Towards adaptive micro-robotic neural interfaces:

Autonomous navigation of microelectrodes in the brain for optimal neural recording

by

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ABSTRACT

Advances in implantable MEMS technology has made possible adaptive micro-robotic implants that can track and record from single neurons in the brain. Development of autonomous neural interfaces opens up exciting possibilities of micro-robots performing standard electrophysiological techniques that would previously take researchers several hundred hours to train and achieve the desired skill level. It would result in more reliable and adaptive neural interfaces that could record optimal neural activity 24/7 with high fidelity signals, high yield and increased throughput. The main contribution here is validating adaptive strategies to overcome challenges in autonomous navigation of microelectrodes inside the brain. The following issues pose significant challenges as brain tissue is both functionally and structurally dynamic: a) time varying mechanical properties of the brain tissue-microelectrode interface due to the hyperelastic, viscoelastic nature of brain tissue b) non-stationarities in the neural signal caused by mechanical and physiological events in the interface and c) the lack of visual feedback of microelectrode position in brain tissue. A closed loop control algorithm is proposed here for autonomous navigation of microelectrodes in brain tissue while optimizing the signal-to-noise ratio of multi-unit neural recordings. The algorithm incorporates a quantitative understanding of constitutive mechanical properties of soft viscoelastic tissue like the brain and is guided by models that predict stresses developed in brain tissue during movement of the microelectrode. An optimal movement strategy is developed that achieves precise positioning of microelectrodes in the brain by minimizing the stresses developed in the surrounding tissue during navigation and maximizing the speed of movement. Results of testing the closed-loop control paradigm in short-term rodent experiments validated that it was possible to achieve a consistently high quality SNR throughout the duration of the experiment. At the systems level, new generation of MEMS actuators for movable microelectrode array are characterized and the MEMS device operation parameters are optimized for improved performance and reliability. Further, recommendations for packaging to minimize the form factor of the implant; design of device mounting and implantation techniques of MEMS microelectrode array to enhance the longevity of the implant are also included in a topdown approach to achieve a reliable brain interface.

i.

DEDICATION

To my mother

For being my anchor while giving me wings to fly.

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CHAPTER 1

INTRODUCTION

Implantable microelectrode arrays play a crucial role in a variety of current and emerging biomedical prosthetic devices such as cochlear implants, visual prostheses, brain-machine interfaces, deep-brain stimulation devices etc. These emerging devices promise to restore function in patients with hearing loss, retinitis pigmentosa, patients with amputated upper-extremities or paralyzed limbs, or Parkinsons' disease. In all of the above applications, implantable microelectrode arrays are the primary portal for communication with single neurons or a family of neurons in a specific region of interest in the brain. Arguably, the single, biggest challenge to cortical (brain) prosthetic devices that remains unsolved is the unreliable brain tissue-microelectrode interface. Current microelectrode technologies fail after a few months or years, which is inadequate for brain prostheses that need to last the lifetime of the patient.

We propose here a novel intra-cortical microelectrode interface that will potentially improve the reliability, fidelity and lifetime of neural interfaces. The interface proposed here is an adaptive movable interface with MEMS microelectrode array that autonomously positions the microelectrodes to maintain optimal neural recording quality. The key innovation for realizing the above goal is based on the technology of MEMS movable microelectrode array. MEMS microelectrode arrays have been in development and have gone through several design iterations and testing for the past 10 years. They have been validated in long-term experiments by implanting the device in the somatosensory cortex of rodents where neural activity was recorded for a period of about 12 weeks. Electrothermal actuators in the device allows for bidirectional translation of the microelectrodes. Further, indigenously developed novel chip-scale flip-chip packaging technique for MEMS devices yields a fully functional packaged device with small form factor. The integration of the above MEMS device with intelligent controls makes this technology the first ever MEMS robotic neural implant that is capable of sensing neural signals,

analyzing neural data and adaptively positioning the microelectrodes in accordance with the quality of incoming neural data.

The overall goal of the control algorithm will be to establish a reliable interface. Reliable interface is defined here as one that which is able to achieve high-quality, long-term recordings consistently. A functional neuron-electrode interface is where the neural signal recordings are of high fidelity with signal-to-noise levels above acceptable thresholds. The control objective is to move the electrode in a one-dimensional search for optimal signal-to-noise values along the electrode track. Three main challenges exist in developing the autonomous system: i) the sources of variability in a recorded signal are largely undefined ranging from immune response, surgical issues, physical displacements of the brain tissue due to breathing, biological variations in neural activity etc. ii) the mechanical properties of brain tissue are unknown and hence an accurate model of the plant (3D space of brain tissue populated with neurons) cannot be defined iii). The brain tissue is a hyperelastic and viscoelastic medium and has a time varying response to microelectrode displacement. Further, directional anisotropies are present in the microenvironment immediately around the microelectrode. Knowledge of the precise position of the microelectrode is difficult due to the complex relaxation behavior of the compressed tissue. Hence the algorithm will incorporate a quantitative understanding of the constitutive mechanical properties of the brain to estimate the position of the electrode. The objective of the control algorithm – is to optimize the signal-to-noise ratio (SNR) of the neural recording by moving the microelectrode. The control algorithm is guided by models that infer the microelectrode position and adapt to the dynamic tissue response.

