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ABSTRACT

Intracortical microwire electrodes record extracellular multi-unit neural activity that is essential to drive neuroprosthesis systems and to develop our understanding of brain functions like learning and memory. Implantation of electrode arrays in the brain is a complex and invasive undertaking that requires excision of skull bone, removal of dura mater and piercing of pia mater meningeal layer. Typically, neuronal spiking events are detected by their relatively large amplitudes as compared to the rest of the signal or by their characteristic shapes. In order to improve microelectrode’s recording performance, researchers have experimented with multiple electrode substrates, insulation materials and implant strategies. However, all the currently available electrode systems have limited clinical applicability because they produce highly variable recording outcomes and most fail to record neural activity beyond one year. It has been hypothesized that this variability is modulated by implantation trauma and tissue response associated with presence of foreign material in the neural tissue. Development of optimal electrode structures and implant strategies has been impeded by an absence of objective techniques to quantify and monitor the state of tissue-electrode interface in order to understand its degradation and effectively compare different electrode platforms.

This dissertation documents the development of novel approaches to help the processes of implantation, neuronal spike detection-quantification, and real-time interface monitoring. It reports an objective and innovative algorithm that utilizes information on multiple electrodes of an array to improve neuronal spike detection and measurement that can be used to quantify the recording performance of the interface. In doing so it also achieves significant reduction in downstream data processing. Minor addition to standard implantation techniques is reported that uses collagenase enzyme enabled disruption of the meningeal layer in order to reduce implantation trauma and electrode insertion forces as well as improve chronic recording. The
technique can also help in the development and deployment of flexible biocompatible electrodes. In the last part of this dissertation, the safety of employing magnetic resonance imaging as a non-invasive imaging modality for real-time tracking of the longitudinal tissue changes around the electrode is demonstrated. It is shown that neural firing events are consistently detected even after numerous imaging sessions thereby pointing to the viability of the tissue close to the uninsulated recording site of the microelectrode. Taken together, these contributions lay the ground-work for a multitude of studies to realistically measure electrode performance and better understand interface degradation thereby enabling improved identification and incorporation of modifications to electrode structure, implantation and monitoring processes with the aim of extending microelectrode functionality.
TABLE OF CONTENTS

LIST OF FIGURES ................................................................................................................. vii
LIST OF TABLES ................................................................................................................... ix
ACKNOWLEDGEMENTS ..................................................................................................... x

Chapter 1  Introduction ............................................................................................................ 1
  Current state-of-art of the microelectrodes ................................................................. 1
  Recording Performance ............................................................................................... 2
  Failure Modes .................................................................................................................. 3
    Encapsulation ............................................................................................................... 3
    Loss of Neurons .......................................................................................................... 5
    Initial insertion damage and dimpling of the Brain .................................................. 6
  Real-time Monitoring Method – Impedance Measurements ...................................... 6
  Motivation for Objective Neural Recording Analysis ................................................. 7
  Motivation for Improvements to Implantation Techniques .......................................... 8
  Motivation for Non-invasive Implant Monitoring ....................................................... 9
  References ...................................................................................................................... 11

Chapter 2  Algorithm for Neural Recording Quantification .................................................... 16
  Abstract ............................................................................................................................ 16
  Introduction ...................................................................................................................... 18
  Material and Methods ...................................................................................................... 25
    Neural Data Acquisition .......................................................................................... 25
    Inter-electrode Correlation (IEC) ............................................................................ 25
      Determination of Correlation Threshold ............................................................... 26
    Different Algorithms for Spike-Detection ............................................................... 26
      Objective Assessments ........................................................................................... 27
    Mean-Spike Comparisons ....................................................................................... 28
      Identification of Mean-Spike Features ............................................................... 28
    Data Selection for Comparative Analysis ............................................................. 28
    Comparison of Signal Processing Techniques for Spike Detection ......................... 29
    Mean-Spike Generation and Feature Analysis ......................................................... 31
  Results .............................................................................................................................. 32
    Correlation Coefficient Threshold ........................................................................... 32
    Trends of Mean-Spike Features .............................................................................. 33
    Results of Objective Assessment ............................................................................ 35
      Effect of Correlation on Simple Thresholding .................................................... 37
      Effect of Correlation on PCA based detection .................................................... 37
      Reduction in False Positives .............................................................................. 38
    Comparative Analysis Overview ............................................................................ 38
    Mean-Spike Shape – Comparative Analysis ............................................................ 38
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>89</td>
</tr>
<tr>
<td>Introduction</td>
<td>89</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>92</td>
</tr>
<tr>
<td>Surgical Procedure</td>
<td>92</td>
</tr>
<tr>
<td>Construction of an MRI compatible electrode array</td>
<td>92</td>
</tr>
<tr>
<td>Electrophysiological Recordings</td>
<td>94</td>
</tr>
<tr>
<td>Magnetic Resonance Imaging</td>
<td>95</td>
</tr>
<tr>
<td>Histology</td>
<td>97</td>
</tr>
<tr>
<td>Results</td>
<td>99</td>
</tr>
<tr>
<td>Electrophysiological Measurements</td>
<td>100</td>
</tr>
<tr>
<td>Tissue damage assessed by MRI T2 maps</td>
<td>101</td>
</tr>
<tr>
<td>MRI-temperature mapping</td>
<td>103</td>
</tr>
<tr>
<td>Histology</td>
<td>104</td>
</tr>
<tr>
<td>Discussion</td>
<td>105</td>
</tr>
<tr>
<td>Conclusion</td>
<td>107</td>
</tr>
<tr>
<td>References</td>
<td>108</td>
</tr>
<tr>
<td>Chapter 5 Future Directions</td>
<td>112</td>
</tr>
<tr>
<td>Algorithm for Neural Interface Quantification</td>
<td>112</td>
</tr>
<tr>
<td>Enhancement: Collagenase-Aided Intracortical Microelectrode Array Insertion:</td>
<td>114</td>
</tr>
<tr>
<td>Effects on Insertion Force and Recording Performance</td>
<td>114</td>
</tr>
<tr>
<td>Monitoring: Feasibility and Safety of Longitudinal Magnetic Resonance Imaging in Rodent Model with Intracortical Micro-wire Implants</td>
<td>115</td>
</tr>
<tr>
<td>Summary</td>
<td>116</td>
</tr>
<tr>
<td>Appendix A Chronic Microwire Electrode Fabrication</td>
<td>118</td>
</tr>
<tr>
<td>Appendix B Surgical Procedure and Histology Details</td>
<td>124</td>
</tr>
<tr>
<td>Appendix C Code used for Implementation of IEC Algorithm</td>
<td>131</td>
</tr>
<tr>
<td>Appendix D Histological Complications</td>
<td>147</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

Figure 1-1: Graphical representation tissue response following microelectrode implantation. ................................................................................................................. 5

Figure 2-1: Graphical representation of microelectrode implant and recorded extracellular multiunit activity. ........................................................................................ 20

Figure 2-2: Conventional Signal Processing Chain. .......................................................................................................................... 21

Figure 2-3: Problem Description. .......................................................................................................................... 23

Figure 2-4: Signal Processing Overview. .......................................................................................................................... 27

Figure 2-5: Objective Data Selection .......................................................................................................................... 29

Figure 2-6: Signal Processing Overview – Comparison Study. .......................................................................................................................... 30

Figure 2-7: Subjective Assessments for determination of optimal correlation coefficient. .......................................................................................................................... 33

Figure 2-8: Results of Subjective Assessments. .......................................................................................................................... 34

Figure 2-9: Representative Mean-spike shapes. .......................................................................................................................... 36

Figure 2-10: Summary of Mean-spike feature analysis. .......................................................................................................................... 36

Figure 2-11: Examples of Mean-spike shapes. .......................................................................................................................... 39

Figure 2-12: Mean-spike Features. .......................................................................................................................... 41

Figure 2-13: Comparison of Spike-Detection Rate. .......................................................................................................................... 42

Figure 3-1: Surgical and Experimental details. .......................................................................................................................... 63

Figure 3-2: Neural recording performance analysis. .......................................................................................................................... 64

Figure 3-3: Examples of insertion force vs. depth profiles for microwire array insertions. .......................................................................................................................... 68

Figure 3-4: Force results for the acute insertion study. .......................................................................................................................... 70

Figure 3-5: Maximum force results from the chronically implanted animals. .......................................................................................................................... 72

Figure 3-6: Signal-to-noise ratio (SNR) and mean peak-to-peak amplitude (mP2P) vs. time. .......................................................................................................................... 74

Figure 3-7: Distribution of peak-to-peak amplitudes of mean waveform. .......................................................................................................................... 76
Figure 3-8: Histology follow-up indicating differences in cellular activation..........................77

Figure 3-9: Histology follow-up indicating extensive damage.............................................77

Figure 3-10: Histology follow-up at depths close to the recording site indicating normal
damage. ....................................................................................................................................78

Figure 4-1: Image distortion and Custom micro-wire electrode assembly to reduce it. ...........93

Figure 4-2: Neural Data Analysis. ..........................................................................................95

Figure 4-3: Histology Image Quantification. ..........................................................................99

Figure 4-4: Extracellular Multiunit Recordings. ....................................................................100

Figure 4-5: Analysis of neural recordings, Signal-to-noise ratio..........................................101

Figure 4-6: Example of T2 variability. ..................................................................................102

Figure 4-7: T2 value analysis..................................................................................................102

Figure 4-8: MRI-based temperature mapping in a gel phantom with "implanted"
microelectrodes. ....................................................................................................................103

Figure 4-9: Histology Analysis. .............................................................................................105
LIST OF TABLES

Table 1: Rater Statistics for Data-sets E1 and E4. ..........................35
Table 2: Summary of feature analysis on mean-spike outcomes............40
Table 3: Peak Force Summary.................................................................71
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Today, implantation of devices in brain tissue for therapeutic purposes has become commonplace. Applications include deep brain stimulation, auditory brainstem neuroprostheses, visual cortical stimulation, and more recently brain-machine interfacing using cortical microelectrode arrays [1-6]. Microelectrodes used in cortical implants enable high-resolution in-vivo recording of neuronal activity to understand complex neural processes. The knowledge thus obtained can potentially be used in developing algorithms for future brain-machine interfaces and for understanding the neurophysiological basis of learning and plasticity.

**Current state-of-art of the microelectrodes**

The ability to record from individual neurons using electrodes implanted in the cerebral cortex was demonstrated as early as 1958 [7]. Since then, a variety of electrodes have been developed. These are inserted in the brain after a standard craniotomy and fixed to the subject’s skull with dental acrylic. Inexpensive, hand-fabricated microwires remain the electrodes of choice for many neural engineering labs. To address the issues of reproducibility and precision, micromachining and photolithography are employed in fabrication of 3-D arrays and planar probes made from silicon or ceramic substrate [8, 9]. On-board circuitry, embedded micro-fluidic channels and post-implant micropositioning capabilities are some of the recently added features that can potentially improve the functionality of the electrodes [10-12]. Some groups have developed flexible electrodes with polymer substrates that can reduce the mechanical impedance mismatch between the brain and the implanted electrodes thereby potentially improving its
neurocompatibility [13]. In addition to the variation in the electrode structures, multiple insertion and anchoring strategies are currently being pursued. The insertion speeds vary from 100µm/min to 600µm/min. The former allow the brain to gently adapt to the insertion, while the latter are aimed at reducing brain dimpling [14, 15]. The implanted electrodes can be anchored to the skull or allowed to float with the brain via a compliant cable [15, 16].

In summary there are a variety of neural probes utilizing an array of materials, manufacturing processes, implantation techniques, and anchoring strategies. With a general lack of understanding on the most optimal electrode designs and implant strategies, chronic neural recording remains much of an art with each group (and sometimes even individuals within a group) adopting their own set of tried and true techniques.

**Recording Performance**

The best measure of electrode performance is its ability to chronically record neural activity. There are reports in the literature demonstrating the functional capabilities of microwires, 3-D silicon type and planar arrays for over 1 year [14, 17, 18]. Although these results are impressive, the average recording yield of these electrodes is less than acceptable for widespread clinical application. According to a recent survey, any given recording site has only 40-60% chance of recording neural activity chronically [19] and all interfaces have a limited recording life.
Failure Modes

There are multiple modes of failure. Some are known and some are postulated. The known failure modes result from the influence of the body on the electrode system. These include material failures of the probe such as fluid infiltration between the insulating layers and the conductor (e.g., delamination), water absorption and/or break-down of the insulating layer, breaks in the electrode leads or the skull mounted connector, or loosening of the acrylic headcap which houses the electrode connector [20]. For the most part, the probability of these types of failure can be limited by proper choice of materials, packaging, and surgical technique.

The other category of failures which relate to the effect the implanted probe has on surrounding tissues and is much more complex, difficult to monitor, and remains largely speculative. There are two main routes by which the brain tissue response to the implanted probe can affect neural recordings: 1) the encapsulation tissue that attempts to wall off the neural probe may block the current path to the neural sources or 2) the nearby neural sources themselves are absent or silent due to relative movement of the electrode, chemical imbalances, or cell-death. It is also postulated that these effects are aggravated by dimpling of the brain due to initial insertion, micromotion of the brain and mechanical impedance mismatch between the stiff probes and softer brain tissue [21].

Encapsulation

Encapsulation is the formation of compact cellular sheath around the electrodes. It is the result of the reactive tissue response involving astrocytes, microglia and other phagocytic cells [22]. Studies have revealed that encapsulation varies with time. The first few hours is dominated
by hemorrhagic necrosis caused by insertion damage which likely activates the local microglia to begin proliferation. Proteins also adsorb to the device surface at this time and are thought to help mediate the ensuing tissue response. Within a few days, a mild reaction of the astrocytes is typically observed while the microglial cells accelerate proliferation. After about a week new capillaries typically form and the necrotic zone becomes diminished as the cellular debris is devoured by phagocytic cells. At the same time the astrocytic response continues to ramp up and at this point collagen fibers can also begin to form around the implant. Significant edema also appears to be present during the early phase. Beyond a month the immune response tends to trail off as a compact encapsulation matrix composed of collagen fibrils, leptomeningeal cells and hypertrophied astrocytes walls off the implant [22, 23]. Recent studies have shown that there are two phases to the implant: acute and chronic. The acute response is dominated by the initial tissue damage (proportional to cross-sectional area of the probe) but the response after 4 weeks was essentially equivalent for all probes tested [24]. Thus, the unavoidable result upon implanting a neural probe is some degree of encapsulation. Figure 1 provides a pictorial view of this process.
Encapsulation is implicated in the deterioration of the electrode performance. It is hypothesized that the higher resistivity of the encapsulation sheath (as compared to the gray matter) impedes the flow of extracellular currents to the recording surface of the electrodes. This explanation although intuitive is contrary to the results obtained from a recent modeling paper that suggests an increase in resistivity might actually boost the sensitivity of the electrodes and enable larger recording of spike amplitudes [25]. Thus the role played by encapsulation in changing the electrode performance over time is not exactly understood.

**Loss of Neurons**

Another potential cause for implant failure might be the loss of neurons around the electrodes. A recent study has shown that the loss of neurons is a result of foreign body response...
and not that of the initial insertion injury. This was observed by comparing the histological results that measured neuronal survivability in chronic implants and stab controls. Chronic implants showed sustained reduction in the nerve fiber and nerve cell bodies. Such depletions were not observed in acute stab controls [26].

**Initial Insertion Damage and Dimpling of the Brain**

Insertion of the electrodes into the brain through the pia-mater is accompanied by compression of the brain tissue. Depending on the speed of insertion, this compression can be as high as a couple of millimeters. Studies related to Traumatic Brain Injury (TBI) have shown that such compression results in neuronal death, release of excitatory neurotransmitters, necrosis of cortical tissue at the site of impact and changes in the blood flow. Proponents of high-speed insertion have always maintained that compression plays a role in deteriorating electrode performance. This idea is strengthened further by a recent report that shows improved long-term recording in high-speed implants characterized by reduced dimpling of the brain as compared to slow insertions that resulted in considerable dimpling [27].

**Real-time Monitoring Method – Impedance Measurements**

Electrode impedance measurement is the only real-time implant monitoring technique that is currently in use. Impedance is measured between the electrode of interest and the skull anchored bone-screw that acts as ground. Measurement is carried out using an impedance meter by sending nano-amp range current waveforms at specific frequency (500kHz or 1kHz) or over a range of frequencies and monitoring the corresponding voltage drop. The ratio of voltage measurement and known current inputs yields impedance. The underlying logic for impedance
measurement is that an increase in glial scar and the associated restriction to current flow will be reflected in impedance changes. Thus scar tissue evolution can be reasonably predicted by impedance measurements. There are technical reports that point to a mixed trend followed by impedance values of the electrodes. Williams [28] reported a steady increase in the microwire impedance to about 200kΩ 10 day post-implant followed by a drop and stabilization to about 100kΩ. Vetter et al found similar impedance increases over the first couple weeks with Michigan electrodes, which then remained stable for the duration of the experiment [29]. However, impedance is not a specific enough measurement of the interface quality and its degradation because it measures impedance between the electrode and the ground/bone-screw and hence may circumvent the glial-scar if it is not tightly attached to the recording electrode sites. Neural cell death may not be predicted by impedance measurements as well.

**Motivation for Objective Neural Recording Analysis**

A major short-coming of all the studies aimed at evaluating the tissue-electrode interface from a histological or impedance stand-point is that they do not establish any relation with the electrodes functional abilities i.e. neural recordings. This is because most of the electrodes used in the previously mentioned histological studies (see “Failure Modes”) are non-functional with no recording sites. Challenges associated with implanting electrode systems having recording sites and percutaneous connectors, high cost and very low availability of wireless electrode systems and lack of agreed upon standards for objectively analyzing long duration neural recording data may be some of contributing factors. One reason may also be that conventional neural recording analysis algorithms have been developed to address problems associated with spike-sorting. In
this approach emphasis is given to understand the origins of a given spiking event as opposed to assessing its quality.

Typically neural recordings are analyzed in an offline setting. Specialized software is used to detect neural spiking events from the raw data. As a first step a subjective amplitude threshold is set to identify a preliminary set of potential neural spikes. These are then sorted using different “feature-space” schemes like principal component analysis (PCA) [30]. Once sorted, the neural spikes are associated with specific behavioral tasks of the animal based on their timestamps. This is a highly subjective process wherein user intervention in selecting parameters of the feature-space like clusters in PCA space as well as time-intervals in raw neural recordings around occurrence of behavioral tasks may result in significant labor and time intensive iterations. From a neural-interface characterization stand-point such processing steps are not required and are not feasible since large quantities of data needs to analyzed. The requirement is to accurately detect neural spikes from the raw data and then quantify the quality of neural spikes by using parameters like signal to noise ratio, peak to peak signal amplitude, rate of neural spike firing etc. Ideally, this analysis should be carried out in an automated and objective manner to enable unbiased neural recording quality comparisons across multiple electrode implant systems (including type of electrode arrays, implantation technique, drug delivery comparisons etc.) Hence in first part of this thesis an algorithm was developed to process large quantities of real-data with “realistic” noise characteristics in an objective and automated fashion.

Motivation for Improvements to Implantation Techniques

Numerous approaches are being pursued by researchers to reduce the immune response of the brain to microelectrode implants. These include development of biocompatible coatings around the electrodes [31], systemic delivery of immunosuppressants [32], and structural
manipulations of the electrodes [13]. The latter involve development of flexible polymer based substrate [13] or use of thin electrodes [33] that have shown to significantly reduce the immune-response and hence may help to improve electrode performance. Such reports are also well supported with modeling studies that show reduced stress in the brain around flexible electrodes [34]. However implantation of these flexible structures requires cutting of the meninges (pia mater) [13] and/or providing temporary backplane support [35] for initial implantation. This in turn increases the initial insertion trauma and compression of the brain that is known to degrade neural recording performance and thus may defeat the purpose of implanting flexible electrodes. Hence in the second part of this thesis a technique is evaluated to structurally weaken the meninges thereby enabling implantation with reduced compression and insertion trauma. It is shown that this technique not only helps in reducing the insertion forces but in also improving the chronic neural recording performance of the electrodes as evaluated by the previously developed neural recording performance algorithms.

Motivation for Non-invasive Implant Monitoring

Histological evaluation remains the gold-standard for studying the tissue-electrode interface. The end-point nature of it results in limited scope for studies that may try to correlate histology results with neural recordings. Hence there is a need to investigate imaging techniques that may provide a reasonable estimation of the interface health without the need to sacrifice the implanted animal. This may allow a first-order correlation between imaging results and neural recording results. Magnetic Resonance Imaging (MRI) provides excellent soft-tissue contrast. Thus it can be used to monitor structural changes associated with glial-scar development, edema, and changes in neural-glial cell densities and link them with the quality of neural recordings. It can also provide a hemisphere-wide view of the brain and help determine any broad scale
difference between the implanted and unimplanted hemisphere. An initial first-step in this process is the evaluation of safety and compatibility of performing MRI with implanted electrodes. This is because MRI involves use of strong static magnetic fields, rapidly alternating magnetic field gradients and most importantly high power pulses of electromagnetic energy at many megahertz frequencies. The latter in particular may induce currents within implanted structures, heat them and cause damage particularly at the uninsulated recording/stimulating tip [36]. Hence the third part of this thesis describes a study that demonstrated the longitudinal safety and feasibility of imaging implanted microwire electrode arrays using MRI in rodent models.

The overall goal of the project was to develop algorithm to quantify functional performance of neural microelectrodes, test methods to potentially improve their functional performance and evaluate approaches to enable a multi-modal view of microelectrode-tissue interface. Thus the work presented in this document addresses the need for development of an automated, objective and robust neural recording quantification approach in Chapter 2. Chapter 3 of this document proposes and evaluates a novel approach of reducing insertion forces and associated trauma involved in electrode implantation. Chapter 4 documents experiments related to chronic evaluation of MR safety and feasibility in microwire electrode array implanted rodent models. Chapter 5 provides a brief description of future directions and conclusions. General methods associated with electrode fabrication, surgery, imaging, histology and algorithm development are presented in Appendix. Complications associated with histological procedures that precluded subsequent staining and analysis are also summarized in Appendix.
References


Chapter 2

Algorithm for Neural Recording Quantification

Abstract

Intracortical microelectrode arrays record multi-unit extracellular spike activity used in deciphering neural basis for learning, plasticity and as command signals for brain-machine interfaces. The first step in these applications is the detection of neural spike activity above the background noise floor; a process complicated by presence of similar non-neuronal signals (in magnitude, spectral, and temporal features) originating from motion artifacts, electromyographic activity, and electric field pickup, especially in awake and behaving subjects. The distal origin and greater strength of non-neuronal signal sources result in their near simultaneous registration on most electrodes, while true neural spiking events are rarely recorded on more than one electrode of an array. This difference is exploited in the inter-electrode correlation (IEC) algorithm that is introduced in this chapter as a means to objectively quantify neural data while reducing the effect of non-neuronal source contribution. The efficacy of the method is first evaluated by comparing outcomes from conventional algorithms that use absolute threshold discrimination and/or Principal Component Analysis (PCA) as a means of identifying neural spikes with the same methods incorporating correlation analysis. A follow-up study is then described that compared the performance of the IEC algorithm with standard differential referencing (DR), and a new technique "virtual referencing" (VR), under different levels of common noise contamination. By evaluating characteristics of the mean-spike waveform produced by each method under different common noise conditions, it is found that IEC consistently offered the most robust means of neural spike-detection. Furthermore it reduced
false-positive detection rates and could therefore reduce downstream spike processing requirements.

