leaves intrinsic amplitude differences intact. In order to do this it is helpful to make use of the other CAPs that have been recorded.

Estimating \( k \)

When applying the preceding normalization technique, the parameter \( k \) must be estimated from the CAP signals themselves. The problem of estimating complicated nerve to electrode transfer functions at four different recording sites has thus been reduced to estimating a single scaling factor, \( k \), relating the two stimulation sites. With simulated data this parameter is known. With experimental signals, however, \( k \) must be estimated from the available data. Since \( k \) is a scaling factor that relates the number of fibers activated at the two stimulating sites, it is tempting to use signal amplitudes to estimate it. The two CAPs recorded by the same electrode but stimulated at the proximal and distal...
sites will have different amplitudes due to both the added dispersion of the 2 cm separation of the two stimulation sites, and the effect of $k$. As the overall propagation distance increases, the dispersive effect of the short distance between the stimulation sites will decrease while the effect of the scaling factor, $k$, will remain the same. It has been useful, therefore, to consider the ratio of amplitudes between the two CAPs recorded by the same electrode as a lower bound on $k$. For the experimental data used in this study, this lower bound ratio increases from the first recording electrode to the last as would be expected. The ratio of the amplitudes of the CAPs recorded by the last channel has therefore been used as an estimate of $k$ in the experimental data analysis of this study.

**Normalization Results**

Figures 5.3, 5.4, and 5.5 show computer simulations of nerve potentials calculated in the same manner as those in chapter 4. In each case, single fiber action potentials are first calculated for the four recording electrodes. The depth and dive angle of the nerve are allowed to vary independently for each nerve-electrode system. Different depths and dive angles give rise to different SFAPs at each electrode, indicative of variations in the nerve-to-electrode transfer function of each site. Differences in SFAPs are the primary consideration in this study. The other major component of the forward model, the distribution of conduction velocities, remains constant for all simulations. Distances of propagation and, hence, distributions of latency are the same for all figures except Figure 5.5, where distances are increased in order to exaggerate the effects of dispersion.

All components of a simulated CAP dataset are shown in Figure 5.3. The four SFAP waveforms of Figure 5.3(a) are generated by keeping the dive angle of the nerve constant for all electrodes and increasing the depth of the nerve linearly as it traverses the electrode array. The four SFAP waveforms, which represent impulse responses of the NETFs of the four recording electrodes, differ mainly in amplitude as the distance between nerve and electrode increases. SFAP shape, largely a factor of the dive angle in this model, is more or less conserved. Although the duration of the single fiber signal grows slightly as the depth of the fiber increases, all four are similar in the size of the two positive peaks with respect to each other and with respect to the large negative peak.

Figure 5.3(b) shows the distribution of conduction velocities used for all simulations in this study. The CVD is a histogram of the percentage of fibers in the nerve with a given velocity of propagation. In the forward model, the CVD is defined over the range from 20 to 90 m/s with 1 m/s wide velocity bins. The CVD was generated by summing two Gaussian distributions, centered at 70 and 55 m/s,
and multiplying by a factor proportional to velocity squared. The distribution is assumed to represent the effective contribution of each fiber velocity class to the compound signal.

The velocity distribution is converted to latency distributions appropriate to each propagation distance of interest. Four latency distributions, corresponding to propagation distances of 19, 21, 23, and 25 cm, are plotted versus arrival time in Figure 5.3(c). Increased dispersion, characterized by the spreading out of the latency distributions as the compound signal travels along the nerve, is evident. The four propagation distances represent activation at the proximal stimulation electrode and recording at each of the four recording sites. Distal stimulation adds 2 cm to each distance so that the last three latency distributions of Figure 5.3(c) are also appropriate to the first three recording electrodes following distal activation. One additional latency distribution for a 27 cm propagation distance (not shown) was calculated to describe propagation from the distal stimulation site to the fourth recording electrode.

The eight simulated CAP signals are calculated by convolving each of the SFAPs with two latency distributions, corresponding to propagation from the distal and proximal stimulation sites. Figure 5.3(d) shows the simulated CAPs plotted versus time from stimulation. Each signal pair simulates the data that would be recorded by a single electrode following proximal and distal stimulation. Differences between CAPs recorded by the same electrode are due to an additional 2 cm of propagation and the scaling factor, \( k \), describing the disparity in activated CVDs. CAPs recorded at different electrodes are additionally affected by the distinct NETF of each recording site.

Figure 5.4 is another example of simulated CAP data. In this case, both the depth of the nerve and the dive angle are allowed to vary between each of the recording sites. The result is that the four electrodes have dramatically different SFAP waveforms. In Figure 5.4(a) it is apparent that not only the amplitude but also the shape of the SFAP changes between channels. Figure 5.4 (b) shows the multichannel CAP data simulated using the SFAPs of Figure 5.4(a) and the CVD from Figure 5.3(b). The compound signals reflect the same dissimilarities as the SFAPs.

The CAP signals of Figure 5.5(a) are derived from the SFAPs of Figure 5.4(a) but with much longer, though still evenly spaced, distances of propagation. Longer distances increase the relative effect of dispersion on the waveforms. The left side of Figure 5.5(a) is a plot of the four simulated CAPs stimulated at the proximal stimulation site. The right hand side of the figure shows the same signals after they have been normalized to remove differences in NETF. The normalized CAPs demonstrate the effect of pure dispersion over large distances. Since increased propagation distance is often used...
to improve the inverse 2CAP solution, we expect to get reasonable results with both the normalized and unnormalized data. Figure 5.5(b) shows the CVD estimates for the unnormalized (raw) and the normalized signals. The actual CVD used to derive the data is also shown as a solid line. As expected, qualitatively reasonable solutions are found for both sets of data. It is clear, however, that the normalized data leads to a solution that more closely matches the actual distribution.

Figure 5.6(a) and 5.6(b) contain CVD estimates for the CAP simulations in Figure 5.3 and Figure 5.4 respectively. Estimates were made before and after NETF normalization. The CVD used in the forward problem is plotted in both cases for comparison. The normalization procedure is seen to overcome even large errors in the CVD estimates. The mild differences in NETF from Figure 5.3 lead to a CVD solution, dash-dot line in Figure 5.6(a), that spans the same range as the actual CVD but lacks the distinct peak at higher velocities. The substantial NETF differences from Figure 5.4 produce a CVD, dash-dot line in Figure 5.6(b), that does not resemble the actual solution at all. The normalized solutions, dotted lines in Figures 5.6(a) and 5.6(b), recover both the shape and the distinctive features of the true distribution in both cases.

Figure 5.7 demonstrates the effect of adding colored noise to the CAP signals to more closely reproduce actual recordings. The CAP simulations from Figure 5.4 were corrupted with low pass filtered, uniformly distributed noise to achieve a peak SNR of approximately 15. Figure 5.7(a) shows the corrupted signals prior to normalization. Figure 5.7(b) illustrates CVD estimation for the corrupt signal before and after normalization. The solid line denotes the actual CVD used in the simulation. Although the normalized solution continues to show a low-velocity peak that does not belong in the distribution, the high velocity peak, completely absent prior to normalization, has returned and is the dominant peak in the CVD.

Figure 5.8 demonstrates the sensitivity of the CVD algorithm to the choice of the parameter $k$. The solid line is the CVD used in the simulation. Accurate estimation of $k$ produces a CVD that closely matches this solution. The dotted and dashed lines are the CVDs calculated when $k$ is overestimated or underestimated by 10%.

Figures 5.9 and 5.10 illustrate the use of the normalization technique on experimentally recorded data. Figure 5.9(a) shows a set of four experimentally recorded CAPs before and after normalization. Similar data sets were acquired from four different subjects using the methodology illustrated in Figure 5.1. NETF differences are apparent in the original data. The factor $k$ was estimated from signal amplitudes to be 0.76 for this data set. Following normalization, the signals exhibit differences
in amplitude and duration that are appropriate to pure dispersion between recording sites. Normalization also has an effect on the CVD estimates. In Figures 5.9(b) and 5.10(a-b) CVD estimates before normalization are shown as a dotted line and the estimates from normalized data are overlaid as a solid line. The “maximum” conduction velocity calculated in the standard way, as the ratio of the propagation distance to the latency of the first positive peak of the CAP, is also indicated in both figures.

Figure 5.11 shows the variations in CVD estimates generated from one normalized data set. CVDs calculated from all ten independent CAP pairs were averaged to yield the mean CVD and plotted as a solid line. The standard deviation of the ten measurements was calculated for each bin and the dotted lines indicate the mean CVD plus or minus one standard deviation.

Discussion

The purpose of this portion of the project was to explore the value of nerve-to-electrode transfer function normalization in 2CAP CVD estimation over a short segment of peripheral nerve. Typically, 2CAP CVD algorithms are performed without normalization, or even consideration of the NETF, and rely instead upon longer distances of propagation to increase the amount of dispersion between the CAP signals. A short segment technique would standardize the choice of recording sites, reduce the subjectivity of nerve length measurements, and minimize inter- and intra-subject variability.

Forward models of compound action potential propagation rely on electrophysiologic assumptions that, although idealizations, are well accepted and experimentally validated. In the past, these models attributed the variability among CAP signals to two distinct factors: differences in the distribution of fiber conduction velocities in the nerve, and differences in the distance of propagation leading to corresponding changes in temporal dispersion. Multiple CAPs recorded from the same nerve have the same CVD and can therefore be used to solve the inverse problem by attributing any changes in CAP shape to dispersion. If the distance between recording sites is small, the amount of dispersion between recordings decreases and consideration of differences in the nerve-to-electrode transfer function becomes crucial. Therefore, the effect of NETF on CAP signals is of particular importance in short segment recordings, as simulated in Figure 5.3. Differences in dispersion can be seen in the latency distributions computed for each propagation distance and plotted in Figure 5.3(c). Each distribution is wider and shallower than the previous one and these qualities are transferred to the CAP when computed as a convolution. Differences in NETF are brought about by varying the distance of the nerve from the electrode in the SFAP simulation. In this way, SFAP shape is more or less conserved
between channels while the amplitude steadily decreases as the nerve dives. The algorithm presented in this paper is aimed at isolating the dispersive factor in order to generate improved estimates of the distribution of conduction velocities. In order to isolate dispersion, the NETFs of all CAP recordings are equalized.

Figure 5.4 is an example of simulated data in which the NETF differences are exaggerated. The amount of dispersion in each of the CAPs is the same as Figure 5.3 but the angle of nerve dive and the depth of the nerve both vary. The effect of NETF differences on the compound signals is immediately apparent. Signal amplitude and duration do not steadily decrease and increase respectively as pure dispersion would dictate. Instead, CAP amplitude is highest at the second channel and drops dramatically at the third and fourth channels. The experimental data of Figure 5.9 also demonstrates unexpected changes in amplitude as the compound signal propagates along the nerve. This suggests that dispersion alone cannot account for the changing CAP size and shape. NETF differences are therefore an important consideration in solving the inverse problem.

Propagation distances have been increased in the simulations leading to Figure 5.5(a) so that the effects of both NETF differences and dispersion can be seen. This figure also demonstrates the use of the NETF normalization procedure. Figure 5.5(a) shows the simulated CAPs before and after normalization. There are obvious differences in shape and amplitude between the raw data (left half of the figure) and the corresponding normalized data (right half). In the normalized data any dependence on NETF has been removed and all four signals have the effective NETF of channel 1. The normalization procedure leaves intact, however, dispersive effects as can be seen from the steadily decreasing amplitude and gradually increasing duration as the CAP propagates [23].

The normalization procedure, in this case, leads to improved estimates of CVD. Figure 5.5(b) shows the CVD estimated before and after normalization compared to the actual distribution that was used in the forward simulation. Long propagation distances increase the overall amount of dispersion and relative dispersion between CAPs so that a qualitatively reasonable CVD can be estimated before normalization. Although reasonable, the raw data CVD overestimates at the peak and drops down to zero much faster than the actual histogram. The normalized CVD, on the other hand, tracks the entire desired solution much more accurately. The small errors that do exist in the normalized CVD come about from quantization errors in the algorithm and the discretization of time and velocity.

The benefits of the normalization procedure can also be seen in Figure 5.6(a and b). Figure 5.6(a) uses the data from Figure 5.3 in which both differences in NETF and differences in the amount of
dispersion between channels are moderate. Even these small variations are shown to produce substantial errors in the CVD estimate. The peak, in this case, is considerably underestimated while the rest of the distribution is somewhat larger than it should be. After normalization, the CVD is much more accurate and closely matches the actual distribution.

The same qualitative results hold for the simulations of Figure 5.4. CVD estimates from these data, which were simulated with substantial differences in NETF between channels and short propagation distances, make the point even more dramatically. In this case the raw data lead to a CVD estimate that bears no resemblance to the true CVD. The general shape of this distribution, broad and skewed towards low velocities, is commonly seen when applying the inverse solution algorithm to data that is corrupted by large amounts of noise or unnormalized data recorded from a short segment of nerve. As in the previous case, normalization produces a more accurate solution.

The results described above are produced by clean data in which the only random components are due to quantization errors. Actual CAP recordings are corrupted by noise from both the physiological system and the recording hardware. Ensemble averaging can be performed to reduce the noise, but it cannot be removed completely. Random noise has been added to the simulated data of Figure 5.4(a) to further assess the performance of the algorithm. The resulting raw data and the CVD estimates are shown in Figure 5.7. The unnormalized data produce a poor estimate of the CVD. Normalization improves the CVD estimate dramatically. The effect of noise is still evident after normalization, however, and can be seen by comparing Figures 5.6(b) and 5.7(b), which differ only in the addition of noise to the CAP signals. Problems caused by noise cannot be solved, and may be worsened, by normalization. The inclusion of low pass filtering after normalization does not appear to further improve the CVD estimate. Improvements in hardware, electrode design and/or technique, such as very-low-noise components or an abrasive or minimally invasive skin preparation, should help to reduce the noise in CAP signals. Additional ensemble averaging when acquiring the data can also be used to reduce errors due to noise.

Noise is carried through the normalization process as part of the relative nerve to electrode transfer functions. The normalized signals can, in fact, have substantially more noise than the raw data from which they were derived. This is because the signals have very little high-frequency content so that their discrete Fourier transforms in this region are determined largely by noise. The ratio of transforms used to calculate the NETFs can therefore be artificially large at certain frequencies above the bandwidth of the CAP signals. These spikes in NETF spectra can lead to corresponding artifacts in the normalized data calculated according to (5.6) and (5.7). The low-pass nature of the signal being
normalized, \( C_{\beta,c,2} \), reduces artifacts at high frequencies but the problem remains in the mid-band, where both the signal and the NETF have substantial energy. It is possible that normalizing all NETFs to that of an electrode over a deeper section of nerve would reduce such artifacts. It would also tend, however, to reduce signal amplitudes. An a-priori decision was made for this study to reference all NETFs to the first electrode, which is generally over the most superficial region of nerve. This decision was intended to maximize the signal amplitude, and hence SNR, of the normalized data and thereby improve CVD estimates.

In this study, many CVD estimates made before normalization contain a broad distribution, often centered at low velocities. This same type of distribution commonly arises when using inaccurate or noisy data. As the signal to noise ratio of simulated data is decreased, the CVD estimate gradually changes to this shape and maintains this shape even as the signal to noise ratio falls below 1. The cause of the consistent shape and low velocity bias of this error is unknown. One possibility is that the steepest descent minimization algorithm is unable to find a global minimum when the objective function is based on noisy data. Alternatively, the objective function itself may be altered so that minimization occurs when the distribution has this characteristic shape. Regularization techniques may be helpful in reducing these problems.