While an adaptive control system will establish an optimal interface for recording, there are engineering challenges at the system level that also impact the reliability of the interface. Reliable neural data recording by implantable MEMS electrodes is dependent on two critical factors: (1) reliability of the interface between the microelectrodes and the brain tissue (2) reliable functioning of the device that is microelectrodes and microactuators over the lifetime of the implant. The latter concern can be mitigated by improved design of the MEMS actuators and

microelectrode system. Failure analysis of the actuators after a few million cycles of actuation is done to determine the operational space for reliable function of the microactuators, microelectrodes and other micromechanical structures associated with them. Further the parameters of activation of the microelectrodes are optimized so that microelectrode can move consistently in both forward and backward direction. The position of the microelectrode in the brain can be then reliably estimated (without taking into account the viscoelastic properties of the brain tissue). Another significant challenge is surgical implantation and mounting of the MEMS device. Mounting is highly customized for different medical applications and devices. For the long-term brain implant, such mounting techniques must accommodate for the animal skull growth, re-growth of skin, and other meningeal layers on the brain etc. Mounting must also be robust enough to protect the packaged MEMS devices from the mechanical impacts due to behavior. Improved packaging and mounting structures can enhance the longevity of the brain implant by creating a stable interface at the surgical site (between the implant and the craniotomy) and inside the brain tissue.

It is our hypothesis that a reliable, high-throughput interface between the brain and implanted electrodes can be realized by using the MEMS movable microelectrode system controlled by an adaptive algorithm that maintains optimal signal quality. At the system level, reliability studies on device operation, recommendations for packaging and surgical implantation are also incorporated in a top-down approach to achieve a reliable interface.

In order to test the stated hypothesis and address the challenges described the following specific aims are proposed:

Specific Aim 1:

Characterization of an optimal electrothermal microactuator and microelectrode for a reliable microelectrode implant: a) determining the optimal parameter set for activation of the microactuators to ensure maximum accuracy in positioning the microelectrodes b) failure analysis to determine the lifetime of operation of the microactuators c) evaluation of design space for

optimal performance i) force requirements at different times after implant ii) structural reliability (with minimal overlapping structures) iii) minimal current consumption and power requirements

Specific Aim 2:

Design and development of adaptive algorithms for establishing a reliable electrodeneuron interface by (a) defining the non-stationarities present in microelectrode recordings over 24-hour duration (b) adapt to signal changes by optimizing movement of microelectrode according to the time-varying constitutive properties of the electrode-tissue interface (c) regulating signal-to-noise by optimal positioning of microelectrodes

Specific Aim 3:

Implementation of adaptive algorithms and in vivo testing (a) packaging, surgery and mounting techniques for MEMS implants (b) software implementation of algorithm for autonomous control (c) in vivo experiments in rodents for validation of control algorithm and characterizing signal quality obtained from adaptive controls

The proposed aims will address the key need of establishing a reliable, high-throughput interface with neurons in the brain. The main contribution of this thesis would be validating strategies to overcome challenges in autonomous navigation of microelectrodes inside a brain tissue that is both functionally and structurally dynamic. The proposed controller will greatly facilitate current 'data deprived' neural signal decoding algorithms that command robotic limbs whose efficiency is largely dependent on good quality neuronal recordings. The adaptive robotic neural recording system will act as an enabling technology that shall provide the means to effectively and reliably link the brain with external devices.

Outline

The rest of the dissertation is organized as follows. Chapter 2 has the results of characterization of the improved peg drive linear ratcheting microactuators. The operating parameters of the MEMS based system that control the dynamics of microelectrode movement were optimized for precise operation in a closed loop control paradigm. Further, analysis of

reliability of the MEMS system for long-term brain implant applications are included. In Chapter 3, experimental observations of the variability in microelectrode recordings over 24-hour duration are presented and the putative causes for such variability in signal quality and the need for developing a control algorithm are discussed. Chapter 4 discusses the rationale, challenges and background for design of control algorithm for autonomous positioning of microelectrodes, presents the proposed approach and a framework for the algorithm. Chapter 5 describes a novel movement paradigm guided by a mechanical model of brain tissue for precise positioning and navigation that adapts to the viscoelastic properties of brain tissue. The mechanical model is incorporated in the feedback loop of the control scheme to minimize the change in relative displacement between the microelectrode and neuron. Chapter 6 discusses the closed loop control scheme with the mechanical model incorporated and the results of testing this control strategy for recording neural activity in the brain. The platforms for software and hardware implementation of the control algorithm are also outlined. Chapter 7 details the challenges in packaging and implantation of MEMS microelectrode arrays over a long term and the progress made so far. In Chapter 8, directions for future research are discussed.

CHAPTER 2

IMPROVED PERFORMANCE AND RELIABILITY OF LINEAR RATCHETING MICROACTUATORS

Introduction

Several MEMS microactuators have been designed and developed for biological applications (N. Jackson et al., 2010; Mukundan & Pruitt, 2009; Muthuswamy, Okandan, Jain, & Gilletti, 2005a, 2005b; Receveur, Lindemans, & de Rooij, 2007). Detailed reviews of a range of MEMS microactuators used for micro and nano-scale manipulations, their capabilities in terms of maximum force, displacement, resolution and frequency can be found in earlier reports (Bell, Lu, Fleck, & Spearing, 2005; Sahu, Taylor, & Leang, 2010). MEMS microactuators with the appropriate design specifications can therefore be chosen based on the requirements of an application. For instance, a polymer V-shaped electrothermal microactuator array was fabricated to measure mechanical compliance of cell in biological media (W. Y. Zhang et al., 2008). Kim et al. (Kim, Liu, Zhang, & Sun, 2008) have demonstrated single cell manipulation using electrothermally activated microgripper with force resolution in the nanonewton range. These examples demonstrate the potential of electrothermal microactuators for precise positioning and manipulation in biological applications. However, several challenges exist in the design, development and performance characterization of these actuators so that they perform with a high degree of repeatability, accuracy and reliability (Baker, Epp, Serrano, Gorby, & Phinney, 2009; Cheng, Chan, Cheng, Hsu, & Lai, 2008; de Boer et al., 2004; Geisberger, Sarkar, Ellis, & Skidmore, 2003; McLain, Lott, Harb, & Howell, 2002; Olney, 2010; J. S. Park, Chu, Oliver, & Gianchandani, 2001). The demands on the microactuator performance are guite varied depending on the specific biological application in question. For instance, long-term implantable applications are significantly more challenging both for the microactuators and the packaging than short-term implantable applications; applications involving cell cultures are typically more controllable than applications involving implantation in vivo. This report focuses on microactuators