Key Words: Spike detection, multi-unit recording, common-noise, inter-electrode correlation, common average referencing
Introduction

Detailed study of multi-unit spike activity from neuronal assemblies is essential to further our understanding of neurophysiology involved in learning and plasticity [1] and developing algorithms for effective brain machine interfaces (BMI). Intra-cortical microelectrode arrays offer the spatial and temporal resolution to record extracellular multi-unit spike activity in vivo that is a fairly accurate measurement of underlying neural activity. Evidence for such assertions comes from studies wherein simultaneous intracellular and extracellular recordings have shown that large inward current associated with sodium intake is observed as a negative deflection in extracellular recordings and the positive deflection is associated with outward currents from the dendritic regions [2]. A further evidence of actual intracellular events being reflected by extracellular recordings is that the first derivative of intracellular action potential is similar to the extracellularly recorded biphasic spike. The first derivative effect is presumed to be caused by capacitance of neuronal membrane. Thus multi-unit extracellular recordings from microelectrodes can be used to predict intracellular events like ionic influx and efflux and hyperpolarization with high fidelity and hence serve as reliable information sources to understand brain’s computational rules. Other forms of neural recordings like local field potentials (LFP) and electroencephalograms (EEG) are at best a gross estimation of the underlying neural activity.

The ability to record from population of neurons using electrodes implanted in the cerebral cortex was demonstrated as early as 1958 [3]. Since then, a variety of electrodes have been developed. They range from inexpensive, hand-fabricated microwires to micromachined planar and 3-D electrodes with silicon, ceramic or polymeric substrate [4-7]. Each of these arrays is implanted in the brain following standard craniotomy. Once implanted, they transducer
extracellular spike activity into voltage signals that are amplified and stored for further analysis (Figure 2.1).
Figure 2.1: Graphical representation of microelectrode implant and recorded extracellular multiunit activity. A) Microelectrode targeting layer IV. Most of the recordings in this thesis are from layer V of the rat-cortex. B) Example of raw-recordings on the electrode. Notice the occasional high-amplitude neural spiking events. C) Zooming on these events shows typical spike shape with depolarization followed by repolarization. Slight difference in spike shapes may suggest that observed spikes originate from different neurons. (Courtesy: J.C. Williams)
Neural spiking events are typically detected using a pre-defined threshold on the recorded electrode followed by template-matching, Principal Component Analysis (PCA), clustering techniques, or Bayesian filters [8-12] to select events with neuronal characteristics (Figure 2.2). Some of these techniques as well as others have been employed in spike sorting applications with goal of associating individual neurons with behavioral tasks [1, 13]. Although considerable sophistication has been achieved in spike-sorting techniques [14], little attention has been given to the operation of spike-detection that precedes sorting.

**Figure 2.2: Conventional Signal Processing Chain.** A) Signal segments are identified by setting an amplitude threshold. Based on the brain region being recorded from, a 1-4 ms signal segment (top right) is captured around threshold excursion. B) Signal segments are classified using Principal Component Analysis (PCA) wherein clusters in 2-D PCA space are considered to be originating from the same source. C) Template matching is also employed in which each detected segment is classified with a set of template-segments to find the closest match.
Experience with behavioral paradigms in rodents [17-19] suggests that in addition to multi-unit neural spike activity, microelectrodes also record electromyographic activity (EMG) from muscles in the body, especially mastication signals (EMG generated by animal chewing), and relatively large signals generated by abrupt animal movements or interference with the recording setup by awake and active subjects. These non-neural signals (defined later) are similar to neural signals in their spectral and temporal characteristics. As shown in figure 2.3, the shape, frequency content and even the projection in 2-D PCA space of neural and non-neural signals has remarkable similarities. Hence spike-detection schemes that involve threshold-based neural-spike detection on an electrode by electrode basis may suffer from false-positive detection thereby negatively impacting downstream spike-sorting operations as well as increasing power and processing requirements of the wireless headstage. In addition, they will provide an inaccurate estimate of the health of the electrode-tissue interface as obtained from quality of neural recordings.

However, there are some differences between neuronal and non-neuronal threshold exceeding events. Recent modeling studies have determined that signal amplitude of layer V pyramidal cells drops substantially with increase in distance between the firing cell and recording site [20, 21]. Hence it can be argued that similar neural-like signals appearing concurrently on distantly (=>250µm) spaced electrodes may not be originating from pyramidal (neural) cells. Given the computational attractiveness of threshold-based detection technique, it is desirable to employ equally simple “signal cleaning” schemes that may enable improvements in spike detection outcomes.
Traditionally, differential recording between electrode of interest and a "quiet electrode" implemented in hardware [17] or post-recording inter-electrode subtraction is employed to ameliorate the situation. However, slight temporal shifts in non-neuronal signals across electrodes can cause asynchronous cancelations thereby resulting in artificial spike-like outputs. Moreover presence of neural spikes on the reference electrode may affect differential recording outcomes. Hence other techniques need to be investigated.
Here, an approach termed as “inter-electrode correlation” (IEC) is introduced that utilizes data from other electrodes of an array in deciding the non/neuronal origin of a threshold exceeding segment. A high degree of correlation between a threshold exceeding segment on a given electrode and that of concurrent segments on other distantly spaced electrodes (≈250µm apart) is considered indicative of the non-neuronal origin of the threshold exceeding segment. The technique does not require a training set or supervision. It is verified by subjective and objective measures that such identification improves performance of standard detection schemes. Following this, another technique termed “virtual referencing” is introduced that tries to obtain a representation of the common-noise recorded across the entire array. This representation is used as a differential reference to clean the signal on electrode of interest from non-neuronal contributions.

Finally, the techniques of “inter-electrode correlation” and “virtual-referencing” are compared objectively with conventional thresholding and differential recording for their ability to detect neuronal spike-like segments from real data acquired from unanesthetized rats. Data-sets with varying degree of common-noise are selected and processed through each of the four techniques. The results indicate that unlike other methods, inter-electrode correlation offers a robust solution whose outcome is not dependent on the quality of data. Moreover, it significantly reduces the number of false-positives (spikes) for subsequent steps like spike-sorting. In the process, it also allows for more accurate estimation of the electrode-tissue interface quality.
Methods

Neural Data Acquisition

The implant procedure, electrode fabrication details and recording conditions are described in detail in [22] and provided in Appendix B. Final implant (2x4 tungsten-iridium microwire array, φ50µm, 250µm inter-electrode spacing) depth was 1mm to target layer V of rat cortex (refer Figure 2.4A). Five minute long neural recordings were obtained from chronically implanted electrodes in awake Sprague-Dawley rats using a commercial multichannel acquisition system (Tucker-Davis Technologies Inc, Alachua, FL). Acquired analog signals were digitized at 25kHz, band-pass filtered between 300 Hz - 5 kHz and downsampled at 12 kHz and then stored for offline analysis. Subsequent processing and analysis was carried out using MATLAB (The Mathworks, Natick, MA).

Inter-electrode Correlation (IEC)

In the implementation of the IEC algorithm, signal segments that exceeded the $3\sigma$ threshold were identified and extracted from the raw recording as candidate neural spikes. For each candidate spike identified on an electrode, concurrent segments from remaining electrodes of the array were also extracted (Figure 4D) and stored in a spike-matrix. Correlation coefficients were then computed between the candidate spikes and these concurrent segments. Thus for an eight electrode array, this resulted in seven correlation coefficients. If any of the coefficients exceeded a threshold (described next), the spike was rejected as not originating from a local neuron and hence eliminated from subsequent analysis.
**Determination of Correlation Threshold**

The value of the inter-electrode correlation threshold was selected after exhaustive analysis utilizing the expertise of a researcher (unbiased rater) with experience in spike detection and sorting. In this assessment, the maximum of the correlation coefficients was evaluated as a discriminant for a selected training set of candidate waveforms (segments exceeding \(-3\sigma\)) from data having different levels of common-noise. A selection of candidate waveforms were presented to the unbiased rater who was charged with categorizing the spike as neuronal or not. Two probability density plots were obtained for the values of the maximum correlation coefficient for events classified by the rater as neural spikes, and those that were not. Receiver Operating Characteristic (ROC) curves which plotted the true-positive rate (determined by rater) vs. false-positive rate as a function of the correlation coefficient threshold. The correlation threshold was then selected to be a value that was around the knee of the ROC curves in all three cases of common-noise. This ensured an optimal trade-off between false negatives and false positives i.e. probability of equal error. These results are presented in Fig. 2.7.

**Different Algorithms for Spike-Detection**

Four algorithms were used to identify neural spike segments (from all threshold exceeding segments) to be considered in the generation of mean spike that may be considered as a good representation of neuronal activity (see Fig. 2.4). In standard thresholding (‘st’) all segments that exceeded \(3\sigma\) were considered, in standard thresholding followed by inter-electrode correlation (‘stC’) only segments that satisfied the low correlation criteria were considered (Fig. 2.4D). In (‘stpca’) and (‘stCpca’), PCA was applied on segments selected by each of the two methods (‘st’) and (‘stC’) respectively. Segments that were present in high density regions of the two-dimensional PCA space were then subjectively selected for further consideration (Fig. 2.4E).
For each of the methods, a mean-spike was generated by taking pointwise average of all the identified spike segments aligned to the depolarizing minima, as shown in Fig.2.4F.

**Objective Assessments**

The goal of objective assessments was to compare mean-spike outcomes from the four algorithms (st; stC; stpca; stCpca) and determine improvement in the process of spike detection by using InterElectrode Correlation (IEC) in an unsupervised manner. Five minute data segments (N = 10) were chosen to emulate neural recording situations typical of a BMI experiment. Mean spike feature analysis was carried out to determine the repeatability of trends observed in subjective assessments (described next).

**Experimental Overview: Signal Processing**

![Experimental Overview: Signal Processing](image)

**Figure 2.4. Signal Processing overview.** A) Electrode Array. B) Simultaneous recordings from 8-channel array. C) Signal segments that exceeded a threshold of 2.5 standard deviations of the signal (green line) were extracted as 3-ms candidate neural spikes. D) The correlation algorithm seeks to eliminate candidate spikes that are highly correlated with the signal segments recorded simultaneously on other electrodes in the array. E) Candidate neural spikes from ’st’ and ’stC’ on 2-D PCA space to identify regions of higher density. F) Mean-spike generation and feature calculation.
Mean-Spike Comparisons

Identification of Mean-Spike Features

Subjective assessments were made on two subsets of the original data recorded 1-day post-implant and hence having distinct neuronal spike activity. The goal was to determine differences between mean-spike obtained from segments that were identified by experienced rater and mean-spike obtained from segments that exceeded the threshold. The rater was asked to rate a signal segment as spike, non-spike or ambiguous based on the shape of the segment on electrode of interest and that of concurrent segments on remaining electrodes of the array. Since the rater identified segments will most likely have higher proportion of true neuronal segments compared to only thresholding, mean spike obtained from the rater was considered as the gold-standard. Trends in features that differed between rater generated mean spike and threshold generated mean spike were identified. These features included peak-to-peak amplitude, width of the depolarizing and the repolarizing phase that differed based on the degree of neuronal and non-neuronal contributions in generation of the mean spike (ref. Figure 2.4F).

Data Selection for Comparative Analysis

The goal of this phase was to compare the ability of different conventional and newly developed techniques in improving spike detection outcomes in data having varying degree of non-neuronal threshold exceeding common-noise present simultaneously across multiple electrodes of an array. The degree of common-noise on a given electrode was calculated in two ways. In one method, an average signal was obtained from all the functional electrodes of an array (virtual reference). The percentage difference in the root mean square value (RMS) of the recorded signal on the electrode of interest and that of the average signal was calculated (Figure
2.5, blue). In the second method, the average inter-electrode correlation coefficient of the entire recording block (5 minutes) was calculated (Figure 2.5, pink) between the signal on the electrode of interest and remaining functional electrodes of the array. Eighteen data-segments exhibiting a range of RMS differences and degree of correlation were selected. These were equally distributed in three groups: low, medium and high common noise as shown in Figure 2.5.

**Data Selection**

![Graph showing data selection criteria for low, medium, and high common noise levels.](image)

**Figure 2.5: Objective Data Selection.** Electrodes with increasing level of common-noise as determined by an increasing correlation with remaining electrodes of the array and correspondingly decreasing difference in RMS value with averaged signal of the array are shown. Eighteen electrodes exhibiting a range of RMS differences and degree of correlation were selected. These were equally distributed in three groups: low, medium and high common noise.

**Comparison of Signal Processing Techniques for Spike Detection**

Four algorithms were used to identify probable neuronal signal segments from raw recordings. These algorithms included Simple Thresholding (**ST**), Differential Recording (**DR**), Virtual Referencing (**VR**) and Inter-electrode Correlation (**IEC**). Each of these algorithms are described below and depicted pictorially in Figure 2.6.

In **ST**, candidate neural spikes were detected by threshold-crossing (set to -3 times the standard deviations of raw signal, Figure 2.6C) and extracted as 3ms (39 sample points: 10
sample points preceding local minima and 29 sample points following the local minima) waveform segments. In DR, raw signal on a functional electrode with lowest RMS value was subtracted from the signal on the electrode of interest. Candidate spikes were detected in the differential signal similarly to ST. In VR, an average-signal generated from the ensemble average recording from all functional electrodes of an array was subtracted from the signal on the electrode of interest. The signal so generated was processed for candidate spikes similarly to ST.

Processing for Inter-electrode correlation was similar to that described previously.

Figure 2.6: Signal Processing Overview—Comparison Study. A) Detailed schematic of the implanted electrode array with approximate dimensions. B) Simultaneous recording from an eight channel array including the resultant ensemble average (used for virtual reference). C) Signal segments that exceeded a threshold of 3 standard deviations of the signal (green line) were extracted as candidate neural spikes (3ms segments). D) The correlation algorithm seeks to eliminate candidate spikes that are highly correlated with signal segments recorded simultaneously on other electrodes in the array. The correlation coefficient between each candidate spike and the corresponding signal segment on each of the electrodes is determined. All spikes with correlation coefficient $R > 0.75$ are classified as non-neuronal and discarded from further analysis. E) At the end of processing of each data set, a mean-spike was computed from all valid spikes detected from each of the four algorithms (simple-thresholding, differential reference, virtual reference, and inter-electrode correlation; ST, DR, VR, and IEC, respectively). Several parameters were obtained from the mean-spikes as indicated for comparison purposes.
Mean-Spike Generation and Feature Analysis

For each of the methods, a mean spike was generated by taking pointwise average of all the identified spike segments aligned to the depolarizing minima, as shown in Figure 2.6E. Mean-spike features were identified and calculated to enable objective quantitative comparison of the mean-spike outcomes from different algorithms. These features included peak-to-peak amplitude, duration of depolarization phase and duration of repolarization phase. Duration of depolarization/repolarization phase was defined as time wherein the magnitude of the mean-spike was less/greater than 10% of negative/positive peak.

These features were selected because modeling studies [20, 21] have shown that spikes with sources (neurons) closest to the recording electrode have highest peak-to-peak amplitude and lowest duration of depolarization phase. Moreover exhaustive subjective analysis undertaken in the author’s lab [23] and as discussed previously has shown that mean-spikes generated from signal segments identified to be neuronal by experienced spike-sorters have a higher amplitude and lower duration of depolarization phase as compared to those generated by segments identified by thresholding or principal component analysis. Thus it was assumed that the algorithm that produced mean-spike features which most closely matched the predictions of modeling studies (high-amplitude and corresponding shortening of depolarization phase) will have the most contributions from true neural spiking events.
Results

Initially ten datasets of five minute long neural recordings from unanaesthetized rats were used to demonstrate the ability of inter-electrode correlation in improving spike-detection outcomes. Then as part of the comparison phase wherein IEC was compared with other signal cleaning schemes, eighteen datasets were used. These datasets had a varying degree of common-noise. Four algorithms (IEC, VR, DR, ST) were used to independently detect neural spiking events in the raw recordings and these events were used to generate a mean-spike. Feature analysis was performed on the mean-spikes to evaluate and compare the efficacy of different spike-detection algorithms.

Correlation Coefficient Threshold

Subjective assessments by an experienced spike sorter (blinded to the details of this study) were utilized to help determine the correlation coefficient threshold for the IEC algorithm. To do this, the sorter was provided three data-sets containing threshold-detected candidate spikes, one from each common-noise category (Fig. 2.5). The rater was then asked to separate what appeared to be individual neural spike events from non-neuronal events. Next, the probability distribution of maximum inter-electrode correlation coefficients was determined for the neural events (rater determined true positives) and non-neuronal events (rater determined true negatives). Since the distributions overlapped, any threshold chosen for the maximum correlation coefficient will likely result in a finite false-positive rate. The maximum correlation coefficient threshold was varied between zero and one and the resulting true-positive detection rate was
plotted vs. false-positive rate for each threshold setting, resulting in the Receiver Operating Characteristic (ROC) curve. As shown in Fig. 2.7A-C, the equal likelihood point (when false-positive rate equaled the falsenegative rate) was between 0.75-0.82 in all three cases. A conservative threshold to distinguish between potentially neuronal (R<0.75) and potentially non-neuronal segments (R>0.75) was chosen. While it was required to utilize a subjective spike-sorter to help determine a good value for the correlation coefficient threshold, all subsequent analysis which was performed on other data sets utilizing the IEC algorithm was strictly objective and automated.

Figure 2.7: Subjective Assessments for determination of optimal correlation coefficient. A-C) Receiver Operating Characteristic (ROC) for a data segment with low, medium and high common-noise. The point of equal error is around 0.8. Hence selection of 0.75 as a correlation coefficient threshold is very conservative and may allow for slightly increased chance of false-detects as compared to missed-detects.

Trends of Mean-Spike Features

Mean spikes were generated from two datasets obtained using recording data from two electrodes one day post implant. They underscored the assumption that neuronal contribution will be the greatest in mean spike generated by rater-identified signal segments and it will be the least in the mean generated from segments identified by simple thresholding. Specifically, modeling
studies have shown that spikes with sources (neurons) closest to the recording electrode have highest peak-to-peak amplitude and lowest duration of depolarization phase [8]. It was observed that amongst all the mean spikes, those generated from rater identified (and hence most neuronal like) signal segments had the highest amplitude and lowest duration of depolarization phase whereas mean spike generated by only thresholding (st) had lowest amplitude and highest duration of depolarization phase. Mean spike generated by (stC) had intermediate features. These results are summarized in Figure 2.8 and Table I.

![Figure 2.8. Subjective Assessments](image)

**Figure 2.8. Subjective Assessments** for determination of trends in features of mean spike that differ on the basis of approaches adopted to select segments that are used in generation of mean spike. A) Graph showing the trend of negative-correlation between mean spike amplitude and duration of depolarization phase for segments identified by the rater (1), and segments identified by simple thresholding (2). B) Representative mean spike outcome for one date-set E4 obtained by applying ’st’, and rater on artificial data-sets
in the mean-spike generated by rater identified segments and threshold. Next, the ability of 
mes produced by rater 
was investigated on large set of real-data using two common detection 
schemes: thresholding and PCA.

Results of Objective Assessment

Real-data from awake and behaving subjects 3-4 weeks post-implant was used (N=10). 
Previously mentioned spike features were extracted and these results are summarized in Figure 
2.10. Figure 2.9A shows representative mean spike shape generated using ‘st’ and ‘stC’. Figure 

<table>
<thead>
<tr>
<th>Dataset $E1$</th>
<th>st</th>
<th>stc</th>
<th>Rater</th>
</tr>
</thead>
<tbody>
<tr>
<td>Segments Exceeding Threshold</td>
<td>1834</td>
<td>1834</td>
<td>1834</td>
</tr>
<tr>
<td>Spikes</td>
<td>1864</td>
<td>1546</td>
<td>1050</td>
</tr>
<tr>
<td>Non-neuronal content</td>
<td>0</td>
<td>318</td>
<td>393</td>
</tr>
<tr>
<td>Ambiguous</td>
<td>0</td>
<td>0</td>
<td>421</td>
</tr>
<tr>
<td>Time Taken</td>
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<td>1 min</td>
<td>37 min</td>
</tr>
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<td>Depolarizing Phase ($-ve$) ($\mu$s)</td>
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<td>442.4</td>
<td>436</td>
</tr>
<tr>
<td>Repolarizing Phase ($+ve$) ($\mu$s)</td>
<td>1094.4</td>
<td>1060</td>
<td>1046.4</td>
</tr>
<tr>
<td>Peak to Peak Amplitude ($\mu V$)</td>
<td>112.5</td>
<td>113.03</td>
<td>134</td>
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<table>
<thead>
<tr>
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<th>st</th>
<th>stc</th>
<th>Rater</th>
</tr>
</thead>
<tbody>
<tr>
<td>Segments Exceeding Threshold</td>
<td>1940</td>
<td>1940</td>
<td>1940</td>
</tr>
<tr>
<td>Spike segments</td>
<td>1940</td>
<td>1606</td>
<td>996</td>
</tr>
<tr>
<td>Non-neuronal segments</td>
<td>0</td>
<td>334</td>
<td>942</td>
</tr>
<tr>
<td>Ambiguous</td>
<td>0</td>
<td>0</td>
<td>421</td>
</tr>
<tr>
<td>Time Taken</td>
<td>1 min</td>
<td>1 min</td>
<td>70 min</td>
</tr>
<tr>
<td>Depolarizing ($-ve$) Phase ($\mu$s)</td>
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<td>459.2</td>
<td>422.4</td>
</tr>
<tr>
<td>Repolarizing ($+ve$) Phase ($\mu$s)</td>
<td>1054.4</td>
<td>982.4</td>
<td>1008.8</td>
</tr>
<tr>
<td>Peak to Peak Amplitude ($\mu V$)</td>
<td>102.0</td>
<td>105.8</td>
<td>143.75</td>
</tr>
</tbody>
</table>

**TABLE I**

**RATER STATISTICS FOR DATA SETS $E1$ AND $E4$**

Thus subjective assessments helped in determining the trends that differed distinctly in 
the mean-spike generated by rater identified segments and threshold. Next, the ability of 
correlation in improving mean spike outcomes so as to approach the outcomes produced by rater 
generated mean-spike was investigated on large set of real-data using two common detection 
schemes: thresholding and PCA.
2.9B shows the resulting mean spikes. Figure 2.9B (inset) shows the spread in 2-dimensional PCA space of signal segments detected using ‘stpca’ and ‘stCpca’ algorithms.

**Figure. 2.9. Representative mean spike shapes** and 2D PCA space spread pre and post-correlation. A Mean spike generated after threshold-detection (red) and that generated after threshold-detection and correlation based elimination (blue) are shown. B) Mean spikes generated before and after PCA. INSET: The square on the left shows the spread of 2D PCA space prior to correlation based elimination of signal segments and the square on the right shows the spread after correlation enabled elimination. Notice the reduction in its spread and improvement in clustering that can aid a sorter in easily identifying spike groups.

**Figure. 2.10. Summary of Mean-spike feature analysis. Clockwise:** Incorporation of inter-electrode correlation resulted in a slight decrease of the mean-spike amplitude. Elimination of non-neuronal events not affected by interface degradation may explain this. The repolarization and depolarization phases decreased significantly thereby making the overall appearance of the mean-spike much more neuronal. The number of events also reduced significantly.
**Effect of Correlation on Simple Thresholding**

Correlation-enabled elimination of thresholded spike-like segments resulted in statistically significant changes (t-test: paired two-sampled for means) in all the characteristics of the resulting mean spike. As shown in Fig. 2.10, the duration of the depolarization and repolarization phase reduced (p < 0.001 and p < 0.01, respectively). The peak-to-peak amplitude also reduced substantially in most cases. This result although contrary to that obtained from subjective assessments wherein amplitude of rater identified mean-spike was higher than threshold generated mean-spike, pointed to the efficacy of correlation-based elimination in removing only the non-neuronal signal (see Discussion).