The normalization procedure has also been applied to clinical short segment data with results that qualitatively validate the usefulness of the technique. Figure 5.9(a) demonstrates the ability of the normalization procedure to isolate dispersion in data recorded in the lab. The experimental data show the effects of NETF differences. The amplitude of the CAPs in Figure 5.9(a) before normalization (left side of the figure) is similar on channels 1 and 2 but drops substantially on channels 3 and 4. These data also demonstrate the variability in CAP shape that can result from small (2 cm) changes in the location of the recording electrode. However, normalization produces CAP data for which the amplitude and duration vary in accordance with dispersion but overall shape is largely conserved.

Estimates of CVD from unnormalized experimental data resemble the low velocity distributions found with very noisy or inaccurate simulations. After normalization these distributions have a bimodal appearance with a sharp peak in the region of the fastest conduction velocities and another, broader peak at lower velocities. Although some researchers have included bimodalities in their CVD estimation procedure, slower components are generally hidden in the noise well after the main peaks of the CAP and statistical methods are required to estimate their contribution. It is believed that the low velocity peaks in the CVD that remain after normalization are artifacts due to the same factors mentioned above for additive noise.
CVD estimates made using this procedure depend on the value of the parameter \( k \) that is used. Although \( k \) was not estimated for the simulations in this study, the accuracy of the estimation technique with simulated data was investigated. The estimation procedure has been shown to give a lower bound on \( k \), i.e., none of the estimates were greater than the actual value, but errors of 5 to 10% were not uncommon. These errors are important to note because the CVD estimation method is sensitive to the value of \( k \) used in normalization. Figure 5.8 illustrates the results of poor estimation of \( k \) on the CVD algorithm. The solid line is the distribution used in the data simulations while the dotted and dashed lines are the results of CVD estimation when \( k \) is overestimated or underestimated by 10% respectively. This figure can be compared with Figure 5.6(b) where an accurate value of \( k \) was used with similar data. The overestimation of \( k \), which can result in normalized CAP's that steadily increase in amplitude along the recording array, seems to lead to a worse solution than underestimation by the same amount. This strengthens our belief that the lower-bound technique of estimating \( k \) is preferable to other estimation techniques or to a-priori presumption. The next chapter will investigate the accuracy of this technique with experimental data and possibly lead to improved methods.

Changes in the shape of CAPs following normalization lead to changes in the CVD estimate for those data. After normalization, clinical CVD estimates of this study (Figures 5.9(b) and 5.10) are similar in shape and span to distributions published for normal subjects. The data in these previously published reports are either from histological measures of nerve fiber diameters or from 2CAP recordings using much longer distances of propagation. It is currently impossible to record single fiber action potentials from surface electrodes, which might be used to establish an appropriate SFAP basis waveform. Even histological studies are based on assumptions about the relationship between nerve diameter and conduction velocity, are highly susceptible to errors during tissue preparation, and require subjective and painstaking measurements of the diameter of every nerve in the bundle. Comparison to well documented studies is therefore a strong argument for the efficacy of the method presented in this paper.

Another important aspect of the procedure outlined in this chapter is that normalization allows ten independent, short segment CVD estimates to be calculated from a single set of data. The multiple estimates can be averaged to yield a mean CVD. Since it includes all available data, the average may be a more accurate measure of the true CVD and may prove more robust to intra-subject variability. The consistency of the multiple solutions with respect to each other can also serve as additional validation of the method itself. Figure 5.11 shows a mean CVD that incorporates data from all ten
CAP pairs. A range of one standard deviation from the mean indicates the consistency of the multiple, short segment estimates. These data suggest that averaging of multiple CVD's would be advantageous in calculating a robust estimate of the velocity distribution given the spacing and number of electrodes available in orthodromic, sensory, median nerve recordings. There are applications of the method presented in this paper, however, for which averaging might not be an option or might not be the best option. Lower extremity nerves, for instance, may allow longer propagation distances overall but be more restrictive in the number of equally spaced recording sites available. It is also possible that single (unaveraged) CVD estimates have a higher sensitivity to the slight changes in nerve conduction that would indicate incremental progression of a disease or a treatment.

Conclusions

In an attempt to accommodate differences in nerve-to-electrode transfer functions between recording sites a method of normalizing multiple CAP recordings has been developed. The normalization procedure incorporates information from CAPs stimulated at two separate stimulation sites and recorded at at least two, equally-space recording sites. Nerve-to-electrode transfer functions are not removed from the recordings in this method; rather all NETFs are normalized to that of the first recording site. Simulations have been employed to show that the normalization algorithm cannot only remove NETF differences, but by doing so can also dramatically improve the CVD estimate from the same CAPs. Experimental data has also been studied. CAPs recorded from an electrode array are shown to demonstrate wave shapes that cannot come about from pure dispersion. Applying the normalization algorithm to the array data results in normalized signals that behave as if additional dispersion is the only difference between them. CVD estimates calculated from the experimental CAPs prior to normalization are qualitatively similar to CVDs from simulations that are heavily corrupted with noise. Following normalization, CVDs from the same data are altered and are comparable to CVDs in the literature estimated from healthy nerves. The normalization algorithm requires the estimation of a parameter, $k$, that describes the relative number of fibers at the two stimulation sites. This parameter is a required input to the algorithm.

There are only two assumptions made in the method presented here that are not required by other investigators. The first is that the factor $k$ can be estimated as discussed above. The second is that all inter-electrode distances, not just two propagation distances, are known. The method developed in this study does not exclude the possibility of equal transfer functions at all recording sites, an
assumption commonly made in the literature. By allowing for differences in NETF, however, this method is a more general approach to the electroneurographic inverse problem.
<table>
<thead>
<tr>
<th>CAP Signal</th>
<th>Time Domain Formula</th>
<th>Frequency Domain Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\alpha,a,1}$</td>
<td>$Q_a {\omega} \ast \phi_1 = n_a \ast \phi_1$</td>
<td>$N_a \Phi_1$</td>
</tr>
<tr>
<td>$C_{\alpha,b,2}$</td>
<td>$Q_b {\omega} \ast \phi_2 = n_b \ast \phi_2$</td>
<td>$N_b \Phi_2$</td>
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<tr>
<td>$C_{\alpha,c,3}$</td>
<td>$Q_c {\omega} \ast \phi_3 = n_c \ast \phi_3$</td>
<td>$N_c \Phi_3$</td>
</tr>
<tr>
<td>$C_{\alpha,d,4}$</td>
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<td>$N_d \Phi_4$</td>
</tr>
<tr>
<td>$C_{\beta,b,1}$</td>
<td>$Q_s {k \cdot \omega} \ast \phi_1 = k \cdot n_b \ast \phi_1$</td>
<td>$k \cdot N_b \Phi_1$</td>
</tr>
<tr>
<td>$C_{\beta,c,2}$</td>
<td>$Q_c {k \cdot \omega} \ast \phi_2 = k \cdot n_c \ast \phi_2$</td>
<td>$k \cdot N_c \Phi_2$</td>
</tr>
<tr>
<td>$C_{\beta,d,3}$</td>
<td>$Q_d {k \cdot \omega} \ast \phi_3 = k \cdot n_d \ast \phi_3$</td>
<td>$k \cdot N_d \Phi_3$</td>
</tr>
<tr>
<td>$C_{\beta,e,4}$</td>
<td>$Q_e {k \cdot \omega} \ast \phi_4 = k \cdot n_e \ast \phi_4$</td>
<td>$k \cdot N_e \Phi_4$</td>
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</table>

Table 5.1
Stimulating Electrodes

Stimulator

Compound Action Potentials

Figure 5.1
Figure 5.2
Figure 5.3
Figure 5.4
Figure 5.5
Figure 5.7
Figure 5.8
Figure 5.9
Figure 5.10
Figure 5.11

![Histogram showing mean DCV and ±1 Standard Deviation](image-url)
Chapter 6. Experimental Data

Introduction

The simulations discussed in the previous chapter helped define methods of stimulus artifact removal and CVD estimation for the work presented in this, and in the following chapters. Simulations were also useful for determining the size and type of errors that might be expected in CVD estimates made from noisy data with varying amplitude stimulus artifacts and different methods of signal processing and artifact removal. This chapter deals with the application of the techniques developed previously to experimentally recorded data in an attempt to estimate CVDs from short nerve segments.

A new hardware system was developed to allow multiple channel CAP acquisition from the same stimuli and to integrate the stimulation and acquisition circuitry. Monopolar or bipolar orthodromic sensory CAPs are recorded from multiple sites on the wrist following stimulation at the finger. Artifacts are removed and CVD estimates are generated with the 2CAP estimation algorithm. Observations of the data lead to the development of a normalization algorithm intended to remove waveform differences that arise from differences in the recording site. Application of the normalization technique requires recording CAPs from different stimulation and recording sites. CVDs calculated from the normalized data more closely match CVDs reported in the literature.

Methods

Hardware

A compact, 4 channel, battery operated stimulation and recording system has been developed for the measurement of evoked CAP signals. The system is based on the idea of integrating independent stimulation, recording, and data analysis components. Each component maintains a high level of functional versatility while PC-based software oversees communication between and control of individual components. There are three main system components: a stimulation circuit, data acquisition hardware, and the user interface. The stimulator is capable of producing complex patterns of constant current stimulus pulses and allows precise control of the amplitude, length, and spacing of all stimuli. The array detector performs four-channel analog signal processing, multiplexing and A/D conversion, and temporarily stores data for upload. Finally, the control and data display software provides a graphical user interface to set measurement parameters, performs digital signal processing,
displays the multi-channel data, and controls the flow of data and the operation of the other two components. The system also contains a built-in database for measurement record keeping. As a whole, the system is capable of making four channel, differential measurements of nerve biopotentials evoked by complex patterns of stimulation. Figure 6.1 shows four channel data recorded with the system and displayed in the system’s control window on a PC. Note that stimulation parameters such as amplitude and duration, as well as recording parameters such as number of channels and sampling rate are all software controlled.

**Recording Methods**

Multiple CAP signals were recorded in order to allow the normalization algorithm to be tested on experimental data. Stimulating and recording electrodes were placed as indicated in Figure 6.2. Four stimulating ring electrodes were placed around the middle finger. The most proximal ring electrode was used as the proximal stimulating cathode. The next ring electrode was used as the proximal stimulating anode. The third electrode was used as a cathode when performing distal stimulation, and the most distal electrode was used as the distal stimulus anode. The cathodic stimulating electrodes were taken to be the point of fiber activation and the distance between them was set carefully to a known length. Figure 6.2 shows both stimulating cathodes but only a single anode.

Recording electrodes were placed both along the wrist and at the anticubita fossa on the ventral elbow. A single, active electrode was placed over the median nerve along the wrist with a pair of indifferent electrodes on either side of the nerve at approximately the same distance from the stimulating sites. The indifferent electrodes were connected together by a small jumper cable and then connected to the positive input of the amplifier circuit. The active electrode was connected, by itself, to the negative input terminal. A reference electrode was placed either on the back or the palm of the same hand and that reference was used for all recording channels. The recording site at the elbow consisted of a single active electrode placed over the nerve, medial to the biceps tendon. A single indifferent electrode was used in this site placed laterally from the active electrode and several centimeters away.

Multiple stimuli were presented to each of the stimulation sites on the finger in order to allow averaging of the resulting waveforms. Recordings were made at at least two locations on the wrist and at least one location at the elbow. The active electrodes of the multiple wrist recording sites were placed carefully so that their separation was equal to the separation of the stimulating cathodes. This careful spacing of stimulation and recording sites allowed the normalization algorithm to be
employed. The addition of at least one recording from the elbow was used to calculate long-distance, or classical CVDs.

**Signal Processing Methods**

CAP signals stimulated at the same location and recorded by the same recording channel were averaged yielding recordings that could be referenced by the site of stimulation (proximal or distal), the recording electrode (1, 2, 3, or 4), and the distance that the action potentials propagated between the stimulating and recording electrodes. Each of the averaged signals was digitally low pass filtered in order to reduce high frequency noise components. The DC offset in each signal was estimated as the average value of the final 1 ms of each recording. In this region the recording has generally returned to baseline. This DC offset was subtracted from the entire waveform. While the recording methodology and the recording electrode placement reduced stimulus artifacts considerably, remaining artifacts were removed through the linear regression technique applied to each signal individually. The resulting ensemble averaged, filtered CAP waveforms were then used in the normalization and CVD estimation algorithms.

The locations of the peaks of the CAP signals, one large negative and two flanking positive, are determined with a peak detection scheme. The onset and termination of each CAP is then determined by examination of approximations of the first and second derivatives of the signal. The location of the CAP onset is windowed and that window is iteratively narrowed to refine the estimate. The general location is identified by a minimum in the first derivative and a large, negative second derivative. The termination location is estimated in an analogous manner. The CAP signals were zeroed before their onset and following their termination.

The normalization algorithm described in chapter 4 was applied to all CAPs recorded from the wrist. This resulted in at least four recordings whose nerve-to-electrode transfer functions were equalized. If more than two wrist recording sites were used, then normalization was extended to the other sites as well yielding six, or sometimes eight, normalized CAPs. CVDs could then be estimated from any pair of these signals. Whenever possible, a pair of CAPs was used that had as large a difference in propagation distance as the wrist electrode array would allow. The most proximal stimulation site and the most distal recording site resulted in the shortest distance, while distal stimulation and the most proximal wrist recording site yielded the array’s longest distance. A combination of those two CAPs could then be used in the 2CAP algorithm.
In addition to short distance CVDs calculated from the array recordings at the wrist, when recordings were made at the elbow long distance CVDs were also estimated. When available, these long distance CVDs were used as a gold standard measurement of the classical CVD. Comparing short distance CVDs with this gold standard allowed for further validation of the normalization procedure.

**Results**

Figure 6.3 shows four wrist recordings from electrodes located 13, 15, 17, and 19 cm from the site of stimulation. Also plotted in the figure are lines representing the arrival time of different signal landmarks at each of the recording sites. Lines corresponding to CAP onset, the first positive peak, the negative peak, and the final positive peak are included. The duration of the same, array-recorded CAPs are plotted versus distance of propagation in Figure 6.4. Duration is calculated as the difference between termination and onset for each of the CAPs. A linear regression has been performed and the best fit is plotted. Figure 6.5 shows the amplitude of actual CAPs recorded along the array. The absolute amplitude of the large negative peaks is plotted as a thick black line. The relative amplitudes of the two positive peaks of the CAPs are also plotted. The amplitudes of the positive peaks are calculated and then they are all normalized to the amplitude of the negative peak of the first channel recording. The results are shown as two thinner lines, one for the first positive peak of each signal and one for the second. The CAPs themselves are also plotted as dotted lines in this figure to indicate where the amplitude measurements were derived.

The similarity of long-distance CVD estimates generated from different CAP pairs is examined in Figure 6.6. Three different pairs of CAP waveforms are used to generate three CVD estimates. The CAPs all result from stimulation at the proximal finger site. The left hand panel of figure 6.6 shows the three CAP pairs after stimulus artifact removal. Each of three wrist recordings is paired with the long-distance (elbow) recording from that stimulus site. A DC offset is included in the long-distance CAP data to clarify the graph. The wrist recordings, 1 through 3, were recorded at propagation distances of 14, 16, and 18 cm respectively. The right hand panel shows the three resulting CVDs. The same three proximally stimulated, wrist-recorded CAPs were used to form three short segment CAP pairs. Each pair was applied to the CVD algorithm. The CAP pairs are plotted in the left hand panel of Figure 6.7 while the resulting CVD estimates from each pair are plotted to the right.