specifically designed and developed for brain prosthetic applications in which the microactuators were used to move microelectrodes and monitor electrical activity of single neurons in the brain of awake, behaving animals. The above application demands that such MEMS devices perform reliably over the life-time of a patient, typically 70 years (Kruger, Caruana, Volta, & Rizzolatti, 2010). The longevity of current microelectrode implants in the brain is highly variable (Chestek et al., 2011; Nicolelis et al., 2003) and there is significant degradation in neural signal quality after a few weeks of implantation (Kipke, Vetter, Williams, Hetke, & Nunamaker, 2004; Suner, Fellows, Vargas-Irwin, Nakata, & Donoghue, 2005). Most current microelectrode arrays are fixed, with no capability to re-position them once implanted. Movable microelectrodes in combination with automatic closed loop controls (Cham et al., 2005) are a powerful technology that gives the ability to position the electrode automatically, provide good guality signal-to-noise ratios (N. Jackson et al., 2010) and improve the yield of neuronal units recorded per electrode (Fee & Leonardo, 2001b) potentially extending the lifetime of microelectrode implants. Earlier reports have consistently demonstrated an improved performance using motorized movable microelectrodes for neuronal recording applications in the brain (Battaglia et al., 2009a; Eliades & Wang, 2008; Fee & Leonardo, 2001b; Gray, Goodell, & Lear, 2007a; Haiss, Butovas, & Schwarz, 2010a; A. Jackson & Fetz, 2007; Venkateswaran, Boldt, Parthasarathy, et al., 2005; Wolf et al., 2009; Yamamoto & Wilson, 2008; Yang et al., 2011). However, the above technologies to move microelectrodes do not readily allow for scaling up the number of microelectrodes to record from ensembles of neurons in the brain, which is often a requirement in brain monitoring applications. Surface micromachining of MEMS (Bustillo, Howe, & Muller, 1998) enables creation of complex, microscale, movable systems that can be batch-fabricated using high throughput standard MEMS fabrication processes. Over the past 10 years, the authors have developed in collaboration with Sandia National Labs, novel MEMS based moveable microelectrode arrays fabricated using the SUMMiT-V[™] process (N. Jackson et al., 2010; Muthuswamy et al., 2005a, 2005b). It is a unique technology that gives the ability to precisely position the microelectrode array after implantation while maintaining comparable chip size and weight as that of the conventional microelectrodes.

The most recent MEMS electrothermal actuators were developed to translate the microelectrode using a Chevron-latch type of mechanism (Muthuswamy et al., 2005b). These devices were successfully implanted in rodents and were able to record neural signal activity for over 13 weeks. The above study revealed some important design requirements for the MEMS movable microelectrodes for successful operation in long-term experiments in the brain. Microelectrode movement consistently led to a significant improvement in the quality of the neuronal signal during the first 3 weeks after implantation. However, after three weeks, in 5 out of 11 cases microelectrode movement did not lead to significant improvement in the quality of the signal recorded (N. Jackson et al., 2010). It was suspected that the most likely reason for this degradation in performance was that the microactuators were not effective in moving the microelectrodes past the glial sheath (a fibrous tissue encapsulation) (Bellamkonda, McConnell, Schneider, & Owens, 2007; Reichert, Polikov, & Tresco, 2005) that typically begins to envelop the implants beyond 3 weeks. Consequently, microactuators with greater force capabilities than the current microactuators are expected to be more effective in moving past the glial sheath. Furthermore, implants in the previous study (N. Jackson et al., 2010) using Chevron-latch electrothermal microactuators contained bulky moving structures like the release locks and the Vshaped central drive shuttle. The drive actuators that moved the microelectrode and the actuators that controlled the release mechanism were overlaid in two different polysilicon layers of the 5layer SUMMiT-VTM process, separated only by 2 µm. As a result, their mechanical structures were complex with potential for failure in the long-term. One of the eight MEMS devices in the previous study that were actuated after implantation in rodents experienced structural failures at multiple places. Thus, a new actuation mechanism where complex mechanical structures were not overlaid on top of each other was desirable to reduce the likelihood for failure in long-term experiments with awake, behaving animals. It is also preferable for a biomedical implant to have minimal power requirements to minimize the number of battery recharges. Thus, the chronic experiments with Chevron-latch microactuator based movable microelectrodes indicated the need

for a newer actuation mechanism that can generate higher forces, have less complex mechanical structures and consume less power.

In addition, the distance moved by the microelectrode after several actuation cycles needs to be predictable in order to accurately determine the location of the implanted microelectrode in the brain at any given time. This is important as the microactuators are further incorporated in a closed-loop control to automate the actuation of the microelectrode implant in the brain (Cham et al., 2005; Wolf et al., 2009) for autonomous prosthetic systems. For a MEMS based system, the microactuator operating parameters (such as voltage levels, frequency of pulses and time-period of activation) directly impact the dynamics of microelectrode movement. Given the inertial and frictional forces inherent in the design of these MEMS devices, different operating parameters can lead to varying degrees of accuracy in the microelectrode displacement. Therefore, an optimization exercise (Montgomery, 2009) is done in the current study to analyze the movement of the microelectrode and determine the optimal parameter set required to operate the microactuator for minimal error in microelectrode displacement.