**Effect of Correlation on PCA based detection**

Changes in spike-features when IEC was used to eliminate potentially non-neuronal signals prior to application of PCA were similar to that seen with threshold based schemes. A statistically significant reduction in duration of depolarization phase and duration of repolarization phase was seen (p < 0.001, p < 0:01 and p < 0:05, respectively). Moreover there was a distinct reduction in the spread of 2D PCA space (Figure 2.9B Inset).

**Reduction in False Positives**

There was a significant reduction in signal segments under consideration for the generation of mean spike after application of inter-electrode correlation. The difference was greater between number of signal-segments selected using ‘st’ and that selected using ‘stC’. An average reduction of 85.94 ± 9.19% (N = 10, p < 0.001) was observed. Results of using PCA
were significant as well. An average reduction of 82.96±11.12% (N = 10, p < 0.001) was observed in the number of signal-segments selected using ‘stpca’ and that selected using ‘stCpca’.

Comparative Analysis Overview

The following are the results of comparative phase of this study wherein four algorithms: simple thresholding (‘ST’), differential recording (‘DR’), virtual referencing (‘VR’) and inter-electrode correlation (‘IEC’iec) analysis were compared for their ability to detect neuronal spikes from data having varying degree of common noise content.

Mean-Spike Shape – Comparative Analysis

Typically, a mean-spike generated from extracellular multi-unit activity recorded in layer V pyramidal cells has a distinct depolarization phase followed by a repolarization phase. The amplitude of the depolarization phase is usually larger than the repolarization phase whereas its duration is shorter. A representative example of mean-spikes generated by the four algorithms for varying degree of common-noise is shown in Figure 2.11.

With low common-noise, there is very little visual difference in the outcomes from ‘ST’, ‘VR’ and ‘IEC’. However, the outcome from ‘DR’ doesn’t have the typical mean-spike characteristics. It has large peak-to-peak amplitude, a broad depolarization phase and a narrow repolarization phase. Neural spikes present on the reference electrode may be a potential reason for this outcome.
Figure 2.11: Examples of mean-spike shapes. Comparison of the shape of mean-spikes obtained by four different methods suggests that mean-spikes generated by inter-electrode correlation and virtual referencing techniques consistently have typical neuronal characteristics (short depolarization phase accompanied by higher peak to peak amplitude) of closely firing neurons. This is especially significant in scenarios with medium and high common-noise levels. Correlation numbers correspond to the average correlation observed across the entire duration of recording between the electrode of interest and other functional electrodes of the array.

All the algorithms have similar appearing mean-spikes in instances of medium common-noise in the raw recordings. Again, the shape of mean-spike generated by ‘DR’ looks least neuronal with a very indistinct repolarization phase. High-common noise produced interesting outcomes as well. The shape of the mean-spike produced by ‘ST’ was the least neuronal. It had large peak-to-peak amplitude, a broad depolarization and a narrow repolarization phase. There was some difference in the shapes from the other three algorithms with ‘IEC’ having the most neuronal feature. Interestingly, both ‘IEC’ and ‘VR’ had comparable visual characteristics in instances of low and medium common-noise however the former seemed better in datasets with high common noise.

Mean-Spike Feature – Comparative Analysis

Feature analysis was done to render quantification to the visual mean-spike outcomes. The goal was to compare values from mean-spike generated by different algorithms and to
determine the existence of trends between spike amplitude and duration of depolarization phase predicted by modeling studies. Specifically, it was assumed that higher ratio of peak to peak amplitude and duration of depolarization phase of the mean-spike would translate into greater contribution from neural spiking events in its (mean-spike’s) generation. Results from feature analysis are summarized in Table II.

<table>
<thead>
<tr>
<th>Low Common Noise</th>
<th>Thres-Detected</th>
<th>Differential</th>
<th>Virtual-Ref</th>
<th>Inter-Elect Cor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dep Phase (uSec)</td>
<td>461.3±18</td>
<td>796.8±212.4**</td>
<td>475.6±19.8**</td>
<td>465.8±17**</td>
</tr>
<tr>
<td>Rep Phase (uSec)</td>
<td>1401.7±268.6</td>
<td>883.6±542.9**</td>
<td>1304±183.5</td>
<td>1254.2±131.7</td>
</tr>
<tr>
<td>Pk to Pk Amp (uV)</td>
<td>110.6±19.8</td>
<td>121.2±17.2</td>
<td>91.5±17.7**</td>
<td>100±12.6</td>
</tr>
<tr>
<td>SpikeSegments</td>
<td>11055±2432</td>
<td>9957±1501</td>
<td>11211±1893</td>
<td>8791±1914**</td>
</tr>
<tr>
<td>Ratio (P2P/Dep Phase)</td>
<td>0.24±0.05</td>
<td>0.16±0.04</td>
<td>0.19±0.04</td>
<td>0.22±0.03</td>
</tr>
</tbody>
</table>

| Med. Common Noise |
|-------------------|----------------|--------------|-------------|----------------|
| Dep Phase (uSec)  | 641.9±197.4    | 575±72.8     | 515±1467.1  | 497.6±49.2     |
| Rep Phase (uSec)  | 1357.5±410.5   | 1355.9±205.3 | 1467.1±63.4 | 1359.2±79.6    |
| Pk to Pk Amp (uV) | 98.2±14.1      | 87.6±15.5    | 70.4±12.2** | 93±10          |
| SpikeSegments     | 7713±1982      | 8088±1979    | 9513±2760** | 4407±2688**    |
| Ratio (P2P/Dep Phase) | 0.16±0.04   | 0.16±0.04    | 0.14±0.03   | 0.19±0.02      |

| High Common Noise |
|-------------------|----------------|--------------|-------------|----------------|
| Dep Phase (uSec)  | 760.3±214.3    | 615.6±124.7  | 514.3±85.9  | 544.6±81.9     |
| Rep Phase (uSec)  | 1083±662.4     | 1600.1±222.8 | 1628.8±183.5| 1308.4±328.5   |
| Pk to Pk Amp (uV) | 73.1±16.5      | 48.3±10.8**  | 44.83±16.9  | 56.6±12.1      |
| SpikeSegments     | 4735±2527      | 4598±3018    | 5823±3636   | 1206±1859**    |
| Ratio (P2P/Dep Phase) | 0.1±0.03    | 0.08±0.04    | 0.09±0.05   | 0.11±0.04      |

Table II: Summary of feature analysis on the mean-spike outcomes generated by all methods under consideration for differing degree of common-noise. ** indicates statistically significant difference (p<0.01) from the outcomes of standard thresholding, ‘ST’.

Figure 2.12 shows a plot of the spread of duration of depolarization phase versus peak to peak amplitude for mean-spikes generated by all the algorithms for all the datasets. These plots help reinforce the visual observations associated with mean-spike shapes. In case of datasets with low common-noise, the shape of the mean-spike resulting from ‘DR’ was atypical. Similarly, the plot shows both a higher peak to peak amplitude and duration of depolarization phase. Such
observations also hold for ‘ST’ generated mean-spike in data-sets with high degree of common-noise. The analysis also reveals that ‘IEC’ generated mean-spikes consistently have the most neuronal characteristic amongst all the methods under consideration irrespective of the degree of common-noise.

![Figure 2.12: Mean-Spike Features](image)

**Figure 2.12: Mean-Spike Features.** Plot of the spread of mean-spike peak-to-peak amplitude versus depolarization phase duration for all datasets considered.

Comparison of Number of Spike-segments

Another important finding of this study was the number of spike-segments identified by each algorithm to be neuronal differed significantly. Figure 2.13 summarizes these results by normalizing the number of identified spike-segments for each algorithm to that identified by ‘ST’. Signal segments identified by ‘VR’ were higher as compared to that of thresholding in all three cases. Asynchronous cancelation and the resulting unpredictable transformation of the raw-signal may have contributed to additional threshold exceeding segments. Moreover the general lowering of the noise-floor may also have attributed to a higher number of threshold exceeding segments. Number of signal-segments identified by ‘DR’ was comparable to ‘ST’ in all three cases. However, the shape of mean-spikes indicates that these segments may not be necessarily neuronal. As with ‘VR’, asynchronous cancellation of threshold-exceeding common segments, a
reduced noise-floor and quality of recordings on the reference electrode may have transformed the original signal on the electrode of interest resulting in a poor outcome. Use of inter-electrode correlation consistently reduced the number of probable neuronal signal-segments. This is a predictable result since in ‘IEC’ the original pool of signal-segments is similar to that of ‘ST’. A useful result however was that the reduction was statistically significant (p<0.01) and it increased with increase in common-noise in the original data (low common-noise: p<0.002, medium common-noise: p<0.001, high common-noise: p<0.0003). Thus use of inter-electrode correlation may help in reducing the headstage requirements and in reducing the data to be considered for further processing.

**Spike Detection Rate Comparison**

![Graph showing comparison of spike detection rate](image)

*Figure 2.13: Comparison of Spike Detection Rate*. Comparison of number of valid spike-segments detected by the four techniques. The original numbers are normalized with respect to those detected by standard thresholding technique.
Discussion

Non-neuronal signals like Electromyogram (EMG) and spurious noise generated by abrupt movements of awake and behaving animals negatively impact the neural-spike detection ability of conventional algorithms like threshold-based detection and principal component analysis. Hence the goal of this study was to propose and objectively evaluate new algorithms for their spike detection efficacy. The inter-electrode correlation algorithm was developed and exhaustive subjective analysis was carried out to determine an optimal correlation coefficient threshold. Some subjective analysis was also done on the raw data to determine a gold-standard for neural spikes recorded from layer V pyramidal cells. It was concluded that neural spikes typically have high-amplitude and low depolarization duration. The merit of incorporating inter-electrode correlation into standard detection schemes of thresholding and PCA were evaluated. It was found to improve the overall mean-spike shape to make it appear more neuronal as well as reduce the number of threshold exceeding events to be considered for down-stream processing. Next, a comparative analysis was done between preprocessing techniques of differential recording, inter-electrode correlation and virtual referencing coupled with standard thresholding for their ability to produce mean-spike outcomes that most closely resembled subjective results and modeling studies. Data with markedly different common-noise content was used. It was found that use of inter-electrode correlation is a robust way of improving neural spike detection and reducing false positives and that its ability is not impacted by degree of common-noise in the raw data. As with previous analysis, by identifying potential false positives, inter-electrode correlation was also able to significantly reduce the number of neural spikes for consideration in downstream processing.
A number of methods have been proposed in the literature for detection of neural spikes from intra-cortical microelectrode recordings. Most of these methods are tested on simulated data that closely resembles real-data. Although such an approach has its advantages, especially in the initial phase of algorithm development, their validation on real-data that has unique noise contributions is acutely desired. Moreover very few reports use data available on other electrodes in an array to improve spike detection and sorting. Some methods like that adopted by Brier et al. [24, 25], have successfully used data from other electrodes in array to reduce noise thereby improving the signal-to-noise ratio. The approach adopted in this report is along those lines in that it evaluates three algorithms (‘DR’, ‘VR’, and ‘IEC’) that use information provided by other electrodes to improve spike detection on a given electrode. A distinguishing factor however with the ‘IEC’ approach is that correlation is calculated only amongst high amplitude (> 3 sigma) neural spike like signal segments. All the proposed algorithms can be implemented seamlessly with minimal processing overhead. The methods, especially inter-electrode correlation thus aims to clean the recorded data and complement the detection capabilities of conventional algorithms. In doing so it enables better true neural spike detection which is useful in objectively tracking the quality of the interface.

**Importance of Subjective Assessments**

Datasets used in this study had a mix of correlated (hence non-neuronal) and uncorrelated signal segments exceeding $3 \sigma$ of the original signal. Two trained unbiased blind raters were asked to identify spike-like segments from these datasets. These spike-like segments were used in generation of a mean-spike. The characteristics of this mean-spike were compared with mean-spike generated by signal segments identified automatically by ‘st’ and ‘stC’. The characteristics of rater-defined mean-spike most closely matched that of layer V neurons. However it took
significantly more time (~50X) to process the dataset manually. Hence, it would be helpful to incorporate a data reduction step that automatically identifies and removes false-positives in situations that require processing of large duration datasets. Inter-electrode correlation can help with such reductions. In scenarios involving wireless headstages on freely moving animals, data reduction is usually achieved by only transmitting signals that exceed a user-defined threshold. By executing onboard inter-electrode correlation a further enhancement in data reduction can be achieved. Finally subjective assessment was useful in identifying features that would point to a presence of non-neuronal content on the electrode. It was observed that duration of depolarization phase is slightly lower in data having reduced non-neuronal content.

**Objective Assessment Outcomes and Interpretation**

The goal of objective assessments was to determine the validity of improvements in the identified characteristics of mean-spike generated by incorporating correlation in standard detection algorithms across multiple real data-sets. Correlation-based elimination had statistically significant impact in reducing the duration of the depolarization and repolarization phase. However, it also resulted in statistically significant reduction of the mean-spike amplitude. This can be explained by appreciating the quality of raw-data used in this stage of the study. Since the data chosen for this part was from electrodes implanted for more than 3 weeks, it is reasonable to expect reduced spike activity and spike amplitudes due to reactive tissue response [26]. In contrast, the non-neuronal signals generated by distant sources would have little or no impact in their amplitudes as a result of reactive tissue response close to the implant. Hence their elimination from the original set of signal segments would result in generation of a lower amplitude mean-spike.
Correlation enabled elimination has significant impact on PCA based detection schemes as well. It was seen that correlated and uncorrelated signal segments were evenly mixed in the scatter plots, especially in the regions of higher density that is usually selected for spike detection and sorting. After application of correlation, the scatter plots had reduced spread with distinctly identifiable dense regions. Hence it can be argued that correlation enabled elimination in signal segments under consideration for Principal Component Analysis can improve the PCA-space region selection process that is integral to spike sorting.

Finally, there was an 85% reduction in the number of threshold and PCA detected signal segments after correlation-enabled elimination. This implies that only 15% of the initially threshold detected signal-segments would require transmission on the wireless link, would require additional processing and sorting. Hence it will have significant impact in relaxing headstage specifications as well as in improving outcomes of subsequent manual/automated sorting operations.

**Comparative Analysis**

The following is the discussion on comparative phase of this study wherein four algorithms: simple thresholding (‘ST’), differential recording (‘DR’), virtual referencing (‘VR’) and inter-electrode correlation (‘IEC’) analysis were compared for their ability to detect neuronal spikes from data having varying degree of common noise content.

**Threshold Detection**

The simplicity of threshold-based detection makes it an attractive first step in many applications. In this study, it performed adequately in instances of low common-noise. But its
performance deteriorated significantly in cases of medium and high common-noise. Thus threshold detection should be supplemented with techniques that would reduce the false-detection of non-neuronal signals. Moreover the threshold selected for this type of detection is completely dependent on quality of raw data. Presence of common-noise originating from animal movement, mastication and other non-neuronal sources may artificially increase the threshold level thereby resulting in missed detections. Hence selection of a lower threshold must be investigated to reduce such occurrences.

**Differential Recording**

Differential recording represents a straight-forward solution to eliminate common-noise. However, the analysis carried out in this report suggests that spike detection outcomes from differential recording are highly dependent on the conditions at the differential reference. It performs poorly when the degree of common-noise is low and the quality of interface is such that detectable neural spike activity is present on the reference. In addition, it was found that threshold exceeding common-noise may have a slight and unpredictable lag across electrodes resulting in sub-optimal cancellation using differential recording. As a result, differential recording has the worst outcome amongst all the methods. Finally, differential recording results in manipulation of the signal at its source preventing true monitoring of the tissue-electrode interface quality.

**Virtual Referencing**

Virtual referencing is similar in concept to common average reference technique employed in EEG recordings [27]. The situations involving appearance of artificial spike-like signals on the electrode of interest because of the presence of neural spikes on the reference
electrode are greatly mitigated in this case. The method may also be more resilient to unpredictable phase lags. The technique also helps lower the overall noise floor of the signal resulting in a lower spike-detection threshold. This was reflected in a consistent increase in the number of spikes detected after virtual reference preprocessing under all conditions of common-noise. The shape of the resulting mean-spike was also fairly neuronal. Thus virtual referencing may be a good approach in tackling problems associated with threshold exceeding non-neuronal signals. However, as in the case of differential recording, the underlying manipulation of the original signal at its source reduces the reliability of this technique in objective monitoring of the tissue-electrode interface quality.

**Inter-electrode Correlation**

Results suggest that Inter-electrode correlation analysis is least affected by the degree of common-noise in raw recordings. The mean-spike shapes had neuronal appearance under all the cases considered in this study. The variance in mean-spike features was also less as compared to other methods. Moreover, use of inter-electrode correlation helped identify and eliminate potentially non-neuronal segments. The normalized number of segments considered in mean-spike generation reduced progressively with increase in common-noise. These results point to the possibility that the contributing signal segments originated from similar sources (neurons only, instead of a mix of neurons and other artifacts). Another advantage of this method is that it doesn’t manipulate the original signal recorded on the electrode. Hence it allows for an objective evaluation of the health of the tissue-electrode interface.
Number of Spikes

Neural spiking events detected from intra-cortical recordings represent useful data that is analyzed further depending on the goals of the experiment. Interesting trends were observed in the number of spiking events detected by each algorithm. Firstly, the number of spikes detected by each algorithm decreased with increase in the level of common-noise across electrodes. This can be due to a couple of reasons. A closer look at the selected data revealed that in most cases datasets with low common-noise were from days immediately (0-7) post-implant whereas datasets with medium and high common-noise were from later days (14-31) post-implant. Hence an overall degradation of the tissue-electrode interface resulting in gliosis and death of neurons may have caused reduction in the spike rate. Moreover, an increase in common-noise may have increased the threshold required for a segment to exceed for detection, thereby resulting in lower number of detected segments. Selection of threshold depending on the degree of common-noise may help in improving detection. Considering spike detection rates amongst the four algorithms, it was seen that spike detection rates of standard thresholding and differential recording were comparable whereas those of virtual referencing and inter-electrode correlation were consistently higher and lower respectively. Since both differential recording and virtual referencing result in the signal being transformed prior to spike detection, the resulting detection may not accurately reflect the true firing activity. In addition, asynchronous cancelation coupled with lowering of the noise floor may result in increasing false-detections. The statistically significant lower number of spike-segments selected by inter-electrode correlation analysis suggests that a large portion of only threshold detected spikes may not be of neuronal origin. The underlying logic behind implementation of inter-electrode correlation is grounded on modeling studies for layer V pyramidal cells. Moreover, the mean-spikes generated by this method had the most neuronal
features. Hence it is reasonable to assume that the probability of the segments selected by ‘iec’ to be neuronal is higher compared to other methods studied in this report.

The proposed ‘IEC’ technique has been applied on microwire electrode arrays. Its extension to other electrode geometries and structures especially tetrodes is yet to be established. Weighting factors based on tissue conductance characteristics and recording site separation may be required to effectively apply correlation based identification of non-neuronal signals in those scenarios. In extremely rare cases, it is possible that true neural spikes do get recorded on two electrodes simultaneously. A refinement of this technique wherein a spike-segment is eliminated only when it is highly correlated across multiple electrodes is being explored. It is possible that neuronal signals are hidden within correlated segments. Development of subordinate techniques to extract them will be useful in further improving the spike-detection efficacy of intra-cortical electrodes. The correlation threshold beyond which a spike is considered non-neuronal can also be adjusted to meet the specific requirements of the experiment. For example, if the goal is to have maximum detection, a higher correlation threshold can be selected whereas if the goal is to have minimal false-positive detections a lower correlation threshold can be selected. Finally, it should be emphasized that the goal of ‘IEC’ analysis is to aid and not to replace the conventional spike detection algorithms.

**Conclusion**

A simple correlation based automated and objective approach has been demonstrated that effectively reduces false positive detection of non-neuronal signal artifacts in intra-cortical microelectrode recordings. Its ability in improving the spike detection efficacy of traditional thresholding and PCA schemes has been evaluated by objective and subjective measures. It is
shown to be a useful and easy to incorporate addition in conventional spike detection algorithms for better interpretation of experimental data.

Moreover an objective comparison of different techniques aimed at removing high amplitude non-neuronal signals from intracortical microelectrode arrays was carried out as well. Results suggest that effectiveness of conventional approaches like differential recording may be highly dependent on experimental conditions. Virtual referencing scheme represents an improvement to the differential recording approach but suffers from similar drawbacks. Use of inter-electrode correlation technique to distinguish between neuronal and non-neuronal signals offers a very robust and reliable solution to the identification and elimination of the latter. Thus it is safe to conclude that any post-processing done (after inter-electrode correlation based elimination) to evaluate the quality of the recorded signal like Signal-to-noise ratio, spike firing rate, peak to peak amplitude of spikes would be a more accurate representation of the interface quality. The algorithm developed and evaluated in this chapter involving threshold based detection followed by correlation based elimination of supra-threshold events is automated and objective. It can hence be used to compare neural recording performance of electrode platforms with differing structures, implant strategies and/or experimental conditions. Example of one such comparison is demonstrated in the next chapter.
References


a robot arm using simultaneously recorded neurons in the motor cortex," *Nat Neurosci*,

multi-channel cortical implantation techniques: manual versus mechanical insertion," *J


artifact-free recording of single unit activity in freely moving, eating and drinking


Chapter 3

Enhancement: Collagenase-Aided Intracortical Microelectrode Array Insertion: Effects on Insertion Force and Recording Performance

Abstract

Intracortical microelectrodes puncture the intact pia mater membrane during insertion, a process which can cause brain dimpling and trauma. To ensure that the device is able to withstand forces during implantation without buckling, the selection of acceptable implant materials and geometries is limited to rigid designs with large cross-sectional areas. Such designs likely increase insertion trauma and potentially exacerbate the chronic tissue response. In this paper a technique that may relax the mechanical requirements of implanted microelectrodes through enzymatic (collagenase mediated) manipulation of the pia mater is quantified experimentally. Measurements of the insertion force profiles were obtained with a load-cell during computer controlled (10µm/sec) insertion of microwire arrays into the cortex of rats. It was observed that collagenase application reduced the peak insertion force experienced by the microwire arrays by almost 40% on average (4.04±2.03mN vs. 2.36±1.17mN; control vs. treated sites). Peak insertion force magnitudes were highly dependent on implant location with anterior sites registering lower peaks than more posterior sites. Chronic neural recording performance (up to 1 month) did not appear to be adversely effected by the collagenase treatment, suggesting the overall safety of the technique. Our data suggests that controlled application of collagenase is a useful method in enabling implantation of thinner microelectrodes, potentially facilitating reduced insertion trauma and lower immune response. Furthermore, due to dependence of insertion force on anatomical location, the intended target region should be considered in implant design.
Keywords— brain-machine interface, neural implants, chronic neural recording, collagenase, microelectrode insertion force, microelectrode array.

Introduction

Chronic implantation of intracortical microelectrode arrays enables high-resolution recordings of multi-neuron activity in awake and behaving animals, leading to better understanding of how the brain processes and encodes information. Of particular interest with chronic recording interfaces is the potential for investigating the neural basis for adaptation, learning, and plasticity. Chronic intracortical interfaces also have clinically relevant application in brain-machine interfacing which may allow individuals to interact and/or control their environment through modulation of brain activity [1].

Neural interfacing technology has advanced significantly in recent decades offering a number of solutions to investigators. These include hand-fabricated microwire designs [2, 3], micromachined arrays [4, 5], and planar thin-film structures with multiple recording sites along the shanks [6-8]. Some designs incorporate on-board circuitry, embedded micro-fluidic channels, and post-implant micro-positioning capabilities [9-11]. More recently, polymer substrates have also been considered due to their perceived biocompatibility advantages, including their ability to release pharmacological agents and better match brain mechanical properties [12, 13].