Figure 6.8 includes information from six inter-stimulation site, long-distance CVD estimates as well as six inter-stimulation site, short-distance CVD estimates. The range of velocity bin values is plotted for both short-distance and long-distance data. The short-distance CVD range is plotted as the lightly
colored filled region and the long-distance CVD range as the darker filled region. In each case, the mean value for each bin is plotted as a solid line.

A. Figure 6.9 shows a long-distance (classic) CVD, the solid black histogram, as well as a CVD calculated from two short-distance CAPs from the same data set, in solid gray. The third CVD plotted in this figure was generated using the most distal CAP recorded from the wrist following proximal stimulation (also used in the other two CVDs in this figure) and the long-distance CAP recorded from the elbow (same recording electrode as the classic CVD) following distal stimulation.

Correlations between inter-stimulus site CVDs and the long distance CVD estimate are illustrated in Figure 6.10. The top plot panel shows the correlation coefficients plotted as a function of $k$. A value of 1 indicates that the two CVDs are identical and a value near zero indicates very low correlation and dissimilar CVDs. The highest correlation occurs when a $k$ value of .64 is used. The bottom plot of this figure shows the CVD results. The long-distance CVD is plotted as a solid black line. In this case the range of bin values generated from all 100 CVD estimates is shown as the light gray shaded region and the best inter-stimulus site CVD ($k = 0.64$) is plotted as a solid gray stair step.

Figure 6.11 shows the effect of normalization on CVD estimation performance. CVDs estimated from the dataset prior to normalization are represented in the top panel. The dark-gray shaded region shows the entire range of all possible long-distance, intra-stimulus site CVDs. The black line within this region is the mean of those distributions. The light-gray region in the background of this figure and its corresponding black line show the range and the mean value of all possible short-distance, intra-stimulus site CVDs prior to any normalization. The bottom panel shows the same results but after normalizing the short segment data with an optimal value of $k$.

Figure 6.12 demonstrates the dependence of the normalized CVD solution on the value of $k$. The top panel shows values of the RMS error between the long distance CVD and the normalized short-distance CVD as a function of $k$. The dotted line indicates the RMS error between the classic CVD and the unnormalized short-distance estimate. The bottom panel of Figure 6.12 shows several normalized CVD estimate ranges. The range of all CVDs calculated from the regions of light gray highlight in the top panel ($0.1 < k < 0.3$ and $0.8 < k < 1.0$) are also plotted in light gray in the bottom panel. Results from the darker shaded region of the top panel ($0.52 < k < 0.6$) are also plotted in the lower graph. The best normalized solution for this data set occurs at $k = 0.56$ and the resulting CVD is plotted as a solid gray line.
Figures 6.13 and 6.14 compare the prospectively estimated value of $k$ to the retrospectively determined optimal value. CVDs produced by normalizing a dataset with both $k$-estimate and $k$-optimal are shown in Figure 6.13 along with the long-distance CVD. Values of $k$-estimate and $k$-optimal are plotted in the top panel of Figure 6.14 for 22 individual sets of data. $k$-estimate is plotted on the left side of the graph and $k$-optimal on the right. A solid line connects the data points that are due to $k$-optimal and $k$-estimate from the same set of data. The ratio of $k$-optimal to $k$-estimate, as indicated by the slope of the line between them, has been determined for all 22 datasets and plotted as a histogram in the bottom graph.

Figure 6.15 illustrates a case where the error between the long-distance CVD and the short segment CVD increased as $k$ values within the physiologically reasonable range were used. The top panel shows the RMS error between the long-distance standard and the average normalized short-distance CVD for 100 different values of $k$. CVDs calculated from this dataset are plotted in the bottom panel. The long-distance solution is plotted in black. Normalized CVDs corresponding to $k = 0.49$ and $k = 1.0$ are shown in light gray, and the normalized CVD resulting when $k = 0.70$ (the maximum of the error function) is plotted in dark gray.

Figure 6.16 demonstrates the application of the cross-correlation method to the same dataset as Figure 6.15 to take only CVD shape into account. The maximum value of the cross-correlation between the gold standard CVD and the normalized short-distance CVD is plotted in the upper panel as a function of $k$. The maximum of the correlation data represents the best duplication of the long-distance CVD shape. The bottom panel shows the normalized CVD estimated with the optimal $k$ alongside the long-distance solution. A second normalized CVD, poorly correlated with the long-distance CVD, is also shown. The long-distance CVD is plotted in black, the normalized CVD corresponding to $k = .55$ is plotted in light gray, and the normalized CVD corresponding to $k$-optimal (0.71) is graphed in darker gray.

Discussion

Short Distance Data

The development of a multichannel hardware system and novel, electrode-array recording methods allow the recording of multiple CAPs from different recording sites due to the same stimulus. When these multichannel recordings are displayed next to each other, waveform differences are apparent. The main types of differences seen- amplitude, duration, and latency- can arise from the dispersion of
single fiber action potentials as they propagate along the nerve. As the simulations of Chapter 4 demonstrate, differences in these parameters due to dispersion can be expected, and there should be some consistency in the change that occurs between equally spaced electrode sites. There are factors besides dispersion, however, that can lead to different wave shapes. These factors—recording site geometry, electrode size, and electrode impedance—will produce differences that do not change consistently along the nerve as with dispersion. Rather, they may lead to opposite effects such as increased amplitude at longer distances or differences in the relative amplitude of the peaks within a single CAP.

**Dispersion**

The main cause of differences between CAP waveforms recorded at different locations is generally assumed to be dispersion. The amount of dispersion that a compound signal undergoes is directly related to the distance that the individual action potentials propagate. As the difference in distance of propagation between two recordings is reduced, so too is the difference in the amount dispersion and the differences in the shape and size of the waveform. Since 2CAP CVD estimation techniques rely on dispersive differences, it is important that recordings are made far enough apart that such differences can be seen.

**Time Shifts**

In data simulations, as shown in Figure 4.7, signals recorded from evenly spaced electrodes along the wrist demonstrate a latency that changes linearly in time with incrementally longer distances. Figure 6.3 illustrates the manner in which latency changes in experimentally recorded CAPs from four, equally-spaced recording sites. Lines corresponding to CAP onset, the first positive peak, the negative peak, and the final positive peak indicate the speed with which each of these waveform components traverses the array. The location of the CAP onset should correspond to the fastest fibers of the bundle arriving at each recording electrode. This line should be unaffected by dispersion since the fastest fibers will be the first to contribute at every recording site. Differences in nerve-to-electrode transfer function can, however, affect the linearity of this line. The lines that track other signal components are susceptible to not only differences in NETF but also dispersion. This is because, unlike signal onset, these landmarks do not necessarily correspond to a particular SFAP arriving at the recording site but rather a feature of the compound signal. As SFAPs disperse, these secondary lines may have nonlinearities due to NETF differences at the electrodes or due to dispersion. Finally, it is expected that, as in Figure 4.7, the line that tracks onset will have the shallowest slope (corresponding to the fastest conduction velocity). Since this line is due to the arrival of the fastest components, its slope
indicates the fastest velocity that occurs within the nerve. Any other feature of the CAPs or individual SFAP that can be tracked must have a slower velocity and, therefore, will result in a steeper slope in a plot such as Figure 6.3.

In Figure 6.3, the line that tracks the onset of the CAPs at each electrode is very close to linear with a slope of approximately 0.7 ms per 6 cm of distance. This slope corresponds to a fastest conduction velocity of around 75 m/s, which is right in line with the normal value for a healthy adult. Differences in the NETF between electrodes are not evident from the examination of differences in onset time in these data since the line is close to linear. This indicates that while NETF may affect the shape and size of CAPs recorded along the array, it does not change the expected time of signal onset at each site.

The arrival times of the subsequent CAP waveform features also maintain a strong linearity as can be seen by looking at the three lines closer to the top of Figure 6.3. As seen in the simulations, the slopes of the lines tend to increase from the earliest occurring signal landmark to the latest. The final line, tracking the second positive peak, has a slope of approximately 1 ms per 6 cm, or 60 m/s. While it may be tempting to think of this number as approaching the slowest velocity of interest, it must be remembered that it does not correspond to the slowest SFAP traversing the array but rather the location of a feature that occurs well before the final SFAP arrives. This can also be seen in the simulations of Figure 4.7 where tracking of CAP onset led to a velocity of 100 m/s and the second positive peak traversed the array at approximately 67 m/s. This is in spite of the fact that the CVD used in the simulations and shown in Figure 4.4 has a very small contribution at 100 m/s and considerable contributing fibers below 67 m/s. It is also important to note that CVD calculations based on simulated data were able to accurately estimate the CVDs, including contributions below 67 m/s.

The difference in slope between the time of onset along the array and the occurrence of other CAP features along the array leads to lengthening of signal duration with longer propagation distance. Figure 6.4 demonstrates this effect for the same set of CAPs plotted in Figure 6.3. Dispersion of the SFAPs along the array will contribute to a linearly changing duration. The deviation of this plot from linear is likely due to difficulties inherent in estimating CAP termination. The distribution of fibers in the nerve is likely to include many smaller, slower fibers whose contribution is too small and too spread out in time to be detected. Since the distribution is a continuum of velocities, it will always be unclear where the cutoff is between slow SFAPs that can be detected and contribute to the recorded CAP and slightly slower SFAPs that do not contribute significantly. Generally the trailing end of the
CAP will fade steadily into the noise of the recording so that the termination point, as determined by a heuristic algorithm, will be sensitive to recording noise. Errors due to this noise are the likely reason for the nonlinearity of the duration data in Figure 6.4.

**Amplitude Changes**

Another effect of dispersion along the array will be a gradual decrease in signal amplitude. In the simulations of Chapter 4, amplitude had a complicated but monotonically decreasing relationship. An important point made by Figure 6.5 is that CAP amplitude does not decrease monotonically, as dispersion would predict, along a closely spaced array of electrodes. While the amplitudes of the first, third, and fourth CAPs seem to be in line with dispersion, the second CAP is much larger in amplitude than the other three. This is true of all three peaks in the second CAP. This change in amplitude is a very strong indication of a difference in NETF between the electrodes. It is likely that the nerve is more superficial to the surface of the skin as it passes under the second electrode than when passing the other three.

**Shape Differences**

Perhaps more important than the change in amplitude indicated by Figure 6.5 is an overall change in CAP shape. If the difference between electrode sites that led to NETF changes were simply a difference in the proximity of the electrode to the nerve, the effect on the CAP would be an overall scaling of the CAP waveforms. If that were the case, the normalization procedure outlined in the previous chapter could be simplified to a scaling of the CAPs with respect to each other. The relative amplitudes of CAP peaks in Figure 6.5, however, indicate that this is not the case. If the CAPs recorded at distances of 15, 17, and 19 cm are simply scaled versions of the first CAP, then all three lines plotting amplitude should lie directly on top of each other. This is almost true for the CAP recorded at 17 cm, indicating the relative amplitudes of its three peaks are similar to that of the first CAP. The two positive peaks of the second (15 cm) and the fourth (19 cm) CAPs diverge to either side of the negative peak amplitude. The conclusion that can be drawn is that it is not simply a scaling of the CAP waveforms, nor a scaling combined with an offset that leads the dramatic changes in amplitude that are seen. It is an overall change in the shape of the CAP at different recording sites that generates the unexpected amplitude differences, the divergence of the amplitudes of different peaks with respect to each other, as well as contributing to differences in latency and duration.
CVDs Calculated From Unnormalized CAPs

Accuracy of Long- and Short-Distance CVD Estimates

Recording enough CAPs to apply the normalization algorithm results in many more waveforms than are required to carry out a 2CAP calculation. Incorporating as much of this extra data as possible into the short-distance CVD estimate might make the CVD more robust and less susceptible to error. When three wrist recording sites, one elbow recording site, and two different finger stimulation sites are used there are a total of eight different CAP recordings in the pre-processed data set. From these eight signals there is the possibility for twelve long-distance CVD estimates (all combinations including one of the wrist recordings and one of the elbow recordings) and twelve short-distance CVD estimates (all combinations including two wrist recordings in which one propagation distance is longer than the other). These combinations include inter-stimulus site CAP pairs that result from stimulation at two different sites. When such pairs are used in the CVD algorithm, they cannot be compared directly due to the scaling factor, $k$, that relates the relative number of fibers activated at the two sites. In order to apply these inter-stimulus site CAPs to the CVD algorithm, one of them must be scaled to reflect that difference. Since $k$ can only be estimated, inter-stimulus site CVD estimates may not be as reliable as intra-stimulus site CVDs, generated from two CAPs stimulated at the same site.

The similarity of long-distance CVD estimates generated from different CAP pairs is examined in Figure 6.6. The three wrist recordings demonstrate the presence of NETF variation, evident from differences in their overall shape and amplitude that do not correspond to simple dispersion. Even though the CAPs from the wrist differ in shape, the long-distance method, which uses wrist CAPs in combination with elbow CAPs, generates three CVD estimates that are very similar. Each of the three CVD estimates have their maximal contribution at the same velocity. They each fall off rapidly at faster velocities and more gradually for slower velocities. Furthermore, all three estimates are zero at very low and very high velocities and vary smoothly over the range of interest as predicted by anatomy. The consistency of these estimates tends to validate the use of averaging multiple long-distance CVDs in order to produce a standard estimate of CVD.

The long-distance results shown in figure 6.6 can be directly contrasted with the short-distance CVDs from the same set of data, shown in Figure 6.7. The short-distance CVDs calculated this way have none of the features used to qualitatively validate the accuracy of the long-distance estimates in the previous figure. The short-distance CVDs do not resemble each other in shape or in the range of velocities that they span; they do not approach zero at the highest and lowest velocities as would be expected; and they do not vary smoothly but rather have many velocity bins with substantially
different contribution from both of its neighbors. The inaccuracy of these estimates can be argued on three, independent points. The three short-distance CVD estimates 1) do not reflect healthy nerve physiology, 2) do not resemble each other as three independent and accurate estimates must, and 3) bear no resemblance to long-distance CVDs estimated from the same nerve. Here we see, as in the simulations, that short distances of propagation coupled with CAP pairs that have noticeable differences in shape results in very poor estimates of the CVD. In Figure 6.8, even with the inclusion of distally stimulated CAPs, the features that indicate a reasonable CVD continue to be contained in the long-distance estimates and absent from the short-distance data.

**Comparison of Long Distance CVD to Short Distance CVD**

When long-distance and short-distance CAPs are both recorded from the same nerve, a classically calculated (long-distance) CVD can be generated for direct comparison to the short segment estimate. A long-distance CVD is shown in Figure 6.9 as the solid black histogram. Unlike the short-distance CVDs in Figure 6.8, this long-distance CVD does demonstrate the properties that have come to be associated with accurately estimated CVDs based on prior electrophysiological and histological research.[34, 36, 82] The main contribution to the distribution comes from fibers in the vicinity of 60 m/s and the fastest contributing fibers propagate at approximately 65 m/s. These numbers match the maximal conduction velocity that would be measured from the first positive peaks of this particular set of CAP data. Furthermore, this CVD does not have wildly varying contributions from neighboring velocity bins such as those seen in the short-distance CVDs of Figure 6.8. A short segment CVD calculated from the same dataset as the long-distance estimate is also shown in Figure 6.9 This short-distance CVD again shows a large contribution from slow fibers and bin-to-bin variations that are un-physiological. It is clear from this figure that the short-distance CVD does a poor job of matching the long-distance solution, which is to taken as a standard.

