STIMULUS ARTIFACT SUBTRACTION FOR CONCURRENT NEURAL RECORDING AND POLARIZING LOW-FREQUENCY ELECTRIC FIELD STIMULATION

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by
Nikolai Chernyy

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The dissertation of Nikolai Chernyy was reviewed and approved* by the following:

Bruce Gluckman  
Professor of Engineering Science and Mechanics, Neurosurgery  
Dissertation Advisor, Chair of Committee

Kevin Alloway  
Professor of Neural and Behavioral Sciences

Corina Drapaca  
Professor of Engineering Science and Mechanics

Steven Schiff  
Professor of Neurosurgery, Engineering Science and Mechanics, and Physics

Michael Lanagan  
Professor of Engineering Science and Mechanics

Mst Kamrunnahar  
Researcher at the Center for Neural Engineering  
Special Member

Judith Todd  
Head of the department of Engineering Science and Mechanics

*Signatures are on file in the Graduate School.
Abstract

Epilepsy is a neurological disorder which affects nearly one percent of the world’s population and is characterized by the spontaneous occurrence of debilitating seizures. One third of epilepsy patients do not respond to pharmaceutical treatments which leaves surgical removal of the seizure generation focus as the primary treatment option. This is still not viable for some individuals as it is impossible to localize the seizure focus. Furthermore, the removal of neural tissue is an irreversible process and the surgery is a high-risk procedure.

Some form of epileptic seizure control with applied electrical stimulation have been demonstrated over the past decades. Specifically, proportional feedback control of polarizing low-frequency electric field (PLEF) stimulation has been shown to effectively suppress seizures in slice preparations. Research carried out in intact rodents and humans indicates that feedback control may be effective for electric control of seizures. This treatment option would be advantageous to patients whose epilepsy is drug-resistant, as the implantable electrodes are relatively small, can be removed, and the stimulation protocol can be tuned to maximize efficacy. One impediment to implementing feedback control is the existence of the stimulus artifact: a spurious, additive electric potential at the recording sites caused by the stimulus current.

In this thesis, we propose a prototype set of electronics for concurrent neural recording and stimulation. These consist of a head-mounted preamplifier, a PC-controlled amplifier bank, and an optically-isolated stimulator. The performance of these electronics permit the recording of the underlying neural activity and the complete stimulus artifact. Furthermore, we present the necessary signal processing techniques for the prediction of stimulus artifacts caused by an arbitrary stimulus waveform. Finally, we show verification of the artifact subtraction system and some associated measurements of in vivo tissue properties.
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Dedication

To June, Victor, Nataliya and Dima.
Direct electrical stimulation of the central nervous system has demonstrated substantial promise for treatment of neural pathologies over the past century. Prominent successes such as deep brain stimulation for Parkinson’s disease and cochlear stimulation for hearing loss have promoted neural stimulation into the realm of accepted and accessible medical procedures. Furthermore, the U.S. Federal Food and Drug Administration has approved the clinical use of neural stimulation devices for the treatment of several disorders as well as off-label exploratory research. The opportunity now exists for the application of electrical neural stimulation technologies to a broader range of diseases as well as improvement in efficacy for existing applications.

Application of feedback control has been proved to improve performance using various metrics across a broad range of technical fields when compared to open-loop (non-feedback) control. When we consider some system being interfaced by a controller, feedback permits updating of the controller output based on measurements of the output of the system which it is controlling. An open-loop controller may update its output based on an internal program, but cannot take into account changes in the system. This ability to incorporate system measurements proves very advantageous as many systems, from electric generators to brains, tend to change with time. In particular, we are very interested in the treatment of epilepsy through direct electrical neural stimulation, therefore, application of feedback control principles to neural systems is a practical approach. We have demonstrated our ability to modulate \textit{in vivo} seizures with polarizing
low-frequency electric fields (PLEF, figure 1.1) using an open-loop paradigm, and we are actively seeking to implement feedback control. [2] An impediment to the implementation of feedback control here is the necessity to measure brain state during ongoing electrical stimulation. Such measurements are often confounded by artifacts caused by stimulation which are not of neuronal origin. Therefore, a framework must be developed to separate neural measurements from stimulation artifact, and permit concurrent neural recording and stimulation, for the successful development of a feedback control system for the treatment of pathologies such as epilepsy.

![Figure 1.1](image)

**Figure 1.1.** Demonstration of seizure modification by polarizing low-frequency electric field (PLEF) stimulation. The top trace shows the complete seizure as recorded differentially, *in vivo*, in a rodent hippocampus while performing sinusoidal stimulation. The bottom plot shows an expanded section overlaid with the stimulus current waveform where the maximum phase locking between ictal spikes and the stimulation current occurs. [2] This phase locking can be seen, at around 16 seconds, as the high probability of ictal spike generation (marked by red circles) during a fixed phase of the stimulation current.

My work with Dr. Bruce Gluckman has been focused on using polarizing low-frequency electric field stimulation to control epileptic seizures in a rodent model of epilepsy. The efficacy of this modulation scheme, employing proportional feedback control, was demonstrated by Gluckman *et. al.* by suppressing seizures in slice preparations. [3] A similar paradigm was implemented and verified in an anesthetized animal preparation by Richardson *et. al.* [4] The current goal
is to implement similar feedback control in intact, behaving animals who have spontaneous epileptic seizures.

I started working in Dr. Gluckman’s research group as an undergraduate research assistant and have continued while working on my doctorate of philosophy. In this time, I have designed several prototypes of neural recording hardware involving animal-mounted pre-amplifiers and an amplifier/filter bank which can be controlled by a PC-based acquisition system. I developed an isolated neural stimulator, both for \textit{in vivo} and \textit{in vitro} applications. These combined neural recording and stimulation systems, which are capable of concurrent operation, are now used for all \textit{in vivo} research in our center. I also developed all of the necessary hardware and software for electrochemical coating and characterization of the electrodes used in all of the \textit{in vivo} experiments in our research center. Finally, I have developed all of the necessary software for stimulus artifact subtraction, qualitative tissue impedance measurements, and for the analysis of multi-unit spike entrainment during on-going stimulation.

This thesis is divided into five chapters that 2.) give a basic overview to relevant neuroscience, 3.) cover instrumentation design, 4.) outline signal processing, 5.) present findings on neural modulation, and 6.) define future research. The neuroscience chapter will cover basic neural function and effects of low-frequency electric field on the excitation/inhibition of neurons. The instrumentation section will define problems associated with electrical measurement of neural activity, electric field stimulation, and the resulting stimulus artifact. The signal processing chapter will cover the novel methods used to predict the stimulus artifact generated by an arbitrary waveform and remove it from recordings to reveal the underlying neural activity during electrical stimulation. The tissue properties chapter will present findings on the nature of the stimulus artifact transfer function and the \textit{in vivo} neural response to polarizing low-frequency electric field stimulation, covering both local field potentials and spike/multi-unit activity. Finally, the future work chapter will focus on the remaining problems associated with implementation of on-line artifact subtraction in an integrated and marketable product.
Chapter 2

Basic Neuroscience – Polarizing Low-frequency Electric Field Stimulation

The study of neuroscience has been greatly advanced over the past century, leading to an enhanced understanding of neuronal function along with insight into various neural pathologies. Additionally, advances in modeling and novel application of electronic devices have allowed the development of neural interface modalities, which can be applied for treatment of neuropathology or for neural rehabilitation. While the design of effective interfaces is a crucial aspect of this thesis, it is important to first understand basic neural function and the neuronal response to electric field stimulation. Basic neuronal models will be described here, followed by a description of network models and polarizing low-frequency electric field (PLEF) stimulation.

2.1 The Neuron

Interest in the human brain can be documented for millennia, but, quantitative approaches to neuroscience did not begin until the latter half of the 19th century. Santiago Ramón y Cajal, who is one of the first pioneers of this field, published many works on the microscopic structure of the human nervous system. His work
revealed that the brain was made up of cells, called neurons, that have a similar cellular makeup to other organs. His observations also lead to some preliminary understanding of the neural interconnections of the human cortex and retina. While his light microscopy technique was advanced for the time, he lacked the electrical tools necessary to gain an understanding of neural function. [5]

Research carried out by A. Hodgkin, A. Huxley, and B. Katz in the 1930-50s furthered the quantitative understanding of neuronal function by analyzing the membrane properties of squid giant neurons. These macroscopic cells were employed due to their relatively large size facilitating experimentation with available equipment. It was later determined that their properties were very similar to those of mammalian neurons. [6] Their findings indicated that neuronal membranes were permeated by ionic channels whose conductivities varied non-linearly with the electric potential difference between the inside and outside of the cell. These membrane conductivities, when combined with time-varying extracellular ionic concentration changes, permit a stereotypical and regenerative excursion in trans-membrane voltage known as an action potential. (Figure 2.1) Here, the action potentials can be thought of as the quanta of neuronal communication.

![Figure 2.1](image_url)

**Figure 2.1.** An action potential generated by a Hodgkin-Huxley neuron model in response to a current injection in the top trace with the gating parameters shown in the bottom trace.

We can begin to approach the Hodgkin-Huxley neuron model (equations 2.2-2.8), where the membrane ion conductances vary non-linearly with trans-membrane voltage potential, by first considering a simpler cell with static membrane ion
conductances. We will assume the cell is in an aqueous solution with concentrations of potassium, sodium, and chloride similar to those found in natural cerebrospinal fluid. Physiological concentrations for potassium, sodium, and chloride are 150 mM (5 mM), 5-15 mM (145 mM), and 4-30 mM (110 mM) inside (outside) of the cell respectively. If the cell is left in solution for an adequate amount of time, the trans-membrane potential $V_m$ will develop in accordance with the Goldman-Hodgkin-Katz equation (2.1) and will not change.

$$V_m = \frac{RT}{F} \ln \left( \frac{p_K[K]_o + p_{Na}[Na]_o + p_{Cl}[Cl]_i}{p_K[K]_i + p_{Na}[Na]_i + p_{Cl}[Cl]_o} \right)$$  \hspace{1cm} (2.1)$$

The net ionic current through the membrane will be zero, as the cell is now in an equilibrium state. Changing an ionic concentration of the bath solution will result in an initial change in $V_m$, followed by a slower change caused by the redistribution of ionic concentration with the inside of the cell, followed by a new equilibrium $V_m$ value once zero net trans-membrane current is reached. Therefore, careful measurement of trans-membrane potential $V_m$, while ionic concentrations are adjusted and equilibrium potentials are reached, can be used to determine the static conductance values of the ionic channels in this hypothetical situation. While static channel conductances make it easier to determine their values, they do not allow the trans-membrane potential $V_m$ to take on interesting forms as seen in figure 2.1.

The experiments performed by Hodgkin, Huxley, and Katz were similar to the previous example: they investigated the trans-membrane voltage and current properties while adjusting the ionic concentration of the bath solution in the hope of determining the conductance properties of the membrane. It became apparent that the channel conductances varied with the trans-membrane potential $V_m$, furthermore, the conductances varied in a non-linear manner (see bottom trace of figure 2.1). It was possible to empirically fit substantial experimentation data and obtain what is now known as the Hodgkin-Huxley model (equations 2.2-2.8). The model contains several gating variables, $m$, $n$, and $h$, which control the trans-membrane conductances of sodium and potassium. The remaining conductance, $g_L$, is considered to be Ohmic and is the source of a leak current, governed by $E_L$ and $g_L$, encompassing all other trans-membrane currents. It was later determined
that the empirical gating parameters had a direct relationship with the protein arrangement of the ion channel structures, something which could not have been proved in the 1950s due to technical limitations. This model can then be driven to generate a spike, or action potential, when the trans-membrane voltage is changed by a sufficient magnitude in a small enough period of time, by injecting current into the cell or abruptly changing the electrochemistry of the surrounding fluid. Furthermore, this model stipulates that the same current injection can have vastly different effects depending on gating parameters, and $V_m$, at the time of injection, and that there is a finite minimum time between subsequent action potentials from the same cell.

\[
C \frac{dV_m}{dt} = I(t) - g_{Na} m^3 h (V_m - E_{Na}) - g_K n^4 (V_m - E_K) - g_L (V_m - E_L) \tag{2.2}
\]

\[
\frac{dm}{dt} = \alpha_m(V_m)(1 - m) - \beta_m(V_m)m \tag{3.3}
\]

\[
\frac{dn}{dt} = \alpha_n(V_m)(1 - n) - \beta_n(V_m)n \tag{2.4}
\]

\[
\frac{dh}{dt} = \alpha_h(V_m)(1 - h) - \beta_h(V_m)h \tag{2.5}
\]

\[
\alpha_m(u/mV) = \frac{2.5-0.1u}{e^{2.5-0.1u}-1} \quad \beta_m(u/mV) = 4e^{-u/18} \tag{2.6}
\]

\[
\alpha_n(u/mV) = \frac{0.1-0.01u}{e^{0.1-0.01u}-1} \quad \beta_n(u/mV) = 0.125e^{-u/80} \tag{2.7}
\]

\[
\alpha_h(u/mV) = 0.07e^{-u/20} \quad \beta_h(u/mV) = \frac{1}{e^{0.1u+1}} \tag{2.8}
\]

One major downside of this model is the treatment of a single neuron as a single structure with a uniform trans-membrane potential over the whole surface. This may be reasonable for a perfectly spherical cell in isolation, however, real neurons form extremely complex shapes resulting in trans-membrane potential gradients (see figure 2.2). Neurons typically have vast amounts of tube-like projections that can carry pulses of ionic current and propagate action potentials. Here, all of the membrane surfaces which are exposed, i.e. not myelinated, act as though they are made of infinitesimally small patches of Hodgkin-Huxley surfaces. When the conductance of one patch changes, whether resulting in an action potential or a slower ionic shift, the conductivity of adjacent patches also changes in accordance with laws of continuity, leading to ionic flow and the propagation of neuronal signals. It is typical that incoming signals travel along projections known as dendrites
and are integrated at the cell body, the soma, whereas the action potential travels down the axon to excite other neurons. [9] The soma’s function as signal integrator naturally makes it an extremely influential region for control of neural signal generation, and therefore, a reasonable target for neural modulation paradigms.