Finally, factors like mechanical wear, fracture/fatigue, humidity of the environment etc. can cause considerable change in the material properties which affects function of MEMS devices (S. L. Miller et al., 1998; Pal & Huikai, 2009; van Spengen, 2003). Prior studies have reported on the reliability of MEMS electrothermal chevron actuators fabricated using the SUMMiT-V[™] process (Plass, Baker, & Walraven, 2004; Sivakumar, Johns, Nava, & Dallas, 2010; Tanner, 2009). In this current study, we report and compare lifetime analysis of the MEMS devices (the earlier Chevron-latch microactuators and the new linear ratcheting microactuators) using resistance measurements across the electrothermal microactuators while in operation. Scanning Electron Microscopy (SEM) analysis of both MEMS devices after four million actuation cycles was done to assess failure mechanisms.

Methods

The MEMS devices reported here were 3mm X 7mm chips that accommodate three microelectrodes capable of bidirectional (forward and backward) movement as shown in Figure 1. Each microelectrode is a polysilicon shank, 50µm wide, 4µm thick and 5mm long. There were four electrothermal microactuators coupled with each microelectrode that operate and control its movement. Spring-type leads make the electrical connection between the moving microelectrode and the stationary bond pad.

In both of the MEMS technologies (Chevron-latch microactuator and the linear ratcheting microactuator) reported here, the microelectrodes were moved using electrothermal actuation. The electrothermal actuators were in-plane 'Chevron' type of actuators. Chevron or bent-beam actuators are used extensively in high force applications and are popular in integrated sensor-actuator MEMS devices (Sinclair, 2000). The basic structure consists of a V-shaped beam anchored at the two ends and connected to a central shuttle at its apex. When an electric current is passed through the beam, it expands due to Joule heating and pushes the apex forward. The displacement is dependent on the length of the beams and the angle of orientation. The force generated at the central shuttle is linearly related to the displacement. An important advantage of this type of actuator is that an array of beams when attached to a single central shuttle can cause a linear increase in the force output.

Chevron-latch actuation

The earlier generation of MEMS microelectrode array was actuated by a Chevron-latch mechanism and is illustrated in Fig. 1. A detailed description of this actuation mechanism was provided in an earlier report (Muthuswamy et al., 2005b). Briefly, there were four disengage actuators that activate the 'release up' and 'release down' locks respectively. The locks hold the electrode in position in the inactive state and release the electrode for movement during the active state. The electrode has two rows of teeth on successive layers of polysilicon constituting the microelectrode, to which the locks latch onto. The electrode was moved stepwise by the

'move down' and 'move up' actuators moving it in the forward and backward direction respectively. The electrothermal heat strips of the 'release' actuators and the 'move' actuators were fabricated in two different polysilicon layers and placed one above the other and they intersected perpendicularly.

Overall, each microelectrode was coupled to six microactuators and failure of even one microactuator can lead to failure in electrode movement. The release latches were large structures tethered to the polysilicon beams at hinge joints (refer to Fig. 1) and hence were susceptible for failure at these joints. Structurally, the design had multiple overlying mechanical microstructures as shown in Fig. 1C which impact the overall reliability and functioning of the device in the long term.



Figure 1. Previous generation of electrothermal actuators. A) A CAD diagram of the electrode actuated by the Chevron-latch type of actuation mechanism. B) SEM image of a microelectrode actuated by Chevron-latch mechanism. The microelectrode is capable of bi-directional movement and a pair of disengage actuators activate the 'release down' actuator that frees the electrode which is moved down by the 'move down' actuator. The electrode moves up by a similar mechanism. The broken arrows indicate the direction of displacement of the actuators. C) SEM image of the Chevron-latch mechanism showing the complex structures of dual counter translation latches and an overlying layer of bulky drive actuators. Enlarged portion shows two rows of teeth (pointing in opposite directions) realized on successive layers of polysilicon constituting the microelectrode to allow bi-directional movement and the point of contact between the latch and the microelectrode.

Linear ratcheting type actuator

The current generation of MEMS microelectrode array reported here for the first time was actuated by a linear ratcheting type mechanism that attempts to address some of the shortcomings of the earlier Chevron-type actuators identified by long-term in vivo testing (N. Jackson et al., 2010). The microelectrode has a linear array of teeth spaced 6.5 µm apart at the edges. The 'pawl', a peg-like structure engages the teeth of the electrode and locks it in position, preventing displacement in rest conditions. The new actuators have a simplified movement mechanism with only one 'pawl' that engages with the microelectrode teeth as shown in the inset in Fig. 2C. A SEM of the drive system with the four microactuators and the microelectrodes are shown in Fig. 2C. It has two pairs of electrothermal Chevron actuator control the forward movement of the microelectrode and 'reverse drive' actuator control the forward movement of the electrode. The central shuttle of the drive and disengage actuators are both connected in an L-shape arrangement to a 'pawl' that engages the microelectrode. A set of timed pulse waveforms was applied to these actuators to operate the pawl and move the linear ratchet/microelectrode in a given direction.



Figure 2. New generation of peg drive microactuators. A) A CAD diagram of the new generation of MEMS microelectrode device with an array of three microelectrodes actuated by linear ratcheting microactuators. The electrodes are electrically connected to the recording bond pad via spring type leads. B) A CAD diagram showing an enlarged image of the electrode actuated by the linear ratcheting type of actuation mechanism with a pair of disengage and drive actuators placed opposed to each other. C) SEM image of microelectrode actuated by linear ratcheting type of actuation. The microelectrode has maximum displacement of 5 mm in steps of about 6.5µm. The inset shows the encircled region that is the contact between the pawl/peg and a single row of teeth on the microelectrode.

Design of waveforms for activating the linear ratcheting microactuators

The transient response of the electrothermal actuators to a step of voltage is modeled

and is shown that the actuator takes finite time to reach steady state displacement (Geisberger et

al., 2003). The time taken by the actuator to reach the steady state displacement is called its

'thermal time constant'. This time constant is based on the actuator design, length of the beams,

polysilicon resistivity and thermal conductivity, gap size to the substrate, air gap conduction, etc.