The third CVD plotted in Figure 6.9 is intended to demonstrate the importance of accounting for the scaling factor, k, when applying the CVD estimation algorithm to pairs of CAPs stimulated at different locations. This CVD was generated using the most distal CAP recorded from the wrist following proximal stimulation (also used in the other two CVDs in this figure) and the long-distance CAP recorded from the elbow (same recording electrode as the gold standard CVD) following distal stimulation. As discussed, the difference in stimulation site will lead to a scaling of the total number of fibers activated and a corresponding scaling of the resulting CAPs. This scaling has been ignored for the generation of Figure 6.9. The inter-stimulus site CVD estimated in this way appears to be a hybrid between the long-distance and the short-distance CVDs. It does not have quite the same
magnitude of low velocity contribution as the short-distance CVD but begins to show the higher frequency peak demonstrated by the long-distance CVD.

The effect of incorporating information about the scaling factor, k, is investigated in Figure 6.10. Inter-stimulus site CVDs are calculated from the same pair of CAPs used to generate the previous figure. In this case, however, the CAP that results from activation at the proximal stimulation site is scaled by a factor, k, prior to estimating the CVD. A full range of physiologically possible values of k is used. The factor k was varied between 0 and 1, a CVD is estimated for each of 100 evenly spaced values. Within this range there should be an optimal k that scales the proximally stimulated CAP an amount that allows it to be directly compared to the distally stimulated CAP. When the optimal k is approached, more accurate CVDs should be generated. The accuracy of the CVD resulting from each value of k was determined by calculating a correlation coefficient between each CVD and the classic CVD estimate. The highest correlation occurs at a k value of 0.64. This is clearly the optimal value of k to use for normalizing this particular dataset when comparison to long-distance results is used as a metric. The performance of the algorithm while varying k is a validation of the approach. The optimal value of k occurs not only within the physiologically possible range of zero to one, but in fact in a physiologically realistic range that matches prior experiments.

Figures 6.9 and 6.10 indicate the importance of incorporating the factor k when using inter-stimulus site CAP recordings to estimate CVD. Figure 6.9 shows that the long-distance, inter-stimulation site CVD comes closer to duplicating the gold standard than the short distance solution does but that the scaling factor between waveforms due to different stimulation sites should not be ignored. Figure 6.10 demonstrates that choosing the correct value of k and incorporating it into the CVD algorithm can improve the inter-stimulation site CVD. While the inter-stimulation site CVD is improved for the optimal value of k (0.64), its coefficient of correlation with the long-distance intra-stimulus site CVD remains below 0.95. This discrepancy may be due to the difficulties inherent in measuring distance previously discussed. The distance between recording electrodes in this situation is relatively easy to measure by stretching a measuring tape along the straight line between the wrist electrode and the elbow electrode. Measuring the separation between stimulating electrodes on the finger, however, and the distance traversed over the hand is considerably more difficult. Since the digit and the hand have so many joints and points of flexion, the nerve must be able to stretch and compress with voluntary motions and changes in position. This makes determination of the path of the nerve under the skin extremely difficult. This is particularly true of the finger where measurements of distance at various angles of extension may differ substantially. This difference is compounded by some uncertainty in the exact point of nerve activation under the electrode, which may itself be a function of joint
position. Ultimately, when calculating a CVD from CAPs stimulated at the same electrode, the error in stimulation site position has little effect because the error is small compared to the distance of propagation and what's critical is that both CAPs are stimulated at exactly the same place, which is inherently true. When different stimulation electrodes are used to produce inter-stimulus site CAP pairs the assumption that the relative location of activation for the CAPs is known breaks down. For this reason, intra-stimulus site CVDs alone should be used when determining the long-distance CVD. The same is not necessarily true of short distance CVDs following normalization. Since normalization by Fourier methods includes data about signal phase, CAP waveforms can be shifted in time as a result of the process. The shifting that occurs is dependent upon the propagation distances that are measured and passed to the normalization function. Normalization can account for errors in distance of propagation measurements relative to the first (shortest) distance. Short distance normalized CAPs activated at different stimulation sites may therefore be compared and used in CVD calculations.

Besides providing insight into the use of inter-stimulus site CAPs, the data of Figure 6.10 can be used to investigate the normalization procedure and to further validate the use of an overall scaling factor. The value of $k$ that led to the most accurate CVD in the case of inter-stimulus site data can be used in the normalization algorithm. The results of this calculation are shown in Figure 6.11. The failure of the short-distance estimates to reproduce the gold standard is evident. If the optimal value of $k$, 0.64, is used to normalize the short-distance CAPs prior to CVD estimation, the lower panel results. In this case the short-distance CVDs do an excellent job of estimating the long-distance solution. Only at the lowest velocities, in fact, does the average normalized CVD fall outside the range of long-distance CVDs. This is dramatic evidence that normalization is an effective method of improving short-distance CVD estimates. The use of $k$ as a factor relating stimulation sites is also supported. The optimal value of $k$ determined from the maximum correlation coefficient is seen to be a very good choice.

**CVDs Calculated From Normalized CAPs**

**Estimation of $k$**

When running simulations to validate the normalization algorithm, the factor $k$ was a parameter of the forward calculation used to produce the CAP signals. The proper value of $k$ could then be used in the normalization algorithm in order to test the efficacy of the procedure under optimal conditions. While values of $k$ known to be incorrect could also be used to examine subsequent errors produced in the CVD estimate, the issue of estimating $k$ was not crucial to these simulations. In the case of datasets that do not include long-distance CAP recordings, the estimation of $k$ becomes an issue. The ratio of
energies in CAPs recorded by the same electrode but stimulated at the two different stimulation sites was used to estimate $k$ in preliminary work. Performance was measured by comparing the normalized CVD to examples of classically calculated CVDs published in the literature for these early studies. While normalization was seen to lead to dramatic improvement in the form and shape of the CVD, no quantitative measure of accuracy was available. The recording of long-distance CAPs from the elbow and the subsequent estimation of long-distance CVDs, however, provide a means for quantitatively assessing the quality of the CVD. Alternatively, since the normalization algorithm is shown to be effective with simulated data, the existence of a long-distance CVD for each data set provides a means for retrospectively checking the accuracy of the estimate of $k$. This was done indirectly in Figures 6.9 through 6.11 by looking at the improvement of inter-stimulus site CVDs as a function of $k$. In order to better understand and improve the method for future iterations, however, a more direct approach of comparing the normalized CVDs to the long-distance solution as $k$ varies may be helpful.

It is the ultimate goal of this work to be able to apply the short segment CVD algorithm completely prospectively, including the estimation of $k$ and the measurement of distances. The estimation of $k$ using discrete-time signal processing techniques is of particular interest. In order to gauge the accuracy of using a ratio of signal energies to estimate $k$, normalized short distance CVDs were calculated for a range of different values of $k$ and compared to the long-distance CVD in each case. Figure 6.12 demonstrates the dependence of the normalized CVD solution on the value of $k$. Normalization was performed for 100 different values of $k$ ranging between zero and one. There is a distinct minimum in the error function as $k$ passes through a value of 0.56, once again within a range that is physiologically reasonable. The RMS error is reduced in this region by almost a factor of six compared to the worst region ($k < 0.3$) and about 4.5 when compared to unnormalized.

The bottom panel of Figure 6.12 shows three ranges of normalized CVDs. The most striking feature of these plots is the narrow range of values demonstrated by twenty individual CVDs each of these regions of large error. Also interesting is the fact that these two ranges of $k$ values produce two distinct, but similarly poor when compared to the solid black long-distance solution, ranges of CVD. CVDs from the darker shaded region of the top panel ($0.52 < k < 0.6$) are considerably different in shape and form from the two previous regions and, as their RMS error values suggest, come much closer to duplicating the long-distance solution. The best normalized solution for this data set occurs at $k = 0.56$. The RMS error between this and the long-distance CVD is approximately 1.15 (in terms of fiber percentage) and the correlation coefficient between the two is 0.9557.
The $k$ value that results in the best normalized CVD when compared to the long-distance estimate, 0.56 in this case, is called $k$-optimal. Finding $k$-optimal requires that long-distance CAPs are recorded and long-distance CVDs are calculated. It is distinguished from, and independent of, its counterpart $k$-estimate, which is intended to be a prospective estimate of $k$-optimal. It is interesting, therefore, to examine the relationship between these two parameters generated from the same dataset and to compare CVD estimates calculated using each. The dataset of Figure 6.12 results in a $k$-estimate value of 0.48, below $k$-optimal but still within the range of physiologically and anatomically reasonable values. The CVDs produced by normalizing the dataset with both $k$-estimate and $k$-optimal are shown in Figure 6.13 along with a long-distance CVD. The CVD corresponding to $k$-optimal is, by definition, a closer match with the classic solution. The CVD generated from $k$-estimate demonstrates the hybrid features seen previously, taking on some aspects of both the unnormalized short segment CVD and the long-distance estimates. The peak of this CVD is in the correct region and the overall shape of the distribution is close to that of the long-distance solution. As in very poor estimates, however, a substantial fiber contribution is present at low velocities. This low velocity contribution is enough to raise the RMS between the normalized and classic CVDs to approximately 3.7 and lower the correlation coefficient to 0.5724 (compare with 1.15 and 0.9557 for $k$-optimal).

In order to further quantify the relationship between $k$-optimal and $k$-estimate, they have both been calculated for a series of 22 individual datasets in which long-distance CAPs were recorded. The results of these calculations are shown in Figure 6.14. The most intriguing aspect of this graph is the fact that the solid lines have a consistently positive, and very similar slope. The value of $k$-estimate always puts at least a lower bound on $k$-optimal for this dataset. This is useful information because of the fact that $k$-estimate is calculated without the need for a long-distance CAP. The majority of $k$-ratio values fall between 1.1 and 1.3 indicating that $k$-estimate is not only a lower bound of $k$-optimal but tends to underestimate $k$-optimal by a consistent 20%. These data point out that it may be possible to get a better estimate of $k$-optimal without the requirement for long-distance CAPs by applying a scaling factor to $k$-estimate. These datasets were compiled from a wide range of recording dates and often had slightly different recording methods. It is hoped that more consistent CAP recording methodologies will lead to a reduced variance in the $k$-ratio and lend additional credibility to its use as a scaling factor to extract an improved estimate of $k$-optimal from CAP data.

**Shifting**

During this phase of the research a large number of datasets were recorded and examined. Each set of CAP data was subjected to a procedure in which multiple CVDs were estimated. Long-distance,
intra-stimulus site CVDs were calculated and averaged to yield a standard CVD for each data set. Short-distance CVDs were generated from all short distance pairs within the dataset to give an idea of the baseline effectiveness of the short segment CVD algorithm. Finally, normalization was performed, and multiple normalized short-distance CVDs estimated, for 100 different values of the scaling factor $k$. The average of the normalized short-distance CVDs was compared with the long-distance CVD in order to assess the performance of the normalization and CVD estimation algorithms for each value of $k$. Assessment was based on either a calculation of error (generally RMS) between the two distributions or through examination of the coefficient of correlation between them.

There were several datasets, such as the one used to produce Figures 6.12 and 6.13, that showed a dramatic minimum in RMS error (or maximum in correlation coefficient) at a physiologically and anatomically appropriate value of $k$. The data of Figure 6.12 might be used as a benchmark because of the sharp minima at $k$-optimal, and the low RMS error (approaching one) at that minima. There were several other datasets, however, that did not exhibit such behavior. Figure 6.15 is due to such a dataset. The most striking aspect of this plot is that in the physiologically reasonable region where $k$-optimal is expected, the error actually increases. Further examination reveals that the error between the CVDs is minimized at a $k$ value of one. This corresponds to no scaling of the CAPs activated at different sites; a highly unlikely result in light of the amplitude differences seen between CAPs stimulated at different sites but recorded by the same electrode. Error throughout the range of $k$ is greater than that in the unnormalized case and considerably larger than any RMS error calculated with the benchmark dataset of Figure 6.12.

In order to understand these results, CVDs from this dataset were examined over the range of $k$ values. Some of these CVDs are plotted in Figure 6.15. The CVDs from $k = 0.49$ and $k = 1.0$, where minima of RMS error occurred, closely resemble the poor CVD estimate ranges from Figure 6.12. They are broad distributions with peaks at lower velocities. After analyzing many datasets, and the CVDs produced from those data, it became clear that this type of behavior commonly resulted from an overall shift in the normalized CVD with respect to the long-distance standard. The shape of the CVD normalized with a $k$ value of 0.70 closely resembled that of the gold standard. The location of the peak of the CVD, however, and the low-velocity tail appeared shifted. The RMS error function plotted at the top of Figure 6.15 shows such large errors because, on a bin-by-bin basis, the similar but shifted CVDs will be considerably different. This is particularly true in the region of the peaks of both CVDs. In a similar manner, the correlation coefficient will be unable to reflect similarities in shifted
CVDs because it is a measure of how close the distributions are to being statistically the same. Even distributions of identical shape that are shifted with respect to each other will fail this test.

There are two possible physiological reasons for CVDs estimated from short segment data to be shifted with respect to the long-distance CVD from the same nerve. The shift could be a result of tapering of peripheral nerve fibers as they reach more distal regions of the body or it could be due to changes in temperature along the limb. Both possibilities would indicate that the shift could only be to lower velocities. The tapering of peripheral nerve fibers has been hypothesized and there is some histological evidence to support it.[24] The more plausible cause of the dramatic shift seen in some datasets, however, is gradients of temperature. Temperature is well known to affect the propagation of action potentials. There is some disagreement in the literature, however, on how to account for temperature differences in electrophysiological studies.[2, 17, 30, 50, 51, 63, 65] Temperature gradients are certainly present between the body core and the most distal regions. An indication of these variations can be achieved by measuring the skin surface temperature at a variety of locations with an infrared temperature gauge or some other method. Besides being a function of how far a location is from the body’s core, temperature also varies with proximity to the skin surface. The measurement of surface temperature, therefore, should give a worst-case value for the operating temperature of the fiber at that point. The exact temperature of the fiber will depend on how superficial it is to the skin. As the nerve becomes more distal in the upper extremity it tends to be more superficial and, in regions such as the wrist or the palm, may be very close to the skin surface. Action potentials in these regions can, therefore, be slowed dramatically.

The 2CAP estimation of CVD investigated here does not account for changes in the velocity of single fiber action potentials as they propagate along the nerve. The assumption is made, in fact, that each fiber propagates at the same speed along its entirety. In the current datasets, CVDs calculated from CAPs recorded close to each other on the wrist are compared to CVDs in which one of the CAPs has propagated all the way to the elbow. The nerve, in the region of the elbow and along much of the forearm, is relatively deep within the tissue and close to core body temperature so that action potentials travel at their optimal speed. In the finger, hand, and wrist, however, the temperature of the tissue is often reduced to several degrees below core body resulting in slowed action potential propagation. CVDs calculated using the data from the elbow will therefore be shifted to higher velocities on average while CVDs determined solely by wrist data will be slower.

In order to account for shifts in the overall location of the CVD and still investigate the influence of normalization on short-distance data, it is necessary to develop a measure of error that is insensitive to
such shifts. The method that has been developed is based on the cross-correlation between the two
distributions. The cross-correlation is a useful metric for two reasons. First, the value of the maximum
of the cross-correlation can be used as a measure of the distributions’ similarity. Second, the location
of the maximum of the cross-correlation function indicates the shift (lag) that is required to make the
distributions line up. It is expected, then, that the physiologically reasonable range of \( k \) will produce a
maxima in the maximum cross-correlation data that is coupled with a shift that can be explained using
temperature arguments.

Figure 6.16 demonstrates the application of the cross-correlation method. In this case, a large value of
the function indicates a strong similarity in CVD shape while allowing for an unknown shift of the
distributions with respect to each other. The peak of the function at a \( k \) value of 0.71 shows improved
results in the physiological reasonable range of \( k \) values as contrasted with figure 6.15. This optimal
value of \( k \) also demonstrates dramatic improvement over both the range of \( k \) close to zero and the
range of \( k \) close to one. The bottom panel of this figure shows CVDs appropriate to these results. The
CVD normalized with the optimal value of \( k \) is seen to require a shift of 3 velocity bins (9 m/s) in
order to be aligned with the long-distance solution.