2.2 Neural Networks

Cognitive function and some neural pathologies are the manifestations of large neural network activity, which may be less sensitive to the functions of individual neurons. Networks are made up of neurons whose connectivity provides inhibition or excitation to other neurons in the network: generally, action potentials of source neurons can cause target neurons to be less (inhibition) or more (excitation) likely to fire action potentials themselves. This interplay between excitation and inhibition can generate profound activity patterns, including various brain rhythms which can be measured by tracking electrical potentials within the brain or on the scalp. [10] It can also be seen that disruptions in the balance of inhibitory and excitatory components of neural networks can have detrimental effects and lead to pathologies such as generation of epileptic seizures. [11] [12]

On a cellular level, a large portion of neuron to neuron communications occurs through synapses. These are locations where axonal projections of the source (pre-synaptic) neuron come in close contact with those of the target (post-synaptic) neuron. The pre-synaptic neuron’s axonal terminals contain vesicles filled with a neurotransmitter, which can be released into synaptic space. This is controlled by changes in electrochemical gradients caused by action potentials, or other waves, traveling down the projection. Once released, the neurotransmitter binds to receptor sites on the post-synaptic neuron causing a change in internal ionic concentration and thereby propagating neuronal communication. Some neurons communicate by gap junctions, which replace the vesicle-synapse-receptor system, and consist of a direct link between neuronal projections and permit ionic flow directly from one neuron to another. This allows for the propagation of action potentials from one cell directly to another cell, and is much more rapid than neurotransmitter-mediated synaptic transmission. [9]

The extracellular environment plays an equally important role in neuronal com-
munication and function. From an energetics standpoint, the maintenance of electrochemical gradients and the firing of action potentials are very intense activities relying heavily on energy and oxygen supplies. Neuronal activity is substantially affected by chemical gradients and by the presence and availability of secondary messenger molecules and ions such as calcium. [13] Finally, it has been shown that neuronal network activity can be modulated through the application of external electric fields. [4]

2.3 Polarizing low-frequency electric fields - PLEF

Qualitatively, applied external electric fields can redistribute ions inside properly oriented neurons causing polarization and an associated deviation in transmembrane potential (figure 2.2). The degree of polarization, per unit applied electric field, is maximized when the cell’s morphology is primarily elongated along a single, or principal, axis (such as a pyramidal neuron) as compared to a cell whose morphology is mostly spherical (such as a basket cell). The polarization effects are further enhanced when the cell’s principal axis is in the same direction as the applied electric field. For example, the radial electric field, near the stimulation electrode, in figure 2.3 will most effectively target the pyramidal cells in the CA3 and potentially CA1. In the case of the pyramidal cell (figure 2.2), it is possible to represent the polarization effects of the apical dendritic tree as one single cable model with a capped end and terminating at the soma. The same generalization can be made about the processes below the soma.

The idealized pyramidal neuron can then be considered a single cable with sealed ends and the soma placed somewhere along the length where the position depends on the projection morphology around the soma. [14] The model for each cable is shown in figure 2.4 where \( n \) is a discrete spatial index parameter. If we consider polarization effects from the dendritic tree only, the impedance of the tissue is continuous, as compared to discrete nodes of Ranvier on an axon, and \( n \) can be replaced with a continuous distance parameter \( x \). We can then write the relationship between the internal and external electric potentials as a differential
Figure 2.2. A two-dimensional projection of a pyramidal neuron in the hippocampus comparing ionic distributions with and without applied electric field along the soma-dendritic axis. The electric field on the right is representative of a single phase of polarizing electrical field stimulation.

equation, set the boundary condition, and find the general solution for the internal potential as a function of the applied external potential. Here, $R$ will be the resistance along the cable, $Y$ is the admittance per unit area of the membrane, a periodic external potential of frequency $f$ and a with cable diameter $a$ and length $L$. The external applied potential is defined by $u$, with respect to a single ground potential. The potential values inside the cable, with respect to the same ground, are defined as $v$, with the axial current inside the cable defined as $I$. SI units are employed in this derivation.

$$u_1(x,t) = \text{Re}\{u(x)e^{2\pi ift}\} \quad (2.9)$$

$$v_1(x,t) = \text{Re}\{v(x)e^{2\pi ift}\} \quad (2.10)$$

$$I_1(x,t) = \text{Re}\{I(x)e^{2\pi ift}\} \quad (2.11)$$
Figure 2.3. A schematic of stimulation electrode implantation and the resulting electric field in a rodent model. The uniform radial electric field is an approximation which is only accurate sufficiently close to the electrode surface. Figure 4.1 contains a more-realistic transverse current density map. The return electrode (not shown) can be implanted several millimeters anterior (parallel trajectory) or a subcutaneous return electrode can be used.

\[ \frac{d^2 v}{dx^2} = RI = R(\pi a Y(v - u)) \quad (2.12) \]

We can rearrange the above equation to contain all of the physiological parameters in a single variable \( q^2 \equiv R\pi a Y \), the squaring is done here to avoid a square root in the solution. The cable is sealed at the ends with no current going in and out, which provides two boundary conditions.

\[ \frac{1}{q^2} \frac{d^2 v}{dx^2} = v - u \quad (2.13) \]

\( I(x, t) = 0 \) at \( x = 0, L \) \quad (2.14)
Figure 2.4. A schematic of a membrane cable. This is an idealization of a single neuronal process or an equivalent of many processes, such as a dendritic tree. \( U(n) \) represent the applied external electric field and \( V(n) \) represent the internal cable potentials with respect to an external ground reference. Here, \( n \) can be considered a spatial parameter.

\[
\frac{dv}{dx} = 0 \quad \text{at} \quad x = 0, \ L \tag{2.15}
\]

We can solve this problem by defining a new variable \( \Phi \) and an arbitrary position \( x' \) which will convert the above into a homogeneous differential equation. We will find solutions for \( 0 < x < x' \) and \( x' < x < L \) separately and enforce that they are continuous. (See appendix A in [15]) The solution for the differential equation 2.16 is a superposition of hyperbolic functions. [16]

\[
\frac{1}{q^2} \frac{d^2 \Phi}{dx^2} = \Phi - \delta(x - x') \tag{2.16}
\]

\[
\frac{d\Phi}{dx} = 0 \quad \text{at} \quad x = 0, \ L \tag{2.17}
\]

Whose solutions are:

\[
\Phi(x, x') = A \cosh(qx) \quad \text{for} \quad 0 < x < x' \tag{2.18}
\]

\[
\Phi(x, x') = B \cosh(q[x - L]) \quad \text{for} \quad x' < x < L \tag{2.19}
\]
With

\[ A = \frac{q}{\sinh(qL)} \cosh(q[x' - L]) \]  
\[ B = \frac{q}{\sinh(qL)} \cosh(qx') \]  

Thus:

\[
v(x) = \frac{q \cosh(q[x - L]) \int_0^x \cosh(qx')u(x')dx'}{\sinh(qL)} + \frac{q \cosh(qx) \int_x^L \cosh(q[x' - L])u(x')dx'}{\sinh(qL)}
\]  

The significance of the above equation is that there is a hyperbolic sinusoidal relationship between the external and internal potentials for a cable model. If morphology of many neurons permits a generation of an equivalent single cable model, where the soma is somewhere along the cable, the above equation defines a general relationship between the trans-membrane shift at the soma caused by the applied electric field. [14] [15] The soma, or any other change in cable thickness, can be mathematically remapped into a change in the spatial coordinate \( x \) thereby restricting the cable to having a constant diameter. This potential shift has a graded modulatory effect on the neuron’s firing threshold, or its propensity to respond to intrinsic inputs. The efficacy of such a protocol has been demonstrated in the hippocampus where the specific cell orientation results in a nearly uniform radial electric field along the CA3 arc (figure 2.3). [2] [17] [18] The competing stimulation paradigm involves timed stimulus pulses, which elicit action potential generation in response to stimulation, however, the response is not graded. [8] [19]

### 2.4 Conclusion

Neurons operate by changing the ionic conductances of their cellular membranes in response to electrochemical gradients inside and outside of the cell. This behavior results in the generation of action potentials when sufficient input signals are
present, and depending on the current state of the cell. Neurons communicate with one another and form neural networks, which balance inhibition and excitation, and can result in very complex emergent behavior. Disruptions in the balance of inhibition and excitation can result in neural pathologies, including epilepsy. Polarizing low-frequency electric field (PLEF) stimulation can be used to modulate neuronal response to intrinsic neural input, thereby, modulating the excitation and inhibition in neural networks. This chapter has also presented some of the classic mathematical models used in neuroscience to interpret and predict the above statements.
Chapter 3

Instrumentation

The turn of the 20th century heralded many new developments in electronics which facilitated new studies in investigative medicines. The availability of the vacuum tube not only improved existing electrocardiogram systems, but allowed for one of the first quantitative studies of neural activity through electroencephalograms. H. Berger was able to use vacuum-tube based amplifiers capable of resolving sub 100\(\mu\)V signals, and published his findings on brain rhythms in 1929. [20] He conclusively demonstrated that changes in cognitive states could be resolved electrographically on the surface of the scalp, implicating electrical fields in neural function. This discovery paved the way for the use of electronics, and electrical stimulation, in the treatment of various pathologies. The clinical use of such stimulation began in the 1940s, however, with limited success. Decades of perseverance have built up on the original experience and have led to the development of several successful neurostimulation treatments like cochlear implants for the restoration of hearing and deep brain stimulation for the treatment of Parkinson’s disease. [21]

While these are great technologies, they suffer a drawback associated with their inability to provide feedback control internally. Responsive commercial neurostimulators have began to appear on the market, however, they implement a threshold/pre-programmed response paradigm, which differs from true feedback control. [22] One major obstacle is the necessity to track neural response during on-going neural stimulation to continuously update the stimulation program parameters. The brain is electrically conductive, therefore, any electric current density generated by stimulation creates additive electrical potentials at recording
sites which have no relationship to the underlying neural activity. In this chapter, we describe the necessary electronics required for concurrent neural recording and stimulation, which facilitate the signal processing required for stimulus artifact subtraction.

Basic principles of electronic measurement and electrochemistry will be presented first covering issues relevant to referential/differential voltage measurements and the tissue-electrode interface. Our novel neural recording electronics will be described next, focusing on reliability and signal integrity. The stimulation electronics will be described next highlighting efficacy and safety. Finally, the interaction of the stimulus artifact and recording system will be covered.

3.1 Electronic Measurement – Ground Reference

The experimental paradigm covering the research in this thesis attempts to track neuronal activity by monitoring electric fields inside neural tissues. A common, and experimentally practical, method is to track the electric potential at various recording sites in the brain. Attempting to determine an absolute electric potential, or voltage is both futile and meaningless as a voltage is defined as a differential quantity (equation 3.1). Multiple voltages can be measured with respect to a single reference voltage, alternatively, pairs of electrodes can be considered with their voltage difference being the quantity of measurement. Issues related to referencing and the definition of a ground potential play a very important role in electrographic tracking of neural activity necessitating their discussion. [23]

\[
V(a, b) \equiv \Phi(a) - \Phi(b) = \int_a^b \vec{E}(r)dr
\]  

(3.1)

It is the standard practice to define a node in an electric circuit as being at ground potential and having “zero voltage.” This node is fixed as ground and the voltages at all other nodes can be evaluated with respect to this ground. While this works well in simulations, there are practical issues associated with referential voltage measurements in real electronic circuits, especially when low-amplitude signals and high-impedance networks are involved. For example, the
voltage measurement apparatus may have some internal reference voltage, where voltage differences between the internal reference and circuit ground can have an impact on voltage measurements of any circuit position with respect to the defined ground voltage. Furthermore, the voltage measurement device will have a finite input impedance necessitating a finite current draw to register a potential difference. This can lead to loading effects which result in a change in voltage, at a node, when the measurement device is connected. The key fact which facilitates deterministic characterization and debugging of electronic circuits is the ability to define a ground potential in a specific and repeatable manner, e.g. a ground connector on the back of an amplifier bank.

The situation is quite different with electrophysiological recordings, especially in behaving animals: there is no optimal position to be defined as ground potential. The relative potential of any position within the brain changes on the time-scale of seconds depending on the changes in ionic gradients resulting from on-going neural activity. Real instrumentation and operational amplifiers have internal references and finite input-impedance which results in a finite input-bias current. This current typically has a non-linear relationship based on the input potential with respect to the internal reference voltage (figure 3.1). Furthermore, this current can drive recording electrode potentials and have a loading effect on the tissue, which can result in erroneous potential measurements.

Measuring electrical potentials in neural tissues is a non-trivial matter. The contributions from all parts of the system, from electrodes to ground selection to amplifier design, have the potential to confound electrographic recordings of neural activity. Improper experiment design can give false statistical measures, i.e. measurement of signal coherence when a common reference electrode is used. [23] Even differential recordings can be influenced by the average potential of the two recording sites leading to an injection of the common-mode voltage into the acquired signal. While these issues make neural recording more difficult, good experiments can be carried out with a vigilant attention to detail.
Figure 3.1. An input-bias current plot of a popular, high-performance instrumentation amplifier. Note the non-linear changes in amplitude as the input potential shifts from the amplifier’s internal ground reference. This is an example of a non-linear system measurement effect as the load (current drawn from) on the measurement system is non-linearly dependent on the measured electrical potential.