The activation waveforms for the microactuators are therefore designed such that each time a voltage is turned on or off for an actuator, there is a delay time that is at least equal to the thermal time constant (labeled 't' in Fig. 3, and henceforth referred to as a 'time period') before the next voltage is applied to activate any of the other microactuators. This 'time-period' ensures the actuator reaches thermal equilibrium and is fully extended or fully retracted due to a change in voltage.



Forward movement of electrode

Figure 3. Activation waveforms applied to the four electrothermal actuators to move the microelectrode. The amplitzude of the waveform depends on the operating voltage defined for the particular actuator. The microelectrode moves by one step i.e. $6.5 \mu m$ in one complete actuation cycle. The time taken for one complete actuation cycle to achieve one forward or one backward step is six time periods or '6t'.

Movement of the microelectrode towards the external environment that is off the edge of the die was referred to as 'forward' movement. Fig. 3A shows the timing of the waveform for actuating the electrode in the forward direction. The waveform was split into six time periods that constitutes a full cycle of operation. The position of the actuators at the beginning of each time period is illustrated in Fig. 4. At the beginning of time period t=1, a step voltage was applied to the 'disengage reverse' actuator that retracts the pawl from the microelectrode. It takes one time period for the actuator to fully retract. The second step voltage was applied at the beginning of t=2, to the 'forward drive' actuator and it was displaced proportional to the applied voltage. The pawl attached to the central shuttle of the actuator caused the microelectrode to move forward in

the direction of the actuator displacement. Further, at the beginning of t=3, the 'disengage reverse' actuator began to cool down and the pawl tethered to the next teeth on the microelectrode. The pawl attached to the 'disengage forward' actuator was angled and still anchored to the electrode. At t=4 a pulse was applied to the 'disengage forward' actuator to release the pawl and after period t=5, the actuator cooled down to re-anchor at the next teeth. Thus, in one full actuation cycle the microelectrode moved one step which is a distance equal to 6.5 µm.



Figure 4. Illustration of microactuator positions in a linear-ratcheting type of actuation mechanism where each panel corresponds to individual time periods in one full actuation cycle waveform. Refer to Figure 3 for the sequence of voltages applied during the respective time period in the actuation waveform. At the end of the cycle, the pawl ratchets over one tooth of the microelectrode moving it forward by one step.

Reverse Movement of the microelectrode

Retracting the electrode towards the die was referred to as the 'reverse' movement. The waveforms for reverse movement were symmetrical to those of the forward movement and follow the same sequence of operation as shown in Fig. 3B. A critical difference is that the forward motion was achieved with the actuator operating in hot condition and the reverse motion was achieved with the actuator operating in cold condition. The reverse drive actuator was placed opposed to the forward drive actuator and hence the pawl pulled on the electrode as the actuator

cooled down. At any point of time in the actuation cycle, there was always one peg anchoring the microelectrode and keeping the spring-leads in tension.

Optimal parameters for the waveform

Operating voltage

Operating voltages for the actuators were determined experimentally. The voltage applied is proportional to the displacement of the actuators and is also proportional to the force output. On the drive actuators, the voltage applied should be sufficient to move the peg to the next tooth location. On the disengage actuators it should be sufficient to fully retract the peg from the teeth in order to allow for the free movement of the electrode. There is a lower threshold voltage level below which the actuator shows no displacement. Voltages were stepped up from 4V in increments of 0.5V until optimal displacements for both kinds of actuators were observed. Observations of peg location and actuator displacements were made using a video recording set-up described below. Voltages above a maximum upper threshold voltage can accelerate the failure of the device and increase chances for wear-out and failure. Hence the actuation voltages were determined based on an optimal voltage level between the lower and upper thresholds of operation.

Operating frequency and time period

The operating frequency of the actuator depends on the time constant of the actuator. When a voltage is applied across the actuator, it reaches a state of thermal equilibrium where the heat loss from the actuator equals the heat generation and the actuator will be at its steady state displacement. Typically, it takes 0.5-1 ms to heat up and about 0.7-1 ms to cool down. Hence, a conservative estimate of the time constant was taken as t=1 ms, so the period for a full cycle was T=6 ms with an operating frequency of 167 Hz. At time constant of t=0.5 ms, a full cycle operation would take 3 ms. Hence, 333 Hz can be the upper bound for the operating frequency of this design. However, for the purpose of actuating the electrodes in the brain, much lower

operating speeds are adequate and arguably even desired. Once the actuator reaches steady state displacement at 1 ms after activation, holding the voltage levels high will have no effect on the actuator displacement and it will remain at steady state.

The electrode can be operated at different speeds in the following ways: a) Increasing the time period 't' for which a voltage is applied to the actuator. Here, the electrode operates slowly as the same actuation cycle would take longer time to execute. Movement efficiency is tested with an increase in time period to *t*=10 ms that results in full actuation cycle operation time of T=60 ms. The increased period of operation could possibly play a role in overcoming stiction issues. b) The actuation waveforms can be applied at different pulse repetition rates (referred to as 'frequencies' here and in the rest of the report). In this case, although one full actuation cycle takes the same amount of time to complete i.e 6*t where t is the time period, the delay between consecutive actuation cycles is varied. Movement efficiency of the microelectrode is tested for frequencies of 0.1 Hz (1 actuation cycle/10s), 1 Hz (1 actuation cycle/s) and 10 Hz (10 actuation cycles/s), that includes the range of speeds required for successful penetration of the brain tissue after the removal of the outer dura mater layer. At these operating frequencies, the electrode is expected to move 6.5 µm every 10 s, 6.5 µm every second or 65 µm every second respectively.