Despite these efforts in the development of state-of-the-art neural-interfacing technology, the ability to maintain chronic neural signal recording remains unpredictable thereby restricting its widespread clinical applicability and use in some neuroscience studies. Examples of recorded unit activity beyond one year have been demonstrated with all of the major interfacing technologies mentioned [3, 14, 15]. However, a significant portion of sites may never record activity and those that do early on typically will degrade in performance over time [16].
The causes of failure for a given recording site are most likely related to gliosis and tissue encapsulation and/or neuronal cell death triggered by the insertion and chronic presence of the implant [17]. Also the initial dimpling of the brain during electrode insertion is hypothesized to aggravate the tissue response by rupturing blood-vessels and damaging support cells and neurons [18, 19]. Finally the implanted probes are much more rigid than the surrounding brain tissue and thus induce shear forces and relative micromotion during normal brain movements and pulsations which may continue to “agitate” and degrade the interface [20].

An ideal neural implant will likely be one that has a limited implantation footprint (low density and small diameter shanks), is mechanically flexible, and employs bioactive intervention strategies to control the tissue response. An implant designed with these characteristics however would lack the mechanical stiffness required to withstand the initial insertion forces. Some proposed solutions have included the attachment of a stiffening backplane [21], micro-incisions of the meningial layers [13], or coating with biodegradable polymers to temporarily stiffen the probe shanks [22]. However these approaches may increase the initial insertion trauma thereby compromising the potential benefits of having a more flexible implanted structure.

Instead of solely optimizing the electrode design parameters, it may also be useful to manipulate the mechanical properties of the main structural barrier to electrode penetration: the pia-mater. Piercing of the pia-mater by electrodes is accompanied by a sharp drop in insertion forces [22]. Moreover, real-time video microscopy of the insertion process shows significant brain compression or dimpling prior to pia-mater piercing [18], which has been implicated as a potential cause of long-term device failure [23]. Thus temporarily reducing the structural integrity of the pia membrane may reduce brain dimpling as well as relax the electrode stiffness requirements. This could enable the implantation of electrode structures that are more flexible and/or have smaller cross-sectional area, thereby reducing the degree of insertion trauma.

At a microscopic level, the pia is primarily composed of flattened connective tissue cells.
and collagen fibrils with varying anatomical thickness[24]. One strategy briefly mentioned by Kralik et al is to apply an enzymatic solution containing collagenase which would attempt to break up the collagen network of the pia [25]. It is thought that collagenase acts to break down the fibrils by unwinding the collagen triple helix and hydrolyzing the peptide bonds [26]. This action may weaken the extracellular matrix of the pia and possibly reduce the force required to insert the microelectrode arrays. However the effectiveness of this technique has not been quantified.

In the current study, the effects of the aforementioned collagenase treatment strategy [25] on insertion forces for different anatomical locations is quantified. A custom-designed load-cell system is described which enabled the determination of the insertion force vs. depth profiles during the insertion of microwire arrays into the cortex of rats. Initially the profiles were obtained in a randomized acute study which compared enzyme treated and untreated sites. Following this a set of rats were implanted chronically and recording performance was tracked for 1 month to evaluate potential long-term effects of the treatment on neural recording performance. The results suggest a significant and consistent reduction in the peak insertion forces without negatively impacting recording performance. Preliminary results from this study were reported in [27]. A follow-up study aimed at evaluating effect of collagenase treatment on the brain surface from a histological standpoint was also carried out.

**Materials and Methods**

**Surgical Procedures**

All animal procedures followed NIH Guidelines for the Care and Use of Animals and were approved by the Penn State IACUC committee. Acute experiments were performed on 7
male Sprague-Dawley rats (~350g, 3.5±0.6 months). Six additional rats (~450g, 6.8±0.7 months) were dedicated to studies aimed at evaluating chronic neural recording performance over 4 weeks. Subjects were anesthetized with an initial dose of ketamine/xylazine/acepromazine (50:5:1mg/kg) with additional anesthesia given to maintain areflexia. The subjects were placed in a stereotaxic frame and warmed with a heating blanket maintained at 37°C. Heart rate and blood oxygen saturation of the animal were monitored with a handheld pulse oximeter.

A midline scalp incision was made to expose the cranial plates. For acute experiments, craniotomies were created at 2-4 mm lateral to the midline at two anterior and two posterior sites located 2-4 mm anterior/posterior of bregma, respectively (see Fig. 3.1A). One site each from the anterior and the posterior sites were chosen for treatment with collagenase enzyme with the contralateral site serving as un-treated controls. The collagenase mixture was the same as that used by Kralik et al [25]. It was composed of 20mg/mL collagenase (Type-I, part #C0130, Sigma, St. Louis, MO) contained in 0.36mM CaCl₂ in 50mM Hepes buffer with equal amount of KY jelly. Prior to treatment, the dura was pierced with a 27G hypodermic needle and further opened with micro-scissors to accommodate the electrode array. A small piece of Gelfoam™ (NC9482920, Fisher Scientific, Pittsburgh, PA) sponge that was taken out of its sterile packing and soaked in the collagenase mixture was placed over treated implant site for ~15 minutes. For control sites, an identical mixture without the collagenase enzyme was applied in the same way. Prior to electrode insertion (see below) the Gelfoam was removed and the sites were thoroughly rinsed with saline to remove any excess treatment mixture. Chronic implantation procedures were essentially identical except that only the two anterior sites were implanted including one control and one treatment site in each animal. In these animals, bone screws were placed in the cranial plates and a dental acrylic headcap was created on top of the skull to house the electrode connectors.
Intracortical Electrode Fabrication

The electrodes were composed of eight tungsten microwires (California Fine Wire, Grover Beach, CA) insulated with polyimide (~50µm total diameter) arranged in a 2x4 array. The microwires were connected to a small custom PCB interface board which was soldered to an Omnetics (Minneapolis, MN) nano-miniature connector. Epoxy was applied to reinforce the microwire attachment to the connector. Wires were then individually fed through a custom jig with a spacing grid and weights were applied to keep the wires straight. Individual wires were separated by ~250µm (or 500µm where indicated; see Figure 3.4 and Table 4A). After the wires were threaded, spacing was maintained with regularly spaced islands of dental acrylic (Teets "Cold Cure", Co-Oral-Ite Dental Mfg Co, Diamond Springs, CA) similar to Williams et al [2]. To render uniform length of the recording tips the assembled array was carefully cut with scissors to place the recording tips ~0.5cm beyond the last acrylic island. A stainless steel ground wire was also soldered to the connector and tied to one of the bone screws to serve as ground (for chronic experiments). Electrodes used in chronic experiments were sterilized by gamma irradiation prior to implantation. More detail on electrode fabrication and surgical procedure is provided in Appendix A.

Insertion Force Measurement System

A schematic diagram of the insertion force measurement system is depicted in Fig. 3.1B. The electrode arrays were anchored to the load-cell via a custom-built light-weight acrylic clamping mechanism. The clamp employed a set screw that was manually tightened against the electrode connector to keep the array fixed during insertion. The load-cell (Honeywell Sensotec model MBL 25g: 0.1% full scale or 0.25mN linearity, 0.03% full scale repeatability; Honeywell,
Columbus, OH) was calibrated to produce an output of 5V for a force of 245.25mN (25g). It is noted that the maximum insertion forces registered during this study were below 10 mN. Despite working in the lower 5% of the specified dynamic range, the sensor was tested with calibrated weights and found to be consistently linear and accurate down to a load of 0.049 mN (5mg). The load-cell output was coupled to an in-line amplifier (model UV) which amplified the signal before routing it for display on a digital multimeter and acquisition by the host computer.

Prior to automated insertion, the entire assembly consisting of the load-cell and the electrode array was slowly lowered to the cortical surface with a manual micromanipulator. After lightly contacting the cortical surface (as reflected by a small change in load cell output) the array was then retracted by 50µm. For final insertion, a custom-built computer controlled stepper-motor setup was used to drive an oil hydraulic micromanipulator (MWO-3, Narishige Scientific Instruments, Tokyo, Japan). This action directed the microwire array at a constant speed of 10µm/sec into the cortex. The final depth of insertion for acute studies was 2mm to ensure full penetration of the array and allow for observation of forces several hundred microns following penetration. The final depth for the chronic studies was restricted to 1mm to enable recording from cortical layer V of the primary motor cortex. Insertions were visually monitored by the surgeon through a surgical microscope. On a small number of occasions (4 out of 28), the electrode array snagged on incompletely removed dura or a portion of the array contacted the skull, resulting in visible bending of the array and exceedingly high insertion force readings. These trials were removed leaving only “clean” insertion runs for analysis.

The analog output signal of the in-line amplifier was sampled at 50kHz and stored on a PC using Datawave SciWorks (Denver, CO) acquisition software for subsequent analysis. A 1-second moving average window was applied to the data to smooth out effects of high-frequency sensor noise, air-flow and other vibrational disturbances. Since the insertion velocity was constant (10µm/sec), sample time was converted to distance so the force values could then be plotted as a
function of penetration depth to obtain insertion force vs. depth profiles.

![Diagram](image)

**Figure. 3.1. Surgical and Experimental details.** (A) Sketch of dorsal skull showing approximate location of implant sites. A=anterior; P=posterior; B=bregma; Λ=lamda. Acute subjects were implanted in both anterior and posterior sites, while only anterior sites (1 and 2) were implanted in the chronic group. (B) Experimental setup. The electrode/load-cell assembly was positioned over the implant site with a standard manual controlled micromanipulator. Electrode array insertion was performed with a fine control oil-hydraulic micromanipulator driven by a computer-controlled stepper motor. Load-cell output was amplified, acquired, and stored in the computer as shown.

**Neural Recording and Data Analysis**

A commercial multi-channel acquisition system (Tucker-Davis Technologies Inc (TDT), Alachua, FL, http://www.tdt.com) was used to collect simultaneous neural recordings from the electrodes of chronically implanted animals. Extracellular voltages from each of the 8 intracortical electrodes were recorded with respect to a ground wire attached to one of the anchoring bone screws.

63
Figure 3.2. Neural recording performance analysis. (A) Two minute sample of raw recording signal shows occurrence of non-neuronal artifacts and mastication. Signals exceeding 300µV were removed. (B) Left: 100ms sample of raw recording containing a highly correlated segment (window) appearing across all the electrodes. These “non-neuronal” segments were detected and eliminated by implementing a cross-correlation algorithm. Right: 30ms sample of raw recording with clear neuronal spike occurring on only one electrode. (C-Top): Segment of raw recording after elimination of non-neuronal contributions (same electrode and recording session as in (A). Threshold for spike-detection = 2.5 times standard deviation of this signal. Noise floor = 2 times standard deviation of the signal that remained after extraction of neural spikes. (C-Middle): Mean-spike formed from all the detected spikes in Top panel (overlaid spikes shown in inset). (C-Bottom) Mean-spike generated from the top one-fifth of the detected spikes (overlaid spikes shown in inset). (D) Representative sample of mean-spikes belonging to each of the four defined categories based on their peak-to-peak values: Category 1 (<40µV), Category 2 (40µV to 80µV), Category 3 (80µV to 125µV) and Category 4 (>125µV).
The signals were boosted with a unity gain FET buffering stage and passed to a lightweight bio-amplifier (Medusa, TDT) which digitized the signals (25 kHz) with low-noise 16-bit A/D converters. The signals were multiplexed and transmitted via a fiber-optic cable to rack-mounted TDT modules, digitally band-pass filtered 300 Hz-5 kHz, down-sampled at 12 kHz, and streamed to disk for offline analysis. Recordings were carried out in regular sessions of 5 mins with the animal either awake or lightly sedated with isoflurane. To help track the state of the interface, electrode impedances were also measured (not reported) at 1 kHz with a Bak™ electrode impedance meter (IMP-1 Electrode Impedance Tester, Bak Electronics, Mt. Airy, MD).

Subsequent analysis of the neural recordings was handled objectively in Matlab (Mathworks, MA) with custom-developed software. Signals were pre-processed to eliminate 20ms segments around large non-neural artifacts (>300µV peak; see Fig. 3.2A) that were likely caused by animal movements and other distant noise sources. Candidate neural spikes were then detected by threshold-crossing (set to 2.5 times the standard deviations of raw signal) and extracted as 3-ms waveform segments. To further eliminate potential mastication and related non-neuronal artifacts from contributing to the neural interface performance measures, an algorithm was implemented to isolate and eliminate similar appearing waveforms that occurred simultaneously on multiple electrodes in the array (see Fig 3.2B, left column). The assumption was that if noise and/or artifact were produced by sources at a distance to the array, then multiple sites would record it. By keeping track of the correlation of signals across the array, segments could be identified in which these simultaneous events occurred. Employing a “correlation algorithm” (see chapter two), each spike was compared to recordings on the other electrode sites to determine the level of correlation. If the inter-electrode correlation was greater than 0.75, the spike was rejected and not used in the estimation of the recording performance for that recording site. A highlighted example of a recording segment that met the criterion for high correlation and therefore was rejected is shown in Fig. 3.2B, left column. The right column of Fig. 3.2B shows an
uncorrelated event identified as a candidate neural spike.

Multiple matrices were employed in quantifying the recording performance of each implanted array. To assess the magnitude of neural signals recorded from each electrode, a mean spike (Fig. 3.2C-middle) was computed from all the detected spikes (inset) that satisfied the previously described criterion. One recording performance metric was the peak-to-peak magnitude of the mean spike waveform (mP2P). This measure could be biased by low-amplitude spikes originating from multiple neural units more distant to the electrode site. Hence a second electrode performance metric (mP2P-20%) was defined that evaluated the P2P amplitude of the mean spike generated from the top 20% of detected candidate spikes based on individual P2P amplitudes (Fig. 3.2C-bottom). Electrodes were classified on the basis of the mP2P-20% as being Category 1 (<40µV), Category 2 (40µV to 80µV), Category 3 (80µV to 125µV) or Category 4 (>125µV), Fig. 3.2D.

The noise floor magnitude was estimated from the remaining signal (after spike and artifact removal; see above) by determining the standard deviation and multiplying by 2. This provided a conservative bound which captured the range of signal peaks typically occurring in between the neural spikes (see Fig. 3.2C top). The signal-noise ratio (SNR) for each electrode was taken as the ratio of the mP2P and the noise floor magnitude.

**Histological Evaluation**

In a follow up study, the effect of Collagenase on surface of the brain was studied from a histological standpoint. Craniotomies were performed in six rats at two anterior sites. After dura removal, one site was applied with collagenase solution and the contralateral anterior site was applied with control solution. The duration of application was same as before. Please refer to Appendix B for details of extraction and histology procedures.
RESULTS

Insertion force measurements during micro-wire electrode array insertion into cerebral cortex were obtained in thirteen (13) rats. Acute studies in seven (7) subjects examined both the effects of anatomy and the collagenase treatment. In 5 out of the 7, one site each from the anterior and posterior sites were chosen for treatment with collagenase enzyme with the contralateral site serving as un-treated controls. One of the remaining 2 subjects was implanted with collagenase applied at all sites (R43) while another received no collagenase at any of the sites (R42). Two anterior sites from R22 and one posterior site each from R21, R42 and R43 did not have “clean” insertions and hence were eliminated from analysis. A follow-up study was carried out in the remaining 6 subjects (implanted only in the 2 anterior sites) to evaluate potential differences between collagenase vs. non-collagenase treated sites in terms of chronic recording performance over one month.

Insertion Force vs. Depth Profiles

Examples of typical insertion force vs. depth profiles are shown in Fig. 3.3. Insertion profiles during the acute experiment were predominately of the type shown in Fig. 3.3A. Prior to pia rupture, the force experienced by the array typically increased steadily until a peak was reached. The initial peak and subsequent drop in the load cell reading coincided with a release of brain compression corroborated visually with a surgical microscope. This release was attributed to pia rupture. The insertion depth at which this initial peak occurred was quite variable ranging as shallow as 500 µm to as deep as 1750 µm for the insertion speed used in this study (10 µm/s). Most of the initial peaks occurred at depths of insertion around 1000 µm. Secondary peaks or rises following penetration were commonly observed and are hypothesized to be caused by
accumulating drag forces as more of the electrode surface area came in contact with cortical tissues.

Distinct insertion peaks were only observed in one out of six subjects (R47) in the chronic study. The reason for this apparent discrepancy between the acute and chronic experiments is not clear, but could potentially be related to the fact that the animals in the latter group were older and larger (acute: average age = 3.5±0.6 months, chronic: average age = 6.8±0.7 months) and thus may have thicker and/or denser meninges [28]. In addition, there was variability in the depth at which the penetration peaks observed in the acute study occurred with most occurring at 1000μm and greater. Since the target depth for the chronic study was only 1000 µm (cortical layer V), it is possible that the electrodes simply did not fully penetrate at this depth. However, recordings with clear neural unit activity obtained immediately following surgery point to eventual pia penetration.

Figure 3.3. Examples of insertion force vs. depth profiles for microwire array insertions. (A) Typical profile for arrays that clearly penetrated the pia during automated insertion (R39, anterior sites). (B) Typical insertion force profile for an array that did not appear to penetrate before the end of advancement (note: insertion depth of only 1mm; R50). Some potential factors contributing to variation in the insertion force profiles observed might include penetration depth (2mm for acutes; 1mm for chronics), age (3.5 months for acutes; 6.5 months for chronics), and/or slight variations in the electrode array tips.
Factors Influencing Peak Insertion Force

The results from the acute experiment examining the effects of collagenase application, anatomical location, and electrode spacing on peak insertion force are presented in Fig. 3.4. It was found that the peak insertion force was a function of both collagenase application and anatomical location. When lumping all implant conditions and anatomical locations together, the 250µm spaced electrodes experienced peak insertion forces similar to electrodes with 500µm spacing (p>0.1). This insignificance was consistent for each of the implant location/condition combinations in this study. One should be careful however to avoid drawing definitive conclusions about the effects of electrode spacing from this study given the small sample size for evaluating this effect. But since the effect of collagenase treatment was much greater than the differences produced by electrode spacing, the different spacing results were pooled for insertion force analysis.
Figure 3.4. Force results for the acute insertion study. (A) Differences in peak force values accounting for the effect of collagenase, anatomy, and inter-electrode spacing. (*R41 was implanted with only a 4-wire single row array). (B) Average of peak forces obtained from same data set in A (including both 250µm and 500µm inter-electrode spacings) shows a statistically significant difference that is a function of both collagenase application and implant location. Bars represent standard deviation.

Collagenase treatment had a significant effect on reducing peak insertion force (Table 4 and Fig. 3.4). Comparing within subjects (same anterior/posterior location and inter-electrode spacing), the mean reduction was 38% ± 23% (Paired student t-test, p<0.01). Most peak force reductions were around 50% with only one occurrence where no reduction was seen (R39p). Including the additional subjects (R42 and R43; see Fig. 3.4A) in which intra-subject
comparisons could not be made, collagenase-aided insertions on average had 35% and 46% lower peak insertion forces for anterior and posterior sites, respectively (Table 4B; Paired two-sample student t-test with equal variance, p<0.05 and p<0.01, respectively).

**TABLE 4A: INTRA-SUBJECT PEAK FORCE COMPARISON (ACUTES)**

<table>
<thead>
<tr>
<th></th>
<th>Collagenase (mN)</th>
<th>Non-Collagenase (mN)</th>
<th>% Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>R21a</td>
<td>1.50</td>
<td>3.06</td>
<td>51.1</td>
</tr>
<tr>
<td>R38a*</td>
<td>1.65</td>
<td>3.73</td>
<td>55.7</td>
</tr>
<tr>
<td>R39a</td>
<td>1.14</td>
<td>1.69</td>
<td>32.3</td>
</tr>
<tr>
<td>R41a</td>
<td>1.11</td>
<td>2.18</td>
<td>49.1</td>
</tr>
<tr>
<td>R22p</td>
<td>4.30</td>
<td>5.20</td>
<td>17.3</td>
</tr>
<tr>
<td>R38p*</td>
<td>3.09</td>
<td>7.61</td>
<td>59.5</td>
</tr>
<tr>
<td>R39p</td>
<td>3.19</td>
<td>2.94</td>
<td>-8.4</td>
</tr>
<tr>
<td>R41p</td>
<td>2.94</td>
<td>5.89</td>
<td>50.1</td>
</tr>
</tbody>
</table>

**TABLE 4B: INTER-SUBJECT PEAK FORCE SUMMARY (ACUTES)**

<table>
<thead>
<tr>
<th></th>
<th>Collagenase (mN)</th>
<th>Non-Collagenase (mN)</th>
<th>% Reduction</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior</td>
<td>1.93±0.91 (n=6)</td>
<td>2.98±0.89 (n=6)</td>
<td>35%</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Posterior</td>
<td>3.26±0.60 (n=5)</td>
<td>6.02±1.93 (n=7)</td>
<td>46%</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Table 4: Peak force Summary

Peak insertion force was also influenced by the anterior/posterior location of the implant site (Table 4 and Fig. 3.4). Quite consistently, the anterior implant sites (sites 1 and 2 in Fig. 3.1A) exhibited lower resistance to electrode penetration as compared to posterior sites in both collagenase-treated and control conditions. In the control condition, the peak insertion force was 2.98±0.89mN for the anterior sites which was about 50% less than the posterior sites (6.02±1.93mN; Table 4B). This difference was highly significant with student t-test p<0.01 (Fig. 3.4B). With collagenase-aided insertion, the mean difference between anterior and posterior peak insertion forces was less (1.93±0.91mN vs. 3.26±0.60 mN) but still significant (p=0.01). Due to the influence of anatomical location on peak insertion force, the electrode placement for the chronic experiment was restricted to anterior sites 1 and 2 (primary motor cortex).
**Chronic Experiment: Insertion Forces**

In order to evaluate potential long-term effects of collagenase-aided insertion on neural recording performance, a follow-up study was conducted in a set of 6 rats. In these subjects 2x4 electrode arrays were implanted into primary motor cortex (anterior sites 1 and 2 of Fig. 3.1A) in both collagenase-aided and control conditions. The implants were advanced to a depth of ~1mm and animals were recovered for post-implant recordings sessions lasting to 4 weeks.

![Graph showing insertion forces](image)

**Figure. 3.5. Maximum force results from the chronically implanted animals.** Differences in maximum force observed during the insertion run shows a consistent reduction as a result of collagenase use. (*due to data acquisition problems in R46, the max force was linearly extrapolated from the insertion force profile for the first 500 µm).

Insertion forces were lower throughout the insertion run for the collagenase-treated site as compared to control. Since peaks did not always occur, maximum insertion force was used for comparison purposes (see Fig. 3.5A). In one subject (R46), technical problems prevented the acquisition of insertion force measurements toward the end of the insertion run and so maximum force was linearly extrapolated to 1mm for both conditions from data obtained during the first 0.5mm. As with the previous acute experiment, the average maximum force was consistently less for collagenase-treated insertion (Fig. 3.5A). Overall, average maximum force was 29% less for
the collagenase-treated insertion condition (2.47 ± 0.47mN vs. 3.47 ± 1.0mN; Paired student t-
test, p<0.015; Fig. 3.5B). The average reduction in maximum force was 26% ± 13% for the
collagenase treated sites when comparing within the same animal.