3.2 Electrochemistry

Neurons operate by changing ionic concentrations inside their cellular membranes, resulting in shifts in electrical fields and chemical gradients. The changes in external ionic concentrations are related to the trans-membrane ionic currents by conservation principles, therefore, tracking extracellular electric fields can have an indication of local neuronal activity. [24] Furthermore, imposing an extracellular electric field, through the generation of extracellular ionic currents, can have a modulatory effect on the neural activity in the area. [25] [17] [18] The issues related to neural sensing (electrophysiological recordings) and modulation (electric field stimulation) are therefore partly rooted in the field of electrochemistry. Here, \( E \) represents a potential difference, as is consistent with nomenclature of the field of electrochemistry. [26]

Let us consider a solid metal electrode as being the donor of electrons in the left hand side of equation 3.2 with both oxidized \( O \) and reduced \( R \) species in solution. If the metal electrode is placed into an aqueous solution and left there for a sufficient amount of time to equilibrate, the potential difference between the electrode and solution will be described by equation 3.3. This is known as the
electrode equilibrium potential and assumes that there is no net current across the electrode–electrolyte interface and that the bulk concentrations of oxidized and reduced species, \([O] \text{ and } [R]\), are identical to their surface concentrations. If the redox reaction described in equation 3.2 has sufficiently fast kinetics, and the reactants are relatively plentiful, the time dependent electrode potential takes a similar form to the equilibrium potential. Here, \(E(t)\) tracks the net relationship between the reduced and oxidized species at the electrode surface. The redox reaction, in solution, operates by exchanging electrons with the metal surface. Therefore, the Faradaic current is described by the Butler-Volmer equation (equation 3.5, i.e. figure 3.2). The parameters \(i_0\) and \(\eta\) are the exchange current (controlled by electrode) and the overpotential (deviation from equilibrium potential) respectively. This equation depends on the instantaneous concentrations of oxidized and reduced species at the electrode surface that can be modulated by migration, diffusion, and convection. [26] These concentrations can be different from bulk concentrations leading to errors in measurements of bulk tissue potentials.

\[
\begin{align*}
O^{n+} + n(e^-) & \Leftrightarrow R \\
E & \equiv \phi_{\text{metal}} - \phi_{\text{solution}} = \frac{RT}{nF} \ln \left( \frac{[O]_{\text{bulk}}}{[R]_{\text{bulk}}} \right)
\end{align*}
\] (3.2)

Figure 3.2. A schematic overview of an interface between an ideally polarizeable electrode. The potential is governed by equations 3.3 and 3.4 even through the reaction in equation 3.2 is not occurring at the surface.

\[
\begin{align*}
E & \equiv \phi_{\text{metal}} - \phi_{\text{solution}} = \frac{RT}{nF} \ln \left( \frac{[O]_{\text{bulk}}}{[R]_{\text{bulk}}} \right)
\end{align*}
\] (3.3)
\[ E(t) = \frac{RT}{nF} \ln \left[ \frac{[O]_{\text{surface}}(t)}{[R]_{\text{surface}}(t)} \right] \] (3.4)

\[ i = i_0 \left[ \frac{[R]_{\text{surface}}}{[R]_{\text{bulk}}} e^{\frac{(1-\alpha)Fn}{RT}} - \frac{[O]_{\text{surface}}}{[O]_{\text{bulk}}} e^{-\alpha F n} \right] \] (3.5)

The dynamics discussed in the previous paragraph describe an ideally non-polarizable electrode: one that can only carry out Faradaic reactions. Conversely, an ideally polarizable electrode operates on the same principle as an ideal capacitor: no electrons cross the electrode–electrolyte interface, all potential changes are due to charging and discharging of the interface layer. We may consider drawing a small, but constant, current from the electrode to illustrate the difference. The ideally non-polarizable electrode will continue to carry out a Faradaic reaction to source the current. The electrode potential will reach a constant value, different from the equilibrium potential, providing there are enough redox reactants available to continuously support the reaction. If the reactant kinetics and mass transport are too slow to support the applied current, the electrode potential will increase in magnitude until enough reactants can be drawn to the surface by migration. The ideally polarizable electrode will simply accumulate charge and the magnitude of the potential will grow linearly with time. Eventually, the electrode will reach a break-down potential where it will start to carry out Faradaic reactions. Real electrodes can come close to the ideals of polarizable or non-polarizable operation, however, they frequently operate in both modes. For example, a platinum electrode behaves almost like an ideally polarizable electrode, however, impurities on the surface will carry out Faradaic reactions and therefore add a small non-polarizable component.

The polarizing low-frequency electric field (PLEF) stimulation used in this research relies on being able to inject currents for relatively long periods of time, as compared to pulse stimulation. This requires a large charge-passing capacity, typically associated with a reduced \( Z_{\text{Faradaic}} \) value (figure 3.3). Furthermore, we study epilepsy and are interested in recording neurological signals at very low frequencies (< 1 Hz), which necessitates non-polarizeable recording electrode for improved signal and noise performance. Electrode size is an important consideration leading to the application of electrically-deposited iridium oxide coatings. [27] In our hands,
coating stainless steel recording (50µm diameter, tips exposed) and stimulation electrodes (125µm diameter, 3mm length exposed) with iridium oxide provides a stable in vivo neural interface.

![Tissue-Electrode Diagram](image)

**Figure 3.3.** A first-order approximation of the tissue–electrode interface. This electrode has both Faradaic and non-Faradaic regimes and a built-in equilibrium potential. A double-layer is formed when polar molecules, such as water molecules in aqueous solution, are brought to the electrode surface and form a polarized layer in response to the applied electric field or junction potential. This layer adds capacitance to the electrode equivalent circuit.

### 3.3 Neural Recording

Our primary design goal for the neural recording system is the safe and accurate reproduction of neural activity in a behaving animal. The recording system must be completely passive when analyzed from the perspective of the neural tissue. [28] A minimal buildup of junction potential must be enforced at the recording electrode interface, associated with a minimal current across the interface. This will ensure safety and fidelity through minimization of electrode current density and signal distortion due to loading effects on the tissue. [29] [27] Additionally, time-varying neural potentials are in the sub 100µV range, during normal behavior, which require proper amplification and filtering for reasonable acquisition.

The electrochemistry of neural sensing dictates that there can be a static (electrochemical, DC) junction potential, on the order of a volt, which relates to the surface chemistry of the recording electrode and the ionic concentration of extracellular solution. [26] Changes in ionic concentration at the surface caused by neural activity may be resolved as sub-millivolt shifts. Additionally, there may be a rel-
atively large current density in the tissue due to on-going electrical stimulation. Finally, it is important to capture a wide frequency band, with near DC on the low end. The first circuit in the signal chain must successfully resolve the neural signals, have enough head-room to capture spurious signals caused by stimulation, and possess a wide bandwidth.

Several popular pre-amplifier (headstage) recording configurations are shown in figure 3.4. The simplest configuration buffers each electrode, and the “animal ground” reference, with respect to the amplifier ground. The reference electrode measurement is subtracted from each electrode measurement, the resulting signal is high-passed, and gain is applied. The first problem with this configuration is the unity gain of the input stage, the voltage buffer, which results in poor signal to noise ratio when the signal goes to the filter/gain stage. It may be difficult to separate neural signal from operational amplifier (opamp) noise in analysis of the acquired data. In our hands, most applied electric fields will cause a relatively large stimulus artifact in proportion to the distance between the reference electrode, which is not necessarily at amplifier ground, and recording electrode. The artifact voltage difference depends on line integral through the applied electric field between the two electrodes (equation 3.1). [30] Another issue will arise any time there is noise on the reference electrode, as it will be coupled into every recording channel which is recorded differentially with respect to the common reference, which is the typical configuration. Noise/spurious signals here are guaranteed due to the lack of an optimal ground position and constantly changing ionic concentrations in the animal. This will cause an erroneously inflated coherence estimate between channels. [23]

Configuration B (figure 3.4) is a modification of configuration A, where feedback is added to address the signal to noise (SNR) problems associated with the initial voltage follower. The inverting input of the operational amplifier is at amplifier virtual ground, which is likely different from the animal reference potential, which will result in current being driven to the recording electrode through the input resistor. Thermal noise limits will limit this input resistor to $< 1 \text{M\Omega}$, thereby reducing the amplifier’s input impedance by at least five orders of magnitude. These loading effects can drive appreciable current through the recording electrodes thereby modifying the ionic concentrations at the recording electrode surface, which then
differ from bulk ionic concentration result in signal distortion. The issues related to stimulus artifact and electrochemical DC offset are made worse due to the reduction of input range by the increased gain. The problem with inflated coherence due to the use of a single reference electrode for all recording sites still remains.

Configuration C (figure 3.4) attempts to mitigate the electrochemical DC offset by AC-coupling the electrode to the input stage. This configuration still loads the recording site at frequencies in the AC-coupling pass-band. The unity feedback suffers from the same SNR problems as configuration A, while replacing it with a resistive feedback network with greater gain introduces the same headroom problems as in configuration B. Problems associated with a common reference still persist.

Configuration D (figure 3.4) employs a truly differential pre-amplifier stage. A three-opamp instrumentation amplifier (i.e. INA116 [Texas Instruments Inc.], AD8224 [Analog Devices, Inc.]) design is depicted, where all three opamps and laser-trimmed resistors are integrated in a single package. We designed custom pre-amplifier/headstages to make use of these chips, instead of using only operational amplifiers as many commercial systems do. High input impedance and very low input bias current are maintained, along with DC-coupling and a relatively wide bandwidth. The amplifier headroom is controlled by the power supply range, and is less sensitive to common-mode artifact. Pairing this configuration with a bipolar electrode sufficiently minimizes stimulus artifact magnitude to permit a gain of up to 100V/V in a reasonable setup. Finally, dependence on a common reference electrode is removed by using only truly differential recording channels with bipolar electrodes to allow for accurate coherence/correlation measurements.

The current pre-amplifier/headstage design employs six instrumentation amplifiers for high-fidelity neural recordings (figure 3.5). The reference electrode is used in an active-ground system to ensure that the animal is within the power-supply range of the amplifier. The active ground system is based on the driven right-leg system where one electrode (A) is measured and another electrode (B) is driven to bring the first electrode (A) to the same potential as the amplifier ground. [28] This is done through the use of proportional feedback control and employing a single operational amplifier. A tri-axial accelerometer is added to the design for accurate head orientation tracking and helps with state of vigilance staging and
Figure 3.4. A figure to show various recording configurations. Configuration A is a voltage buffer which reproduces the low-amplitude neural signal with respect to amplifier ground. Configuration B is a voltage feedback amplifier, referenced to amplifier ground with a fixed gain. Configuration C is a voltage buffer, similar to configuration A, with a built-in high-pass (DC-blocking) filter. Configuration D performs fully differential recordings and is the most ideal setup presented here.
seizure detection. [2] Several pass-through channels are added to permit a direct connection between stimulators and the stimulating electrodes.

A custom amplifier bank consisting of twelve digitally-controlled channels (figure 3.6) was designed to acquire data from the headstage and track stimulation parameters. The amplifier features a fully differential input stage with AC/DC coupling, a gain stage, and an anti-aliasing stage prior to digitization. Control of the gain and DC offset is done using digital potentiometers which are interfaced by the acquisition and control software. National Instruments PCI-based acquisition cards were used in conjunction with LabView (National Instruments, Inc.) to acquire the neural data and generate the stimulation program waveform.

The total signal chain was benchmarked for correct frequency response and performance comparison to a Dagan commercial amplifier. The frequency response of the headstage and amplifier combination is shown in figure 3.7. This figure is based on real data, which illustrate an appropriate high-pass cutoff frequency ($\approx 0.12 Hz$) and a very flat pass-band. This headstage and amplifier combination was benchmarked against a Dagan commercial amplifier, as shown in figure 3.8, which resulted in nearly an order of magnitude improvement in noise performance.
Figure 3.5. A schematic of the pre-amplifier. This version includes six differential channels, a tri-axial accelerometer, four stimulation pass-through channels, and an active ground system to bring the animal’s “ground” potential close to the amplifier’s ground potential.
Figure 3.6. A schematic of a one amplifier channel featuring an input stage with selective AC or DC coupling, a gain stage, and a 4-pole Sallen-Key anti-aliasing filter. signalIN is typically connected to a channel coming from the pre-amplifier or stimulator and signalOUT is connected to the PC acquisition system. Both of the potentiometers (POTs) are controlled digitally through software providing digital offset control in DC mode and digital gain control.
Figure 3.7. An impedance spectrum of the combined amplifier and headstage computed using the same analysis as the transfer function estimation procedure. These data are collected with the headstage gain fixed at 20 V/V, amplifier gain set to 50V/V and amplifier input switched to AC mode. The shape of the high-pass filter is shown for relevance to later analysis. The low-pass filter is a fourth-order Sallen-Key topology with -3dB frequency of 1kHz.

Figure 3.8. A comparison of the input referenced (gain compensated) noise level time-series and power spectra of the custom amplifier and heastage configuration relative to the commercial Dagan amplifier alone. The gain compensation is performed by dividing the recorded data by the signal trains gain. The result is the equivalent noise at the amplifier input required to generate the recorded data provided we are operating with an ideal amplifier. The Dagan amplifier performs nearly an order of magnitude worse than the custom amplifier.

3.4 Neural Stimulation

Safe and effective neural stimulation instrumentation is vital to research on neuro-modulation in animal models. As with the recording system, problems can arise at the stimulation site when junction potentials are high enough to cause irreversible electrochemical reactions, or, at a high enough stimulus current density. [29] A
stimulator must then be designed to accurately generate a programmed current while controlling electrochemical junction voltages caused by tissue impedance, polarization, or changes in the animal’s potential with respect to the instrumentation. The stimulator design should be aimed at minimizing power for greater stability and to facilitate stability and eventual higher levels of integration.