Reliability in movement of the electrode

In order to optimize the operating parameters for the activating waveforms a design of experiment analysis was done. A general, full factorial design was used with frequency, time period and direction of movement as the main factors. Frequency has three continuous levels of operation – 0.1 Hz (slow), 1 Hz (medium) and 10 Hz (fast), and time period has two levels of operation – 1ms (fast) and 10 ms (slow). It is also necessary to characterize movement of the electrode when it moves forward versus when it moves backward. Thus, the third factor would be the direction of movement with two discrete values - forward and backward movement. The experiment is run with three replicates for each set of factor levels. Replication helps in estimating internally the errors due to high order interactions among factors that usually occur while

conducting an experiment. There were two response variables for the experiment- 1) the mean error between the expected movement of the electrode and the actual movement as measured by the optical set-up (described below) 2) the standard deviation of the error that gives us an estimate of how variable the error is in a given run.

A design test matrix for the three full factorial design experiments with three replicates was determined. Design Expert[™] 8.0.5 was used to determine the run orders and the design matrix for the experiment. The experiment has a randomized test sequence.

Optical set-up for measuring microelectrode displacement

A custom characterization set up was built to observe movement of the electrodes under different actuation schemes described above. A set up with an optical microscope of 120x magnification and a c-mount adapter for a digital camera was used for characterizing the movement of the electrodes on a probe station. TTL pulses from the computer generated by Tucker Davis Technologies' signal processing interface (TDT technologies, Boca Raton, FL) were fed into a custom drive signal generation circuit. This circuitry allowed the manipulation of voltage, pulse width and frequency of the drive signals as required. The movement of the electrodes was recorded as a video sequence with a frame rate of 3 fps. Upon completion of the runs in the experimental design, videos characterizing the movement of the electrodes were obtained. The frames in the captured video were analyzed using a custom image processing algorithm in MATLAB that measured the displacement of the electrode between consecutive frames. A series of data points that show the displacement profile of the electrode with time is created. The error between the distances moved by the electrode as measured by the optical set-up and the expected movement of the electrode due to the given operating parameter set was examined. The expected waveform was calculated from the frequency of actuation. For example, at 1 Hz the electrode is expected to move 6.5 µm every second. An error series was calculated for each run and was defined by the equation below. The mean and standard deviation of this error series for each run was determined.

Error = <u>Expected Movement – Actual Movement</u> <u>Expected Movement</u>

Force measurements

Force measurements were made using a flip chip packaged MEMS device secured to the tower of a stereotactic frame. The device was then lowered by using a 5 DOF micromanipulator. A load cell (Wipotec, Germany, model: *MTC 10/30 – ZER*) was used to make the force measurements. It has a maximum weighing range of 2 g and has a sensitivity of 0.1 mg. The MEMS chip was positioned above the load cell in the vertical direction such that the edge of the MEMS package was at a distance of about 2 mm from the load cell surface. The microelectrodes were extended off the edge of the die by approximately 1 mm. The microelectrode tip and the load cell surface were visualized under a stereo-zoom microscope with a digital camera connected to a LCD screen. The microelectrode was actuated with operating voltages as determined above until the tip of the electrode pushed on the load cell surface. The load cell was connected to the computer via a serial port and force measurements were made once every 1 µs.

Resistance measurements to test long term electrical functionality and mechanical wear

The actuators were operated over four million cycles of actuation at 10 actuation cycles/second. The voltage was set at the operating voltage level at which the actuators are normally operated which is the safe zone of operation, lower than the level at which they undergo plastic deformations. The current through the actuator under displacement was measured using a digital multimeter (M3510A, Picotest Corp., Taiwan) connected to the computer via a serial port. Resistance measurements were made at randomized intervals of time during the four million cycles. The duration of every measurement lasted for half an hour during which a reading was recorded every 25 ms. Thereby; the resistance of the actuators was sampled during steady state displacement and zero displacement.

Failure analysis of microactuators

SEMs of the MEMS device were analyzed for any structural changes or failures, presence of debris, oxidation etc. After four million cycles of operation, the actuator strips were imaged to determine mechanical wear and physical deformations that would disrupt the actuation mechanism.

Results

The linear ratcheting actuators required lower voltages (5.8 V-6.5 V) and have finer displacement resolution (6.5 μ m) compared to the earlier Chevron-latch electro-thermal microactuators which required 9 V-10 V and had displacement resolution of 9 μ m. The current drawn by the linear ratcheting microactuator was 22 mA per cycle and hence the power consumption was approximately 143 mW/cycle. This was an 85% reduction in power consumption from the earlier Chevron microactuators which drew 100 mA per cycle of current and consumed power of 1W.

Optimal parameters for the new actuation scheme

Operating Voltage

The linear ratcheting actuator required approximately 5.8 V and 10 mA dc to fully disengage the pawl from the electrode. The forward drive actuator required approximately 6.5 V and 12 mA to ratchet over one peg slot, resulting in a linear displacement of 6.5 μ m per actuation. The reverse drive actuator required 7 V to move back one step. The differences in voltage requirement was because the reverse drive actuator exerted force when it was cooling down and hence the force applied on the electrode returned to zero as the actuator returned to the zero displacement position. The upper threshold voltage for disengage and drive actuators was 10 V. This was found by increasing the activation voltage until the heat strips of the actuators burned out. Hence, activation voltages in the range of 6-7 V were well within the upper threshold decreasing the chances for wear-out and failure.

Optimal frequency and time period of activation waveforms

Design Expert[™] provided statistical models that captured the effect of three factors – (1) frequency of the activation waveform (2) time period of the activation waveform and (3) direction of microelectrode movement and their respective interactions on the two response variables - (1) mean and (2) standard deviation of error in the movement of the microelectrode. The mean and standard deviation values for each run followed a normal distribution as identified by the normal

probability plot of the studentized residuals as shown in Fig. 5. Statistical analysis using analysis of variance (ANOVA) was done from the factors that most significantly affect the two response variables. In the ANOVA table generated by Design ExpertTM, the interaction term of frequency and time-period had significant impact on the mean error response with a *p*-value of 0.0003. Thus, frequency or time period factors individually do not influence the error rates significantly. In the ANOVA model for standard deviation response, only the direction of movement factor had significant impact with a *p*-value of 0.0331.