**Chronic Experiment: Neural Recording Results**

Spontaneous neural activity was monitored during regular sessions for 4 weeks in three (3) of
the subjects (R46, R47, R50). Two of the subjects (R48, R51) were implanted normally but did
not recover from anesthesia and a third subject (R44) had headcap failure and so no recording
performance data are reported for these subjects. For a given animal, data from all days and all
recordable channels was pooled in determining the statistical significance of differences in mean
SNR and mean spike amplitudes for collagenase-treated and control conditions (R46: n =
157/164; R47: n = 133/157; R50: n = 126/124; electrode-days for collagenase-treated/control
sites).

**Signal-to-Noise Ratio.**

Average signal to noise ratio (SNR) across the array for both collagenase-treated and control
insertions are plotted against time (1 month) for the three subjects in Fig. 3.6A. On average, the
SNR of collagenase treated sites was only slightly better than control sites (2.4 vs. 2.3; 6%
difference). Evaluating each subject individually, the differences in average SNR between
collagenase-treated and control arrays were not significant in two subjects (p>0.1; R46, R47), but
highly significant in the third subject (p<0.001; R50).
Figure 3.6. Signal-to-noise ratio (SNR) and mean peak-to-peak amplitude (mP2P) vs. time. (A) SNR comparisons of neural recordings between the collagenase and control sites reveals statistically significant difference in one implant (R50). Comparable SNRs were calculated for the other two cases. (B) Comparison of peak-to-peak amplitude of mean-spike (mP2P) on electrodes implanted in control and collagenase treated sites reveals statistically different outcomes favoring collagenase treatment in two animals (R47, R50).

Peak-to-Peak Amplitude of the Mean Spike (mP2P).

Sub-threshold neural activity contributed by neurons at some distance to an electrode will likely increase the estimated noise floor [29]. Thus an electrode with significant background activity, actually indicative of healthy neural tissue, could yield lower than expected SNR. Additional metrics of interface performance were therefore explored which quantified the magnitude of nearby neural spike activity. The average mP2P values for each of the electrode arrays are plotted as functions of time in Fig. 3.6B. On average, the mP2P values were greater for
collagenase-treated sites compared to controls (67\(\mu\)V vs. 58\(\mu\)V; 16% difference). Evaluating each subject individually, there was no significant difference in mP2P values in R46 (p>0.1), while the remaining subjects (R47 and R50) had highly significant differences (p<0.001; Two-sample student t-test).

**Individual Electrode Performance.**

To evaluate the spectrum of performance of the individual electrodes within an array, each electrode was assigned to one of four categories based on the peak-to-peak magnitude (mP2P-20%) of the mean waveform formed by the top 20% of candidate spikes. This type of metric was chosen because it was thought to be more influenced by neurons closest to the recording site. A representative sample of mean spike waveforms belonging to each of these categories is shown in Fig. 3.2D. As can be seen in the figure, Category 3 and 4 (mP2P-20% > 80\(\mu\)V) exhibit classic neural features that include distinct depolarization and repolarization phases. The average percentage of electrodes belonging to each of the categories was calculated for the beginning (weeks 0-2, corresponding to early reactive response[30]) and end (weeks 3-4, corresponding to late reactive response [30]) of the experiment for each subject. These results are summarized in Fig. 3.7. In the first two weeks, lower percentage of electrodes implanted in treated sites belonged to Category 3 and 4 when compared to control in R46 (40% vs. 60%). The opposite trend was observed in R50 with higher percentage of electrodes in collagenase treated sites belonging to Category 3 and 4 (78% vs. 34%), with both sites performing equally well in R47. In the latter half of the study, the differences in performance between the treated site and control were reduced in R46 (33% vs. 43%), whereas the treated sites outperformed control in R47 and R50 (R47: 100% vs. 96%. R50: 82% vs. 19%). Overall, the mP2P-20% values in the first two weeks was slightly larger for collagenase treated sites as compared to control (106±51
μV vs. 102±46 μV, p >0.05). Interestingly the difference became significant over the last two weeks of the study with treated sites recording higher amplitude spikes (112±47 μV vs. 101±44 μV, p < 0.05).

![Weeks 0-2](image)

**Figure. 3.7.** Distribution of peak-to-peak amplitudes of mean waveform of the top-fifth detected spikes (mP2P-20%) on individual electrodes over the duration of implant. Higher percentage of electrodes implanted in collagenase treated sites belongs to Category 3 and 4 in R47 and R50. In R46, control site electrodes record better initially, but the differences reduce after 2 weeks.

**Histological Evaluation**

In all cases, significant astrocyte and microglia activation was observed on the surface of collagenase exposed sites as compared to the control sites. Figure 3.8 depicts these observations on collagenase exposed surfaces (left) and control surfaces (right). In some instances the tissue
was entirely degenerated around the collagenase exposed sites (Figure 3.9, left) whereas no such damage was observed on the control sites (Figure 3.9, right) at a similar depths.

An interesting result was observed in the only specimen (R47) wherein clear microwire tracks were visible at depths closer to the recording sites (Figure 3.10). This subject had clear neural recordings and comparable astrocyte and microglia activation was noticed around both control and collagenase treated implant sites. Thus the damage associated with crude enzyme
application may be restricted to superficial structures of the brain.

**DISCUSSION**

The goal of this study was to evaluate the efficacy of collagenase enzyme treatment in reducing mechanical constraints for intra-cortical electrode design. It involved the measurement of insertion force with a custom-developed load-cell system during microwire electrode array
insertion into rat cortex. Comparing both control and treated hemispheres in the same subjects, an approximate 40% reduction in peak insertion force was observed for the collagenase treated implant sites. Interestingly peak insertion forces also showed dependence on anatomical location with the anterior locations (~1-2 mm anterior of bregma) registering lower insertion force peaks than the posterior ones (~2-3 mm posterior of bregma). Chronic studies also indicated that collagenase treatment did not negatively impact electrode performance. On the contrary collagenase treatment may actually provide slight enhancement on long term performance although further studies are needed to verify this.

Several recent studies have measured insertion forces during intracortical electrode implantation to ensure the structural strength and integrity of newly designed implants [22, 31] as well as to analyze implant biomechanical properties [32, 33]. This is the first reported study wherein a conscious attempt was made to evaluate the effect of structural manipulation of the pia mater on implant biomechanics with a follow up on its impact on electrode recording performance. The insertion force vs. depth profiles and peak force magnitudes obtained in the current study were similar to previous reports. Exact details vary slightly depending on electrode design and insertion technique, but in general insertion forces grow to a peak of 1-10 mN over the first 0.5-1.0 mm followed by a visible reduction in insertion force presumably due to pia membrane rupture.

**Electrode Design Implications**

To ensure electrodes are not damaged during the insertion process, they must be designed to withstand the peak insertion forces. Specifically, the theoretical buckling force of the electrodes should be greater than the peak insertion force. For probes with rectangular cross-section like most silicon-based devices, buckling force is directly proportional to the width of the
electrodes [13]. Therefore a 40% reduction in the maximum insertion force can allow for a 40% reduction in the width and hence the cross-sectional area of the electrodes. Thus after application of collagenase, electrodes having a cross-sectional area of 3000 µm² (typical for commonly used Michigan probes [34]) can be reduced to 1800 µm² and still be able to withstand the forces during implantation. Following calculations similar to that performed by Wise et al [34], decreasing the cross-sectional area of the electrodes as allowed by collagenase application will result in damage to roughly 108 neurons and 864,000 synapses as compared to 180 neurons and 1,440,000 synapses in the control case. Similarly, for microwires (modeled as cylinders) a 40% reduction in the buckling force requirement would yield a 23% reduction in the cross-sectional area.

In addition to cell and tissue displacement, the immune response to implanted microelectrodes appears to be influenced by electrode dimensions [35, 36]. A recent report indicated that reducing microwire diameter by half (from 24-µm to 12-µm) produced significantly less gliosis as evidenced by GFAP expression at 4 weeks [35]. Insertion of the 12-µm wire in that study required the application of PGA which more than doubled the initial electrode diameter. Use of collagenase may reduce the amount of PGA coating required for implanting thin electrodes, potentially reducing the acute immune response [36]. These advantages can be extended to flexible probes as well wherein coatings or stiff backplanes are used to impart the required structural strength.

**Importance of Implant Location**

Another interesting and useful finding of this study was the dependence of peak insertion force on the anatomical location of implant. Anterior locations consistently required lower forces for electrode penetration as compared to their posterior counterparts. Such differences in forces based on anatomical locations have been reported by others in extracted brains [37]. Moreover
the reduction in peak insertion force after collagenase application in the posterior sites was slightly higher as compared to the anterior sites (46% vs. 35%; see Table 4B). Variations in the thickness and/or density of the collagen-network of the pia-mater, variations in ultrastructure of the pia-mater at different regions of the brain, differences in the vascular network etc. could be likely explanations [24, 38]. The intact pia over the posterior locations during non-collagenase insertions appears to be more prominent due to the higher insertion forces seen relative to the anterior locations. After application of collagenase, the insertion force differences between anterior and posterior implant sites were reduced but still present. Since the duration of exposure to the collagenase treatment was constant, it points to a less complete dissolution of the collagen network for the posterior sites. An interesting hypothesis to be evaluated in future studies is whether increasing the time of exposure to the collagen treatment can further reduce the variation in insertion force between implant locations. Nonetheless it is clear that anatomical differences in insertion force exist and should be taken into account in the design of neural implants.

**Potential Role of Insertion Trauma and Electrode Performance**

In five out of six instances in which direct intra-subject comparisons could be made, the depth at which the initial insertion peak occurred was less for the collagenase treated site with respect to the contralateral control site. This indicates collagenase-aided insertion may also have the added benefit of reducing brain compression (or dimpling). The amount of brain dimpling and overall trauma caused by insertion is thought to be critical in mediating long-term recording performance. One recent study compared a manual (slow) insertion technique with an impact insertion technique [23]. The results indicated far superior performance with the use of a custom developed high-rate mechanized insertion approach that was presumably caused in part by less brain dimpling. However a controlled study exploring the influence of brain dimpling and initial
insertion trauma on the later immune response and/or recording performance is yet to be conducted. The collagenase treatment protocol could provide an interesting approach to conducting such a controlled study in which the level of insertion trauma might be influenced at some level.

Neural recording functionality from chronically implanted electrodes suggests the collagenase treatment method is safe. Comparisons of signal-to-noise ratios yielded similar performances amongst the collagenase-treated and control sites. In two of the three subjects the amplitude of the mean-spike waveform obtained from top one-fifth of the spikes recorded on individual electrodes yielded better results for collagenase-treated sites as compared to control sites. Additional studies need to be performed to determine whether this result will be significant across subjects. It may be that local loosening of the pia-mater resulting in reduced dimpling and initial insertion trauma could lead to long-term improvements in recording performance. The loosening of the collagen network might also increase the flexibility of the surface blood vessels during insertion allowing for lesser vessel damage. The promising outcome at this point is that the treatment does not apparently seem to adversely influence chronic recording performance.

**Histological Evaluation**

Elevated immune response and large scale damage of tissue observed on the surface of the brain can be attributed to the composition of collagenase powder used in these experiments. It was crude in nature and it was secreted by anaerobic bacteria Clostridium histolyticum (Collagenase Guide. Sigma-Aldrich Labs). It contained sulhydryl proteases, polysaccharidases and lipases in addition to collagenase (clostridiopeptidase) for effective tissue dissociation by attacking not only collagen, but also other proteins, polysaccharides and lipids in the extracellular matrix. It also contained Clostripain which is known to be toxic and damaging.
This damage can be reduced by using chromatographically purified collagenase that is substantially free of proteases and Clostripain, is cell-culture tested and sterile filtered. Example of purified collagenase like CLSPA offered by Worthington Biochemical Corporation that contains collagenase A and B and is used for controlled degradation of collagen should be experimented as suitable substitutes to improve safety of the collagenase application protocol.

CONCLUSION AND FUTURE DIRECTIONS

A unique method aimed at relaxing the design constraints of intra-cortical electrode arrays has been demonstrated. It enables a reduction in peak insertion forces, thereby making it possible to use dimensionally smaller and even flexible probes. This may in turn reduce the tissue response and improve chronic performance. Future studies involving a cocktail of enzymes and a follow-up with more comprehensive histology will further our understanding regarding the underlying mechanisms for force reductions and what influence this may have on the tissue response. The insertion force measurement system could also be utilized in future studies to evaluate the effect of parameters such as opening angle, insertion speed and electrode spacing leading to the optimization of electrode design to minimize insertion trauma.

References


Chapter 4

Monitoring: Feasibility and Safety of Longitudinal Magnetic Resonance Imaging in Rodent Model with Intracortical Micro-wire Implants

Abstract

The purpose of this paper is to investigate: (1) the feasibility of carrying out longitudinal magnetic resonance imaging (MRI) studies in animals with implanted micro-wire electrodes adapted for MRI compatibility; (2) the effect of MRI studies on the quality of neurophysiological recordings; (3) the use of MRI to study the extent and recovery of tissue damage due to electrode insertion and (4) histological tissue damage due to MRI. There was no evidence of chronic neural damage caused by repeated MRI by any of the measures used, nor any statistical difference in the quality of the electrophysiological recordings between animals that had undergone MRI scans and those that had not.

Keywords: Biomedical MRI, biomedical electrodes, intracortical microelectrodes, neurophysiology, MRI heating, Immunohistology

Introduction

A number of different invasive and non-invasive platforms exist for analyzing and activating the central nervous system. Invasive techniques typically involve implantation of metallic electrodes whereas non-invasive analysis techniques include modalities such as magnetic resonance imaging (MRI) and computed tomography as well as surface electrode based sensing.
Valuable insights can be gained by combining both invasive and non-invasive schemes. For example, combining functional MRI (fMRI) and intracortical recording has yielded important information about metabolic mechanisms that are most highly correlated with recorded neural activity[1]. Similarly, combining fMRI with electrical microstimulation has improved understanding on the extent of neural activation [1, 2]. In future, combining MRI with invasive recordings may help better estimate the extent of damage due to stroke and trauma [3] or enable multimodal visualization of neural changes associated with learning and task execution [4].

An important step towards combining these technologies is an evaluation of chronic safety and compatibility. In addition to the very strong static magnetic fields (1.5-21 tesla) required for MRI, the technique also uses high power pulses of electromagnetic energy at frequencies in the many hundreds of megahertz range, and rapidly alternating magnetic field gradients. Induced currents and associated tissue heating and damage, especially at electrode tips[5], are a major concern. Modeling studies, experiments using gel phantoms, and in-vivo studies have been carried out for implanted neurostimulators [6], coronary catheters[7], stents[8], pace-maker leads[9] and straight wires[10]. These studies were used to estimate the effect of the strong static magnetic field alone, or while exposed to typical MRI protocols on the surrounding tissue. Despite differences in field strength, imaging protocols, temperature measurement methods, specimen under examination and the overall setup, a common finding was that heating effects are maximal at the un-insulated device tip where it contacts the tissue. Since these wires are much larger than those used for neurophysiological monitoring, the current study was performed to determine whether such heating effects were present in a microelectrode setup, and whether detrimental effects of MRI on neurophysiological measurements would be present.
Studies wherein neural tissue is sensed both by the implanted electrodes and MRI itself present both the aforementioned opportunities and unique challenges. These challenges are markedly different from those associated with stimulation based implants because sensing requires viability of the neural tissue in close proximity to the electrodes. Such requirements are inherently more stringent using, for example, microelectrode arrays recording extracellular multiunit activity (EMUA) since the sensing zone is limited to a couple of hundreds of microns from the un-insulated metallic electrode tip [11, 12]. It is also necessary that the electrodes are MRI-compatible while maintaining a high quality of neural recordings. Previous work presented by Santiesteban et al., [13] addressed important issues related to short-term compatibility of silicon microelectrodes. In that report, identification and replacement of appropriate components of a silicon microelectrode system resulted in virtually artifact-free MR images and stable recordings of EMUA, although chronic studies were not performed.

In order to extend the work of Santiesteban et al. the project described here provides a quantitative assessment of the longitudinal stability and quality of EMUA, comparing animals that have undergone extensive MRI scanning (N=4) vs. control animals (N=3) that were not scanned. The current work uses an MR-safe and compatible chronic microwire based microelectrode system capable of such longitudinal monitoring. In addition, the MRI scans were used to assess the initial extent of tissue damage as a result of microelectrode insertion, and the resolution of the damage as a function of time. Estimates of heating from the microwires were assessed in phantoms using the proton resonance frequency (PRF) MRI method. Preliminary end-point histology analysis was also performed to determine the extent of tissue damage.
Materials and methods

Surgical procedure

All animal procedures followed the NIH Guidelines for the Care and Use of Animals and were approved by Penn State University’s institutional animal care and use committee (IACUC). After administration of the initial anesthesia (ketamine/xylazine/acepromazine (50:5:1 mg/kg) the rat was cleaned via application of ethanol and iodine, and its head was shaved and stabilized in the stereotaxic frame. Areflexia was maintained in the frame by gaseous administration of isoflurane (1-1.5% in 0.4 l/min of O₂). A midline scalp incision was made to expose the cranial plates. A craniotomy opening was created roughly 2 mm lateral to the midline and 2 mm anterior to the bregma. The dura was pierced with a 27 gauge hypodermic needle and further opened with microscissors to accommodate the electrode array. The pia-mater was kept intact. Electrodes were inserted into the brain using a computer-controlled micromanipulator at a speed of 10 µm/sec. The final implant depth was 1 mm to target layer V of the cortex. Once implanted, the site was irrigated by a saline-soaked gel-foam. Bone-screws were placed in the parietal plates. Finally a dental acrylic headcap was formed to enclose and stabilize the exposed cranial plates, bone screws and electrode array.

Construction of an MRI compatible electrode array

The microwire electrode assembly typically used in our laboratory, described in [14, 15], was found to give large image artifacts due to the presence of materials with a significant magnetic susceptibility, as shown in Figure 4.1(a). Individual components were tested by embedding in agarose gel and measuring image distortion (data not shown) and it was found that, in particular, the presence of the standard Omnetic connector and bone-screws produced a large
image distortion. Therefore, a modified assembly was developed to replace this connector with a custom-made in-house version using a plastic interconnect (Series 310, MIL-MAX, Oyster Bay, NY) with the electrodes soldered into the pin bases. Brass or polyetheretherketone (PEEK) were used as bone screws. The implant consisted of four 50 μm diameter tungsten electrodes insulated with polyimide (California Fine Wire) arranged in a 2 x 2 array. Each electrode was spaced 1.25 mm apart (separated by dental acrylic) and soldered to individual pin connectors. A fifth pin connector was soldered to a stainless steel ground wire and attached to the other four. The uninsulated tip of each microwire electrode served as the recording site. Solder joints were insulated and secured with epoxy and cyanoacrylate glue. Figure 4.1(b) shows the electrode assembly and connector: images acquired with the modified microwire array are shown in Figure 4.1(c) with much reduced image distortions compared to Figure 4.1(a).

![Figure 4.1: Image distortion and Custom micro-wire electrode assembly to improve it.](image-url)

(a) Image distortion induced by metallic bone screws and connectors. No feature can be identified. Spin Echo Sequence: TR = 1sec, TE = 6.75ms, NT = 4. Matrix = 128x128, FOV = 60x60mm. Expt. Time = 512 sec. (b) [Top] Micro-electrode array used in the study. [Bottom] Close-up of the connector piece that was constructed for multiple connection/disconnection cycles. Interface piece to connect the nano-miniature ommnetics connector of the headstage with the head-cap embedded custom connector for extracellular multi-unit activity monitoring. (c) Improvement in image quality after replacement of bone-screws and connectors with compatible equivalents. TR = 3500msec, TE = 20msec, Averages = 2, Acquisition matrix = 128x96, FOV = 21x21mm, Slice Thickness = 0.4mm, Total Acquisition time = 672 sec, Resolution = 164μmx220μm.
Electrophysiological recordings

Recordings were carried out in sessions of five minutes with the animal either awake or lightly sedated. Initially, animals were lightly sedated with isoflurane to facilitate connection of the head-cap embedded pin-connectors with the interface adapter (Figure 4.1(b), bottom, right). Once connected, extracellular voltages from each of the four intracortical electrodes were recorded with respect to a ground wire attached to one of the anchoring bone screws. The signals were boosted with a unity gain field effect transistor (FET) buffering stage and passed to a lightweight bio-amplifier (Medusa, TDT) which digitized the signals (25 kHz) with low-noise 16-bit A/D converters. The signals were multiplexed and transmitted via a fiber-optic cable to rack-mounted TDT modules, digitally band-pass filtered (300 Hz-5 kHz), down-sampled at 12 kHz, and streamed to disk for offline analysis. In order to track the state of the interface, electrode impedances were also measured (data not shown) at 1 kHz with a Bak™ electrode impedance meter (IMP-1 Electrode Impedance Tester, Bak Electronics, Mt. Airy, MD).

Data analysis was similar to that described in [14-16]. Briefly, candidate neural spikes were detected by threshold crossing (set to three times the standard deviations of the raw signal) and extracted as 3 ms segments. To further eliminate potential mastication and related non-neuronal artifacts from contributing to the neural interface performance measures, correlation coefficients were calculated between threshold detected segment of an electrode and simultaneous segments from other functional electrodes of the array. The rationale was that a truly neural spike would be recorded on only one electrode of an array whereas non-neuronal spike generated as a result of animal chewing or movement would be registered across multiple electrodes and hence be highly correlated. A correlation threshold of 0.75 was chosen to distinguish between neural and non-neuronal signals.
Figure 4.2: Neural data analysis. (a) A four-minute duration raw recording data. (b) A representative zoomed-in segment that shows threshold at 3 times the standard deviation, candidate neural spikes that exceed this threshold and noise-floor at 2 times the standard deviation. (c) Mean-spike generated by overlay of threshold exceeding segments (Inset) as discussed in methods section.

The threshold detected signal segments that remained were sorted in ascending order of their signal amplitudes. Only the top one-fifth of these signals was considered for further analysis since they would most likely originate from regions closest to the electrode site and hence from a region most likely to be affected by MR exposure. A mean-spike was generated by overlaying the top one-fifth of the threshold detected signals aligned to their depolarizing minima and their peak-to-peak amplitude was determined. The noise floor magnitude was estimated by determining the standard deviation of the remaining signal and multiplying it by two. The ratio of the mean-spike peak-to-peak amplitude and noise floor was considered as the signal-to-noise ratio (SNR). Figures 4.2(a) and 4.2(b) show a raw recording segment and figure 4.2(c) shows the generated mean-spike. Cumulative SNR comparisons were made between MR exposed rats and controls at Week 1(Day 0-7), Week 2(Day 8-14), Week 3-4(Day 15-28) and Week 5-6(Day 29-43). SNR of all individual electrodes was calculated and then averaged over the time duration under consideration.

Magnetic resonance imaging

All MRI experiments were performed using a Varian Direct Drive console, a 7 tesla Magnex horizontal bore magnet, and a gradient insert with inner diameter 12 cm and maximum
gradient strength 400 mT/m. A quadrature birdcage coil, inner diameter 6.9 cm, was used for both transmission and reception. Animals were imaged at days 1, 8, 15, 29 and 43 days post-implant. The animals were anesthetized in a chamber using isoflurane and were transferred to an acrylic cradle that was custom-built to stabilize the animal head and body inside the magnet. Once in the magnet, anesthesia was maintained via a nose-cone delivering 2% isoflurane and 0.5 l/min oxygen. During scanning, the animal’s temperature and respiration rates were monitored (SAI Model II, Brooklyn, NY) using a rectal probe and pressure transducer, respectively, to ensure that they remained constant.