It is unreasonable to assume that the average electric potential of the neural tissue will stay constant when compared to the instrumentation ground potential. The concept of mean potential, or an animal ground reference, is poorly constructed: there is not optimal position in the animal whose electric potential does not shift with respect to an external reference. It is then a poor assumption that the stimulator can effectively measure the animal’s ground reference and compensate the stimulator outputs to maintain a programmed current flow and prevent any spurious voltage shifts. One solution is to electrically isolate the output stage of the stimulator from the rest of the equipment, thereby allowing it to float with the animal, or track changes in electrical potential at the stimulation electrode sites. This can be accomplished using an optocoupler, such as a LOC110 (Clare Inc.), which can communicate a voltage or current signal between a pair of circuits without any electrical coupling. The stimulator output stage can be powered by batteries to maximize such isolation. Signals generated on the isolated stimulator and monitored on the non-isolated side can be tied to the input terminals of non-isolated CMOS operational amplifiers. This would slightly reduce the isolation as compared to a purely optical system, however, the coupling between the isolated and non-isolated side will depend primarily on the opamp’s input impedance, and will typically be greater than 50GΩ for low-power CMOS devices.

The LOC110 consists of an infra-red light emitting diode (LED) and a pair of matched photodiodes. One photodiode can be used in a feedback/servo operation allowing for very fine control of current generation in the second photodiode. Combining this with a precision voltage reference like the REF 1004-1.2 (Texas Instruments Inc.) permits a fully optical transmission of a control waveform to the isolated side, which is insensitive to changes in isolated power as the batteries lose stored energy. The bandwidth of this communication channel is approximately 40kHz allowing for a very versatile isolated stimulator design.

The stimulator described here operates in current program mode, that is, the
output current is proportioned to the program voltage by a constant factor. Electrochemical reactions at the electrode-tissue interface are primarily controlled by junction voltage, therefore, the pair of junction voltages must be monitored. This reduces the possibility of irreversible electrochemical reactions as the program current amplitude is increased. The full-cell potential is tracked, as shown in figure 3.9, and compared to a pair of voltages set by potentiometers. The operation of this clipping circuit is shown in figure 3.10, where the current delivered to the load is reduced to restrict the full-cell potential to a maximum of $\pm 1V$. When these thresholds are reached, current can flow through one of the two diodes into the current mixing port of the main output opamp reducing the actual output current to prevent a further increase in full-cell potential.

Finally, a subcircuit is added to prevent long-term electrode polarization. This can occur from a relatively small program offset current which can drive electrochemical reactions in one direction over a long period of time. This will change the electrode chemistry and charge distribution resulting in a non-zero voltage, at zero current, between a pair of electrodes which were initially identical. This can result in a reduced stimulation current capacity as the voltage threshold will be reached with smaller stimulation current due to the polarization potential. One solution is to low-pass filter the full-cell voltage ($-3\text{dB@}0.01\text{Hz}$) and use it to slowly source current from the mixing node at the input to the main output opamp. The result is that DC offsets whose length is on order of 100 seconds are canceled and electrode polarization is mitigated.

The issue of power consumption must be addressed. Minimizing the power consumption allows for the isolated stimulator side to operate for longer periods of time without changing batteries and interrupting experiments. Additionally, the eventual goal for this system is a highly-integrated implantable product, where changing batteries is very difficult. The use of CMOS opamps which can run off low voltage sources (2.7V total supply) like the TLV2374/2 (Texas Instruments Inc.) has proved to be beneficial. We are able to run our stimulators, at about 50% duty cycle with an average of $40\mu A$ stimulus waveform amplitude, for one to two weeks using a pair of standard AA batteries.
Figure 3.9. A schematic of the isolated stimulation subcircuit which was designed. This portion of the stimulator ensures that the output of the current-programmed device is within the maximum allowable voltage limits and that there is no persistent DC offset. The electrode de-polarization subcircuit measures the voltage between stimulating electrodes through a low pass filter, inverts the value, and adds current to the mixing port on the drive opamp. The tunable resistor R10 is used to more closely match the impedance of the stimulating electrodes. The voltage clipping circuit generates a pair of voltage potentials by using buffers U3A and U3B connected to potentiometers R3 and R4. The two opamps U3C and U3D behave like analog comparators and add current to the mixing port (right terminal of R2) on the driving opamp, through diodes D1 and D2, when the set potentials are exceeded. The input signal is provided optically at the optocoupler site and is converted into a program voltage by U4A.
Figure 3.10. Traces showing the voltage clipping circuit in action when the current controlled stimulator is connected to a parallel resistor and capacitor load. The units for the voltage trace are unit volts while the current has been arbitrarily scaled for clarity. The clipping limits have been set to ±1V, and clipping can be verified as the required $V_{stim}$ to drive the programmed $I_{stim}$ reaches the clipping limits.

3.5 Validation

The goal of validation is to determine if the designed electronics operate within safety and performance specifications to ensure that the experiments carried out with those electronics provide valid data. The recording system components, amplifier and headstage, are first tested independently to verify signal gain, noise performance, and frequency response by connecting their inputs to a bench-top signal generator and monitoring the output signals during a parametric sweep. The amplifier and headstage are then connected and the whole system is tested together in the same manner. Next, the head-stage is connected to a virtual rat\footnote{A piece of battery-powered electronics which provide isolated, high output impedance signals of $1mV - 100\mu V$ scales to mimic the source signals available in the animal} and it is verified that the generated signals are adequately resolved by the amplifier. The impedance of the virtual rat will make any significant leakage current apparent. Driving a signal between the virtual rat ground and amplifier ground is done next to verify the headstage/amplifier common mode rejection ratio.

The stimulator is initially tested and calibrated by connecting a known resistance across the isolated outputs and linking it by a large (but finite, > $100k\Omega$) resistance to the amplifier ground. The stimulator is tested for linearity between the program voltage and output, gain, and total harmonic distortion. The program voltage is then increased to verify that output clipping occurs when the predetermined voltage difference at the stimulator output is reached. Finally, the whole system (stimulator, amplifier, and headstage) is tested together by connecting irid-
ium oxide stimulating electrodes to the stimulator output and iridium oxide/screw recording/ground electrodes. The exposed areas of all electrodes are then placed in phosphate buffered saline solution, which is connected to amplifier ground by a silver/silver-chloride electrode, and stimulation output is verified along with the recorded stimulus artifact.

3.6 Concurrent Operation – Stimulus Artifact

Feedback control necessitates that both systems, neural recording and stimulation, work concurrently. Neural tissues are electrically conductive, which results in spurious electric fields generation from the stimulus current density (equation 3.6). These electric fields result in potential differences, or artifacts, that are recorded in the same exact manner as those generated by neural activity (equation 3.7). These artifacts can be orders of magnitude larger than the neural recordings. The ability to distinguish artifact from neural activity is paramount for the successful implementation of in vivo feedback control. It will be demonstrated later that the required artifact subtraction can be done with on-line signal processing, however, the acquisition instrumentation must be appropriately designed with sufficient fidelity and common-mode rejection ratio (CMRR) to capture the complete artifact and neural signal.

\[ \mathbf{E} = \sigma \mathbf{J} \]  

\[ V_{\text{recorded}}(t) = V_{\text{neural}}(t) + V_{\text{artifact}}(t) \]  

3.7 Conclusion

Neural interface instrumentation has been clinically available for nearly a century. Accurate measurement and stimulation systems must be designed for safety and must take into account difficulties related to grounding/reference, electrochemistry, and analog performance. The design of feedback control systems requires concurrent electrographic neural recording and stimulation, which results in some
difficulties. These difficulties can be addressed through appropriate signal processing when the instrumentation has been designed correctly to capture the whole signal, neural and artifact, with high fidelity and low distortion. Specifically, we have used these hardware systems to publish on improved state of vigilance discrimination and on seizure entrainment. [31] [2]

Our hardware allows for the inclusion of bi or tri axial acceleration along with conventional EEG data. These extra data improve linear discrimination between states of vigilance as compared to using EEG data alone. [31] This improvement can then be used in a state machine to potentially improve methods for seizure detection which will be sensitive to the state of the animal.

Our hardware also allows for the concurrent neural recording and stimulation which facilitated investigations of seizure dynamics in the presence of polarizing low-frequency electric field stimulation. In vivo seizures, in animal models, are characterized by large-scale electrographic events which have sharp features known as ictal spikes. We find that these spikes can be entrained, or phase locked, to sinusoidal electric field stimulation in open-loop mode. [2] This is an observation of seizure modulation, which provides insight into feedback controller design for seizure suppression applications.
The long-term goal of the research described here is the development of a feedback-enabled system to suppress epileptic seizures. The neural interface modality employs polarizing low-frequency electric field (PLEF) stimulation to modulate neural excitation in a graded and reversible manner. Unlike pulse stimulation, this paradigm requires low frequency (< 100Hz) electric fields to be maintained in neural tissue, which are proportional to the stimulation current density. The same current density confounds measurement of neural activity by creating an additive stimulus artifact potential, which can be orders of magnitude larger than the underlying activity (figure 4.1).

\[ V_{\text{rec}}(t) = V_{\text{artifact}}(t) + V_{\text{neural}}(t) \]  

Methods to circumvent the problem of stimulus artifact, which have been available for nearly half a century, can be split into either hardware or software categories. The hardware implementations have mainly focused on efficient methods to disable, or blank, the amplifier input stage during the stimulation. These blanking methods were necessitated by the amplifier performance of the time: the input stages would become saturated by the stimulus artifact and it would take some time (10-1000ms) for them to return to useful function. It was more effective to shield the amplifiers during the stimulus artifact and settle for recording neural activity after the artifact passed. The eventual availability of relatively high-performance computers led to novel artifact suppression software
Figure 4.1. A schematic representation of the stimulation current density and the relative position of the recording electrodes in a region of the rodent hippocampus. Blue arrows indicate current flow during one phase of stimulation. The recording system acquires both the potentials related to neural activity and the stimulus artifact and does not readily distinguish between the two.

algorithms. [37] [38] These methods can be generally thought of as template fitting and depend heavily on a priori knowledge of the artifact waveform. For example, removal of artifact caused by very fast stimulation pulses has been done by subtraction of an optimized template and interpolation of the data.

These approaches are not sufficient for stimulus artifact removal in a PLEF paradigm due to the use of arbitrary stimulus waveforms and the necessity of recording neural activity during electrical stimulation. Conversely, the relatively lower frequency content of PLEF, as compared to pulse stimulation, facilitates the design of instrumentation that can accurately capture the whole artifact and underlying neural activity. This allows for the use of novel signal processing techniques to design a filter whose frequency response matches that of the neural tissue and permits for the prediction of the stimulus artifact voltage waveform which results from an arbitrary stimulation current waveform.

This chapter will describe the signal processing techniques involved in the determination of the frequency domain relationship between the applied stimulus current and the artifact voltage over some frequency band. This is done by constructing an optimal interrogation waveform (discussed in section 4.4) and compar-
ing the stimulus current and artifact voltage spectra using multi-taper techniques to derive a transfer function. This transfer function can be computed at discrete times or continuously, where its frequency response can be used to design an infinite impulse response (IIR) filter. The designed filter can be directly applied to an arbitrary stimulus waveform to predict the resulting artifact.

4.1 Spectral Estimation – Fourier Transform

Spectral estimation is a very powerful tool in time-series analysis as it investigates the existence of periodicity in the data. The Fourier transform projects a finite, bounded time-series onto a set of sinusoids, which are orthonormal basis functions. The continuous transform (equation 4.2) can be applied to well-behaved functions to bring them into Fourier space to simplify mathematical operations such as convolutions. The discrete Fourier transform (equation 4.3) is more applicable to analysis of sampled data, and can be used to determine the projection (amplitude and phase) of a signal onto discrete frequency components. Certain care must be taken to ensure the Fourier transform results are accurate, regardless of whether the discrete or continuous transform is employed. Specifically, the issue of data taper/window functions must be addressed.

\[
F(f) = \int_{-\infty}^{\infty} f(x)e^{-j2\pi fx}dx \tag{4.2}
\]

\[
F_k = \sum_{n=0}^{N-1} f_n e^{-jkn/N} \tag{4.3}
\]

The Fourier transform is defined over infinite time; using a finite integral or sum is a simplification when it is known that the time-series value is zero everywhere outside of the range of integration/summation. It is possible that the finite time-series being analyzed contains all of the relevant data and is zero everywhere else, such as a single response from an accelerometer, however, it is more likely that the finite time-series is a piece of a longer time-series. Taking the Fourier transform of a short time-series is identical to taking the Fourier transform of the long time-series that has been multiplied by a square function that is unity over the range
of the short time-series and zero everywhere else. By a fundamental property of
the Fourier transform (equation 4.4), this time-domain multiplication results in a
frequency-domain convolution with the Fourier transform of the square function.