Figure 5. Normal probability plot of studentized residuals for A) mean error responses and B) standard deviation responses shows that the residuals satisfy the normality assumption for the model generated by ANOVA. All the responses should fall in a line and satisfy the 'fat pencil test'.

The model output graphs showed the effect of the significant factors on the model response variables. In Fig. 6, the mean error response is minimal and is approximately zero for a frequency of 1 Hz and time period of 1 ms. The expected and the actual waveforms for the above parameters of operation completely overlapped in both forward and backward direction of movement as shown in Fig. 7. The second model graph in Fig. 6B shows the standard deviation of the errors in various runs as a function of the direction of movement of the microelectrode. The SD of error response while the microelectrode is moving forward had lower variation when compared to the same while the microelectrode is moving backward. This was consistent with the

observation that forward movement of the microelectrode was more uniform while the backward movement of the microelectrode resulted in skips and jumps that makes the error variations large.



Figure 6. Model output variables after ANOVA showing the effect of the most significant factors on the two response variables a) mean error and b) standard deviation of error. A) The interaction graph shows the mean error for different combinations of frequency and time period as tested in the experimental design. The frequency of 1 Hz and 1 ms has the least mean error. B) Only the direction of movement significantly affects the standard deviation where backward movement has larger standard deviation whereas forward movement is more consistent, with smaller standard deviation in errors.



Figure 7. Movement profiles at optimal activation parameters. A) Forward movement profile of an electrode actuated at 1 Hz frequency with 1 ms time period. B) Backward movement profile of an electrode actuated by the same waveform parameters. In both cases the expected and the actual movement profiles track each other closely.
The expected and actual movement profiles of an electrode actuated at 0.1 Hz with 10 ms time period is shown in Fig. 8 that illustrates the skipping behavior observed in backward movement. In this case, the electrode skipped a few actuation cycles at approximately 500 s while the electrode was stuck. Repetition of the actuation waveforms allowed it to overcome the stiction at that position and it again began moving backward. Fig. 8B shows the backward movement profiles of an electrode where the electrode jumped more than one peg per actuation resulting in larger displacement than expected. The kind of movement profiles shown in Fig 8 where the expected and actual movement significantly differed from each other were observed in about 1 out of every 5 trials conducted.



Figure 8. Movement profile at non-optimal movement parameters. A) Backward movement profile of an electrode actuated at 0.1 Hz with a time period of 10ms. At 0.1 Hz the electrode is expected to move 6.5 μ m every 10s. The electrode got stuck after being displaced by about 200 μ m and repeated pulsing of the actuators allows it to recover movement. B) Backward movement profile of an electrode actuated at 0.1 Hz with a waveform of 1ms. The electrode jumps through more than one step per actuation cycle resulting in a faster retraction of the electrode than expected.



Time (seconds)



The force generated by the Chevron-latch microactuator on one microelectrode is shown in Fig. 10. The peaks and troughs correspond to each actuation cycle. The peak force is generated when the release locks are activated and the forward microactuator moves the microelectrode down. The trough in the force data corresponds to the instant when the release locks hold the electrode in position before the beginning of the next actuation cycle. As shown in Fig. 9, the average peak force generated by the MEMS microelectrode is approximately 99.5 ± 7.5 μ N. The MEMS devices used in the earlier chronic in vivo study (N. Jackson et al., 2010) had microactuators with 4 thermal strips and they generated a force of approximately 200 μ N since the force scales linearly with the number of thermal strips on the actuator.

The force generated by linear ratcheting type of actuator on a microelectrode is shown in Fig. 9. The actuators have two thermal strips as shown in Fig 2. As the electrode is actuated, a peak force of 250 μ N is registered when the drive actuator moves the electrode down by one step and the electrode tip touches the load cell. Each actuation cycle is for 60 ms as the time period *t*=10ms and the waveforms for actuation are as shown in Fig. 3. The force output varies in accordance with the actuation cycle and the peak is recorded every time the microactuator moves

the microelectrode. At the end of the actuation cycle, the microelectrode is held in position by the disengage microactuators and exerting a resting force of approximately 150 μ N corresponding to the microelectrode being pressed against the load cell in the new position. After the initial twelve actuation cycles, the microelectrode has already moved approximately 80 μ m and a marginal decrease in the peak output force is observed as a result of buckling in the electrodes.



Figure 10. Force output of linear ratcheting type of actuation. The force varies during the actuation cycle and the peak force output is when the microactuator acts on the microelectrode. The microelectrode is actuated over a total of 20 actuation cycles and the average peak force output is recorded.

Resistance measurements for lifetime analysis

Electrical resistance measurements were made over four million cycles of operation of the microactuator. Resistance across the reverse drive actuator in the linear ratcheting type device and release down actuator in the Chevron-latch type device were measured while the actuators were in operation. They were operated at 6.5V and 9V respectively. The mean and standard deviation of all the resistance values were calculated and plotted as shown in Fig. 11. The linear ratcheting type actuator showed fairly constant resistance across four million cycles of operation as evidenced by the smaller error bars in Fig. 11. The Chevron-latch actuator showed deviations in resistance across four million cycles of operation with large variations across individual readings.



Number of actuation cycles (in millions)

- Reverse actuator in the linear ratcheting type
- Release down actuator in the Chevron type

Figure 11. Resistance measurements to measure reliability of operation at a frequency of 10 actuation cycles/second. The reverse actuator of the linear ratcheting device shows constancy in electrical measurements. The release down chevron actuator shows large variations in individual measurements that were recorded over a period of one hour while the actuator was in operation.