T2 maps were calculated from six spin-echo images acquired with different values of TE (10, 20, 30, 40, 50, and 60 ms, TR=3500 ms for all datasets). T2 values are known to be different for different tissues. They represent the time it takes for transverse spins to dephase due to energy exchange with surrounding nuclei. T2 is defined as the time it takes for transverse signal to drop to thirty seven percent of its original value as a result of this dephasing. Forty contiguous coronal slices were acquired with an acquisition matrix of 128 x 96 and a field of view of 2.1 x 2.1cm giving an in-plane resolution of 164 µm x 220 µm. The slice thickness was 400 µm and two signal averages were acquired. The T2 values were calculated on a pixel-by-pixel basis by fitting a single exponential to the signal intensity as a function of TE. The total data acquisition time was approximately 75 minutes. T2 values were compared for identical regions in the same hemisphere as electrode implantation vs. the contralateral hemisphere using a one-sided t-test assuming equal variance (this process was repeated three times by different individuals). Analyzed regions were slightly farther (≈1mm) from the electrode tips to eliminate inclusion of areas with image artifacts caused by microwires.
Temperature measurements in a gel phantom using MRI were based on the proton reference frequency method [17]. A four-electrode array was implanted into an agarose gel containing 100 mM NaCl to mimic physiological conductivity. A twenty-second train of RF pulses (600 pulses, power 1 kW) was applied to the phantom, before a slice-selective gradient echo image was acquired in a plane containing the four electrodes. The phase change in the image is proportional to the change in temperature from a baseline image. This process was repeated thirty times. The temperature was estimated at different points close to, and far away from, the electrodes to determine whether the presence of electrodes induced higher temperature changes.

**Histology**

The animals were euthanized within 1-2 days after the end of the final imaging session. Rats were anesthetized with an overdose of ketamine-xylazine-acepromazine (KXA) and perfused intracardially with 0.5 mg/ml heparinized phosphate buffered saline (PBS) (120 ml at 70 mm Hg), followed by 4% paraformaldehyde (100 ml at 100 mm Hg) and finally again by PBS (120 ml at 70 mm Hg). The electrodes were carefully extracted from the brain by removing the skin and bone around them without disturbing the implant site. The brain region of interest was carefully cut, immersed in optimal cutting temperature (OCT) compound and frozen by placing it for two minutes in isopentane cooled in dry-ice. Frozen brain samples were stored at -80°C. Brain tissue samples were sliced horizontally into 10 µm thick sections in a cryostat maintained at -20°C. Sections at successive depths were double labeled with primary antibodies mouse anti-glial fibrillary acidic protein (GFAP) (an intermediate filament protein in astrocytes, Chemicon) and rabbit anti-Iba1 (a cytoplasmic antigen in microglia and macrophages, Wako). Fluorescently conjugated secondary antibodies were donkey anti-mouse-Cy2 and donkey anti-rabbit-Cy3.
(Jackson ImmunoResearch). All sections were counterstained for nuclei with Hoechst dye (5 
µg/ml, Sigma).

Fluorescent images were acquired using a Hamamatsu Orca-ER digital camera mounted 
on an Olympus BX61Epi-Fluorescence Microscope with a 10X objective. Sections closest to the 
electrode tip insertion depth of 1 mm were chosen for imaging. All sections were imaged in a 
single session, and the exposure time was set below saturation and kept constant for each channel. 
All imaging and acquisition parameters were controlled using the software package SlideBook 
4.1. Fluorescence intensity as a function of distance from the electrode was calculated in slices 
that were closest to the exposed electrode tip. Slices stained for GFAP/Iba1 were selected. Images 
were rotated to center the electrode footprints to perform subsequent quantitative analysis using 
MATLAB. Initially, a sector was defined by considering the electrode tip as its center and 
defining the sector angle and final radius in such a way to avoid hollow spaces created by large 
blood vessels and adjacent electrode tips. The entire sector was spanned by incrementing in steps 
of one pixel starting from an inner radius identified by the edge of the hollow space created by the 
electrode to the final outer radius value. For each increment the mean-intensity of the pixels 
added to the sector (i.e. pixels at the circular edge of the sector) was calculated. In this way a 
single intensity value was obtained for each pixel increment away from the center of the sector. 
Thus the magnitude and spatial spread of astrocytes and microglia was estimated. Refer to figure 
4.3.
Figure 4.3: Histology Image Quantification. (Left) A slice of tissue around the electrode track is analyzed by defining center, angel, inner and outer radius of a sector. (Right) Intensity profiles for astrocyte (GFAP), microglia (Iba1) response corresponding to this slice are shown. Microglia intensity peak is observed in close proximity to the electrode whereas astrocyte intensity peak is slightly distal to it.

Results

Four rats were chronically implanted with micro-wires. Three rats survived the entire study duration of six weeks whereas head-cap failure on day 16 required euthanization of the remaining rat. Regular MR imaging sessions (1, 8, 15, 29 and 43 days post-implant) were carried out to measure potential changes in brain T2 values. Over the implant duration, tissue viability close to the recording tips was estimated from electrophysiological recordings that were obtained on a near daily basis and processed to determine recording performance. Electrophysiological recordings from three control rats with no MR exposure that were part of a separate study [14] were used for baseline comparison of recording performance. End-point histology was also attempted.
Electrophysiological Measurements

(a) R16
(b) R17
(c) R18
(d) R19

Figure 4.4: Extracellular Multiunit Recordings (a) A collection of 780ms recording segments acquired on the last day of the experiment. Spiking activity is observed on all the functional electrodes as indicated by (*). (b) Neural looking mean-spikes generated from recordings on the functional electrodes on the last day of the experiment.

Spontaneous extracellular multiunit neural activity was recorded in 16 electrodes (four in each animal) for 6 weeks post-implant. Out of 16 electrodes, 12 recorded distinct neural activity prior to MR exposure. One electrode lost good neural recording during the course of the implant and showed an abrupt rise in impedance that was attributable to wire-breakage. The remaining eleven electrodes recorded distinct neural activity (as determined by the shape of the mean-spike) throughout the course of the experiment. Figure 4.4(a) shows typical recording traces with examples of spiking activity on the last day of the experiment and Figure 4.4(b) shows shape of mean-spikes generated. These clearly suggest the presence of neuronal activity in close proximity to the electrode even after periodic MR exposure. A comparison between cumulative SNR trends of neurophysiological recordings acquired from animals that underwent MRI
scanning vs. controls (no scanning) at multiple post-implant times is shown in Figure 4.5. There was a statistically significant difference (p<0.05) in the SNR at week two post-implant.

![Figure 4.5: Analysis of neural recordings, Signal-to-noise ratio. Comparison of signal-to-noise ratios of functional electrodes between MR-exposed and control implants reveals minimal differences. Statistically significant difference (* p<0.05) observed only in week two. No abnormal decrease in SNR of MR-exposed rats is observed, thereby pointing to a relatively stable interface not affected by MR imaging.]

**Tissue damage assessed by MRI T2 maps**

Figure 4.6(a) and (b) shows T2 maps from consecutive slices through two different animals, one implanted in the left hemisphere and the other in the right. The locations of the micro-wires are easily visible as dark lines in the image (indicated by ovals). The results of statistical analysis are shown in Figure 4.7, comparing the T2 values in the implanted hemisphere vs. those in the contralateral hemisphere. There was no significant difference in T2 values between the two hemispheres.
Figure 4.6: Example of T2 variability. A) T2 maps from MRI Rat16 at Day 7 post-implant shows elevated values proximal to the implant location (circled). B) T2 maps from MRI Rat18 at Day 30 post-implant shows no difference between implant and control hemispheres. TR = 3500msec, TE = 10,20,30,40,50,60msec, Averages = 2, Acquisition matrix = 128 x 96, FOV = 21x21mm, Slice Thickness = 0.4 mm, Total Acquisition time ~= 75 mins, Resolution = 164 µm x 220 µm. Ovals indicate the site of electrodes.

Figure 4.7: T2 value analysis. Summary of T2 values in all the image slices that spanned the electrode arrays in all animals. There was no significant difference between the implanted and contra-lateral control hemispheres in the susceptibility free regions proximal to electrodes. P Values (one-sided t-test assuming equal variance) Day 0: 0.2, Day 7: 0.7, Day 14: 0.4, Day 28: 0.8, Day 42: 0.4. Combining all imaging sessions for all animals: 0.6.
MRI-temperature mapping

As outlined earlier, one of the major concerns in performing MRI in the presence of electrodes is the possibility of inducing tissue heating close to the electrodes. Figure 4.8(a) shows a magnitude MR image through a plane containing the four electrodes implanted into the agarose gel. Application of a long train of RF pulses causes the gel to heat up slowly, as shown in Figure 4.8(b). However, it is also apparent that there is no additional heating present at locations (i) and (ii) which are very close to the electrodes compared to locations (iii) and (iv) which are much further away. These results show that, at least for the particular micro-wire configuration used in this study, that electrode-induced heating is not a concern. It should be noted that the RF energy deposition in this experiment is far greater (1-2 orders of magnitude) than would be used in a typical imaging study.

Figure 4.8: MRI-based temperature mapping in a gel phantom with "implanted" microelectrodes. (a) A magnitude image taken through a slice perpendicular to the long axis of the microelectrodes. The positions of the four wires can be identified. (b) Temperatures derived from successive phase maps of the phantom using the temperature-dependent proton reference frequency during RF heating from the MRI coil. Results are shown for two regions very close to the microelectrode array, (i) and (ii), and far away from the array, (iii) and (iv). The identical nature of the heating behavior shows that there are no heating effects produced by the interactions of the RF field with the microelectrode array.
**Histology**

Figure 4.9(a) shows immunofluorescent microscope images from control and implanted hemisphere in an animal in which two electrode sites E3 and E4 were clearly evident in the slices at depths close to the final implant depth of 1 mm. The unimplanted control hemisphere had homogeneous immunoreactivity for GFAP (green) and Iba1 (red), indicating astrocytes and microglia, respectively. In contrast, the implanted hemisphere had elevated immunoreactivity associated with astrocyte and microglia activation proximal to the electrode site (Figure 4.9). Fluorescence intensity was measured at varying distances from the electrode (Figure 4.9b). In the control region the fluorescence level was homogeneous across the section, while in the implanted region it was highest close to the electrode and diminished to normal levels within 50-100 um from the electrodes.
**Figure 4.9: Histology analysis.** A) Section stained for astrocyte and microglia activation acquired at a magnification of 20X. (Left) Section from control hemisphere, (Center, Right) Section around electrode in R18. B) Intensity analysis of the same slices acquired at a magnification of 10X. Unimplanted control hemisphere showing stable intensities associated with astrocytes (GFAP), and microglia (Iba1) activation. Electrode sites 3 and 4 show slightly elevated values for microglia and astrocytes very close to the electrode that return to normal values within 50µm.

**Discussion**

The use of MRI in microelectrode studies is increasingly common. For example, MRI has been used to locate explanted electrode tracks [18], to locate microelectrode recording sites in-vivo with high resolution [19], and to help in target localization prior to surgical implantation of microelectrode arrays [20]. Some reports have successfully explored the simultaneous use of microelectrode recordings and MRI with the goal of understanding the relationship between fMRI
signals and underlying neural activity[1, 21] while microelectrode stimulation in conjunction with fMRI has been used to map functional connectivity in the brain [2]. Finally, studies have been performed using specialized microelectrode setups in monkeys [22] (glass-guided platinum-tungsten electrodes) and rats [13] (silicon microelectrodes) to establish their acute compatibility.

The goal of this study was to establish the long-term, chronic MR compatibility and safety of microwire electrode arrays which form a significant portion of microelectrode based recording and stimulating experiments. This can be particularly helpful in multi-modal studies that plan to monitor the state of tissue-electrode interface over the course of the implant, and/or studies that seek to track changes in functional connectivity and adaptability of the brain using magnetic resonance imaging and microelectrode based neurophysiological monitoring. To achieve this, an MR compatible microwire setup was developed and tested in-vitro for extent of image artifacts and temperature changes around the electrodes. Chronic in-vivo tests included implantation of the microwire arrays in rats followed by MRI sessions over a period of six weeks.

Microelectrodes typically record neural activity from tissue within a couple of hundred microns from the electrodes[11, 12]. Hence an estimate of tissue viability is the quality of extracellular neural recordings sensed by the implanted microelectrodes. Multiunit extracellular recordings were carried out throughout the duration of the experiment including on days before and after MRI sessions. The quality of the electrophysiological recordings was not statistically different between imaged animals and controls. One of the concerns with combined MRI and microelectrode recordings is potential tissue heating local to the electrodes. It has been well established that temperature increases result in irreversible and exponential damage to neural and glial cells. Moreover, temperature changes coupled with damage to endothelial cells of the brain and exposure of serum proteins across the blood-brain barrier causes edema, amplifies cell
damage [23], and in turn severely degrades electrophysiological recordings. The unchanged quality of recordings, together with preliminary histological results, strongly suggests that MR exposure did not induce irreversible changes to neural and glial cell health.

MRI can also be used to follow both the short-term and chronic state of neural tissue post implantation. Short term, T2 measurements are a good indicator of edema evolution. Edema is one of the distinctive complications of acute hyperthermia and flow of serum proteins across a compromised blood-brain barrier [24]. In the experiments shown here, small differences in T2 value between the implanted and control hemispheres were restricted to first week post-implant. Longer-term, T2 measurements of the brain are known to be associated with glial and neuronal densities [25, 26]. Histology studies of intracortical microelectrode arrays have demonstrated significant glial activation and reduction of neuronal population close to the implants. However, such changes have only been reported in regions very close (<<1 mm) to the implants. Therefore, it may be possible to use very high-resolution MRI to non-invasively follow the processes of gliosis and astrocytosis which are believed to be responsible for the ultimate degradation in neural recordings that eventually occur in all electrophysiological recordings. Such experiments may require improvements in the MR compatibility of the electrodes themselves in order to reduce further any image artifacts, or else magnetic susceptibility compensated MR sequences might have to be incorporated [27]

Conclusion

This study demonstrates the longitudinal feasibility of performing Magnetic Resonance Imaging in rodent models implanted with microwire electrodes. Neurophysiological recordings were carried out to detect extracellular multiunit action potentials, brain tissue T2 measurements
were obtained by regular magnetic resonance imaging session and preliminary histological evaluation was also done. Comparable neurophysiological outcomes between MR-exposed and control rats, as well as comparable T2 values between implanted and control hemispheres were observed. They point to the possibility of safely using MRI as a non-invasive imaging modality to probe the tissue-electrode interface and undertake studies that leverage strengths of both invasive and non-invasive brain sensing techniques. Future studies will use MRI on intracortical microwire implanted brains to measure tissue parameters like Anisotropic Diffusion Coefficient, employment of magnetic susceptibility compensated MR sequences and detailed histological evaluation to detect any damage that can be attributed solely to MR exposure.

References


Chapter 5

Future Directions

Lack of chronic functionality of intracortical microelectrode arrays is arguably the single-most important reason for its limited success in clinical and research domains. There is an enormous scope for improvements to extend their useful life-time. These include development of biocompatible coatings, incorporation of neural-growth factors to promote growth of neurons close to the electrode tips, improvements to surgical protocols and design of electrode structures with reduced footprint.

Before the benefits of such improvements can be evaluated and compared to determine the most optimal approach it is necessary to develop a tool-set that can render quantification to electrode recording quality. It is also necessary to develop techniques that can provide a multi-modal view of the electrode-tissue interface so that the results of these techniques can be used to better understand the underlying post-implant neural remodeling. Currently, the impact of the latter in influencing electrode functionality is unknown.

Algorithm for Neural Interface Quantification

The goal of this algorithm is to detect neural spiking events in an automated and objective manner. Events so detected can be used to measure recording quality by determining signal to noise ratio, peak to peak signal amplitude, rate of spiking events etc.
In its current form, the algorithm has only been tested in microwire electrode arrays. These arrays are widely spaced (≥250µm) allowing for direct application of results from modeling studies. Microfabricated electrode structures made from silicon or ceramic substrates, tetrodes etc can have closely spaced (≤100µm) metallic recording sites. Thus a refinement to the correlation algorithm that takes into account inter-electrode spacing in determining an optimal correlation threshold is required.

The performance measures calculated by this algorithm are stand-alone. That is, their dependence on degree of gliosis or neuron survivability hasn’t been established. As a first step in this direction an effort should be made to determine the relation between performance measures and impedance levels. For example, changes in peak to peak amplitude should only be dependent on neuronal survivability close to the electrode and hence show little or no correlation with changes in impedance levels. Similarly, the noise in signal to noise ratio is indicative of distantly firing neurons and may be modulated by the extent of glial scar. Hence changes in noise levels should exhibit higher correlation with changes in impedance levels. Such dependencies if observed would provide greater degree of validation to the proposed algorithm. Histological analysis would provide the best validation. In this scenario, higher peak to peak amplitude is observed in subjects having greater neuron density close to the electrode tip and an inferior signal to noise ratio in subjects with higher degree of gliosis. Thus it is important that follow-up studies be carried out that attempt to establish a link between the performance measures of the developed algorithm and the outcomes of impedance measurements and histology analysis.

Finally, correlation-based elimination of threshold-detected spikes can have significant implications in reducing the over-head associated with wireless headstages. Currently, wireless head-stage placed in an animal’s head or body-pack transmits all events that exceed a predetermined threshold to a base-station. The transmission bandwidth constraints impose an
upper limit to the number of channels/electrode sites that can be analyzed in real-time by the head-stage and be transmitted wirelessly. By incorporation of inter-electrode correlation analysis on the head-stage, a number of threshold-detected spikes can be rejected as false-positives thereby eliminating their need for transmission and thus freeing up the transmitter for transmission/processing of more channels.

Enhancement: Collagenase-Aided Intracortical Microelectrode Array Insertion: Effects on Insertion Force and Recording Performance

Goal of the collagenase study was to explore its use in reducing implant associated insertion forces and trauma in order to facilitate use of flexible and thinner electrode arrays. The study also showed improved neural recording quality as determined by the previously developed algorithm.

In addition to collagenase, use of other enzymes like lipase, dispase etc or a cocktail of these enzymes can be experimented in further reducing insertion forces. Moreover a thorough analysis needs to be carried out to understand the exact mechanism for disruption of meninges by these enzymes as well as to determine any damage to the brain tissue. Results of these studies can help determine refinements to the application protocol, like changes to the concentration and duration of enzyme exposure.

As important aspect of this study was the development of an automatic force measurement setup. The current analysis was concentrated only around comparison of peak-insertion forces. This system is extremely sensitive and is capable of detecting minute forces
associated with electrode’s contact with the brain surface. Hence it is possible to accurately measure the distance traveled by the electrode between its first contact with the brain surface and the peak of insertion force. This distance is a good indicator of the magnitude of brain compression or dimpling. Thus studies can be envisioned wherein brain compression and quality of neural recordings are examined to determine the impact if any, of the former on the latter. Currently the evidence of brain dimpling in negatively affecting neural recording quality is indirect at best.

**Monitoring: Feasibility and Safety of Longitudinal Magnetic Resonance Imaging in Rodent Model with Intracortical Micro-wire Implants**

Magnetic Resonance Imaging is a non-invasive, non-ionizing imaging technique that offers excellent soft-tissue contrast. It has been used to extensively study brain remodeling associated with traumatic brain injury and stroke. However use of MRI with implanted microelectrodes had not been attempted in survival studies. Part of the reason is that MRI is contraindicated with metallic implants since alternating magnetic fields present during MRI can induce currents in metallic structures resulting in excessive heating and damage at the uninsulated surface like electrode tip. Hence the purpose of this study was to merely test the ability of MRI in imaging implanted electrodes and to determine any damage caused by sustained MR exposure. Favorable results obtained from it should pave the way for many studies involving multimodal tracking of the device tissue interface.

For example, it has been recently found that surgical brain injury can result in functional metabolic deficits in the implanted hemisphere. Specifically it was found using Positron Emission Tomography (PET) that $^{18}$F-fluorodeoxyglucose uptake in the implanted hemisphere was
significantly lower. Similarly studies involving MR imaging of the brain after a micro-knife cut have shown hemisphere-wide elevated values of Anistropic Diffusion Coefficient (ADC) with correspondingly heightened astrocyte activation. Similar studies can be carried to detect hemisphere wide changes in metabolites as a result of microelectrode implantation using Magnetic Resonance Spectroscopy or changes in ADC values, T1 or T2 values using Magnetic Resonance Imaging. Again, these measurements can be used to study their impact on the quality of neural recordings. Since MRI is used in detecting damage associated with stroke and traumatic brain injury, it is possible to implant electrodes in different/downstream processing centers of the brain to determine the affect of this damage to other parts of the brain that is not readily observed with MRI.

It should be emphasized that the present study was carried out with electrode implanted in the motor cortex. The safety associated with implants in other parts of the brain like auditory cortex wherein the orientation of the electrodes with respect to the alternating magnetic fields is different from that of the motor cortex hasn’t been evaluated. Current study was carried out using metallic microwire electrodes. The resulting distortion prevented image-analysis within 1mm from the electrode, which is the region undergoing most changes due to implantation. Hence use of silicon microelectrodes having minimal metal content can be tested to reduce image distortion and potentially enable MR analysis of regions very close to the electrode.

Summary

The work presented in this dissertation aimed to address the challenges of developing and testing methods that can allow for better comparison of different electrode-platforms and the changes thereof for longitudinal functionality. It presented an algorithm for efficient and realistic
electrode performance measurement, demonstrated the use of it in evaluating and comparing differences in surgical protocol and finally tested the safety and feasibility of a non-invasive technique in observing post-implant neural remodeling. It thus lays the ground-work for a multitude of future studies to realistically measure electrode performance and better understand interface degradation thereby enabling improved identification and incorporation of modifications to electrode structure, implantation and monitoring processes with the aim of extending microelectrode functionality.
Appendix A

Chronic Microwire Electrode Fabrication

This document describes the fabrication of a 4x2 configuration of a microwire electrode-array that is used in chronic experiments. It can be extended to other array configurations as well.

Attachment of Omnetics Connectors to PCB boards

Custom PCB boards with footprints that matched the Omnetics Connector and thru-holes that matched microwire diameter were designed and used in this phase.

Figure 1: Custom PCB and Omnetics Connector. (Left) Arrangement of the pieces to elucidate small dimensions, (Center) Close-up of the board and connector, (Right) Alignment and attachment of PCB and connector to make the connector-board assembly. Make sure that the printed side of the connector is opposite to the ground trace (trace connecting hole closest to the connector and parallel footprints)

1) Align and solder PCB footprints to the male ends of the connector. Make sure that the center footprints are accurately aligned. This reduces misalignment error at the periphery. Precise application of solder-flux on the PCB footprints or to the Omnetics Connector prior to aligning and soldering is recommended but not required. Solder the two pieces together.
2) Use a digital multi-meter (DMM) to check for connectivity and shorts. Make sure that no two pads are shorted together and that all thru-holes are connected to their respective points of the Omnetics Connector.
3) Apply a thin layer of epoxy to only cover the footprints and the male end of connector. Do not cover any thru-hole (especially the ground hole closest to the connector) of the
PCB. This imparts stability and strength of the connector-PCB assembly that is required in subsequent steps.