![Sinc function graph](image)

**Figure 4.2.** A trace depicting a sinc function.

\[ F\{h(t) \cdot g(t)\} = H(f) \ast G(f) \] (4.4)

The above equation describes the discrete Fourier estimate \( \hat{F}(f) \) which is the
product of the true spectrum \( F(f) \) convolved with the sinc function. The result-
ing spectral estimate at \( f \) contains the true value, along with contributions
from adjacent frequencies due to the broad nature of the sinc function (figure 4.2).
These contributions are known as spectral leakage and must be minimized for an
accurate spectral estimate. \cite{39} The ideal case would convolve the true spectrum
with a Dirac delta function, which would result in minimum leakage by definition.\(^1\) The equivalent time-domain window of a frequency-domain delta function
is a half-cosine window spanning the time-series analyzed and zero everywhere
else. While the spectral leakage properties have been addressed, the time-series
data near the edges of the window are weakly weighted by the half-cosine windows
function resulting in effectively less data being analyzed and an increased variance.
It is possible to trade-off one type of variance for another, and effectively minimize
overall variance, when some information about the time-series is available \textit{a priori}.

\[ \hat{F}(f) = \frac{\sin(f)}{f} \ast F(f) \] (4.5)

\(^1\)This will not be zero in the discrete case due to finite sampling period and therefore a finite
delta function width and height.
4.2 Parceval’s Theorem, Concentration, and Slepian Tapers

Given a function \( H(f) \) with unit period in \( f \), Parseval’s theorem equates the function’s power in the time and frequency domains. Integration and an infinite sum may render this theorem impractical for certain applications, however, useful mathematical properties are evident when the set of frequencies is made finite. [40]

\[
H(f) \equiv \sum_{n=-\infty}^{\infty} h_n e^{j2\pi nf} \tag{4.6}
\]

\[
h_n = \int_{-1/2}^{1/2} H(f) e^{-j2\pi nf} df, \quad n = 0, \pm 1, \pm 2, \ldots \tag{4.7}
\]

This usefulness can be observed by restricting the integral in equation 4.7 to \( \pm W \) where \( W < 1/2 \) (recalling \( H(f) \) has unit period). This has the complementary effect on reducing the indexes \( n \) with non-zero \( h_n \) values. A concentration parameter, \( \lambda \) can then be defined as the ratio of the power of some function in bandwidth \( W \) over the total function power.

\[
h_n = \int_{-W}^{W} H(f) e^{-j2\pi nf} df, \quad 0 < W < \frac{1}{2} \tag{4.8}
\]

\[
\sum_{n=N_0}^{N_0+N-1} |h_n|^2 = \int_{-W}^{W} |H(f)|^2 df \tag{4.9}
\]

\[
\lambda \equiv \frac{\sum_{n=N_0}^{N_0+N-1} |h_n|^2}{\sum_{n=-\infty}^{\infty} |h_n|^2} \tag{4.10}
\]

The problem of finding functions limited to bandwidth \( W \) that maximize the concentration \( \lambda \) is identical in the frequency and time domains as Parseval’s theorem only stipulates that the functions must have a Fourier representation. The functions, \( U_k(N,W;f) \), which maximize \( \lambda_k \) are known as the discrete prolate spheroidal sequences (DPSS), or Slepian tapers, and have some very attractive properties beyond their concentration. (See figure 4.3.) These functions are doubly orthogonal with respect to each-other, which makes them useful as data tapers. By design, they are also eigenfunctions of the Dirichlet kernel, whose convolution
with a function is defined to be the function’s index-limited discrete Fourier transform. Following Thomson’s work: [41]

$$\lambda_0(N, W) > \lambda_1(N, W) > \ldots > \lambda_{N-1}(N, W) > 0$$ (4.11)

$$\int_{-W}^{W} U_i(N, W; f)U_j(N, W; f)df = \lambda_i \int_{-1/2}^{1/2} U_i(N, W; f)U_j(N, W; f)df = \lambda_i \delta_{ij}$$ (4.12)

$$\int_{-W}^{W} D_{N}(f')U_k(N, W; f')df' = \lambda_k(N, W)U_k(N, W; f)$$ (4.13)

where

$$D_{N}(f') = \frac{\sin(N\pi[f - f'])}{\sin(\pi[f - f'])}$$ (4.14)

and

$$(D_{n}*f)(x) = \sum_{n=N_0}^{N_0+N-1} f(n)e^{inx}$$ (4.15)
Figure 4.3. A plot of the first few most concentrated discrete prolate spheroidal sequences (top) and their spectral concentrations (bottom). These functions maximize power concentration giving a finite bandwidth and are eigenfunctions of the Dirichlet kernel.

4.3 Multi-Taper Harmonic Analysis

It is difficult to determine the variance of a spectral estimate when a single taper is used and one discrete Fourier transform operation is performed without \textit{a priori} knowledge of the spectrum shape. Some limits can be determined by analyzing propagation of variance on equation 4.16, which will depend on the covariance of $x(t)$ and $e^{-j2\pi ft}$, which is in essence the spectral estimate which is being sought. This covariance measure cannot be defined for a single frequency, unless variance data for each individual sample in $x(t)$ are known, but its average can be estimated for all frequencies given the distribution of the time-series $x(t)$. It is much more typical to have a time series $x(t)$ that has uniform variance, or error, at each time. As a result, $Var[\hat{F}(f)]$ can only be estimated as uniform across all frequencies leading to an overall measure of spectrum estimation goodness but will not be useful in determining which frequency detections were more statistically significant.
in a given time-series. This is because each spectral estimate \( \hat{F}(f) \) is a mean of time-domain measurements \( x(t) \) weighted by a complex sinusoid.

\[
\hat{F}(f) = \langle x(t)e^{-j2\pi ft} \rangle
\]  

(4.16)

Figure 4.4. A figure demonstrating a multi-taper spectral estimate, \( \mu(f) \), of a square wave in noise in the top frame and the associated Thomson F-statistics in the bottom frame. The F-statistic represents a measure similar to signal-to-noise and thereby re-shapes the spectrum making line components easier to identify. Note the broad peaks in the \( \mu \) estimate around the line detections, they are elevated above the background spectrum and are the result of the wider taper bandwidth.

The double orthogonality, in time and frequency domains, of discrete prolate spheroidal sequences (DPSS) is a very useful features in spectral estimation applications. Applying multiple DPSS tapers to a single time-series, then performing a Fourier transform on each tapered time-series, provides multiple independent estimates of the single time-series spectrum and allows direct estimation of variance at each frequency. This provides the necessary data for statistical analysis of spectral estimation and identification of line spectra based on the spectral variance at a given frequency. The disadvantage associated with multi-taper techniques is the width of their frequency domain representation, which is broader than a half-
cosine or Hanning window. [39] This broader bandwidth, $W$, results in smearing of line components in the spectral estimate, even if they can be individually resolved using the Thomson F-statistic test. [41]

Given a time series $x(t)$ and $k$ tapers, the individual eigenestimates (Thomson’s notation) are computed by taking a discrete Fourier transform of the time-series multiplied by the time-domain representation of each taper $v^{(k)}(t)(N,W)$ (equation 4.17). [41] The parameters $N$ and $W$ are the number of data points and the normalized bandwidth product respectively, and are used in the DPSS taper generation algorithm. A weighted average is performed to compute the spectral estimate $\hat{\mu}(f)$ at each frequency (equation 4.18). $U_k(N,W;f)$ is the Fourier transform of the $k$-th DPSS taper $v^{(k)}(t)(N,W)$ evaluated at frequency $f$. This weighing factor $U_k(N,W;0)$ is zero for odd DPSS tapers, as they are perfectly symmetric and have no component at zero frequency, thereby removing their estimates from the analysis, and is greater for tapers with higher concentration values, giving their estimate slightly more significance. The F-statistic (equation 4.19) is computed by taking the ratio of the estimated power at a given frequency, to the estimate variance. This statistical measurement provides a signal-to-noise ratio of the detection and follows a F-distribution, or a ratio of two $\chi^2$ distribution per ratio of power and variance, with $(2, 2k - 2)$ degrees of freedom. The determined F-statistic value can then be employed to compute a measure of statistical certainty for a spectral estimate, $\hat{\mu}(f)$, at a given frequency. [41]

$$y_k(f) = \sum_{t=0}^{N-1} [x(t) \cdot v^{(k)}(t)(N,W)]e^{-j2\pi f[t-(N-1)/2]}$$ (4.17)

$$\hat{\mu}(f) = \frac{\sum_{k=0}^{K-1} U_k(N,W;0)y_k(f)}{\sum_{k=0}^{K-1} U_k(N,W;0)}$$ (4.18)

$$F(f) = \frac{(K - 1)|\hat{\mu}(f)|^2 \sum_{k=0}^{K-1} U_k(N,W;0)^2}{\sum_{k=0}^{K-1} |y_k(f) - \hat{\mu}(f)U_k(N,W;0)|^2}$$ (4.19)
Figure 4.5. A graphical demonstration of the Thomson F-statistic computation given different signal-to-noise (SNR) levels for the same signal. The top frames demonstrate the complex eigenestimates with their mean and variance while the bottom frames show the estimated $\mu(f)$ and F-statistic around the signal’s dominant frequency. In each case, 10Hz sinusoid of unity amplitude (0dB) is added to a white noise signal to achieve the SNR value above each column. As the SNR is increased, the blue eigenestimates more closely align, leading to an estimator variance reduction, and a more accurate measurement of the sinusoid.

4.4 Transfer Function Estimation

4.4.1 Impedance Model

The goal of transfer function estimation is to determine the relationship, $T(f)$, between the stimulation current ($I_{stim}$, figure 4.6) and the artifact voltage as seen in electrographic recordings. This relationship will then be used to predict and subtract the artifact waveform, revealing the underlying neural activity and any neural response to stimulation. We will use $\eta(f)$ to represent all other contributions to $V_{rec}$, which are not linearly dependent on the stimulus current. This value has been found to small over appreciable time windows (> 10s). Ideally, the transfer
function $T(f)$ would be based primarily on the tissue impedances ($Z_{t1}, Z_{t2}, Z_{t3}$, figure 4.6), however, it is clear that there are four electrode–tissue interfaces in the stimulation/measurement path. The current flow across the recording electrode–tissue interfaces is very small (<1pA) and does not lead to an appreciable potential change across the interface, which does not substantially contribute to the analysis. Furthermore, tracking the current flowing through the stimulation electrode circuit, both Faradaic and non-Faradaic, desensitizes the analysis to non-linear interface effects of the stimulation electrodes, as only current flow is measured, and allows the artifact generation network (figure 4.6, equation 4.21) to be modeled as a linear system. The goal of the transfer function estimation algorithm is to define an optimal $T(f)$ which spans a range of frequencies which will cover any stimulation waveform used in feedback control experiments.

**Figure 4.6.** A schematic model of the stimulation and recording system. The electrode interfaces are modeled in the same manner as 3.3. The impedance elements $Z_f$ and $Z_t$ represent the Faradaic electrode impedance and tissue impedance respectively. $C_{dl}$ represents the double layer capacitance and $E_j$ represents the a possible half-cell electrode potential. This is another representation of figure 4.1. The stimulus current, along with the current from neural sources, flows through the impedance network resulting in $V_{rec}$ consisting of components due to the neural activity and stimulus artifact. The goal in the artifact subtraction procedure is to differentiate between these two signal sources.
\[ V_{rec}(t) = V_{artifact}(t) + V_{neural}(t) \quad (4.20) \]
\[ V_{rec}(f) = T(f) \cdot I_{stim}(f) + \eta(f) \quad (4.21) \]

### 4.4.2 Stimulus Waveform

Successful artifact subtraction requires optimal estimation of the transfer function \( T(f) \), which is tied to physical tissue properties (figure 4.6). Our initial in vivo investigation on the nature of this artifact transfer function revealed that it is smooth in our frequency range of interest \((< 100Hz)\), therefore, it is sufficient to evaluate its value at a finite set of frequencies and interpolate when the matched IIR filter is designed. This can be done by constructing an interrogation waveform which will be used to stimulate the neural tissue to compute \( T(f) \), but is completely different from the stimulus waveform used in experiments whose artifacts we seek to subtract. These two waveforms can be used simultaneously, as will be discussed later. A natural choice for the interrogation waveform is a sum of sinusoids as given in equation 4.22.

\[
I(t) = \sum_{n=0}^{M-1} A_n \sin(2\pi f_n t + \phi_n) \quad (4.22)
\]

This distributes the waveform power into line components in the frequency domain and ensures that each frequency, with appreciable power, is consistent through the entire windows being analyzed. \(^2\) The set of frequencies employed must be numerous enough to span the frequency range and allow for an accurate 3\(^{rd}\) or 4\(^{th}\) order IIR filter design, but have sufficient space between frequencies as to overcome the 2W bandwidth limitations of DPSS tapers. Furthermore, first order frequency mixing combinations must also be more than 2W away from any frequencies included in the analysis, as they can appear due to 2\(^{nd}\) order non-linear tissue response. [42] For example, if 3Hz and 5Hz are in the set \( f \), then the following frequencies must be excluded: 6Hz (3+3), 10Hz (5+5), and 8Hz (5+3).

\(^2\)Consistency here implies that the sinusoid at each frequency maintains a constant amplitude and initial phase over the whole analysis.
Figure 4.7. A figure showing a typical interrogation stimulus waveform time-series in the top panel and its power spectrum in the bottom panel. This waveform is constructed by summing sinusoids whose frequencies span the frequency range over which $T(f)$ is to be defined. The statistical properties of the time-series are similar to Gaussian noise, however, the power is concentrated at distinct frequencies instead of being spread over the spectrum evenly.

Excluding these frequencies from the stimulus waveform, and therefore the analysis, would minimize non-linear effects when the data are fit to a linear model. If the frequencies were not excluded from the above case, the nonlinear frequency-mixing effects from 3Hz+5Hz stimulation may get confused with linear effects from the 8Hz stimulation. Higher order nonlinear effects appear to be small enough to be neglected in this analysis. Finally, the set of phases $\phi_n$ is uniformly distributed over $(-\pi, \pi)$ and the set of amplitudes $A_n$ is uniformly distributed over $(0.1k, k)$ to give the signal $I(t)$ similar statistical properties to Gaussian noise and to maintain an over-all RMS power set by parameter $k$.