**Conclusions**

Examination of many sets of multi-CAP data and the CVDs calculated from those data have led to
insights into the performance of the CVD estimation algorithm with and without normalization.
Long-distance CAPs have been recorded from the elbow along with short segment array data. The
long-distance data provides a CVD estimate that takes advantage of added dispersion in the signals
and is theoretically a more accurate assessment of the nerve. This long-distance CVD has been
directly compared to the short segment estimate in a large number of datasets. These comparisons
have demonstrated that normalizing short segment CAPs can produce dramatic changes in the
resulting CVDs. These changes are further shown to be dependent upon the value of \( k \) used in the
normalization procedure. Ranges of \( k \) values have been used to normalize the same sets of data in
order to retrospectively determine the \( k \) that leads to optimal performance as measured by the
correlation to the long-distance estimate. Observing CVDs that result from short segment normalized
data for many values of \( k \) has uncovered a second test-specific parameter that must be considered.
The absolute location of the long- and short-distance CVDs on the velocity axis with respect to each
other is often shifted even thought their shapes are very similar. Comparing CVDs without
accommodating this shift can therefore lead to poor performance. Correlations between the short
segment normalized CVD and the long-distance were calculated while allowing the position of the distributions to vary. This analysis quantifies the agreement in shape between the long-distance CVD and the new method. When the CVD shapes are compared, normalization is seen to improve the short segment estimate significantly.
Figure 6.1
Figure 6.2

Stimulating Electrodes

Recording Electrodes
Figure 6.4

Duration of Signal (ms) vs. Distance of Propagation (cm)

- CAP Duration vs. Propagation
- LMSE Linear Fit \( m=0.0475 \),
Figure 6.5
Figure 6.6
Figure 6.7
Figure 6.8
Figure 6.9
Long Distance CVD Estimates
Intra- and Inter-Stimulus Site

Figure 6.10
Figure 6.11
Figure 6.12

RMS Error Between Long-Distance CVD and Normalized CVD

Coefficient $k$

- $0.1 < k < 0.3$
- $0.8 < k < 1.0$

Conduction Velocity (m/s)

- $0 < k < 0.6$

Normalized CVD Range
- $0.1 < k < 0.3$
- $0.8 < k < 1.0$

Long-Distance CVD

Normalized CVD
- $k = 0.56$
- $0.52 < k < 0.6$

Percentage of Fibers
Normalized CVD $k = 0.48$ (k-estimate)

Normalized CVD $k = 0.56$ (k-optimal)

Figure 6.13
Figure 6.15
Maximal Cross-Correlation Between Long-Distance CVD and Normalized CVD

$k = 0.55$

$k = 0.71$

Conduction Velocity (m/s)

Percentage of Fibers

Normalized CVD
$k = 0.55$

Normalized, CVD
$k = 0.71$

Figure 6.16
Chapter 7. Clinical Validation

Introduction

In order to further validate the CVD estimation approach developed in the preceding chapters, a small clinical study was designed. One challenge of developing such a study is to define a quantitative manner to assess the method. Ideally, the true CVD of each nerve being tested would be known and the results of the new method could be compared directly. Unfortunately no such ‘true’ measure of CVD exists. Alternatively, an independent CVD estimate that does not rely on the assumptions of the short-segment method could be used to corroborate, if not confirm, the accuracy of the new technique. Histological estimates of CVD, which ultimately destroy the tissue, have been used previously to substantiate electrophysiological methods but are only feasible in situations where the nerve is to be excised for other reasons or in acute animal studies.[18, 35] To facilitate clinical validation of the new method on healthy human subjects a nondestructive metric is required. The long-distance CVD estimate was selected as a benchmark for this study because of its history as the most widely used and most often referenced method. It is also convenient to collect data to be applied to the long-distance CVD at the same time that short segment data is recorded.

Subjects / Methods

Subjects

Twenty human subjects were recruited for this study. Subject recruitment, the experimental protocol, and subject compensation were all performed under guidelines of the Committee on the Use of Humans as Experimental Subjects of the Massachusetts Institute of Technology (COUHES; protocol No. 2551). Data was acquired from both hands of every subject for a total of 40 datasets. Subjects with a personal history of median nerve entrapment, axonal neuropathy, or nervous system disorders may have conduction velocities that fall well outside of normal ranges and were either not recruited or excluded prior to testing. Subjects with a history of allergic reactions to cosmetics and/or lotions, or with skin disorders on the arms or hands such as psoriasis, exema, etc. were also excluded due to possible adverse reaction to the skin preparation protocol. There were no exclusions based on gender, age, or ethnicity.
The experiment required a single visit to the Massachusetts Institute of Technology General Clinical Research Center (CRC; protocol 445). The total time commitment of each subject ranged from one hour to ninety minutes. The majority of the time required for this experiment was devoted to subject preparation and electrode placement. The actual acquisition of data required only 5 to 10 minutes per hand tested. There were no special preparation or diet requirements prior to a subjects' visit to the CRC. Nor were there any obligations following the single visit. Subjects were compensated $25 for each hand tested. Since every subject consented to bilateral testing, every subject was compensated a total of $50.

**Experimental Methods**

**Subject Preparation**

Subjects were asked to read and sign an informed consent form including the standard language of the COUHES and the CRC of MIT. The testing procedure was briefly described in the form and the subjects given an opportunity to ask any further questions pertaining to the study. All subjects were asked to fill out a brief health history questionnaire that included questions pertaining to general well-being and specifically to median nerve health (see appendix).

Each subject laid comfortably on their back for the remainder of the test with the hand to be studied resting, palm-side-up, beside them. The middle finger, the ventral portion of the wrist, the back of the hand, and the antecubital fossa were thoroughly cleaned with an alcohol and pumice skin preparation pad. Particular attention during this skin preparation step was devoted to the elbow recording site at the antecubital fossa. This region was scrubbed until it remained red. Disposable Ag/AgCl electrodes were applied to the skin for both stimulation and recording. Four stimulating ring electrodes, two cathode anode pairs, were placed around the middle finger. The most proximal ring electrode was placed first, proximal to the first interphalangeal joint. A template was used to determine the proper location for the next electrode, 3 cm distal to the first. These two electrodes were used as the stimulating cathodes for proximal and distal stimulation of the finger. Two more ring electrodes were placed to serve as stimulating anodes. One anode was located between the two cathodes and the other was the most distal of the four electrodes. Six rectangular recording electrodes were positioned on the forearm and two at the antecubital fossa. The first active wrist-recording electrode was placed by eye over the nerve, just proximal to the crease of the wrist. The second active electrode at the wrist was placed using the same 3 cm cardboard spacer that separated the stimulation sites. A pair of electrodes was placed laterally to either side of each active electrode to serve as indifferent inputs. These two straddling electrodes were placed at least one cm from the active electrode on the sides of the wrist.
The exact location of the electrodes was dependant on the size and shape of the subject’s wrist. A third active electrode was placed at the anticubital fossa, medial to the biceps tendon. A single indifferent electrode was located laterally, several centimeters away from that active recording site. A single reference electrode, twice the size of the other recording electrodes, was placed on the back of the subject’s hand. This reference electrode was used for all differential recording channels.

**Data Acquisition**

**Clinical Study**

A new stimulation and recording hardware system was developed and implemented for this clinical study. The stimulator produces constant current pulses in response to an external trigger. The magnitude of the pulses is adjustable, with 2% resolution, up to the maximum of the compliance range. The compliance limit is approximately 20 mA depending on the impedance of the stimulating electrodes. The recording hardware is a four-channel system. Two channels are tied together at their inputs so that three independent channels can be recorded and one of those channels is recorded on two channels. Each recording channel has a two-pole lowpass analog filter with a cutoff of approximately 5 kHz and three single-pole highpass stages with cutoffs of 120 Hz. The highpass stages are located in-between three amplification stages and serve to avoid saturation of the system through amplification of DC signals. One of the three amplification stages is adjustable to four settings resulting in an overall gain of 5.1k, 106k, 127k, or 206k. Both the stimulation and recording hardware are connected to a National Instruments DAQCard 1200 data acquisition PC card. Software running on a laptop computer delivers stimulation triggers, initiates data acquisition, sets the appropriate data acquisition card gain, and graphically displays the data as it is acquired. Virtual instrumentation allows the user to control the length of each time series that is acquired, the number of channels that are recorded, and the total number of stimuli to be presented. The data is written to a text file as it is acquired. When the complete ensemble has been recorded, the ensemble-averaged data is also saved to a file. A testing mode, in which a single stimulus is presented and the resulting waveforms are displayed without being saved, is also provided.

After placing all stimulating and recording electrodes on the skin, connections were made to the hardware. The pair of indifferent electrodes straddling each wrist recording site was electrically coupled together with a short wire with small alligator clips on each end. Each pair of straddling electrodes, as well as the off-nerve electrode at the elbow, was then connected to the indifferent input of each channel. Over-nerve recording electrodes were connected to the active amplifier inputs and stimulating leads were connected to the appropriate ring electrodes on the finger. Single stimulus
pulses were applied to one of the stimulation sites while monitoring the resulting signals from the recording site at the elbow. The active electrode at the anticubital fossa was removed and replaced, several times if needed, until the amplitude of the elbow CAP seemed to be maximized. In addition to readjustment of electrode position, several arms required the skin preparation step to be repeated. Satisfactory CAPs were eventually recorded from both elbow of all subjects. Similar adjustment of the active electrodes at the wrist was only required on one hand of a single subject. All other wrist recording electrodes easily recorded large amplitude CAPs from the initial placement site.

The proximal stimulation site was used first in all cases with the cathodic lead connected to the most proximal electrode. Several test stimuli were presented and the responses displayed in order to ensure that all electrodes were connected properly, that the stimulation and recording equipment was working, and that the skin preparation was adequate. Once reasonable signals were being acquired from the hand to be tested, a series of 128 stimuli at a rate of approximately one Hz was initiated. Each individual response from every recording channel was saved. After acquiring an ensemble of time series responses to proximal stimulation, the stimulating leads were moved to the more distal stimulating site where another ensemble was recorded. After acquiring the CAP data, a skin-surface temperature measurement was made from the wrist using an infrared temperature probe. This entire procedure was repeated on the contra-lateral side.

**Temperature Manipulation**

Three additional tests were carried out in an attempt to better understand the relationship between the optimal shift required to align the normalized CVD with the long-distance solution and the temperature of the subject’s skin. For these tests, the preparation procedure outlined above was implemented and electrodes placed on the subject’s hand and arm. A dataset was acquired in the same manner as before. Without removing the electrodes or disconnecting the leads, the subject’s arm was wrapped with an electric heating pad and the heating pad was turned on. The temperature of the skin on the hand and the index finger was monitored until it approached core body temperature at which time the heating pad was removed and a second dataset was acquired.

**Data Analysis**

An ensemble average of all responses was calculated for each channel and these signals were used as the compound action potential waveforms (CAP’s) for that subject. The long-distance CVD for each subject’s data was estimated from the CAP recorded at the anticubital fossa and each of the forearm recordings following activation at the proximal stimulation site. An unnormalized, short-distance
CVD was estimated from the two forearm CAP’s that occur following both proximal and distal stimulation.

Flowchart 7.1 illustrates the retrospective analysis procedure of the short segment technique. The normalization algorithm, which utilizes all CAP’s recorded from the forearm, was implemented and a normalized, short-distance CVD estimated from the resulting, normalized signals. Normalization was performed and a short-distance normalized CVD generated for a range of values of the parameter $k$. Each short-distance normalized CVD was shifted, as described previously, to maximize the correlation between it and the long-distance solution. The coefficient of correlation between the long-distance CVD and the normalized, shifted, short segment CVD estimate was calculated for each value of $k$. The highest correlation was used as an indication of the best normalized short segment estimate. Each dataset, therefore, has associated with it two test-specific parameters, the optimal value of $k$ and the optimal shift, that lead to the best CVD that can be estimated over a short-distance. Overall performance of the algorithm on a dataset is gauged by the coefficient of correlation that results when the optimal value of $k$ is used for normalization of the signals and the optimal velocity shift is applied to the calculated CVD.

After the study was complete and the data had been analyzed, the test-specific parameters generated by retrospective analysis were examined. The optimal value of $k$ for each dataset was compared with the estimate of $k$ generated a-priori from CAP waveform energy. This comparison led to an improved method of estimating the factor $k$ that could be applied prospectively given only the short segment data. The velocity shift that is required to align the best normalized solution with the long-distance CVD was compared to the skin temperature measured during data acquisition. A lack of any correlation between temperature and the optimal shift led to further investigation and provided insight into a correlation that was unanticipated, but one that affords another improvement to a prospective, short segment CVD algorithm. This improvement, along with the enhanced manner of estimating $k$, was implemented in a short segment CVD algorithm and that algorithm was applied prospectively to all datasets from the clinical study. Flowchart 7.2 shows a flowchart of how the prospective analysis was applied.

The correlation between the short segment solution and the classical, long-distance solution was quantified by calculating the normalized correlation coefficient between the two. These coefficients indicate the probability that two histograms represent samples of the same underlying distribution. Correlation coefficients between the long-distance CVD and the short-distance (unnormalized) CVD
were also calculated. Histograms showing the distribution of correlation coefficients themselves were then generated.

**Results**

Figure 7.1 shows examples of the data acquired for this study and the resulting CVD estimates. The top left-hand panel is a set of CAP data acquired from one of the study’s subjects following stimulation at the proximal stimulation site. The top trace in this figure is the CAP recorded from the anticubital fossa with a measured recording distance of 37.5 cm. The lower two traces were recorded from the wrist. The wrist electrodes were measured to be 13 and 16 cm from the proximal stimulating cathode for this dataset. The top right-hand panel shows the same CAP data after removal of the stimulus artifact and digital filtering. The bottom panel illustrates the CVDs that are generated from the data above. The solid black CVD is a long-distance CVD that was estimated using the CAP recorded from the anticubital fossa and each of the two CAPs from the wrist (short-distance recordings). Two CVDs were generated using the long-distance CAP and each of the short-distance CAPs and those CVDs were averaged to yield the long-distance CVD shown. The CVD represented by a dotted line is the short-distance CVD for these time series data. The two bottom CAPs shown in the top right-hand graph were used to produce this CVD estimate.

The effects of normalization on the short-distance CVD are illustrated in Figure 7.2. The top panel shows the short-distance CAP data from Figure 7.1. The two CAP waveforms recorded from the wrist are plotted following stimulus artifact removal in gray. The CAP plotted in black is the resulting second wrist recording after the dataset has been normalized. The first wrist recording does not change after normalization because all CAPs are normalized to that electrode site. The normalized data in this figure produced the best short-distance normalized CVD solution that could be estimated from the dataset as a function of the test-specific parameter, k. The bottom panel of this figure shows the long-distance CVD and the best, normalized CVD. Similarities between the shape of the long-distance solution (solid line) and the short-distance normalized solution (dotted line) are apparent. The short-distance normalized CVD that is shown results from normalization with k-optimal but there is no shift incorporated so that, while the shapes are very similar, the correlation between these distributions as they are plotted is quite low.

When the optimal shift is also applied, the resulting short-distance normalized CVD can come extremely close to duplicating the long-distance solution as illustrated in Figure 7.3. The top panel in this figure shows the same two CVDs as Figure 7.2 (thin solid line and thin dotted line) but also
shows the best short-distance normalized CVD shifted to line up with the long-distance estimate. The normalized CVD (thick solid line) and the long-distance estimate are highly correlated in this case. The bottom panel is a histogram of the correlation coefficients between the classic CVD and the short-distance normalized CVD after shifting for all datasets in the study.