**Attachment of microwires to the connector-board assembly**

1) Under microscope, fill the connector thru-holes with solder-flux using a 2µl pipette. This helps in filling the holes with solder in the next step.
2) Fill the holes with solder using a thin soldering rod.
3) Cut eight pieces of 4inch long microwire (0.0014inch ø) from the spool.
4) Burn 0.25inch of insulation at one end and 1-2mm of insulation at the other end using a gas lighter.
5) Now place the end of microwire with 1-2mm insulation removed on the thru-hole that is currently filled with solid solder. Apply compression force that is just enough to slightly bend the wire, but not displace it from the top of the solder filled thru-hole.
6) Carefully bring the soldering iron close to filled thru-hole. Place it in direct contact with the solid solder.
7) The heat from the soldering iron melts the solder and allows penetration of the wire through it. Make sure that only the exposed part of the wire penetrates through the molten solder to create a connection with the PCB. The insulation will prevent connection if the wire is inserted too far into the thru-hole. The wires will fall out in a later step if they are inserted too shallow.
8) Remove the soldering iron to harden the solder and thus embed the exposed part of the microwire in the thru-hole.
9) Repeat steps 5-8, on alternate thru-holes for each side, thus connecting only four microwires on each side of the connector. Similar attachment patterns could be used for other threading configurations (eg. 2x8 or 4x4 array patterns).
10) Again check the continuity between the solder-filled thru-hole and the end of microwire using a DMM.

**Figure 2: Formation of connector-board-microwire assembly. (Left) Connector thru-holes filled with solder, (Right) Attachment of microwires to the thru-holes.**
Array Formation

1) Place a thin layer of plastic-wrap on the guide holes of the jig. This prevents clogging of the jig guide holes by dental acrylic.

2) Attach the connector-board-microwire assembly to the manipulator.

3) Use Teflon coated microforceps to thread microwires into the guide holes by piercing through the plastic-wrap. Guide-holes are spaced 250 µm apart. Hence thread microwires through the guide-holes based on the spacing requirements of the array.

4) Once the wire is threaded through the hole, hang a weight one inch below the jig surface. This weight will provide tension and keep the microwires aligned when applying acrylic to form the array.
   a. The weights should be placed high enough on the hanging microwire that when the manipulator is lowered to its minimum height the weights do not touch anything below
   b. It is necessary to make certain the weights are hanging in the same pattern as the threaded microwires when threading a 4x4 array. This step is not required for 2x4 or 2x8 arrays. It is difficult to untwist the wires once the weights are attached, but the untwisted wires will prevent many construction errors of the final electrode array. A strip of tape was used to keep each microwire hanging in its proper place.

5) After hanging the weights, lower the manipulator such that the microwire-connector interface is close to the jig.

6) Now apply acrylic so as to form a supportive island. Let it stand, dry and harden for 10-15 minutes. This will create the initial 2x4 array spaced at 250 µm.

Figure 3: Array formation, threading of microwires through the jig. (Left) Assembly attached to the manipulator, (Center) Appearance of threaded microwires prior to attachment of weights, (Right) Close-up of the jig with plastic wrap removed. It clearly shows how the microwires are threaded through guide-holes of the jig in the 2x4 array.
7) Elevate the manipulator by a centimeter and form another island. If the plastic wrap does not separate from the acrylic island, carefully separate the two without touching the array.

8) Repeat the previous step for two-three islands, each spaced 1-1.25cm apart.

Figure 4: Array formation, straightening of threaded microwires through the jig. (Left) Weights suspended to each microwire to straighten it. (Right) Close-up of the lowered assembly. Dental acrylic is applied to strengthen connection of microwires to the PCB.

Figure 5: Array formation, formation of acrylic islands to form straight, equally spaced microwire arrays. (Left) Three nearly equally spaced acrylic islands. Notice the small shape of these islands. It is absolutely essential that these features be as small as possible. (Center, Right) Weights suspended to each microwire to straighten it.
9) After the third island is formed and hardened, cut the wires between the weights and the jig or the lowest island and the jig, depending on how far the manipulator can raise. A quick snip with sharp medical scissors will provide best results. Remove array from
10) Similar to the microwire method, attach ground wire to the connector. Use the thicker Medwire for this purpose.
11) Apply a thin layer of epoxy on the entire PCB. This provides mechanical strength to the solder joints and also serves as additional electrical insulation.
12) Check impedance. The value should not be too low (<100Ω) or too high (>1MΩ)

![Image of soldered ground wire](image.png)

Figure 6: Attachment of thicker ground wire to the electrode array.

Packaging and Sterilization

1) Cut rectangle pieces 3x1 inch in dimension. Cut another piece 1x0.5inch in dimension.
2) Attach the smaller piece at one end of the larger piece using epoxy.
3) Attach a hollow Teflon tube to remaining part of the larger rectangular piece using a scotch tape.

![Image of protective tube arrangement](image.png)

Figure 7: Custom protective tube arrangement to prevent electrode array from bending
4) Gently introduce the electrode array into the tube such that the microwires are safely encased by the hollow tube while the connector end rests on the smaller rectangular piece.
5) Attach the connector end to the piece using a scotch tape.
6) Place the encased array in a sterilization bag.
7) Electrodes are sterilized by gamma irradiation (dosage 5 Megarad) at the Radiation Science Center.

Figure 8: Final package with electrodes meant for sterilization prior to chronic surgery
Appendix B

Surgical Procedure and Histology Details

SURGICAL PROCEDURE

Animal Preparation

All animal procedures followed NIH Guidelines for the Care and Use of Animals and approved by the Penn State IACUC committee (IACUC Protocol #20920). A custom-built plastic knockdown chamber was used to initially anesthetize the rats. Isoflurane vapor at 5% concentration was delivered with oxygen at a flow rate of 0.8L/min using Cyprane Tech V, Eagle Eye Anesthesia, FFV. While anesthetized, the animal’s head was shaved and cleaned with alternate application of iodine and 70% ethanol. It was then transferred to a surgical stereotaxic frame (myNeuroLab.com, Product #463001) and its head was stabilized using ear-bars, front teeth clamp and nose-cone. Nose-cone was also used to deliver isoflurane anesthesia at 2.5% vapor concentration at 0.4L/min oxygen flow-rate to maintain areflexia. A final application of iodine and 70% ethanol was made on the shaved head to ensure sterility. A heating blanket maintained at 37°C was placed beneath the animal to maintain its body temperature. A pulsoximeter (Nonin, 8500AV) probe was clamped onto one of the hind legs to monitor pulse rate and blood oxygenation percentage. Isoflurane flow rate was maintained at 0.4L/min throughout the surgery, while the percent vapor was adjusted to maintain a pulse-rate range of 250 to 350 beats per minute, a breathing rate of 30 to 48 breaths per minute, and a blood oxygenation of at least 95%. Depth of anesthesia was monitored through the toe-pinching reflex every 15 to 20 minutes.
Surgical Details

Survival surgeries required the use of sterile techniques. All the surgical tools were autoclaved at 121°C for 20 minutes. Surgery began with a midline scalp incision made with a scalpel to expose the cranial plates. The fascia covering the cranial plates was trimmed away with scissors until where the jaw muscles began. The remaining fascia was scraped away with a scalpel blade. Craniotomies were created 2-4mm lateral to the midline and 2-4mm anterior and posterior to the bregma by drilling through the skull using a hand-held manual drill. Saline-soaked Gelfoam (Pharmacia & Upjohn Company, 09-031503) was placed in the craniotomies to maintain moisture. Depending on the nature of the experiment, either one or two craniotomy sites were opened further using rongeurs (Fine Science Tools, 16221-14) to approximately 2x3mm in dimension. A section of the dura mater was then lifted upward with a 27G needle, before an incision was made through it with micro spring scissors (Fine Science Tools, 15000-08) across the craniotomy in the anterior to posterior direction. The sliced dura mater was cut again in the lateral directions and peeled aside with microforceps to expose the pia mater. Bone-screws were placed in the remaining two or three craniotomies to prepare for implant insertion.

Implant Insertion

The implant (see Appendix A for more details) was secured in a custom-designed vice connected to a three-axes micromanipulator and lowered to the implantation site, allowing the electrode tips to barely touch the surface. The electrodes were then inserted through the pia mater and into the cortex at a speed of 10μm/sec. Insertion was carried out by outputting TTL pulses under computer control to a custom-built stepper motor setup that drove an oil hydraulic micromanipulator (Narishige MWO-3). The final implant depth was restricted to 1mm to target
layer V pyramidal cells. The ground wire was then tied around one or both of the posterior bone screws and Gelfoam was used to cover the implantation site. The headcap was built from the skull to the base of the connectors with dental acrylic in order to enclose the exposed cranial plates and secure the implant to bone screws.

Recovery and Post-Implant Monitoring

After surgery, the animal was allowed to recover in the cage till he was fully awake and able to move around. Soft/crushed food and water were provided initially. The implanted animal was monitored daily by measuring its weight and any abnormal behavior that it may demonstrate. Electrode impedance measurements were made using a meter from Bak Electronics Incorporated. 1KHz was used as the test signal. Electrophysiological recordings were also done.

HISTOLOGY

Surgical preparation for perfusion

At the end of the experiment rats were heavily anesthetized with a ketamine-xylazine cocktail (30 mg ketamine per kg) through intraperitoneal injections. An initial cut was made near the abdomen and scissors were used to cut the skin between the abdomen region and mouth. Another incision was made through the fascia directly above the xiphisternum, which then protruded outwards. Microforceps were used to grasp and lift the xiphisternum away from the heart, as blunt-tipped scissors was used to cut through the sternum bones from the bottom to the top of the rib cage. The rib cage was then pulled apart and held in that position with a retractor (Fine Science Tools, 17012-11) at the separated sternum. The diaphragm was pushed downward,
while the pleura were trimmed away to allow full access to the lungs and heart. The right lung was peeled back with forceps to expose the spinal cord and descending aorta. The descending aorta was clamped to the spinal cord with a hemostat to prevent flow to the lower extremities and limit perfusion to the upper body. The heart was stabilized by holding it with forceps at the apex, while a catheter guided by an 18G needle (Abbocath-T, Abbott, G715-A01 4535-18) was inserted into the bottom of the left ventricle. Once the catheter and needle were in the heart, the needle guide was removed and the catheter was guided through the left ventricle into the aorta. When the catheter tip could be seen through the transparent aorta, the catheter inlet was connected to the outlet of the pressurized perfusion system. The right atrium was then lacerated with a scalpel blade to create an opening through which the used perfusion fluids would escape.

**Pressurized perfusion system and perfusion fluids**

The primary goal of this step was to perfuse and fix the animal by removing body fluids and passing fixative through its system under standardized conditions of fixation volume and time. The pressurized perfusion system was built using three polyethylene bottles (McMaster-Carr, 4280T35), a disassembled sphygmomanometer, male and female polypropylene barbed tube fittings (McMaster-Carr, 51525K133 and 51525K263), clear polyurethane tubing (McMaster-Carr, 5195T63), and luer-style stop cocks (McMaster-Carr, 7033T112).

Pressure was finely controlled by manually pumping the sphygmomanometer bulb. Fluid flow was controlled through the opening and closing of the valves on the stop cocks. Each rat was perfused with 120 mL of heparinized phosphate balanced saline (PBS) (0.5 mg/mL) at 70 - 80 mm Hg, 100 mL of 4% paraformaldehyde for 6 minutes at 100 – 110 mm Hg, and 120 mL of PBS at 70 – 80 mm Hg. The flow of concentrated heparinized PBS through the rat removed blood deposits possibly present due to hemorrhaging caused by implantation and other trauma. A
shorter fixation time prevented excessive cross-linking of tissue, which allowed for more binding sites in immunohistochemical labeling. A standardized fixative volume and fixation time, created by the removal of fixative through a second perfusion with PBS, made successive histological results comparable.

**Brain Extraction and quick-freezing**

After fixation, the brains were frozen to facilitate subsequent sectioning. The rats were decapitated with scissors, their heads were carefully skinned and muscle tissue removed with scalpel blade so as that only skull remained. The skull bone around the brain was carefully removed with skull flap cutter (Fine Science Tools, 16050-15) to expose the ventral part of the brain. The brain was then carefully separated from the skull and the implanted electrodes. This was extremely difficult since on numerous occasions, brain tissue got torn-off and got attached to the electrodes. The extracted brain was then dried with Kimwipes to prevent surface fluid from expanding abnormally and thereby damaging the surrounding tissue. Brain section containing foot-prints of implanted electrodes were carefully cut from the rest of the tissue using a scalpel blade. It was then placed in 15mm x 15mm x 5mm cryomolds (Sakura, Intermediate 4566) filled with OCT compound (Tissue Tek, 4583), with the implantation sites facing downward. The cryomolds containing the brain tissue and OCT compound were then immersed for at least 1 minute in isopentane (VWR International, EMMX0760-1) that had been chilled with dry ice to -140°C. After freezing, samples were wrapped in aluminum foil, labeled, and placed immediately back onto dry ice until they could be stored in a -80°C freezer, as thawing and refreezing would destroy the histology.
**Tissue sectioning and immunohistochemistry**

The brain tissue samples were sectioned axially into 10-micron thick sections with a -19° to -20°C cryostat and placed on glass slides. Sectioning began on the tissue furthest away from the implantation sites. Sections were saved in intervals of 40 to 100μm while the corpus callosum was still visible within the surrounding cortical tissue. Serial sections were taken once the sections only contained cortical tissue. Sections that were not immediately labeled were stored at -20°C. The slides were then rinsed with 1X PBS for 5 minutes, followed by each section being circled with a PAP pen (Rockland Immunochemical, RLKHP001) for easy identification in the future. 200μL of blocking buffer (10% donkey serum in PBS with 0.1% triton) was applied to each slide of sections in a humid chamber at room temperature for 1 hour. Sections were then treated with 200μL of their respective primary antibodies and incubated in a humid chamber at 4°C overnight.

Selected slides from various depths of electrode insertion were labeled with two primary antibodies in the following combinations: anti-GFAP and anti-Iba1, anti-NeuN and anti-Iba1, anti-Agrin and anti-Iba1, anti-Occludin and anti-Iba1, and anti-NeuN and anti-NSE (neuron-specific enolase). Three 1X PBS with 0.1% triton washes for 20 minutes each were followed by the application of 200μL of their respective secondary antibodies conjugated to cyanine dyes. All antibodies were diluted with 10% serum. Hoechst dye (1:1000, Sigma) was used as the counterstain and was added to the secondary antibodies at a concentration of 0.5μg/mL. For each batch of slides labeled, one slide of tissue was treated with only secondary antibodies to serve as a control for monitoring non-specific bindings. The sections were incubated in a humid chamber covered with aluminum foil for 1 hour. Another three 1X PBS with 0.1% triton washes for 20 minutes each followed. 1.5”-thick coverslips were mounted over each section with 3 to 4 drops of
Aqua-Poly/Mount (Polysciences, Inc., 87001-902). Slides were then stored at 4°C before being imaged.
Appendix C

Code used for Implementation of IEC Algorithm

Top Wrapper

% Commented by Kunal: 11/30/08
% This is a master code that calls two functions: RunThresholding and RunCorrelation.
% The former detects candidate spikes by simple thresholding and stores them in files
called spike_stores whereas the latter works on spike_stores and checks to % determine the
degree of correlation amongst different electrodes. As a % result, the contribution of non-neural
spikes in the calculation of % electrode performance matrices like SNR, PK-to-Pk amplitudes is
% significantly reduced
%
clear all
close all
clc;

daysCell = {'1', '2', '4', '5', '6', '10'}; % Enter days for which processing is to be done
dataTypes = {'B1L','B2R'} % Enter information about the blocks. For Bilateral studies it
is advisable to follow the format
% RXXDYYBZL/R. R,D and B stand for Rat, Day
% and Block respectively. XX == Rat Number,
% YY == Day past implant. Day0 is the day of
% implant. Z == Block i.e either block 1,2 etc
% for the TDT. L/R is either left or right
% side.

ctr=1
for i=1:length(daysCell)
    for j=1:length(dataTypes)
        dataSetCellctr = strcat(daysCell(i),dataTypes(j)); % Day, block and anatomy info is
        % concatenated.
        ctr = ctr+1;
    end;
end;

for fileCtr = 1:length(dataSets)
    % f=fullfile ('E:','R46_RawDataD0_D13','R46D11B2R.mat'); %Select the file to be
    loaded
    a = ['R47D',char(dataSets(fileCtr)),'.mat']; % The entire file name
f = fullfile ('C:', 'Documents and Settings', 'kjp170', 'Desktop', 'CollagFig3', 'a'); % The entire path info.
[pathstr, name, ext] = fileparts(f);
success = RunThresholding(f); % returns a one if there is no error while running the RunThresholding function.
if (success == 1)
    name1 = [name, 'E1', '_spike_block'];
f2 = fullfile(pathstr, [name1 ext])
disp ('Running Correlation');
successCorr = RunCorrelation(f2);
end;
end;

Threshold Based Detection of Events.

function success = RunThresholding(fname) % fname is file name with the path.
try
    mastPosThres = 150; % Voltage value beyond which it is definitely mastication. Recommended 500uV.
    mastNegThres = -300; % Voltage value beyond which it is definitely mastication below zero. Recommended -750uV.
    zeroWindow = 250; % Length of time (in terms of number of samples) that should be zeroed out when extremely large signals are detected.
    prePeak = 10; % Number of samples prior to the negative peak of the candidate spike.
    postPeak = 29; % Number of samples after the negative peak of the candidate spike.
    % Thresholding algorithm version 2.
    % Changes:
    % Try to automate as much as possible.
    % Use a variable for file name and the Row_Index and have these entered only once.
    % Get rid of all unnecessary code.
end;
% Windowing of only 3 ms and not 4 ms.

%f=fullfile ('C:','Documents and
%Settings','Administrator','Desktop','ChinmaysAlgo','R50','RawData_Onex
%Standard','R50D31B2R.mat'); %Select the file to be loaded. Not used if
%Topwrapper is run earlier.
f=fname;
load(f); % Raw Data with all electrodes is loaded.
[pathstr,name,ext]=fileparts(f);

for Row_Index=1:8 %Which row we are running on
    % Row_Index is displayed
    s1=['R_Good_Test=',name,'(Row_Index,:);'];  % Equivalent to R_Good_Test =
    % name(Row_Index,:);

    eval (s1);
    % Mastication Detection and Zeroing. This block is no longer used.
    % thres_index = find(R_Good_Test>mastPosThres); % Arbitrary Threshold to be
decided everytime
    % diff_thres_index = thres_index(2:end) - thres_index(1:end-1); % Distances
    % between consecutive index values
    % if(length(diff_thres_index) > 1)
    %    for n = 1:length(diff_thres_index)
    %        if((thres_index(n) > zeroWindow) &&(thres_index(n) < (length(R_Good_Test)
    %            - zeroWindow)))
    %            R_Good_Test(((thres_index(n)) - zeroWindow):(thres_index(n))) = 0; %
    %            Additions to block out the data. 20ms prior.
    %        else
    %            R_Good_Test(thres_index(n)) = 0;
    %        end
    %    end
    % end

    % thres_index_neg = find(R_Good_Test < mastNegThres); % Similarly for negative
    % mastication threshold
    % diff_thres_index_neg = thres_index_neg(2:end) - thres_index_neg(1:end-1);
    % when is this used? IT IS NOT USED.
    % if(length(diff_thres_index_neg>1))
    %    for n = 1:length(diff_thres_index_neg)
    %        if((thres_index_neg(n) > zeroWindow) &&(thres_index_neg(n) <
    %            (length(R_Good_Test) - zeroWindow)))
    %            R_Good_Test(((thres_index_neg(n)) - zeroWindow):(thres_index_neg(n))) =
    %            0; % Additions to block out the data. 20ms prior.
R_Good_Test((thres_index_neg(n)):(thres_index_neg(n)) + zeroWindow)) = 0; % Additions to block out the data. 20ms post

% else
% R_Good_Test(thres_index_neg(n)) = 0;
% end
%
% end
%
R_Good_Test_Truncated = R_Good_Test((R_Good_Test~=0)); % remove all zero elements

Next, find the 3 sigma threshold on the reduced data set

std_dev_threshold = 3*std(R_Good_Test_Truncated); % Three standard deviation

% Now find the indices that are below -3 Standard Deviations. This is your % negative crossing. On the left side of this the index try to find the % negative crossing (i.e. going from positive to negative) and on the right % side find a positive crossing (i.e. going from negative to positive) % followed by negative crossing (i.e. going from positive to % negative).

negative_3STD_Index = find(R_Good_Test_Truncated < -std_dev_threshold);

% GROSS Left negative crossing, that is going from negative to positive
left_neg_cross_index=zeros(1,length(negative_3STD_Index)-1); % INITIALIZE!!
actual_index = 1;
for m = 1:(length(negative_3STD_Index) - 1)
    if (R_Good_Test_Truncated(negative_3STD_Index(m)) < 0) % This condition is always true.
        actual_index = negative_3STD_Index(m);
        while ((R_Good_Test_Truncated(actual_index) < 0) && (actual_index > 1))
            actual_index = actual_index - 1; % since we are going left
        end
        left_neg_cross_index(m) = actual_index;
    end
end;

% GROSS Right negative crossing, that is going from negative to positive
right_neg_cross_index=zeros(1,length(negative_3STD_Index)-1); % INITIALIZE!!
actual_index = 1;
for m = 1:(length(negative_3STD_Index) - 1)
    if (R_Good_Test_Truncated(negative_3STD_Index(m)) < 0)
        actual_index = negative_3STD_Index(m);
        while ((R_Good_Test_Truncated(actual_index) < 0) && (actual_index < length(R_Good_Test_Truncated)))
            actual_index = actual_index + 1; % since we are going right
end
  right_neg_cross_index(m) = actual_index;
end
end;

% Detection of the lowest value between the two crossings
% Initialize peak_neg_value and peak_neg_value_index

peak_neg_value=zeros(1,(length(negative_3STD_Index)-1));
peak_neg_value_index=zeros(1,(length(negative_3STD_Index)-1));

a = 1;
for k = 1:(length(negative_3STD_Index) - 1)
  [peak_neg_value(k),peak_neg_value_index(k)] =
  min(R_Good_Test_Truncated(left_neg_cross_index(k):right_neg_cross_index(k))); % Peak negative value between two zero-crossings and its corresponding index.
  peak_neg_value_index(k)=peak_neg_value_index(k)+left_neg_cross_index(k)-1;
end;

% If there are more than one peak negative values between the left and
% right zero crossings, then those values are considered as one.
c = 1;
for b = 1:length(peak_neg_value_index)
  if((b < length(peak_neg_value_index)) && (peak_neg_value_index(b + 1) -
  peak_neg_value_index(b)) < 2)
    b = b + 1;
  else
    actual_neg_value_index(c) = peak_neg_value_index(b);
    b = b + 1;
    c = c + 1;
  end
end
actual_neg_value_index=actual_neg_value_index(1:c-1);

Start_Spike_Index=zeros(1,length(actual_neg_value_index)-1);
End_Spike_Index=zeros(1,length(actual_neg_value_index)-1);
% For a uniform window for each spikes. 1ms before the
% actual_neg_value_index and 3 ms after the actual_neg_value_index

for m = 1:(length(actual_neg_value_index) - 1)
  if((actual_neg_value_index(m) > 10) && (actual_neg_value_index(m) <
  (length(R_Good_Test_Truncated) - 30)))
    Start_Spike_Index(m) = (actual_neg_value_index(m) - prePeak); % 0.75 ms prior to Peak Negative
  end
end
\text{End\_Spike\_Index}(m) = (\text{actual\_neg\_value\_index}(m) + \text{postPeak}); \ % 2.25 \ ms 

after Peak Negative

\text{else}
\begin{align*}
\text{Start\_Spike\_Index}(m) &= 1; \ % \text{This is a fix. Not sure of the results.} \\
\text{End\_Spike\_Index}(m) &= \text{prePeak} + \text{postPeak}; \ % \text{TO DO Check this properly}
\end{align*}

with some code.

\text{end}

\text{end}

\%SNR Calculations

\text{Spike\_Matrix} = \text{zeros}((\text{length(\text{actual\_neg\_value\_index})-1}), (\text{max(\text{End\_Spike\_Index})+1})); \ %\text{INITIALIZE}!!