4.4.3 Algorithm

The optimal transfer function $T(f)$ can be estimated based on discrete windows where the neural tissue was stimulated with an interrogative waveform defined in equation 4.22, or the estimation can be done continuously. The discrete estimation
method will focus on analyzing several presentations of the interrogation waveform, where the optimal fit will be determined across all windows. This method requires that any other stimulation program to be suspended while the interrogation waveforms are presented. The continuous method involves using longer, sliding windows and a Kalman-like update protocol. The longer windows allow for a smaller amplitude interrogation waveform, less than $1/10^{th}$ of the experimental stimulation waveform amplitude, permitting the estimation of $T(f)$ during other stimulation programs.

The discrete estimation procedure involves presenting the interrogation waveform $N$ times, with the frequency content of each presentation held constant, but with a redistribution of initial phases and amplitudes in the sum of sinusoids. Multi-taper spectral analysis will be performed on input current and recorded voltage, for each recording channel, at the frequencies contained in the sum of sinusoids interrogation waveform. The result will be $N$ artifact input (stimulus current) and output (recorded voltage) measurements per frequency per channel.

These data are then fit to the linear model in equation 4.23. Various methods were attempted, with the optimal performance being fit by a 4D (matching a complex (2D) $T(f)$ and complex (2D) $\eta(f)$) least-squares method which minimizes $R^2$. The $R^2$ manifold (equation 4.25) appears to have a single extrema allowing for quick detection using a downhill simplex method. [43] Alternatively, a gradient method can be used if more information is known if the $R^2$ derivative can be analytically computed. [44] [45] Taking the $\mathbf{\hat{T}}(f) = \mathbf{\hat{V}}(f)/\mathbf{\hat{I}}(f)$ as the initial guess typically gets to the minima quickly. The values of $T(f)$ determined by minimizing $R^2$ can then be used to compute IIR filter coefficients. This method proved to be extremely robust in MATLAB simulations, where the transfer function was effectively computed in the presence of uncorrelated random noise. A transfer function estimation of a resistor-capacitor system, based on real recorded data, is shown in figure 4.8. Here, the agreement between the results and a SPICE simulation of the same system is good and the small discrepancies can be explained by the tolerances of the components used to build the resistor-capacitor element. An in vivo transfer function, which was computed using this algorithm, is shown in figure 4.9.

Continuous $T(f)$ estimation can be done by presenting a low-amplitude inter-
Figure 4.8. Traces showing a computed transfer function of a resistor-capacitor circuit and the transfer function generated by SPICE simulation. The data match well except for a small discrepancy at the lower frequencies where the maximum difference is less than 5%. This error is less than what is predicted by considering the tolerances of the real resistors and capacitor used for this circuit, which were 5% and 10% respectively.
Figure 4.9. Traces showing a computed transfer function between iridium oxide stimulating electrodes and a 50µm bipolar recording electrode in vivo. Of particular interest are the filtering effects between DC and 10Hz. There are data showing both high-pass and low-pass behavior for recording electrodes and even a change from one to the other in an animal over time. The shape of this plot cannot be attributed to filtering properties of the high-pass amplifier stage which is set to 0.3Hz.

\[ \overline{V}(f')|_{f' = f_n} = T(f') \cdot \overline{T}(f') + \eta(f') \]  

(4.23)

\[ V\text{ar}(w) = \left[ \sum_{k=0}^{K-1} |y_k^{(V)}(f) - \hat{\mu}^{(V)}(f) U_k(N, W; 0)|^2 \right]_{\text{window}=w} \]  

(4.24)

\[ R^2 = \sum_{w=0}^{M-1} \frac{|\overline{V}(f) - T(f) \cdot \overline{T}(f) - \eta(f)|^2}{V\text{ar}(w)} \]  

(4.25)

The IIR filter computation can be done directly by solving a set of Yule-Walker equations using the estimated \( T(f) \) data at the frequencies which make up the interrogation waveform. [46] The IIR filter is selected over FIR as it allows for the representation of continuous systems, such as RC circuits, with fewer coefficients than an equivalent finite impulse response filter (FIR). This is technically a regressive process where the number of steps involved depends on the filter order required. The filter designed is guaranteed to be stable and realizable, however, it
may not match the complex (real and imaginary components of \( T(f) \)) data perfectly. Filter order selection can be done empirically and will likely depend on the frequency range which \( T(f) \) covers. The less than 100Hz range covered by PLEF is affected primarily by a low-frequency dispersion, around 10Hz, which is adequately modeled by a 2\textsuperscript{nd} or 3\textsuperscript{rd} order filter. Higher bandwidths may see more complex frequency response requiring higher order. Care should be taken not to set the filter order higher than necessary as the Yule-Walker algorithm will attempt to create a better fit for noisy data leading to undesirable results.

\[
\sigma^2_{n-1} \equiv Var[T_{n-1}(f)] \quad \text{existing estimate} \quad (4.26)
\]

\[
\sigma^2_n \equiv Var[T_n(f)] \quad \text{incoming estimate} \quad (4.27)
\]

\[
\hat{T}_{n+1}(f) = \frac{\sum_{i=0}^{10} \frac{T_{n-i}(f)}{\sigma^2_{n-i}}}{\sum_{i=0}^{10} \frac{1}{\sigma^2_{n-i}}} \quad (4.28)
\]

### 4.4.4 Algorithmic steps

We assume that two time series, \( I_{stim} \) and \( V_{rec} \), are available. They are the stimulus current waveform and the differential recording waveform respectively. Their relationship is illustrated in figure 4.6.

**Discrete interrogatory stimulation case**

1. Identify the \( N \) interrogation stimulation windows in \( I_{stim} \), an additional sync channel can be used for this or the properties of \( I_{stim} \) can be analyzed.

2. Compute \( \mu \) and \( F \)-statistic estimates for each window for \( I_{stim} \) at the frequencies which make up the sum-of-sinusoids interrogatory waveform. The \( F \)-statistic can be computed for all frequencies and the peaks can be identified to find the sum-of-sinusoids components if this list is not known \textit{a priori}.

3. Compute \( \mu \) and \( F \)-statistic estimates for each window for \( V_{rec} \).
4. At each frequency which makes up the interrogatory waveform, numerically fit optimal $T$ and $\eta$ to $\{V\} = T \cdot \{I\} + \eta$ where $\{V\}$ and $\{I\}$ contain $N \mu$-estimates from all stimulation windows at the frequency being analyzed.

5. The computed $T$ values at each frequency which makes up the interrogatory waveform are used to design an IIR filter to have the same frequency response.

6. The designed IIR filter is applied to an arbitrary current waveform, whose bandwidth is within the bandwidth of the interrogatory waveform, to predict the stimulus artifact which is seen in $V_{rec}$.

**Continuous interrogatory stimulation case**

1. The interrogatory stimulation is continuous, therefore consecutive non-overlapping windows are selected and $\mu$ and $F$-statistic estimates are performed on $I_{stim}$ and $V_{rec}$. The $F$-statistic can be computed for all frequencies on $I_{stim}$ and the peaks can be identified to find the sum-of-sinusoids components if this list is not known *a priori*.

2. Each time a window is processed, a new value for $T$ can be computed at each frequency making up the interrogatory waveform. This takes into account the latest spectral estimates and the previous estimates following the update scheme in equation 4.28.

3. The computed $T$ values at each frequency which makes up the interrogatory waveform are used to design an IIR filter to have the same frequency response.

4. The designed IIR filter is applied to an arbitrary current waveform, whose bandwidth is within the bandwidth of the interrogatory waveform, to predict the stimulus artifact which is seen in $V_{rec}$.

### 4.5 Validation

The signal processing techniques involved in stimulus artifact subtraction were validated on a number of levels, ranging from MATLAB (The MathWorks, Inc.) simulation to *in vivo* tests. The first step was in simulation, where all system
parameters were known *a priori*. An arbitrary IIR filter was designed to model the relationship between the stimulus current and the recorded voltage, noise was added, and the a matched filter was designed by computing the transfer function based on the generated data. This algorithm worked very well with approximately 80dB artifact subtraction capability when using the matched filter to remove artifact generated by the arbitrary filter. This was measured by comparing the artifact prior to removal and the residual artifact.

Next, the algorithm was used to perform artifact subtraction where the stimulus current to recorded voltage relationship was set by a bench-top resistor-capacitor system. The component target values, and tolerances, were known but the distribution of noise and the actual component values were unknown. Again, the algorithm was able to identify the component values closely to the real values (figure 4.8) and artifact subtraction was successful.

The next validation test involved suspending a pair of stimulation electrodes and a recording electrode in phosphate-buffered saline. The transfer function between stimulus current and recorded voltage was computed and then used to design a filter to subtract arbitrary stimulus artifact. This subtraction achieved nearly 60dB which places the residual artifact below the noise floor of the electrochemical system.

Once the previous three validation tests proved successful, artifact subtraction experiments were carried out in deeply anesthetized animals. A large dose of anesthetic was delivered to suppress any kind of neural activity in response to the interrogatory stimulation or the stimulation whose artifact was to be removed. The results were similar to those in solution.

Finally, artifact subtraction tests were carried out in awake, behaving animals. The efficacy of artifact subtraction was determined by comparing the pre-subtraction and post-subtraction spectral estimates in the EEG signals at the stimulation frequency. The level of artifact subtraction was similar to the previous two cases providing that the EEG data were not confounded by substantial noise or motion artifacts.
4.6 Artifact Subtraction Demonstration

The first step in quantifying the effectiveness of this artifact subtraction system was to analyze its performance on simulated data. The interrogatory stimulus waveform was generated as in figure 4.7 and filtering it using an arbitrary, but stable, “tissue” IIR filter of order 2 or 3. The result was combined with white noise to achieve an SNR defined by the ratio of RMS power in the stimulus over RMS power in the noise. The algorithm was used to come up with “computed” IIR coefficients of the same order or one higher. An arbitrary waveform was then passed through both set of filters, the results subtracted, and the remaining power after subtraction was considered residual artifact. The result was approximately a 60dB reduction in residual artifact. In vivo experimentation yielded similar results, however, it was much more difficult to quantify as the frequency spectrum of the underlying activity is unknown a priori. Figure 4.10 demonstrates the effects of artifact subtraction on in vivo time series. A comparison of the line spectra at the artifact frequency has yielded close to 50dB subtraction.

![Figure 4.10. Plots showing time-series artifact subtraction, in vivo of a sinusoidal stimulation and a square stimulation. Note that the pre/post artifact subtraction EEG traces overlap when there is no stimulus current, but readily diverge when the stimulation is turned on.](image-url)
4.7 Conclusion

Spectral analysis is an extremely powerful tool in the scope of neural interfaces. The use of multi-taper estimation techniques give statistical robustness to spectral estimation and permit detection of the ideal relationship between stimulus current and stimulus artifact as seen by various recording sites. This, in turn, allows for the implementation of a digital filter, which matches the frequency response of $T(f)$, to predict and subtract stimulus artifacts and the observation of the underlying non-linear tissue response by removing the linear artifact. We have previously published these methods as a conference paper at the IEEE EMBS. [47]
5

Artifact Transfer Function and Neural Modulation

The hardware and algorithms described in the previous two chapters permits suppression of stimulus artifact which indirectly measures tissue properties when the transfer function between the stimulus current and recorded artifact voltage is computed. The linear artifact subtraction allows for the investigation of tissue modulation by polarizing low-frequency electric fields. Additionally, tracking changes in transfer function, both as functions of time and frequency, may shed light on changes in tissue properties and how they relate to activity. The next two sections will cover the observed neural activity during stimulation and the time-frequency variability in in vivo transfer function estimates.

5.1 Implantation Methods

All procedures were carried out under the supervision and with approval of the IACUC at George Mason University and The Pennsylvania State University, where these experiments were performed. Stimulation electrodes were constructed by exposing 3\text{mm} of polyimide coated 250\mu m diameter stainless steel wire and electrically depositing iridium-oxide. Recording electrodes were constructed by bonding a pair of polyimide coated 50\mu m diameter stainless steel wires, exposing the tips, and electrically depositing iridium-oxide. Adult male Sprague-Dawley and Long Evans rats were deeply anesthetized and placed into a stereotaxic frame (Angle
A stimulation electrode was implanted with an end target of \( AP = -5.15, \ ML = +5.35, \ DV = -7.6, \) mm referenced to bregma, with a second stimulating electrode implanted in parallel 2mm to the anterior. Bipolar recording electrodes were implanted at \( AP = -2.5, \ ML = \pm 2.0, \ DV = -3.3 \) and \( AP = -3.5, \ ML = \pm 3.0, \ DV = -3.3. \) Stainless steel 0-80 screws were placed over the cortex at \( AP = +1,-4, \ ML = \pm 3.0, \) and \( AP = -7, \ ML = 0. \) These screws were used for differential recording and animal grounding. Four animals participated in the evoked potentials study, twelve in the multi-unit activity study, and ten were used for all aspects of transfer function estimation.

Four animals were used in the mean evoked potential study, four animals were used in acute validation of transfer function estimation under anesthesia\(^1\), four animals were used in the transfer function time-dependence study, and ten animals were used in the spike entrainment study. Animal selection was based on the presence of physiological recordings which was determined prior to experimentation and any stimulation. Four of the four animals demonstrated evoked potential generation in multiple electrodes. Three of the four animals demonstrated time-dependence in transfer function while the fourth animal’s recordings developed substantial motion artifact which confounded the analysis prohibiting the use of that animal’s data. All but two animals demonstrated significant spike entrainment. Those two animals lost all recording channels before the full set of experiments could be concluded and their data was removed from the analysis.