Distributions of the subject-specific parameters, k and shift, that lead to the optimal short-distance solution are plotted in Figures 7.4 and 7.5. The top panel of Figure 7.4 is a plot showing the values of k-estimate and k-optimal for each dataset in the study. A linear least mean squares regression is also plotted as a solid line. The squared sum of residuals ($r^2$) for this data is 0.34. The bottom panel shows a histogram of the ratio of k-optimal to k-estimate for all datasets in the study. The mean of the distribution is 1.24 and the standard deviation is 0.1287. Figure 7.5 illustrates the distribution of optimal shifts that are determined from retrospective processing of the data. Overlaid on the bar graph is a line plot of a normal distribution that has the same mean (11.46 m/s) and the same standard deviation (4.8 m/s).

Figure 7.7 includes plots of CVDs estimated before and after manipulating the temperature of the skin. The top panel compares the long-distance CVD (dark stair step) to the normalized short segment CVD without adjusting peripheral skin temperature. The lower panel once again shows the long-distance and short segment CVDs calculated from data acquired from the same subject using the same electrode placement but after heating the arm until the skin approached core body temperature.

**Discussion**

This study was designed to compare the new method to a long-distance technique and to yield a better understanding of certain subject-specific parameters that must be estimated in the application of the new method.

**Retrospective Analysis**

The results shown in Figure 7.1 are typical of the datasets taken for this study and indicate the need for the advanced, normalization algorithm. The CAP signals plotted at the top of the figure are very good CAP recordings considering the extremely sensitive electronics, the small amplitude of the signals of interest, and the large magnitude of the stimulus pulses that are used. The stimulus artifacts in the left-hand panel are non-saturating and return quickly to baseline, well ahead of the arrival of the CAP waveform. The CAPs themselves, even the smallest CAP recorded from the elbow, are recorded with a high signal-to-noise ratio so that these ensemble-averaged waveforms (N=128) are very clean.
The stimulus artifact removal algorithm, because of the small size and limited temporal extent of the artifacts, is able to cleanly remove the artifact components without affecting the shape of the CAPs. Even the CAP in this dataset recorded from the elbow has a signal to noise ratio sufficient to allow effective preprocessing of the waveform and application to the 2CAP algorithm. Signals of this quality were consistently recorded from subject’s elbows by vigorously scrubbing the skin with a pumice pad, repositioning the recording electrode to find and then maximize the signal, and by averaging an ensemble containing twice as many CAPs as ensembles from recording sites at the wrist.

Given the high quality of the data, the short-distance CVD calculated from these signals prior to any normalization should also be of high quality. The bottom panel of figure 7.1 shows, however, that this is not the case. The short-distance CVD in no way resembles the long-distance solution. The short-distance CVD demonstrates properties that are commonly seen in CVDs calculated from noisy data or from datasets in which the stimulus artifacts are incompletely removed. The first of these properties is the overall shape of the distribution, which is heavily weighted to lower velocities. The short-distance CVD also has a single bin whose contribution is significantly different from the surrounding bins. This type of discontinuity is also common among CVDs from noisy data but goes against physiological models of nerve conduction. This figure shows quite clearly that the careful acquisition of clean short segment data is not enough to ensure that accurate short-distance CVDs can be calculated. At a minimum, additional signal processing such as a normalization algorithm will be necessary to improve short-distance CVDs.

The effect of the normalization algorithm on the time series data and on the CVD estimates is illustrated in Figure 7.2. The most noticeable problem with the unnormalized time series in the top panel is that the second CAP recording from the wrist is larger in amplitude than the first. This is counterintuitive given the model of a compound signal as a temporally dispersing superposition of single fiber components. It is not surprising, given this behavior, that the short distance CVD calculated from those two CAPs is of questionable quality. The CVD estimation algorithm tries to determine the most probable distribution based on pure dispersion as the only difference between waveforms. The properties that make the unnormalized short-distance CAPs unlikely to return an accurate CVD do not appear to be present on the normalized short-distance CAP that is plotted on the same graph. After normalization, differences between the first and second CAPs are more appropriate to pure dispersion. The amplitude decreases from the first CAP to the second and the durations, from onset to termination and of the main negative peak, are noticeably longer. The changes produced by normalization on the CAP pair also have a dramatic affect on the short-distance CVD. As
demonstrated in the bottom panel of Figure 7.2, the normalized CVD is not only much more similar to the long-distance solution in shape, it also no longer contains the large low velocity component or the discontinuities typical of poor CVD estimates. The short-distance normalized CVD in this case is, in fact, almost identical to the long-distance CVD apart from an overall shift. The best, normalized CVD is determined by allowing the CVD to shift with respect to the long-distance solution and recording the minimum error (where the two CVDs line up). The minimum error is then plotted for all values of the scaling factor k. The value of k that results in the minimum error is saved as k-optimal for that dataset. In this case, a k value of 0.77 produced the best results.

**Estimation of k-optimal**

The values of k that produced the best short segment CVDs for all datasets in this study are plotted as a histogram in Figure 7.4. This figure shows that there is no standard value that can be applied to all datasets since optimal values of k range from 0.56 to 0.97. A scatter plot of the optimal values of k for each dataset versus the value of k estimated for that dataset by the ratio of signal energies method is also shown in Figure 7.4. This scatter plot includes a linear least mean square regression and indicates the squared sum of residuals for these data. The correlation between the optimal and estimated values of k is extremely low. While k-estimate is seen to consistently underestimate k-optimal, there is no linear function that can effectively relate the two. This rules out the possibility of improving k-estimate through multiplication by a scaling factor as was discussed in the previous chapter. The use of relative signal energies may, therefore, not be an appropriate estimator of k for use in prospective short segment CVD.

Another potential method for estimating k is by mandating a specific relationship between the normalized CAP waveforms. The effect of normalizing a CAP dataset with different values of k is to change the relative amplitude, and therefore signal energies, of the normalized waveforms. Small values of k will cause the normalized dataset to progressively get larger in amplitude from the first (most distal) recording site to the last (most proximal) site. Such behavior due to dispersion is an impossibility. There is a lower bound on k, therefore, that is identified by normalized CAPs that are the same amplitude along the electrode array. Since each recording electrode will see a CAP with slightly more dispersion than the previous electrode, normalized CAP amplitude is expected to decrease along the array. This indicates a k slightly larger than the lower bound. It has further been determined empirically that the ratio of energies in the first two optimally normalized CAPs is similar among all datasets in this study. After normalization with the optimal value of k, the ratio of signal energies between the CAPs recorded from the first and second recording sites has a mean value of

-217-
0.7737 and a standard deviation of 0.15. It may be possible, therefore, to use this ratio to recursively estimate \( k \) a-priori.

**Temperature and an Overall Velocity Shift**

Short-distance CVD often duplicates the shape of the long-distance solution while having a substantially different distribution mean. Important diagnostic information may therefore be intact, and encoded in the CVD shape, even though the correlation coefficient between the two is low. For this reason the short-distance CVD has been allowed to shift along the velocity axis when determining how closely the short-distance solution duplicates the long-distance estimate. Normalized CVDs have therefore been assessed by the highest correlation that results as the CVD is shifted in velocity with respect to the long-distance estimate. The shift that is necessary to produce that maximal correlation has also been recorded. As an example, shifting the short-distance CVD of Figure 7.2 three bins (12 m/s) to the right will cause the distributions to line up and the correlation coefficient to increase to over 0.98.

Variations in temperature occur throughout the human body. Temperature not only decreases from the core to the surface of the skin but also from the torso towards the more peripheral parts of the body. The median nerve in the hand and forearm, being both relatively superficial and peripheral, is susceptible to decreases in temperature that may be as large as 10°C. The lower temperature will affect action potential propagation and, therefore, the distribution of conduction velocities that is determined from the CAP data. The effect of temperature on the conduction velocity of individual fibers, and hence on the CVD, may be most noticeable when comparing short-distance results to long-distance because of a large difference in the average temperature of the nerve between stimulating and recording sites. Temperature differences are therefore hypothesized to result in the overall shift of the short-distance CVD. This hypothesis is strengthened by the fact that the optimal shift determined from data in this study is consistently positive, indicating that propagation velocities are slower in the periphery where the temperature is lower.

If variations in temperature along the arm are responsible for the shift of the CVD, then there should be a strong correlation between the optimal shift required and the temperature of the skin at the time of testing. Figure 7.6 shows a scatter plot of the optimal shift calculated for each dataset versus the difference from core body temperature at the wrist recording sites. The temperature variable is calculated as a percentage below core body temperature. A least mean square linear regression is also plotted. This plot demonstrates a very low correlation between shift and temperature and the squared
sum of residuals for the data is 0.0666. This indicates that variations in temperature are not responsible for the difference between the short-distance normalized and long-distance CVDs.

**Temperature Manipulation Study**

To further investigate the effect that temperature might have, a series of experiments were performed in which two datasets were collected from the same hand of a subject while the temperature of the limb was actively adjusted. The first dataset was acquired without any special preparation so that it should be equivalent to any other dataset acquired for this clinical study. After collecting those data the electrodes were left on the skin and connected to the recording hardware while the arm was warmed with an electric heating pad. Warming continued until a temperature sensing perfusion monitor placed on the index finger came within one degree Celsius of core body temperature. At that time a second dataset was acquired. Figure 7.7 compares the long-distance CVD to the short-distance normalized CVD for both datasets, before and after warming of the arm. The top panel shows CVDs generated from the initial dataset without temperature manipulation. The bottom panel is from the second set of CAPs after raising the peripheral temperature. In both panels, long-distance CVDs are plotted as solid black lines and the short-distance normalized CVDs as solid gray. The most interesting aspect of these data is that, not only is a shift still required when the entire arm is warmed close to core body temperature, but the same amount of shifting is required as when the arm is several degrees colder. This indicates quite strongly that changes in temperature do not necessitate the shift, and that there is another factor that is responsible. This factor is something that is not subject-specific, since different shifts have been required for different datasets from the same subject, but is likely to be test-specific, and a function of the experimental setup since the same approximate shift is required for multiple datasets acquired without moving the electrodes.

One factor that fits these criteria is the measurement of distances. Determining the exact path of a peripheral nerve is very difficult. For this reason measurements are commonly made in a straight-line manner, over the surface of the skin, in between the stimulating and recording sites. This type of measurement is particularly error-prone over the hand. It is not uncommon to make repeated measures of distance from the stimulating electrodes to the wrist that differ by as much as two centimeters (approximately 15%) depending on the extension of the wrist. This amount of error in distance measurements will directly affect the CVD that is calculated. Over the entire distance from the finger to the elbow the same absolute error will have a much smaller relative effect so that the long-distance CVD will not be as sensitive. A simulation has been performed to assess the affect of errors in the measurement of propagation distances on the estimated CVD. The results of that
simulation are shown in Figure 7.8. The top panel shows the actual CVD used to generate the data as a solid line and two CVDs estimated from the data as dotted lines. The CVD estimates were calculated with the propagation distances underestimated and overestimated by 18%. The bottom panel shows quite clearly the strong correlation between error in distance measurements and the shift that is required. These data indicate that a positive shift is required whenever the distances are underestimated. This result fits with the experimental data from this study, in which positive shifts were the norm. It is reasonable to expect that distances measured in a straight line over the surface of the skin will represent the minimum length that the nerve actually traverses. These measurements would then, in general, be underestimates of actual distance and lead to the need to incorporate a positive shift in the CVD.

One problem with distance measurement errors leading to shifts in the short-distance CVD is that it is very difficult to account for the error prospectively. Unlike temperature, which can be recorded and corrected for, the straight-line distance over the surface of the skin is the best method of estimating distances noninvasively. One possibility is that this method tends to underestimate the actual propagation distance by a given percentage. The distribution of shifts in Figure 7.5 has a strong mode and median at 12 m/s. Comparing this number with Figure 7.8 indicates that distance measurements made for this study were typically underestimated by more than 15%. One way to examine this possibility would be to reanalyze the data after adding 15% to the short distance measurements. Another technique might be to attempt to back-calculate more accurate distances using information from the long-distance recording. If the assumption is made that the negative peak of the long-distance CAP represents a standard velocity and that components of the compound signals traveling at that velocity also coincide with the negative peak of the short-distance CAPs, then distances to the wrist electrodes can be back calculated. This technique was performed on every dataset collected for the clinical study. The effect of the back calculation of distances on the clinical study data is illustrated in Figure 7.9. The top panel shows the correlation coefficient between the long-distance CVD and the short-distance normalized CVD for all of the clinical study datasets. The histogram in black is the performance of the algorithm using distances as they were measured. The overlaid gray histogram is the performance using back calculated distances. The bottom panel of this figure shows the effect that back calculating distances has on the optimal shift for the clinical trial datasets. The black histogram is the optimal shift required when analysis is performed using measured distances. The gray overlaid histogram represents the shift needed when back calculated distances are used. The important aspect of these results is that the performance of the normalization and CVD estimation algorithm, as measured by the correlation to the long-distance CVD, does not deteriorate when
distances are back calculated but that the shift is reduced dramatically to a distribution centered at zero. These results, in combination with the simulations of Figure 7.8, indicate quite strongly that underestimation of propagation distances between the finger stimulation electrodes and recording electrodes at the wrist can explain the necessity for a shift in the CVD algorithm.

The experiment in which temperature was manipulated in order to investigate the cause of the shift led to a rejection of the hypothesis that temperature effects were responsible. There was an additional and unexpected result, however, that is worthy of mention. Figure 7.10 shows distributions of conduction velocities calculated from one pair of datasets. Distributions plotted in black are calculated from the control dataset in which the skin temperature was not manipulated. The CVDs plotted in gray are generated from a dataset of the same subject following raising of peripheral skin temperature to near core body temperature (37°C). The top panel of this figure is a comparison of the long-distance CVDs calculated before and after temperature manipulation. The lower figure shows the same comparison for the short-distance normalized CVDs. As discussed above, the overall shift of the CVDs to lower velocities is likely due to errors in the short distance measurements. What is more interesting is the effect that the raising of limb temperature has on the shape of the short-distance CVD. The long-distance distributions are very similar in shape regardless of the distal skin temperature. This would indicate that overall nerve propagation itself was minimally affected. This is likely to be the case, in fact, for the majority of the nerve path which lies between the wrist and the elbow. In this region the nerve is less superficial and therefore much closer to core body temperature regardless of the temperature of the room or the surface of the skin. In short-distance recordings, however, the entire tested segment lies in the hand and wrist, regions where the nerve is more superficial and closer to skin temperature. In this region manipulation of temperature might have a more profound affect. The short-distance CVDs of Figure 7.10 seem to verify this and show a substantial change in the shape of the CVD after heating of the arm. It is intriguing that the maximum velocity of contributing fibers does not appear to be altered. This is in line, however, with research that has shown that conduction velocity in peripheral nerve plateaus near core body temperature. The possibility of a nonlinear relationship between temperature and velocity would explain the uneven shift of low velocity fibers to higher velocity bins in the gray CVD of the bottom panel. The effect shown in the figure has been seen, to some degree, in each of the temperature-manipulated datasets. This may provide a means of examining the behavior of the algorithm under an intervention that models neuropathy and creates actual changes in the CVD over a relatively short amount of time. These results also indicate that the overall shift in CVDs calculated over a short distance may be of
little consequence in the development of a CVD diagnostic and that shape changes, regardless of the mean of the distribution, may be more informative of peripheral nerve health.