\text{Peak\_To\_Peak} = \text{zeros}(1, \text{length(\text{actual\_neg\_value\_index})-1}); \ %\text{INITIALIZE}!!

for \ m = 1:(\text{length(\text{actual\_neg\_value\_index})-1})
\begin{align*}
\text{Spike\_Matrix}(m,:) &= \text{Start\_Spike\_Index}(m):\text{End\_Spike\_Index}(m); \\
\text{Peak\_To\_Peak}(m) &= \text{max}(	ext{R\_Good\_Test\_Truncated}(\text{Spike\_Matrix}(m,:))) - \\
&\text{min}(	ext{R\_Good\_Test\_Truncated}(\text{Spike\_Matrix}(m,:))); \\
\end{align*}
\text{end}

\%\text{Saving data for each row for future use}
\text{mast\_red} = (\text{R\_Good\_Test} \neq 0); 

\text{name1} = \text{strcat(pathstr,} , '\text{E}', \text{num2str (Row\_Index)}, '_\text{spike\_store}', \text{ext}); \ % \text{TO DO Can this name be converted to spike\_matrix instead of spike\_block.}
\text{save (name1,}\ '\text{Row\_Index}', 'Spike\_Matrix', 'mast\_red'); \ %\text{Thus spike store is saved for each electrode that has data from suprathreshold event and corresponding events from remaining electrodes%}
\text{end};
\text{success} = 1; \ %\text{returns 1 if there are no errors in the execution of the thresholding module%}

\text{catch}
\text{success} = 0; \ %\text{returns 0 if there is an error. This will then require checking of the data-set and line by line debugging%}
\text{end};
Inter-electrode Correlation based elimination of False-positive detections

function success = RunCorrelation(fname)

try
  % BUNCH OF DEFINES
  corr_vector_threshold=0.75;

  % This file does scanning on intra electrode correlation and uses maximum
  % and minimum values of correlation to draw upon thresholds
  %
  % Version 2.0 Streamlined storage of spike_block. Now this file creates the
  % spike block and then it operates on a clean memory.

  % Version 2.1 changes
  % Added 2 extra correlation thresholds. The first one combines linearly ALL
  % correlation coefficients and forms a "correlation index". Thus, we are
  % only eliminating places where there is sufficient correlation between ALL
  % electrodes. The second threshold adds and subtracts weights from the
  % different correlation coefficients, the weights being conditioned on the
  % geometry of the electrodes. Then we eliminate as mastication signals
  % spikes that show high correlation between at least 3 electrodes.

  % clear all;

  % Select the file to be loaded
  % f1=fullfile ('C:','Documents and Settings','Administrator','Desktop','ChinmaysAlgo','R46','R46D14B4RE1_spike_block.mat');
  % Select the file to be loaded
  % f1=fullfile ('C:','Documents and Settings','Administrator','Desktop','ChinmaysAlgo','R47D1B1RE1_spike_block.mat');
  % f1=fullfile ('C:','Documents and Settings','Administrator','Desktop','ChinmaysAlgo','R46D30B1LE1_spike_block.mat');

  f1 = fname;

  [pathstr,name,ext]=fileparts(f1);
  name1=name(1:end-14);  % TO DO Is there a way to make 14 as a define.
  disp (strcat('operating on data set:',name1));

  f2=fullfile(pathstr,[name1 ext]);
  disp ('Loading all electrode data');
  load (f2);  % Data from all the electrodes is loaded. Raw Data is loaded.

  s3=['clear ',name1(1:end),'_TS'];  % The TimeStamps are removed since they are not used at all.
  eval (s3);
tic
for Electrode_Index=1:8
    name2=strcat(name1,'E',num2str(Electrode_Index),'_spike_store');
f3=fullfile (pathstr,[name2 ext]);

disp (strcat('Loading data from electrode #',num2str(Electrode_Index)));
load (f3);  % Loading Spike Block for a given electrode.

[pathstr,name,ext]=fileparts(f1);
name1=name(1:end-14);   % TO DO  Make this a constant.

%To get from R_Good_Test to R_Good_Truncated
s2=['R_Good_Test_Truncated1 = ',name1,'(:,mast_red);']; % Truncated data set from all electrodes is loaded. For a given electrode, if a segment is truncated then the corresponding segment from all the electrodes are removed as well while doing correlation analysis.
eval (s2);

Spike_Block=zeros(size(Spike_Matrix,1),size(Spike_Matrix,2),size(R_Good_Test_Truncated1,1))  % No. of Spikes, Length of Spike, No. of Electrodes.
for i=1:size(Spike_Matrix,1)
    Spike_Block(i,:,:)=R_Good_Test_Truncated1(:,Spike_Matrix(i,:))';    % Notice the Transpose Operation. For each spike detected on a given electrode, corresponding segments from all electrodes are loaded into the Spike_Block.
end;

[no_spikes,spike_length,no_electrodes] = size(Spike_Block);
corr_coeff_matrix = zeros(no_spikes,no_electrodes,no_electrodes);   % Correlation of spike segment on each electrode with respect to corresponding segment on every other electrode
corr_vector = zeros (no_spikes,no_electrodes);  % One needs to only consider the electrode of interest and not all electrodes.
for i=1:no_spikes
    one_group=squeeze(Spike_Block (i,:,:));
a=corrcoef(one_group);  % If Spike_Length == Rows and No_Electrodes == Columns, then correlation of each column wrt every other column is calculated.
corr_coeff_matrix(i,:,:)=a;
    %Set all diagonal elements to zero
    for j=1:no_electrodes
        corr_coeff_matrix(i,j,j)=0;
    end;

%Looking at only one row of the correlation matrix
    corr_vector(i,:)=corr_coeff_matrix(i,Row_Index,:);  %Row_Index Info comes from Spike_Block.
end;
end;

% Setting a Threshold for correlation matrix detection
% I am assuming we look at only one of the rows. This will depend on the
% Row_Index and we look at that particular row, and select a maximum value
% of allowed correlation

%%%%%%
% code to pick threshold%%%%
%%%%%%

% assume threshold

% assume we are using the correlation vector to form a threshold

% find the maximum value of the correlation vector for each reading
corr_vector_max=max (corr_vector,[],2);
corr_vector_boolean=corr_vector_max>corr_vector_threshold;
no_spikes_post_correlation=no_spikes-sum(corr_vector_boolean);

% %Additions to v_2_1
% % Using 2 additional metrics to form a correlation threshold
% %
% % Metric 1: Adding/Subtracting Weights to the correlation vector to form
% % the
% % correlation vector weighted. These weights are selected according to
% % the
% % electrode geometry.
% weights_row=[0 0.2 0.2 0.1 0.1 0.1 0 0];
% weights_row=repmat(weights_row,no_spikes,1);
% weighted_corr_vector=corr_vector-weights_row;
% % get threshold from the weighted corr_vector
% % 2 means the sorting is done according to dimension 2 i.e. columnwise
% weighted_corr_vector_sorted=sort (weighted_corr_vector,2);
% % 6 here indicates we are taking the 3rd highest value. 7 would mean taking
% % the second highest value, 8 would be the highest etc.
% weighted_corr_vector_threshold=weighted_corr_vector_sorted(:,6);
% % Set the threshold for method 2:
% corr_vector_threshold2=0.6;
% % Find the no of spikes remaining after this method of correlation
% corr_vector_boolean2=weighted_corr_vector_threshold>corr_vector_threshold2;
% no_spikes_post_correlation2=no_spikes-sum(corr_vector_boolean2);

% % Metric 2: Forming a linear weighted combination of the correlation matrix
% weights_vector=[0 0.05 0.05 0.1 0.1 0.2 0.2 0.3];
% % weights_matrix=repmat (weights_vector, 1,no_spikes);
% weighted_comb=corr_vector*weights_vector;
% Set the threshold for method 2:
% corr_vector_threshold3=0.6;
% Find the no of spikes remaining after this method of correlation
% corr_vector_boolean3=weighted_comb>corr_vector_threshold3;
% no_spikes_post_correlation3=no_spikes-sum(corr_vector_boolean3);
%
% count=0;
% for i = 1:no_spikes
%   %if ((max(corr_vector(i,:)))>corr_vector_threshold)
%   %reject the reading
%   %Spike_Block(i,:)=zeros(spike_length,no_electrodes);
%   %count=count+1;
% end;
% end;

% NOTE THE AS OF TODAY 11/30/08, CORRELATION ACCORDING ONLY
% METHOD ONE
% IS CALCULATED. OTHER METHODS ARE FEASIBLE AND ARE INTUITIVE
% BUT THE WEIGTHS ETC SHOULD BE BETTER ESTABLISHED BY
% THINKING ABOUT
% THE TISSUE PARAMETERS. SOME MODELING KNOWLEDGE IS
% REQUIRED.

%Now, we construct a NEW spike_matrix and a new matrix consisting of
%start_index and end_index
disp ('Constructing a new spike matrix acc. to threshold 1');
Spike_Matrix_PC=zeros(no_spikes_post_correlation,spike_length);
count=0;
for i=1:no_spikes
  if ((corr_vector_boolean(i)==0))
    count=count+1;
    Spike_Matrix_PC(count,:)=Spike_Matrix(i,:); % This matrix has spikes that are
    %below the correlation threshold.
  end;
end;
no_spikes_post_correlation_array(Electrode_Index) = no_spikes_post_correlation;
no_spikes_array(Electrode_Index) = no_spikes;
if (no_spikes_post_correlation > 0)

R_Good_Test_Truncated=R_Good_Test_Truncated1(Row_Index,:); % To DO
Considering only one electrode now. Row_Index and Electrode_Index should match. Have a test
for that.

%Finding new values of SNR after correlation
Peak_To_Peak_PC=zeros(1,no_spikes_post_correlation); % INITIALIZE!!
for m = 1:no_spikes_post_correlation
Peak_To_Peak_PC(m) = max(R_Good_Test_Truncated(Spike_Matrix_PC(m,:))) - min(R_Good_Test_Truncated(Spike_Matrix_PC(m,:))); 
end 

Sort_Peak_To_Peak_PC = sort(Peak_To_Peak_PC); 
Top_20Per_Sort_PC = Sort_Peak_To_Peak_PC(floor(0.8*length(Sort_Peak_To_Peak_PC)) + 1):length(Sort_Peak_To_Peak_PC)); % +1 is a fix. 
Mean_Top_20Per_Sort_PC = mean(Top_20Per_Sort_PC); % Top 20 Mean obtained directly from individual Peak to Peak Signal amplitudes. 

n = 1; 
Minimum_Sorted = min(Top_20Per_Sort_PC); 
for m = 1:(no_spikes_post_correlation-1); 
if(Peak_To_Peak_PC(m) >= Minimum_Sorted) 
Top_20_Spike_Block_PC(n,:) = R_Good_Test_Truncated(Spike_Matrix_PC(m,:)); 
n = n + 1; 
end 
end 
mean_waveform_Top20_PC = mean(Top_20_Spike_Block_PC); % Mean wave-form of Top 20% of detected spikes. 
clear Top_20_Spike_Block_PC; 

pk_2_pk_meanwaveform_Top20_PC = max(mean_waveform_Top20_PC) - min(mean_waveform_Top20_PC); 
Start_Spike_Index_PC=Spike_Matrix_PC(:,1); % TO DO Is there a better way of doing this like size(Spike_Matrix,2) 
End_Spike_Index_PC = Spike_Matrix_PC(:,end); %I think this is the code you are looking for.. 
Spike_matrix_min_PC = min(min(R_Good_Test_Truncated(Spike_Matrix_PC))); 
Spike_matrix_max_PC = max(max(R_Good_Test_Truncated(Spike_Matrix_PC))); 

Mean_Spike_PC=zeros(1,(max(End_Spike_Index_PC-Start_Spike_Index_PC))); % INITIALIZE % The Mean of the Spike Matrix i.e. our Mean Waveform 
for m = 1:(max(End_Spike_Index_PC-Start_Spike_Index_PC)) % Number of Data Points at the sampling rate of 12.5KHz. 3 ms window. 
Mean_Spike_PC(m) = mean(R_Good_Test_Truncated(Spike_Matrix_PC(:,m))); 
% Calculating mean along 39 data points. 
end 

% Calculation of SNR According to Donoghue's Paper 
Noise_Matrix_PC=zeros(no_spikes_post_correlation,max(End_Spike_Index_PC-Start_Spike_Index_PC)); %INITIALIZE 
R_Good_Test_Truncated = double(R_Good_Test_Truncated); %TO DO Example of explicit type conversion. 
for m = 1:no_spikes_post_correlation 
Noise_Matrix_PC(m,:) = (Mean_Spike_PC - R_Good_Test_Truncated(Start_Spike_Index_PC(m):(End_Spike_Index_PC(m)-1))));
Row_Noise_Matrix_PC = reshape(Noise_Matrix_PC,1,[]);
Noise_Std_Dev_Actual_PC = std(Row_Noise_Matrix_PC);
Brown_SNR_PC = (max(Mean_Spike_PC) -
min(Mean_Spike_PC))/(2*Noise_Std_Dev_Actual_PC);

% Hybrid SNR Approach

m=floor (no_spikes_post_correlation/2);
temp = Start_Spike_Index_PC(2:m)-End_Spike_Index_PC(1:m-1);
temp=temp(temp>0); %I think this line is required since there are a few occasions
when a spike is accounted for more than once. Hence, we might be including signal in
calculations otherwise. Please check this since it involves a moderate worsening of the Hybrid
SNR value obtained.
temp_sum=sum(temp); % TO DO Does this determine the length of the noise
signal.

%Noise_Signal_Matrix=zeros(max_length,floor(((length(actual_neg_value_index)))/2));
Noise_Signal_PC=zeros(1,temp_sum);
clear temp; %Since it is used again.
sum_index=0;
start=1;
for m=1:floor (no_spikes_post_correlation/2)
    temp=(Start_Spike_Index_PC(m+1) - End_Spike_Index_PC(m))+ 1;
    if (temp>0) %not required if you use
        sum_index=sum_index+temp;
    end;
    start=sum_index+1;
end;
Hybrid_Std_Noise_PC = std(Noise_Signal_PC);
Hybrid_SNR_PC = (max(Mean_Spike_PC) -
min(Mean_Spike_PC))/(2*Hybrid_Std_Noise_PC);

Mean_Spike_Block_PC(Row_Index,:) = Mean_Spike_PC;
Spike_matrix_min_Block_PC(Row_Index,:) = Spike_matrix_min_PC;
Spike_matrix_max_Block_PC(Row_Index,:) = Spike_matrix_max_PC;
Mean_Spike_max_PC(Row_Index,:) = max(Mean_Spike_PC);
Mean_Spike_min_PC(Row_Index,:) = min(Mean_Spike_PC);
pk_2_pk_Mean_Spike_PC(Row_Index,:) = max(Mean_Spike_PC) -
min(Mean_Spike_PC);
Brown_SNR_Block_PC(Row_Index,:) = Brown_SNR_PC;
Hybrid_SNR_Block_PC(Row_Index,:) = Hybrid_SNR_PC;
Mean_Top_20Per_Sort_Matrix_PC(Row_Index,:) = Mean_Top_20Per_Sort_PC;
mean_waveform_Top20_block_PC(Row_Index,:) = mean_waveform_Top20_PC;
pk_2_pk_meanwaveform_Top20_block_PC(Row_Index,:) =

pk_2_pk_meanwaveform_Top20_PC;

else
    disp('VERY VERY BAD ELECTRODE. CHECK RAW DATA')
    Hybrid_SNR_PC = 0;
    Mean_Spike_Block_PC(Row_Index,:) = 0;
    Spike_matrix_min_Block_PC(Row_Index,:) = 0;
    Spike_matrix_max_Block_PC(Row_Index,:) = 0;
    Mean_Spike_max_PC(Row_Index,:) = 0;
    Mean_Spike_min_PC(Row_Index,:) = 0;
    Brown_SNR_Block_PC(Row_Index,:) = 0;
    Hybrid_SNR_Block_PC(Row_Index,:) = 0;
    Mean_Top_20Per_Sort_Matrix_PC(Row_Index,:) = 0;
    mean_waveform_PC(Row_Index,:) = 0;
end

clear Noise_Signal_PC;
clear Spike_Block;
clear Spike_Matrix;
clear Noise_Matrix_PC;
clear Row_Noise_Matrix_PC;
clear corr_coeff_matrix;
clear mast_red;
clear R_Good_Test_Truncated;
clear R_Good_Test_Truncated1;
clear pk_2_pk_meanwaveform_Top20_PC;
toc

% plot(mean_waveform_Top20_block_PC(Row_Index,:));
% figure;
end;

name8 = strcat(name1, '_PerformCoeff',ext);
save
(name8,'Brown_SNR_Block_PC', 'Hybrid_SNR_Block_PC', 'Mean_Spike_Block_PC', 'Mean_Spike_max_PC', 'Mean_Spike_min_PC', 'Mean_Top_20Per_Sort_Matrix_PC', 'mean_waveform_Top20_block_PC', 'no_spikes_array', 'no_spikes_post_correlation_array', 'pk_2_pk_Mean_Spike_PC', 'pk_2_pk_meanwaveform_Top20_block_PC', 'Spike_matrix_max_Block_PC', 'Spike_matrix_min_Block_PC', 'corr_vector', 'corr_vector_boolean', 'corr_vector_max', 'corr_vector_threshold');
clear Brown_SNR_PC;
clear Electrode_Index;
clear Hybrid_SNR_PC;
clear Hybrid_Std_Noise_PC;
clear Mean_Spike_PC;
clear Noise_Std_Dev_Actual_PC;

clear Noisy_Signal_PC;
clear Peak_To_Peak_PC;
clear Mean_Top_20Per_Sort_PC;
clear Sort_Peak_To_Peak_PC;
clear Top_20Per_Sort_PC;
clear Row_Index;
clear Spike_matrix_min_PC;
clear Spike_matrix_max_PC;
clear a;
clear count;
clear ext;
clear f1;
clear f2;
clear f3;
clear i;
clear j;
clear m;
clear name;
clear name1;
clear name2;
clear no_electrodes;
clear no_spikes;
clear no_spikes_post_correlation;
clear pathstr;
clear s2;
clear s3;
clear spike_length;
clear start;
clear sum_index;
clear temp;
clear temp_sum;
clear one_group;

success = 1;

catch
    success=0;
end;
Experimental Overview: Signal Processing

Signal Processing overview. A) Electrode Array. B) Simultaneous recordings from 8-channel array. C) Signal segments that exceeded a threshold of 2.5 standard deviations of the signal (green line) were extracted as 3-ms candidate neural spikes. D) The correlation algorithm seeks to eliminate candidate spikes that are highly correlated with the signal segments recorded simultaneously on other electrodes in the array. E) Candidate neural spikes from 'st' and 'stC' on 2-D PCA space to identify regions of higher density. F) Mean-spike generation and feature calculation.
Figure 3: Signal Processing Overview. A) Detailed schematic of the implanted electrode array with approximate dimensions. B) Simultaneous recording from an eight channel array including the resultant ensemble average (used for virtual reference). C) Signal segments that exceeded a threshold of 3 standard deviations of the signal (green line) were extracted as candidate neural spikes (3ms segments). D) The correlation algorithm seeks to eliminate candidate spikes that are highly correlated with signal segments recorded simultaneously on other electrodes in the array. The correlation coefficient between each candidate spike and the corresponding signal segment on each of the electrodes is determined. All spikes with correlation coefficient $R > 0.75$ are classified as non-neuronal and discarded from further analysis. E) At the end of processing of each data set, a mean-spike was computed from all valid spikes detected from each of the four algorithms (simple-thresholding, differential reference, virtual reference, and inter-electrode correlation; $ST$, $DR$, $VR$, and $IEC$, respectively). Several parameters were obtained from the mean-spike as indicated for comparison purposes.
Appendix D

Histological Complications

Attempts to perform histological analysis of rat brains that were implanted with functional electrodes proved to be extremely complicated and prone to failure. The most prevalent complication was associated with the process of extraction. In this process an attempt is made to gently separate the entire brain from skull. Prior to the final separation attempt; skin, muscle and bone from around the brain except the top part is carefully removed. The goal of the final separation step between the brain and the skull is to remove the implanted electrodes (attached to the skull via acrylic head-cap) from the brain without causing any damage or disruption of the surrounding brain tissue. Such a separation can then allow for histological evaluation of the tissue very close to the electrodes that may have elevated immune response compared to unimplanted region.

However, in most cases this separation was not a clean one. As shown in Figure 1 (left), the brain tissue had variable degree of attachment with the electrodes. This resulted in tearing and separation of tissue from the remaining brain. As a result the region of interest was disrupted (Figure 1, right), thereby preventing any meaningful histological analysis.
Figure 1: Example of disruptive extraction. (Left) The brain tissue was tightly adhered to electrodes on numerous occasions. Lumps of tissue were trapped within the array as shown by circles. (Right) It resulted in tearing of that region from the brain tissue precluding any histological analysis of the tissue around the implant. Rectangles show regions of brain with missing tissue.

Discussion

To the best of author's knowledge, no study in current literature has carried out a systemic and multi-sample histological analysis of brain tissue with chronically implanted microwire electrodes. In fact no study has even been done with recording electrodes that require anchoring to the skull. All studies aimed at understanding the chronic tissue response have only been carried out with floating planar microelectrode arrays made from silicon. Thus these attempts to carry out histology on chronic microwire electrodes lack a published precedence. The observed adherence of tissue around the implant may imply multiple things. It may mean that unlike planar electrodes, the surface of microwire electrodes with uncontrolled roughness may be more amenable to tissue adherence and be more compatible. That may potentially explain improved recording performance with microwire electrodes as compared to silicon electrodes. It may also be possible that unlike planar electrodes where the encapsulation region is restricted to less than 50µm around the structure, tissue reaction with microwires may be such that it encapsulates the entire array thereby forming a sheath around the array that isolates it from the
healthy brain tissue. Although in light of limited reaction observed in instances of uneventful extraction, this seems like an unlikely possibility.

Another possible reason for the observed tissue attachment may merely be procedural. The protocol for perfusion and fixation was borrowed from lab involved in histology of planar probes. It may be possible that blood-vessel disruption and revascularization associated with microwire implant may impede processes of perfusion and fixation in the damaged zone thereby requiring a change in pressure and/or volume of fluids used to achieve desired perfusion and fixation. A modification that can be employed irrespective of the changes in perfusion and fixation protocols would be to gradually dehydrate the tissue using sucrose solution of progressively higher concentration. Such dehydration can shrink the tissue thus increasing the possibility of its detachment from microwire surface. In summary it is worth noting that brain extraction in microwire implant models is not similar to planar microelectrode implants. Hence the above mentioned changes should be tried and a dedicated protocol be developed prior to embarking on a full-fledged study aimed at understanding the role of immune-response to chronic recording functionality of microwire arrays.
VITA

Kunal Paralikar

Kunal J. Paralikar was born in Baroda, India. He attended the Bright High School and Rosary High School for his secondary and higher secondary education respectively. He received the Bachelor of Engineering degree in electrical engineering in 2001 from the Maharaja Sayajirao University of Baroda, Baroda, India, and received both the M.S.E.E. degree in 2003 and PhD degree in Bioengineering in 2008 from The Pennsylvania State University, University Park. From 2002 to 2003, he was a Research Assistant at the Communications and Space Sciences Laboratory, The Pennsylvania State University, where since 2004, he was a Research Assistant in the Neurotechnology Research Laboratory. During summer 2007, he was a Technology Intern in the Sensor Technology Group, Medtronic Neuromodulation, Minneapolis, MN. His current research interests include multimodal investigations of the tissue–device interface, neuroprostheses, and medical–device compatibility.