\subsection*{5.2 Evoked Potentials}

The research presented here typically involves sinusoidal current waveforms for stimulation, which is a very narrow field of polarizing low-frequency electric field (PLEF) stimulation. One strength of this approach is the very narrow frequency-domain representation of the stimulus waveform, a single frequency \( f_0, \) which then restricts the frequency content of any non-linear responses to harmonics of \( f_0. \) [42]

\[\text{For example, a 10}Hz\text{ stimulus current will elicit a response and potential artifact at}\]

\[\text{\footnotesize{\textsuperscript{1}}These animals underwent acute surgery under deep anesthesia which was characterized by a loss of synchronized EEG activity. This was done to validate \textit{in vivo} transfer function estimation and artifact subtraction performance without significant background neural activity}}\]
10Hz, and non-linear tissue responses at $n \cdot 10Hz$ frequencies for integer $n$. These harmonics manifest themselves in recorded data during low-frequency ($< 100Hz$) stimulation programs of sufficient intensity as to induce neural modulation. In these situations, it is typical to see recorded waveforms, after artifact subtraction, which are not purely sinusoidal but share the same period as the ongoing sinusoidal stimulation.

*In vivo* experiments were carried out to investigate the frequency dependence of these evoked responses to sinusoidal stimulation. Several frequencies were selected and a stimulation program was designed where the paradigm consisted of three sinusoidal cycles at a fixed frequency followed by five seconds without stimulation. This was repeated fifty times for each frequency, using the same stimulus amplitude for all presentations. The stimulated data for each frequency were isolated and the onset of each set of three sinusoidal cycles was identified. Stimulation artifact subtraction was performed and all presentations were averaged for each frequency. The resulting mean waveforms from the contralateral recording site, for each frequency, are plotted in figure 5.1. A similar type of local field potential entrainment can be seen in the same animal, but on a recording site which is ipsilateral to the stimulation (figure 5.2). Finally, a similar plot is shown for another animal in figure 5.3, which is representative of two other animals which underwent this type of local field entrainment experimentation.

It does not appear that the observed neural response to modulatory stimulation increases monotonically with frequency. This gives more weight to the hypothesis that the source of the response waveforms is neuronal and not some inadvertent coupling between the isolated stimulation and recording systems. One of the downsides of completely relying on recorded local field potentials (0.3-1000Hz) is the temporal integration associated with low-pass filtering, which would make it difficult to resolve any single/multi-unit neural activity and making it difficult to further investigate these effects.
Figure 5.1. Plots showing mean evoked potentials in a behaving animal in the local field potential (0.3-500Hz) band. Each box represents the average LFP response (blue trace), after artifact subtraction, to 50 presentations of the stimulus current (red trace) from the contralateral side. Each single stimulation consists of three sinusoidal cycles at a fixed frequency followed by 5 seconds of silence. It can be noted that the evoked response does not increase monotonically with frequency, i.e. 11Hz response is greater than 13Hz. This type of response is representative of other animals who participated in the same experiment. This experiment has been reproduced in three animals with similar results.
Figure 5.2. A plot showing the same traces as figure 5.1, during the same experiment, with the recording taken from an electrode ipsilateral to the stimulation.
Figure 5.3. A plot showing the same electrode position as figure 5.2, and following the experimental procedures as in figure 5.1, but from a different animal.
5.3 Multi-unit Analysis

The pre-amplifier/headstage used in this in vivo research (chapter 3) is designed to perform fully-differential DC-coupled electrophysiological measurements without any explicit filtering. Therefore, it was possible to route the headstage output to a second amplifier which would operate in parallel with the custom local-field amplifier. The second amplifier was tuned to have an increased gain and a passband of 600-6000Hz, facilitating the recording of multi-unit activity from bipolar 50µm electrodes while also recording local field potentials from the same electrodes, all concurrently with electrical stimulation.

A time-series of the multi-unit and stimulation waveforms can be seen in figure 5.4. Enlarged plots of a selection of detected spikes is shown in figure 5.5. The data were acquired at 15kHz, therefore, each window represents approximately 40 data points. The multi-unit data were analyzed using accepted methods to identify multi-unit spikes: maxima and minima > 3.5 standard deviations from the mean were identified and the points of inflection were marked as the locations of multi-unit spikes. The temporal locations of the detected multi-unit spikes were then mapped to the phases of the stimulation waveform during which they occur. The distribution of spike phases, relative to the stimulation waveform, and the time of their occurrences are plotted in figure 5.7. A schematic of the phase detection process is shown in figure 5.6. It appears that the structure of the phase-locking changes throughout the stimulation, starting with a lack of clear trend, to a single preferential phase, to a bi-modal distribution. Finally, the detected spike locations can be used to generate a representative point-process time series which is made up of Kronecker delta-functions at the spike locations. The spectral techniques discussed in the previous chapter can be used to identify line-spectra in the point-process time series (figure 5.8). [48] The transient detections at 15Hz, and harmonics, indicate a significant phase locking of spike times to the 15Hz stimulus waveform.

It is understood that local field potential measurements are tracking spatial and temporal integration of single/multi-unit activities. [23] [24] More specifically, a deflection in the local field potential is a representation of the activity of many neurons located in close proximity to the electrode and firing action potentials in
Figure 5.4. A plot overlaying high frequency (600-6000Hz) differential in vivo neural recordings, the stimulus current, and detections of multi-unit spike activity. The red circles mark signal inflections which are more than 3.5 standard deviations from reference. The stimulation is a 50\(\mu\)A sinusoid at 15Hz.

a short period of time. Figure 5.9 plots the multi-unit spike detections, local field potentials after artifact subtraction, and the stimulation waveform at the onset of the stimulation in figure 5.4. There is minimal phase entrainment and subsequently any activity at the stimulation period is minimal. The situation is vastly different at the end of this stimulation session when there is clear phase locking at two phase ranges (figure 5.10). It appears that there is some correlation between the phase-locking of multi-unit activity to stimulation, and the resulting periodic waveform which remains after artifact subtraction. This further supports the hypothesis that the sinusoidal PLEF stimulation is indeed modulating neural tissues and that the remaining periodic waveform, during stimulation, is of neuronal origin.
Figure 5.5. Plots showing enlarged multi-unit spike detections from the data shown in figure 5.4. Each box is 2.5mV high and is 2.5ms across. Only one detection is marked per window at the center. These detections are chosen randomly from all detections and it is possible that the each time window may have additional unmarked detections in this plot. However, all detections are marked in figure 5.4.
Figure 5.6. A schematic of the process for computation of spike detection phase given a stimulation waveform. Data from figure 5.4 is employed here, where the phases of each spike detection relative to the stimulus current is shown in figure 5.7.

Figure 5.7. A plot showing the phase of detected multi-unit activity (figure 5.4) relative to the ongoing stimulation current. Initial phase locking appears at 250 degrees, while a second mode at about 75 degrees can be seen later. The red bar indicates active stimulation.
Figure 5.8. A plot showing the Thomson F-statistic, computed using 0.1 second windows, of the multi-unit spike point process as detected in figure 5.4. A value of 14 approximately corresponds to a p-value of 0.005. The magenta bar indicates active stimulation carried out at 15Hz.
Figure 5.9. A plot overlaying local field potential (0.3-500Hz) differential \textit{in vivo} neural recordings, the stimulus current, and the detections of multi-unit spike activity (point process marking only the location of spike detections) based on high-frequency data. This corresponds to the initial stage of figure 5.4 where the main phase locking was at 250 degrees (figure 5.7). The stimulus waveform consists of a $15\text{Hz}$ sinusoid with $50\mu\text{A}$ amplitude.

Figure 5.10. A plot overlaying local field potential (0.3-500Hz) differential \textit{in vivo} neural recordings, the stimulus current, and the detections of multi-unit spike activity (point process marking only the location of spike detections) based on high-frequency data. This corresponds to the latter stage of figure 5.4 where phase locking was evident at 75 and 250 degrees (figure 5.7). The stimulus waveform consists of a $15\text{Hz}$ sinusoid with $50\mu\text{A}$ amplitude.
5.4 Transfer-function Time-dependence

The linear artifact model (equation 5.1) used for this research draws similarities between the transfer function and an impedance as it relates the applied stimulus current to the recorded stimulus artifact voltage. It is comparable to a standard four-port impedance/resistivity measure in that a pair of electrodes are used to drive a current and a second pair records the generated potential difference, a Kelvin sense. The key difference is that the geometric relationship between the electrodes, while being fixed, is unknown. Furthermore, the iridium oxide coatings on the recording and stimulation electrodes allow them to closely resemble ideally non-polarizable electrodes, which closely approximates nearly-Ohmic potential sensing when the pre-amplifier input bias current is sufficiently low ($< 10\mu A$ in our instrumentation). A computed in vivo transfer function is shown in figure 5.11, the shape of this transfer function cannot be attributed to the amplifier and headstage, whose relevant frequency responses are shown in figure 3.7.

Figure 5.11 represents a typical in vivo transfer function estimate between the applied stimulus current and a bipolar recording electrode. The observed frequency dispersion is not caused by the frequency response of the amplifier and is not reproduced when the transfer function is estimated with identical electrodes in either phosphate-buffered saline, or physiological (0.9%) saline. Plotting the loss-tangent frequency relationship (figure 5.12) indicates that neural tissue behaves like a poor (lossy) dielectric at frequencies above 5Hz and is slightly inductive at lower frequencies, which can be verified by the phase of figure 5.11. [1] The former observation on the prevalence of Ohmic losses in the presence of dielectric properties in the frequency range relevant to PLEF stimulation is consistent with other investigations of neural tissue impedance. [49] The same analysis was performed on a comparable electrode configuration, and using the same type of stimulation/acquisition hardware, in physiological saline solution (figure 5.13), phosphate buffered saline (figure 5.14) and deionized water (figure 5.15). It appears that there is an inductive component at lower frequencies, however, the phase of the in-solution transfer function is zero above 5Hz, as compared to capacitive phase in from the in vivo transfer function. This is likely explained by the presence of membranes/molecules in tissue which act as dielectrics.
Figure 5.11. A plot of the detected in vivo transfer function data and a 2\textsuperscript{nd} order infinite impulse response filter fit. The transfer function appears to be dominated by an inductive regime below 5Hz, and a capacitive regime above.

\[ V_{rec}(f) = T(f) \cdot I_{stim}(f) + \eta(f)^2 \approx 0 \] (5.1)

The interrogatory stimulation used for transfer function estimation is relatively low-amplitude which allowed transfer function estimation to be carried out for several days in multiple chronically-instrumented animals. High temporal resolution transfer function tracking is shown in figure 5.16 with the day/night cycle and animal acceleration overlaid. Figure 5.17 tracks ratios of the transfer function data at fixed frequencies and indicates that the rough shape of the transfer function is maintained while its mean magnitude shifts. This was a non-seizing animal who experienced substantial shifts (30 – 40%) in transfer function magnitude, and therefore relative shifts in tissue impedance, on a time scale of hours and without correlation to gross motor activity. A similar analysis was carried out on a seizing animal, as shown in figure 5.20, and similar gross shifts in transfer function were noted. Unfortunately, the available data are not sufficient to correlate

\textsuperscript{2}This is assuming long enough time-windows for spectral estimation so that any neural activity and background noise are statistically uncorrelated with the applied stimulus current. Furthermore, the power contained in the interrogation waveform used to compute \( T(f) \) is sufficiently small as not to cause any apparent neural entrainment. This is beneficial as \( T(f) \) seeks to model the passive properties of neural tissue.
\[ T(f) \approx A \cdot f^{0.5} \approx B \cdot f^{-1.25} \]

Figure 5.12. A plot showing the frequency dependent loss tangent of the \( T(f) \) data (figure 5.11). \([1]\) The loss peak is close to 5Hz when the IIR filter data are used. The frequency-dependent magnitude of the complex portion of the transfer function is used due to the presence of inductive regime in the low frequency range and the logarithmic plot format.

Figure 5.13. A plot demonstrating the computed transfer function between a pair of iridium-oxide stimulation electrodes and one bipolar iridium-oxide recording electrode in physiological sodium-chloride solution (0.9\%). These data were recorded using the same headstage and amplifier combination whose relevant frequency response is shown in figure 3.7.

transfer function estimates/tissue impedance to probability of seizure. It is reasonable to assume that such shifts in impedance are likely to have an impact on
Figure 5.14. A plot demonstrating the computed transfer function between a pair of iridium-oxide stimulation electrodes and one bipolar iridium-oxide recording electrode in phosphate buffered saline (1x PBS) solution. These data were recorded using the same headstage and amplifier combination whose relevant frequency response is shown in figure 3.7.

non-synaptic communications in the brain, specifically intrinsic electric fields, and would therefore have an impact on various neural processes. [25]

Significant shifts in transfer function magnitude have been observed on much shorter time-scales during seizure events which occurred during episodes of open-loop stimulation. In the case of figure 5.21, a seizure was recorded during on-going stimulation at 45Hz. A Kalman filter was designed to optimally track the amplitude and phase of the sinusoid at 45Hz in the recorded voltage data from a bipolar electrode. This allowed for very fast updating as the data were sampled at 2000Hz, and the covariance estimate in the Kalman filter permitted the computation of an F-statistic and therefore a significance test in the high-resolution Kalman estimate. The result was a visible shift in transfer function, and therefore impedance, of around 25% on a timescale of seconds as the seizure progressed.