**Prospective Analysis**

Insights gained from the preceding analysis of clinical data have been incorporated into an algorithm to perform short segment CVD analysis prospectively, without relying on the long-distance CAP recording or the long-distance CVD estimate to generate the best solution. This prospective algorithm has been applied to the clinical datasets collected for this study. Once again, the long-distance CVD has been used for comparison and to quantify the performance of the algorithm. A flowchart describing the prospective analysis process is shown in Flowchart 7.2.

The test-specific parameters, k and an overall shift in velocity, are of principal concern in a prospective application of short segment CVD. A manner of recursively estimating k from the ratio of energies in the normalized CAPs was implemented. The value of k that results in equal amplitudes between normalized CAPs is first determined as an initial value. The ratio of the energies in CAPs from the first two recording electrodes was calculated. The value of k used in the normalization algorithm was incrementally and recursively increased until the ratio of energies fell below 0.77, a value determined empirically from the retrospective analysis.

The simulations shown in Figure 7.8 have been used to generate a method of correcting propagation distances. First, the shortest distance that was originally measured over the surface of the skin was increased by 20%. Underestimating distances by this amount has been shown to result in a shift in simulated data that corresponds to the average shift required in the retrospective analysis. It was then assumed that the initial positive peaks in the pair of CAPs recorded at the first electrode site and stimulated at different places on the finger correspond to SFAPs of the same conduction velocity. This assumption allows a back calculation of the separation between stimulation sites to be performed. The separation of stimulating sites thus calculated is then used to extrapolate the remaining required distance measures. The method described here requires only the short segment data and makes no assumptions about the maximum velocity represented in the CAPs. The one assumption that is made by this technique, that is based on both retrospective and simulated data analysis, is that the distance that the nerve travels through the hand is consistently underestimated by measuring over the surface of the skin.

Increasing measured propagation distances and back calculating the separation of stimulating sites should reduce the need for a shift in velocity in order to align the short segment CVD with the long-
distance solution. Prospective short segment CVDs calculated this way have therefore been compared directly to long-distance CVDs without allowing a velocity shift. The results of this comparison are illustrated as a histogram in the top panel of Figure 7.11. The dark histogram is the correlation between the short-distance, unnormalized CVDs and the long-distance solution. The overlaid histogram is the correlation between the prospectively normalized short-distance CVD and the long-distance CVD. This panel shows that the prospective normalization algorithm substantially improves the performance of short segment CVD estimation. Typical examples of CVD estimates with and without normalization and their corresponding long-distance CVD are shown in Figure 7.12. The long-distance CVD is plotted in solid gray in each panel. The unnormalized short segment CVD is plotted as a dotted line and the prospective normalized solution is plotted as a solid black line. The top panel represents very good performance of the algorithm. The coefficient of correlation between the long-distance CVD and the prospective solution is 0.94. The lower two panels are from datasets with poorer prospective results illustrating the range of performance of the algorithm. The lower left panel was plotted from a dataset that resulted in a correlation of 0.43 and the right hand panel dataset produced a correlation of 0.08.

The prospective short segment CVDs shown in Figure 7.12 demonstrate that, as in the retrospective analysis, the shape of the prospective CVDs in the lower panels have a similar shape to their long-distance counterparts although their correlation is unremarkable. The same comparison has therefore been carried out while allowing the short segment CVD to shift in velocity with respect to the long-distance solution until the two are aligned. This approach measures a correlation coefficient that reflects the similarity of the shape of the distributions independent of absolute position. The lower panel of Figure 7.11 compares the correlation of short segment CVDs to long-distance CVDs when shifts in velocity are allowed. The dark histogram results from the unnormalized short segment CVD and the overlaid histogram from the prospectively normalized short segment solution. Allowing the distributions to shift with respect to each other improves the correlations in both cases. The use of the prospective normalization algorithm, however, results in short segment CVDs whose shape is highly correlated with the shape of the long-distance CVD. A velocity shift remains the main cause for poor performance (low correlation with long-distance CVD) of the normalization algorithm. The CVDs of Figure 7.12 illustrate this. The prospective CVD of the top panel does not require a velocity shift in order to be aligned with the long-distance solution. The correlation coefficient between the two therefore remains 0.94. The lower left panel CVD does benefit from a simple velocity shift and the correlation coefficient is increased to 0.96 and the lower right dataset improves to a very respectable 0.88.
The analysis illustrated in Figure 7.12 for three sets of clinical data is extended to all datasets from this study in Figures 7.13 through 7.17. Each of these figures shows multiple panels of similar data. Results from a single subject are plotted side-by-side. Long distance CVDs are plotted as a solid black line and unnormalized short segment CVDs as a dotted black line in each panel. The short distance CVD calculated from prospectively normalized data is overlaid in each case as a solid gray line. Two correlation coefficients are also reported for each set of data. The short distance normalized correlation coefficient (SDNCC) represents the correlation between the long distance CVD and the completely prospective short distance normalized CVD. The short distance normalized and shifted correlation coefficient (SDNSCC) is the best possible correlation between the prospective short distance CVD and the long distance CVD if they are allowed to shift with respect to each other along the velocity axis. The SDNCC measures the overall accuracy of the prospective method while the SDNSCC is an indication of how similar the shapes of the short distance and long distance CVDs are. Both of these values are reported for every set of data in the study and are shown in each panel of Figures 7.13 through 7.17.

The CVDs shown in Figures 7.13 through 7.17 are considered to be normal long and short distance distributions from healthy individuals. Statistical properties have been calculated in an attempt to make these data more usable to future researchers. Figures 7.18 through 7.23 show the arithmetic mean, the median, the mode, the standard deviation, the coefficient of skewness, and the coefficient of kurtosis respectively for both the long-distance and the short distance CVD following prospective normalization.

**Conclusions**

The clinical study outlined in this chapter was undertaken in order to continue to test methods of short-segment CVD estimation in a more formal and more controlled environment on several healthy subjects. Short segment CVD estimates have been generated whose shape is strongly correlated with the shape of the long-distance solution. This study has also helped to better understand the need for an overall shift in the short-distance CVD that is required to align that distribution with the long-distance estimate. The shift, which is consistently from lower velocities to higher, is likely due to underestimation of the distances of propagation through the hand and wrist. Experiments to alter the temperature of the limb have indicated, however, that short-distance CVD may be able to discern physiological differences in CVDs to be used in peripheral nerve diagnostics. Furthermore, factors related to the shape of the CVD might be more important than its absolute position. This type of
application may be appropriate and effective, therefore, regardless of an overall shift with respect to
the long-distance solution. A prospective analysis of the clinical data using techniques generated
through examination of retrospective results has revealed the ability of signal normalization to
dramatically improve the estimation of CVD over a short segment of nerve.
Figure 7.1
Figure 7.3
Figure 7.4

Top graph: Scatter plot showing the relationship between k-estimate and k-optimal values.

Bottom graph: Histogram showing the number of occurrences for different k-ratio values (N=34).
Figure 7.5
Figure 7.6

LMS Linear Regression
\( r^2 = 0.0666 \)
Comparison of Long-Distance and Short Segment CVD
Prior to Temperature Manipulation

Comparison of Long-Distance and Short Segment CVD
After Raising Peripheral Skin Temperature

Figure 7.7
Distances Overestimated

Distances Underestimated

Percentage Error in Distance Measurements

Figure 7.8
Figure 7.9

Measured Distances
Back-Calculated

Correlation Coefficient

Optimal Shift

Number of Occurrences

N = 38
Comparison of Long-Distance CVDs
Before and After Adjusting Temp

Comparison of Short-Distance Normalized CVDs
Before and After Adjusting Temp

Figure 7.10
Figure 7.11
CVD:
Unnormalized:
Short Segment:
CVD,
O------
U---
CX.
---
*U.
---
+KU-
30
40
50
60
70
80
Velocity (m/s)

Figure 7.12
Figure 7.13
Figure 7.14
Figure 7.15
Figure 7.16
Figure 7.17
Long Distance CVDs

Short Distance CVDs

Arithmetic Mean of CVD (m/s)

Number of Occurrences

- Long Distance CVDs
- Short Distance CVDs

Figure 7.18
Figure 7.19
Figure 7.20
Figure 7.21
Figure 7.22
Long Distance CVDs
Short Distance CVDs

Figure 7.23
Chapter 8. Summary / Conclusions

Brief Summary

The goal of this work was to improve the accuracy of previous techniques for CVD estimation to the point where they could be applied to compound signals recorded over a short segment of nerve. The challenge of this project was to isolate the effects of dispersion in multiple CAP signals and eliminate the effects of other factors so that differences between the closely spaced CAPs are dominated by the small amount of differential dispersion between them. In order to accomplish these goals a new method of stimulating and recording multiple CAPs and an algorithm for normalizing the resulting waveforms were developed and incorporated into the conduction velocity distribution estimation procedure.

Meeting the goals of this work required developing a better understanding of the way in which compound action potential signals are generated, how they propagate, and how the tissues between the nerve and the recording electrode can alter their shape. It is also necessary to be aware of the effect of different stimuli on both the evoked action potential and on the signals that are recorded some distance from the stimulation site. Considerable work was done to develop and refine the hardware and software systems used in the stimulation and acquisition of multichannel data. Improvement of the data acquisition system over several iterations contributed substantially to the reduction of both broadband noise and stimulus artifacts in the CAP recordings. Hardware improvements were not enough, however, to completely eliminate these problematic effects. Algorithms were therefore developed to filter the recorded signals and to reduce any remaining stimulus artifacts from the time series in an automated fashion.

The efficacy of the new methods developed for this study was assessed using simulated compound signals. Simulated data was generated based on a double propagating dipole model of the single fiber action potential. Single fiber potentials generated by this model were dependent upon the orientation of the nerve fiber with respect to the recording electrode and on the separation distance of the propagating dipoles. Distributions of the conduction velocities of the single fiber potentials were defined from published data and combined with the simulated single fiber potential to generate compound signals. After ensuring the efficacy through simulations, the accuracy of the techniques with respect to the long-distance CVD was studied using experimental action potential data gathered
from human subjects. Initial experimental studies were performed with variations in recording methodology in an attempt to identify the most appropriate electrode configuration, ensemble size, and stimulus intensity. Various signal processing and CVD estimation algorithms were implemented and refined along with the data acquisition techniques and ultimately a complete experimental procedure was defined. The procedure that was developed served as a protocol for a clinical study of twenty human subjects (forty hands). The clinical study directly compared CVD estimations calculated from short segment data with an estimate based on long-distance data acquired at the same time and due to the same stimuli.

**General Conclusions**

**Estimation of Relative Nerve-to-Electrode Transfer Functions**

It has been confirmed through simulations and experimental studies that relative transfer functions can be calculated from multiple CAP data given the additional estimate of a coefficient relating the number of fibers activated at each stimulation site.

The transfer function between a peripheral nerve fiber and a surface recording electrode has been the subject of theoretical studies for more than half a century. These studies have generally been based on a-priori knowledge of the extracellular action potential from in-vitro recordings. The in-vitro action potential is assumed to exist at the surface of the in-situ nerve fiber and is then extrapolated throughout the surrounding volume conductor. This type of research has been driven by the incorporation of new and more refined information about the volume conductor itself, leading to presumably more realistic results. These theoretical studies have been very useful in furthering the understanding of bioelectric phenomena and in the development of volume conducted action potential simulations such as the one used in this project.

Empirical estimates of these transfer functions present a much larger challenge. This is due to the fact that individual single fiber action potentials have, to this date, never been recorded from surface electrodes. The form of single fiber action potentials recorded from the skin surface can only be surmised and/or estimated from the compound signal. Such estimates are inherently noisy and contain errors associated the experimental method and the assumptions that are made. Even if a method were developed whereby a single fiber response could be entrained and repeatedly recorded, the amplitude of that response will be hundreds of times lower than the noise floor of the most sensitive recording hardware so that tens of thousands of individual action potentials would need to be averaged to yield
a single estimate. The situation is further complicated by the fact that the shape of the single fiber action potential at the surface of the skin will vary dramatically with the configuration of the electrodes, the exact location of the recording site, and the preparation of the skin underneath the electrodes. These variations, along with the complicated and involved techniques that would be required, make the recording of single fiber action potentials as part of a clinical procedure completely infeasible. This infeasibility is due to factors that are close to the physical limits of modern electronics and are not likely to be overcome in the foreseeable future.

The present work has, for these reasons, focused on the estimation of relative functions that relate the transfer function at one electrode to the transfer function at another. It is the unique recording methods developed for this work, along with the assumptions of the forward model of CAP production, that allow these transfer functions to be estimated. In particular, the linearity assumptions of the forward model and some basic anatomic assumptions regarding the nerve being studied reduce the description of an evoked CAP to: 1) the relative number of nerve fibers within the bundle at the site of stimulation, 2) the distance that the action potentials propagate (dispersion), and 3) the nerve to electrode transfer function (NETF) at the recording site. Furthermore, multiple equally-spaced stimulation and recording sites generate pairs of CAP recordings from the same nerve that share the same NETF and other pairs that result from the same amount of dispersion. Comparison of these CAP pairs leads directly to a Fourier description of the relative transfer function between one recording electrode and each of the others.

What is missing from this model is knowledge of the relative number of fibers that are activated at each stimulation site. This number has been described in this work as a proportionality constant between the most proximal and more distal stimulation sites. Determination of this coefficient through both prospective and retrospective methods has been an ongoing theme of this research. The most promising prospective method to date is based on the comparison of total signal energy between two CAPs stimulated at different locations. This technique has been empirically shown to consistently underestimate the actual coefficient. The ratio of the actual coefficient to the estimate for the data of the controlled clinical study is distributed with a mean of 1.23 and a standard deviation of 0.12. One might expect to be able to utilize this ratio to make prospective estimates of the coefficient with the same accuracy.

**Incorporation of Transfer Function Information into the CVD Algorithm**

Normalization of multiple CAP waveforms prior to CVD estimation allows information about the relative nerve-to-electrode transfer function to be incorporated into a CVD estimation procedure.
Incorporation of this information has been shown to improve the accuracy of CVD estimation in data simulations.

The estimation of relative nerve-to-electrode transfer functions is only the first step in improving the accuracy of CVD calculations over a short segment of nerve. That information must somehow be incorporated into the CVD algorithm in order for improvement to be realized. In this project, incorporation of NETF information has been accomplished through a normalization procedure. Normalization applies the relative transfer function between, say, electrodes 1 and 2 to a CAP recording made at electrode 2. The result of this approach is to transform the original CAP, which had the dispersion and NETF appropriate to a signal recorded at electrode 2, to a signal with the same amount of dispersion but with the NETF of electrode 1. The same technique can be used along an array of equally-spaced recording electrodes so that all recordings have different amounts of dispersion but identical transfer functions between the nerve and the recording electrodes.

Quantitative simulations and qualitative analysis of experimental time series data have verified that the normalization of CAP waveforms in this manner is possible and that such normalization can indeed improve CVD estimates. Simulated signals representing CAPs recorded from different locations along a single nerve bundle were generated. These CAPs result from the same CVD, which is translated to a different latency distribution at different recording locations. In addition to the differences in the distribution of latencies, the CAPs are also simulated with different single fiber action potential components. Different SFAPs may represent differences in the depth of the nerve under the skin, the geometry of the nerve with respect to the recording electrode, or characteristics of the electrode-skin interface. All of these effects lead to noticeable differences in the amplitude, phase, and shape of the simulated CAPs. Normalizing the CAPs in the manner developed for this project eliminates the major differences between signals from different electrodes so that all CAPs are similar in shape to the original CAP corresponding to the first electrode. Minor differences that are associated with dispersion between recording sites remain, however. Simulations allow verification that the normalized CAP from the second electrode does indeed represent the SFAP contribution of the first electrode with the latency distribution of the second. Experimental data cannot be tested as stringently but rather is examined to ensure that differences after normalizing are appropriate and realistic effects of dispersion. Finally, CVDs are calculated from the data both prior to and following normalization. In simulated data, normalizing the NETF among electrodes improves the CVD estimate even in the presence of strong noise or stimulus artifact components.
Conduction Velocity Distribution Estimates Following Normalization

Incorporation of relative NETF information by normalizing the CAP waveforms is capable of dramatically improving the distribution of conduction velocities estimated from data acquired over a short section of nerve. The improvement has been verified by comparison with a long-distance CVD estimate from the same nerve in a small clinical study involving forty datasets from twenty different subjects. Besides depending on a test-specific parameter necessary for normalization, the short-distance normalized CVD is generally shifted in velocity with respect to the long-distance solution but of very similar shape. This shift is likely due to errors in the measurement of nerve propagation distances over the surface of the skin.