It appears that the in vivo transfer function between stimulus current and recorded potentials from electrodes in neural tissue, and therefore relative tissue impedance, is not completely Ohmic in the DC-100Hz range relevant to polarizing low-frequency electric field stimulation. This observation that neural tissue possesses a complex impedance is not new and has repeatedly been published over the
Figure 5.15. A plot demonstrating the computed transfer function between a pair of iridium-oxide stimulation electrodes and one bipolar iridium-oxide recording electrode in deionized water ($17M\Omega \cdot cm$). These data were recorded using the same headstage and amplifier combination whose relevant frequency response is shown in figure 3.7. Low frequency transfer function data were not statistically significant and therefore are not shown here. This estimation problem was due to the difficulty of passing appreciable current through deionized water.

past half-century. [50] [51] [49] Furthermore, this impedance measure can change on time-scales of hours in normal homeostasis as well as seconds during extreme events such as seizures. Impedance shifts similar to ours have been confirmed by other groups. [52] The impact of these impedance shifts on non-synaptic neural communications \textit{in vivo} is unclear, however, it is plausible given the impact of tissue impedance on modeling studies. [53] [24] We have previously published these findings as a paper at the IEEE EMBS. [54]
Figure 5.16. A plot of the transfer function magnitude shifts at 12.7Hz (top) and the average transfer function magnitude shift across all frequencies (bottom). The correlation between these two traces indicates that the whole transfer function shifts with time while the relative shape of the transfer function (figure 5.11) is static. The superimposed light/dark cycle is at 50 when the room lights are on and the animals are more likely to be asleep. There are appreciable shifts in transfer function magnitude over time scales of hours which are statistically independent from animal movement, figure 5.19 shows these same data versus acceleration. Percent change is defined as the ratio of $T(f)$ at any divided by the average $T(f)$ over all data and multiplied by 100.
Figure 5.17. A ratio plot of the transfer function at three frequencies based on data from figure 5.16. These ratios describe the shape of the transfer function data and vary less than both the mean transfer function data and specific values. This indicates that the transfer function shifts in magnitude but roughly maintains its shape.
Figure 5.18. A plot of the relationship between the magnitude of the transfer function at 5.9Hz and the magnitude at 12.7Hz based on the data in figure 5.16. It appears that the relationship is fairly linear which gives more credibility to the claim that the transfer function shape does not change as much as the mean amplitude, e.g., the transfer function gets multiplied by the same constant across all frequencies.
Figure 5.19. A plot of transfer function magnitude, at 12.7Hz, of the data shown in figure 5.16 with respect to logarithm of acceleration root-mean-square power. The logarithm scale is chosen because the distribution of acceleration appears to follow a power-law.
**Figure 5.20.** A similar analysis to figure 5.16 showing a transfer function spectrogram in a different, seizing animal using less-frequent transfer function estimates. Seizure times are plotted at the bottom, however, the temporal resolution of the transfer function estimation is too low to determine a meaningful statistical relationship.
Figure 5.21. A trace of an *in vivo* seizure (bottom trace) which occurred during electrical stimulation at 45Hz along with a measurement of the transfer function at the stimulation frequency (top, blue) and the associated Thomson F-statistic (top, green). The animal was anesthetized with isoflurine during this recording, whereas all other data presented are from awake behaving animals. There is a substantial shift in transfer function amplitude, which is a relative measure of tissue impedance, as the seizure evolves. The dashed red line in the top plot is the F-statistic significance level for $p < 0.005$. 
5.5 Conclusion

The stimulus artifact subtraction system permits novel investigations into the \textit{in vivo} properties of neural tissues. Modulation of neuronal excitability can be observed through entrainment of neural activity to ongoing stimulation, while intrinsic tissue properties can be investigated through continuous stimulus artifact transfer function estimation. The former observation is consistent with previous observed modulatory effects \textit{in vitro} and \textit{in vivo} and may be applicable to feedback controller stimulus program design. \[18\] \[4\] \[17\] The latter observation of shifts in relative tissue impedance may be useful as an input to a feedback control algorithm. Finally, the techniques used in these observations are not mutually exclusive, permitting concurrent tracking of neural response and tissue impedance.
Chapter 6

Future Work

The future research goals are twofold: to create an integrated system for scientific/industrial stimulus artifact subtraction and to further explore the sources of the shape and time-dependence of the stimulus artifact transfer function. As it exists now, effective use of the artifact subtraction system requires specialized skills in signal processing rendering its use out of reach of many experimenters. It would be beneficial to create a device which can be rack mounted along other hardware, which would incorporate all of the necessary hardware and signal processing, that would require minimal maintenance and troubleshooting. The latter goal focuses on the nature of the observed in vivo artifact transfer function and aims to identify the origins of its dynamics in real time. A better understanding of the time and frequency dependence may shed light on tissue properties and possibly help interrogate cognitive states.

6.1 Hardware Integration

Technical merits are fundamentally important to research instrumentation, however, usability and reliability will have a substantial impact on the quality of research the instrumentation can support. Neural engineering is an interdisciplinary field combining the efforts of physicists, engineers, mathematicians, biologists, neuron scientists, and others; it is unreasonable to expect every experimenter to be trained as an electrical engineer to operate their equipment. Conversely, electrical engineers may lack necessary expertise in experimental design while they may have
full control over the equipment. Successfully-integrated systems must be intuitive to be operated by non-experts in electronics and must contain enough intelligence to give a rough estimate of how well they are operating.

### 6.1.1 Usability

A popular standard for neural engineering instrumentation is the combination of rack-mounted hardware with a software interface which operates on a standard personal computer (PC). This is the case for both *in vitro* configurations as well as chronic *in vivo* configurations sold by several large vendors. The typical *in vivo* configuration will consist of a pre-amplifier/headstage to be worn by the animal, with rack-mount amplifiers and stimulators, which are connected to dedicated equipment for signal digitization and synthesis, or to equivalent digitization/synthesis boards inside the PC. The control software can be fully custom, or a combination of LabView (National Instruments, Inc) and Simulink/MATLAB (MathWorks, Inc.). The usability of these devices depends on the complexity of the software interface and the difficulty in maintaining/changing the physical connections between pieces of equipment.

The artifact subtraction system must be re-designed in such a way as to place all non-headstage electronics into a single case which can be rack-mounted. This will involve the amplifier, stimulators, signal processing, test circuits, and digitization/waveform synthesis hardware. A USB 2.0 interface can be used to move data between the hardware and the acquisition PC, as well as for system control. Additional ports for power and headstage connection can be placed on the back, with only basic indicator lights on the front. This will shift the focus of experiment complexity to the software interface, which can be developed in LabView or Simulink/MATLAB. The benefit of the minimalistic hardware approach is a reduction in confusion as the experimenter will only have the necessary information available to them, as defined by the software interface. In this scheme, the headstage and rack-mounted amplifier will provide all the necessary hardware, in addition to a PC, to design neuro-modulation feedback experiments.
6.1.2 Quality Control

A potential downside of having an integrated system is the limited ability for the user to determine if the system is operating correctly. Furthermore, the goal is usability by non-specialists, which would exacerbate the problem. The stimulus artifact subtraction system must therefore include basic intelligence to determine if the acquired signals are valid and if the artifact subtraction is operating reasonably well. These two functions are primarily intended give guidance to the user, potentially to determine if their data needs to be viewed by an expert, they are not meant to be conclusive criteria for assessment of experimental results.

Determination of a neural recording signal is “good” requires expert readers and is not a fully subjective process, implementing this type of screening in the stimulation artifact subtraction system would be extremely difficult. A more attainable target is to define criteria which would indicate that a signal is “bad” and should be disregarded. Electrode impedance is often used as such a metric in scalp EEG, however, it requires measurement electronics which are too large to implement on the headstage. More reasonable criteria can include tracking the signal amplitude to mark for dead channels, to estimate the power spectrum to identify power line noise, and monitor for correlation with accelerometer channels to help identify movement artifacts. Liberal and conservative thresholds can be empirically set to give the experimenter an rapid indication of which channels may be performing poorly.

The efficacy of the stimulus artifact subtraction can be measured directly by quantifying residual artifact based on the stimulus and recording power spectra, or indirectly by seeing how well the transfer function data fit the given filter model. The former method can be implemented by interpolating the post-subtraction around frequencies which have non-zero stimulus power, then comparing the interpolation to the subtraction result. The differences at the stimulated frequencies will give a rough indication of efficacy, but do not take into account non-linear tissue response at the stimulation frequencies. The latter method wholly depends on having an the accurate representation of tissue properties by a fixed filter order. This must be validated \textit{a priori} for a given experimental paradigm, and is much easier to implement once the filter order is established. This measure can be combined with the cross-correlation between the stimulus waveform and the recorded
channels to give an indication of the artifact power and the artifact subtraction efficacy by comparing results prior and post artifact subtraction.

Finally, the integrated system should have some on-board diagnostics. The system must be able to couple each recording channel to an internal source signal and verify its operation. Internal circuitry which can simulate a stimulus artifact must also be present, permitting the internal calibration of the transfer function estimation and stimulus artifact subtraction system. A similar circuit should exist for testing the headstage, which can then specify the operation of the whole recording/stimulation system. Lastly, there needs to be a method to ensure that internal voltages are at appropriate levels, supply currents are reasonable, and track the AC noise on the DC supply voltages. Failure of any on-board diagnostic must be notified to the user through a separate channel as compared to qualitative measures of recorded signals.

6.2 Transfer Function Shape/Time-Evolution

The transfer function estimation problem is analogous to a tissue impedance problem as it determines the frequency-domain relationship between a stimulus current and recorded differential potential. The electrodes are not expected to move during chronic experiments, therefore, the shape and time-dependence of the transfer function has some relationship with neural tissue properties, which are presently unclear. The data presented in this thesis seem to indicate that neural tissue has a complex impedance and that the impedance changes with time, the former statement is partly supported in other recent research. [49] A brief survey of neural modeling literature indicates that using a constant, Ohmic value for brain conductance is currently the norm.

The first steps in assessing the importance of tissue impedance, and its time-evolution, is a better understanding of the physics involved. Even the shape of the frequency spectrum, over some frequency band, may be indicative of the underlying process of dispersive effects of neural tissue. This could provide insight into ionic flow in tissue and may have an impact on non-synaptic neural communication. [25] Experiments can be carried out to determine which changes in physiological parameters correlate with shifts in transfer-function/tissue-impedance. It may be
facile to observe impedance shifts with changes in animal hydration, however, it would be very interesting to determine if there is a relationship between impedance values and probability of epileptic seizure generation. We observe nearly diurnal impedance shifts and have published on diurnal changes in seizure probability, the possible relationship between the two processes may be useful in the development of an active seizure prediction mechanism. [31]

6.3 Conclusion

The stimulus artifact subtraction system shows some promise in the development of feedback-enabled neural interfaces, however, it lacks refinement and the underlying stimulus artifact physics are not fully understood. Integration of the system would make it more useful and accessible to non-engineers, and would therefore broaden the potential neural interface research that can be carried out employing feedback control. An improved understanding of the transfer function/stimulus artifact dynamics would provide a measure of brain impedance and may be used as a measure to track the probability of commencing an epileptic seizure. Combining the ability to do feedback control and brain impedance measures will hopefully lead to emergent interface technologies and improved therapeutic strategies.
Non-Technical Abstract

The brain is made up of cells called neurons which communicate by manipulating concentrations of ions and chemicals called neurotransmitters. Some neurons act to suppress or enhance other neurons, and are known as inhibitory and excitatory cells respectively. Networks of these inhibitory and excitatory cells are in a precise balance, and a loss of this balance can lead to crippling diseases such as epilepsy.

Approximately one percent of the world’s population is afflicted with some form of epilepsy. At least one third of those cases cannot be managed through medication, which may present neurosurgery as a necessary treatment. This surgery involves the removal of the brain area which is responsible for epileptic seizure generation if such an area can be identified. This procedure irreversible and is considered to be extremely high risk. Recent advances in neural stimulation electronics have shown promise in delivering effective treatment to this unresponsive third of patients, without the removal of brain tissue and at a reduced risk.

Epilepsy is a dynamic disease where seizures occur sporadically, furthermore, each seizure has a progression where the manifestation may change with time. Applying principles of feedback control would therefore be advantageous to minimize overall stimulation time, present a more effective stimulation program, and extend the operation of implantable neural stimulators. Feedback control, in the scope of neural stimulation, involves continuously updating the stimulation program based on recorded neural activity. The difficulty is due to the presence of a stimulus artifact, which is a result of stimulus current passing through electrically conductive neural tissue, and is added to the recordings of neural origin. A framework must
be designed to remove the stimulus artifact from neural recordings to permit the development of feedback control algorithms.

A set of prototype electronics capable of concurrent neural recording and stimulation will be presented. Furthermore, the necessary signal processing techniques which separate neural activity from stimulus artifact will be described. Finally, the efficacy of such techniques, and some preliminary data will be presented.
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Vita

Nikolai Chernyy

Education

- The Pennsylvania State University – State College, PA
- George Mason University – Fairfax, VA
  B.S. Computer Engineering – 2004

Professional Experience

- Center for Neural Engineering—State College, PA
  Graduate Research Assistant: 2004–present
  - Developed robust techniques to facilitate neurological recording during ongoing electrical stimulation. Designed instrumentation to study feedback-enabled control of epileptic seizures and navigation-related brain rhythms. Implemented a system for electrochemical deposition and characterization of iridium oxide thin films for improved implantable electrodes.

- Center for Neural Dynamics—Fairfax, VA
  Undergraduate Research Assistant: 2003–2004
  - Designed a complete set of novel instrumentation for chronic neurological recording in freely-behaving animals.

- Dataprise Inc.—Rockville, MD
  Senior Network Engineer: 1999–2001
  - Carried out on-site installation of and upper-tier support for Cisco-brand network equipment.

- Sysnet Inc.—Silver Spring, MD
  Network Engineer: 1998–1999
  - Maintained core infrastructure for a medium-sized internet service provider consisting of BSD UNIX servers and various network equipment.

Distinctions

- TBII engineering society member, former web-development officer