Simulations have been very important in this work, and in prior studies in this area, because they allow complete quantitative characterization of algorithm performance under a variety of conditions. Ultimately, however, any techniques developed must be applied to clinical data and their performance must be assessed in that, more realistic, paradigm. Assessing the accuracy of CVD estimation in an experimental setting is difficult for several reasons, but primarily because questions inevitably arise concerning the gold standard CVD of the nerve being tested. Any measure of the distribution of conduction velocities within a nerve bundle will be an estimate. Even histological studies, in which the nerve cross-section is studied and fiber diameters meticulously measured, rely on assumptions regarding the relationship between velocity and size and on the effects of tissue preparation. In-situ estimates, in particular noninvasive techniques applied to healthy tissue, are even more uncertain because the nerve cannot be displaced or disturbed in order to somehow ensure the accuracy of the measurement. In order to provide proof that the techniques being pursued in this study are applicable to clinical data, however, some standard must be used.

The standard that has been chosen is an estimate of CVD from the same nerve, using the same estimation algorithm but incorporating long-distance data. This standard was chosen because it represents a classical method of CVD estimation that has been used for more than twenty years. During that time, CVD estimates from long-distance data have been compared to histological data from the same nerve and shown to be comparable. The classic method has also been used to show that CVD can be a very sensitive indicator of the onset of diabetic peripheral neuropathy. For these reasons, and because the long-distance CVD method is the most accepted technique in the field, it has been used in this study to quantify the accuracy of short-distance CVD estimates.

Assessment of the techniques developed to incorporate NETF information into CVD estimates was performed on sets of data in which short distance and long distance CAPs were recorded from the
same nerve. Classic CVD estimates were made from one CAP recorded at the wrist and another at the elbow. These estimates were used as the standard for each dataset. Short distance CVDs were calculated from two CAP waveforms recorded close to one another at the wrist. Relative nerve-to-electrode transfer functions were estimated and the normalization algorithm was applied to the short-distance data for a range of values of the test-specific parameter that relates the number of fibers activated at each of the two stimulation sites. The accuracy of the short-distance CVD was determined through direct comparison with the long-distance solution and the calculation of the coefficient of correlation between them.

Early experimental data analysis indicated that normalization performed with the proper coefficient would consistently produce CVD estimates with a similar shape to the long-distance CVD but which were shifted in velocity. Without surmising the reason for the shift, short-distance CVDs continued to be compared to the long-distance solution but they were allowed to move along the velocity axis until they were optimally aligned with the classic solution before the error between the two was determined. The overall outcome of this type of comparison was a CVD generated from short-distance, normalized data. Also returned from this analysis is the coefficient relating the total number of fibers under the two stimulating electrodes that is used during normalization and the shift that must be included in the short-distance CVD to produce the optimal results. When these two test-specific parameters are determined retrospectively from knowledge of the long-distance solution, the short-distance normalized CVD shows dramatic improvement over the short-distance CVD without normalization and is related to the classic solution with a correlation coefficient greater than 0.95. The coefficient relating fiber numbers at the stimulating sites is a product of the relative NETF estimation algorithm. Its distribution and techniques for its estimation are discussed above. The shift that is required to align the classic and the normalized CVDs, however, was an unexpected development. Although the original approach was to incorporate the shift into the analysis without explaining its cause, the need to understand its origin quickly arose. The primary hypothesis was that lower temperatures in the more peripheral regions of the nerve (where the short-distance data was recorded) could lead to the shift in CVD. A small study performed with multiple temperature measurements and control of temperature with a heating pad indicated, however, that this was not the cause. An alternative hypothesis is that errors in the measurement of distances over the surface of the skin, particularly in the region of the hand, could produce the overall shift in CVD. This possibility was strengthened by showing considerably stronger correlation between the shift and a parameter distilled from distance measurements than between the shift and the measured temperature. An attempt to retrospectively correct for this measurement error produced a dramatic change in the distribution of
the optimal shift, to a mean and mode of zero, without affecting the correlation coefficient between the classic and the normalized CVDs.

**Clinical Short Segment CVD Estimation**

The clinical study performed as part of this research has been instrumental in showing that incorporating NETF information into a short segment CVD calculation can indeed improve the accuracy of the estimate. It has also produced a large database of multiple CAP recordings. This database has been used to develop new methods of prospectively determining the test-specific factor \( k \) and reducing the need for a shift in velocity. These methods can be performed in the absence of long-distance information and have been applied to the short segment recordings from the clinical study database. The results, when compared to the long-distance CVD, show that the normalization of nerve-to-electrode transfer functions along a recording array dramatically improves the correlation of the short segment CVD to the long-distance solution. Furthermore, those results can be further improved if, instead of measuring absolute correlation, the CVDs are allowed to shift with respect to each other and a correlation between their shapes is used as a metric. In either case, normalization leads to superior performance of the estimation of CVDs from a short segment of nerve. Short segment CVD has been identified as a technique that will bring CVD estimation from the research lab into the clinical setting. The improvements to developed and studied in this work clearly show that short segment CVD is not only feasible to perform, and accurate when compared to an established method, it is a completely realizable clinical procedure given technology that is readily available today.

**The Importance of this Work**

The value of the distribution of conduction velocities as a clinical assessment or diagnostic has been established in prior work dating back more than two decades. At that time, much of the work in this area was performed using laboratory and computer equipment that would be considered antiquated by today’s standards. This project was attractive, in fact, because of the length of time since CVD studies had been seriously undertaken and because of the clinical potential of CVD that those studies established. In the intervening period there have been a few sparse attempts to improve upon the original work; to lessen the experimental exactitude or to reduce the computational burden of CVD estimation. All of these attempts have fallen below expectation by either becoming too complicated and involved to be applied easily or sacrificing accuracy because they rely on an overly simplified model of nerve conduction. The techniques originated by Cummins, Dorfman, Leifer, and Barker in
the late 1970’s therefore remain the most referenced, most respected, and most often applied methods of noninvasive CVD estimation in peripheral nerve.

Since the time that the original work was performed the computing power available to electrophysiological researchers and clinicians alike has grown astronomically. Any desktop PC would outperform a machine that, at that time, would have taken up an entire room. Similarly, a device that would fit in the palm of your hand and cost tens of dollars to produce could easily match the computers that CVD researchers used in the 1970’s, which may have cost tens of thousands. A revolution of sorts has also occurred in the area of surface electrodes. While metal electrodes with colloidal electrode paste continue to be taped to the skin of subjects in many research labs, the strong trend is towards disposable, peel-and-stick electrodes. This technology is flexible, scaleable, easy to use, has electrical characteristics as good or better than metal disk electrodes, and is inexpensive to buy from any distributor of electrophysiological measurement equipment. These innovations in surface electrodes have been extended in some cases to electrode arrays mounted on a flexible backing that can maintain a known distance and spacing between electrodes while at the same time supporting flexible circuitry for electrode connections and for the mounting of electronic components, such as preamplifiers, very close to the recording surface.

The combination of the two technologies mentioned above, low cost computing power and flexible circuit electrodes, open up considerable opportunities for advancement in biopotential measurement and electrodiagnostics. Ideally, a clinician will be able to apply a single integrated electrode array to a subject’s skin, connect the array to a handheld computing device, press a measurement button, and thereby generate accurate noninvasive estimates of the peripheral nerve CVD. This vision is a reality today for measurements of peripheral distal motor latency, which can be recorded with a single set of electrodes at the wrist. The classic method of CVD estimation has, until now, not been possible on the type of device platform described, however, due to the requirement for long distances between multiple recording sites. The work reported here combines classic methods of CVD estimation with novel innovations in computation and recording and thereby shows the feasibility of a clinical device for estimating peripheral nerve CVDs.

Besides leading toward this vision, however, the project at hand has also contributed to the field of electrophysiology and neuromuscular diagnostics by hopefully generating and awakening interest in a measurement technique that has been largely forgotten. Measurements such as CVD will not gain acceptance among the clinical community, regardless of their potential, without maintaining some amount of ongoing interest among both researchers and clinicians. The hope is that publication and
presentation of parts of this work will not only be a reminder of the usefulness of CVD analysis but also demonstrate that it is clinically realizable and generate thoughtful discussions regarding its further improvement.

**Future Work**

**Algorithms**

While the techniques presented in this document represent an improvement over previous methods of CVD estimation, that improvement does not come without a price. The price in this case is the existence of the test-specific parameters that are required to generate short-distance CVDs that are substantially equivalent to the long-distance solution. The first of these parameters is the factor $k$ that represents the relative number of fibers activated at the two stimulation sites. A method of estimating this factor from the total energy in multiple CAP recordings has been used in the clinical study described in chapter 7 but retrospective analysis of the data indicated that this estimate required a ‘fudge factor’ in order to be effectively applied. Further analysis of the clinical study database led to improved estimates of $k$ that could be utilized prospectively and that produced very good CVD estimates. The second test-specific parameter required by the current algorithm, is an overall velocity shift incorporated into the short-distance CVD at the end of the analysis. The need for this shift was determined empirically after observing that the short-segment CVD was often a shifted version of the long-distance estimate. Evidence has been presented indicating that the shift is highly correlated to errors in the measurement of distance across the surface of the skin and a technique for back-calculation based on arrival time analysis of the short segment CAPs has shown promise.

Future work in this area should initially concentrate on further improving the estimates of these test-specific parameters or eliminating the need for them altogether. Since $k$ is an integral part of the model used in developing the normalization procedure, eliminating it is likely to be impossible. One should be able, however, to continue to refine the way in which $k$ is estimated. Statistical methods may be useful, for instance, in empirically determining the best estimator of $k$ given certain aspects of the CAP recordings. The test-specific shift may prove to be much less crucial. The shape of the short-segment CVD matches that of the long-distance estimate before the shift is applied. If it is the shape that is altered during pathology, and that provides the best diagnostic discrimination, then the shift itself may not be necessary. Rather, statistical parameters relating to the shape of the CVD may be used to provide diagnostic information. This is particularly true if CVD studies are to be performed longitudinally on the same subject to track changes or improvements in peripheral nerve function.
Nevertheless, the need for applying a shift at the end of the short-segment CVD algorithm may not be required.

**Hardware**

One way to move the development and acceptance of short-segment CVD forward is to implement it on a different hardware platform and to use custom electrode arrays to ease placement issues. The next generation of hardware should be self-contained and more compact. The stimulation and recording circuitry should be housed in the same enclosure. They might also be put on the same printed circuit board although doing this in the past has led to substantially more capacitive coupling, and therefore larger artifacts, on the recording channels. The entire system should be transitioned to a microprocessor-based architecture to eliminate the reliance on an external PC or laptop computer. Additional functionality, such as the measurement of electrode impedance before and after data acquisition to assess the electrode connections, could be included on the new platform. This next generation CVD hardware system should remain completely battery powered in order to maintain portability. It will require substantial work on the power supply circuitry to provide the required voltages and currents for stimulation, recording, and processing hardware from a reasonable number of smaller batteries (the current setup uses five 9-Volt batteries in addition to the batteries for the laptop).

The other major hardware advance that should be pursued is the incorporation of, at least, the recording electrodes into an integrated electrode array that would fix the distances between them and ease electrode placement. The integrated electrode array would be connected to a flexible Mylar substrate that could also support the deposition of conductive tracks and the attachment of integrated circuits just as a rigid circuit board does. It is also possible that the stimulating electrodes could be integrated on the same substrate or onto a platform of their own. This raises some challenges, however, due to the larger size and odd shape of the finger ring electrodes. One motivation for incorporating both stimulating and recording electrodes would be to provide a means of automatically determining the distance between sites. If the electrodes were in fact connected by a flexible circuit board that slid or stretched to accommodate different sized hands, one might imagine an electronic method of determining distances based on the determination of the resistance of an electronic trace between the electrodes that has a known resistivity. This type of distance measurement would, besides being automatic, be less susceptible to user error. Coupled with a more stringent protocol for distance determination that specifies the proper angle of the wrist and finger joints, this advancement
may lead to more robust CVD estimation and counter the measurement errors that are thought to shift the short-distance CVD in velocity.

**Clinical Studies**

When this work was started, it was understood that the implementation of CVDs as a peripheral nerve diagnostic would require more than just technical innovation. Making CVD measurements more simple and straightforward, as has been attempted in this project, is only the first step to their eventual acceptance and widespread use. Considerable work will also be required to convince clinicians that the benefits of performing CVD analysis are worth the extra time, the purchasing of additional equipment, and additional training of their staff. Publishing of this document and of the other papers and abstracts associated with this work will provide the first stage of technical acceptance of these techniques. While the technical aspects of this work are crucial to moving forward with the development of short segment CVD, however, they will be of interest to a select few clinicians. What will influence the neuromuscular medical community in general is the execution of well-designed clinical studies aimed at applying the method to specific pathologies.

The decision was made during this project to pursue a clinical validation of short-segment CVD that concentrated on that CVDs correlation to a standard rather than its behavior under intervention or pathology. This decision was made because a comparison to an established technique was viewed as a necessary step at some point in the method’s development and it made sense to perform this step before moving to diseased or altered nerves. Having established in this work a correlation between short-distance CVD and the long-distance CVD, however, performing similar studies on subjects with disease or with models of disease is a probable next step. At one point data was acquired during induced hypoxia of the nerve in an attempt to model a disease state. It was found that the physiological changes associated with restricted blood supply to the arm were on a time course much faster than the time required to collect a complete data set. CAPs recorded at the beginning of a test were different from CAPs recorded near the end. The assumption of an ergodic ensemble were therefore violated and the data was unusable. The temperature experiments described in the previous chapter may offer a manner of intervention that would overcome this shortcoming. Temperatures are easier to maintain over a longer period of time, long enough for a full set of data to be acquired. Those experiments also demonstrate changes in the short-segment CVD due to alterations in temperature that match expectations. Further investigation of this effect should involve the production of CVD alterations during warming of the limb and a subsequent return to the original shape after the arm
cools again. It may also be interesting to observe gradations of the effect as the temperature is increased gradually.

Besides performing studies of peripheral nerve intervention such as manipulating temperature, it will be necessary to extend the clinical studies to subjects, both symptomatic and asymptomatic, with a history of peripheral neuropathy or diseases known to affect the peripheral nerves. At this stage the recording of long-distance data might be eliminated. Full control groups would be included in the protocol so that comparisons could be made between the short-distance CVD of healthy controls and of subjects with nerve detriment. Challenges of these studies will not only be to calculate CVDs from datasets of diseased individuals where the signal to noise ratio is reduced, but also to determine the best discriminators between the entire set of normal short-segment CVDs and the sets of altered short-segment CVDs corresponding to each particular disease state. Higher-level statistics or multivariate analysis may prove useful in discriminating between healthy and altered CVDs and this type of analysis would be yet another step towards a short-segment CVD diagnostic.
References