Failure analysis in microactuators

The linear ratcheting type of actuator remained functional even after four million cycles of operation. No structural deformations were observed after 4 million cycles of operation. The Chevron-latch actuator failed to operate beyond four million cycles of actuation. A scanning electron microscope (SEM) analysis was done to determine the reasons for failure. SEM micrographs documenting the different mechanisms of failure in these MEMS devices are shown in Fig. 12. Mechanical wear was the dominant failure mode as seen here. Pin joints, substrate hinges/tether points and hubs were critical points of failure as observed in Fig. 12A. Failure by fracture due to fatigue was a dominant failure mode largely due to the tethering of the release lock (a large mechanical structure) by a thin beam which induces large stresses and results in fatigue and wears at the joint over 4 X 10⁶ continuous actuation cycles. An enlarged view of the broken hinge can be seen in Fig. 12B. Fig. 12C and Fig. 12D show failure in the electrothermal strips of the microactuators. Oxidation and plastic deformation of the beams can be observed in

the beams on the left hand side of Fig. 12C. The electrothermal strips on the right hand side have melted. In Fig. 12D, mechanical failure in the electrothermal strips has occurred due to creep and there is ductile separation. Fig. 12E illustrates deformation in the beam that orients the release lock with the teeth of the microelectrode, as a result of which the teeth and locking structure are misaligned. Each of the above failure modes can be catastrophic to the operation of the device.



Figure 12. SEM micrographs showing the different failure modes in Chevron actuator after four million cycles of operation. A) Fracture at a hinge joint due to fatigue. B) Magnified view of the fatigue failure in the region highlighted in A. C) Illustration of oxidation and plastic deformation in the electrothermal strips of the actuator. D) Creep and plastic deformation in the electrothermal actuator beams due to large stresses as they operate the large V-shaped pawl in the move down actuator. E) Plastic deformation in the beam that orients the release-lock structure and the teeth of the microelectrode leading to misalignment that hinders the movement mechanism of the microelectrode.

Discussion

In the new devices with linear ratcheting microactuators, the microelectrodes have a single set of teeth along the length on either side (compared to two successive layers of teeth on each side of the microelectrode in the Chevron-latch actuators) that results in more reliable grasping of the microelectrode by the actuators. Further, overlying thermal strips were eliminated and have significantly reduced the polysilicon surface areas that come in contact during actuation. The bulky dual counter translation latches that were prone to fatigue failure in the earlier actuation mechanism are replaced by a simple pin-like design in the new actuation mechanism. Therefore, the new generation of microactuators and microelectrodes are expected to be less prone to mechanical failure in the long-term.

The current generation of devices with the linear ratcheting actuators is a low power design with lower supply voltages that is preferable for long-term implantable applications. Table 1 summarizes the design improvements in the linear ratcheting type of actuation mechanism.

Device attributes	Chevron latch mechanism	Linear ratchet mechanism
Voltage levels for actuation	9 V	5.8-6.5 V
Actuation step	9 µm	6.5 μm
Full stroke length	5 mm	5 mm
Maximum frequency of operation	333 Hz	333 Hz
Minimum duration of activation cycle	3 ms	6 ms
Power consumption	1 W/cycle	143 mW/cycle
Force generated per heat strip	50 μN	111 μN

Table 1. Comparison between the two types of actuation mechanisms

The linear ratcheting type of actuation produces force per heat strip that is twice as large as the previous Chevron-latch type of design with almost 30% decrease in the voltage levels applied for actuation. The average force output increases linearly with the number of actuator legs. The new MEMS devices have three linear ratcheting actuators as shown in Fig 2, where the left and the right microelectrodes have drive actuators with five heat strips each and the middle microelectrode has microactuators with ten heat strips. Thus, these devices are capable of generating a maximum force output of approximately 550 μ N (on the left and right microelectrodes) and 1 mN (on the middle microelectrode) respectively. We have determined that the force required to penetrate an ex-vivo brain using polysilicon microelectrode moved at 10 μ m/s was about 200 μ N (unpublished data). However, the forces required to penetrate the glial sheath in long term implantation are unknown and can only be estimated from acute measurements in the brain. (Bellamkonda et al., 2007; Kas et al., 2006; Sharp, Ortega, Restrepo, Curran-Everett, & Gall, 2009a). Force output in the current generation of devices has increased by approximately 3 times on the left and right microelectrodes and 5 times on the middle microelectrode. The increased force is expected to enable movement in the brain for a longer duration of time.

The results from experiments to optimize the operating parameters of the activation waveforms in the linear ratcheting microactuators indicate that frequency or time period alone does not influence the error between the actual and expected microelectrode displacement profiles. It is the interaction of these two factors that determines the error values. In about 2/3rd of the runs to test microelectrode displacement, the error values are within the 30% range. During forward movement of the microelectrode, the error values increase to approximately 30% error during the initial phase as the microelectrodes just begin to move, before settling down to a steady-state value. This steady-state error value is the cumulative error from the previous cycles. Inertial and static frictional forces appear to play a significant role in determining microelectrode displacement errors during the initial phase when the microelectrodes are about to move. However, once the microelectrode is in motion, there appears to be no further errors in displacement as indicated by the steady-state values for the cumulative errors. This is in contrast to variations in the error seen when the electrode is moving backward. Although the range of error percentages is comparable to the range seen during forward movement, the standard deviation of error is large for the runs when the electrode is moving backward. This is also verified by the statistical model where the standard deviation of error is only influenced by the direction of movement of the microelectrode and are seemingly unaffected by frequency or time period of the activation waveforms or their interaction. The large variations during backward movement of the

electrode can be attributed to the slipping issues discussed earlier. However, the slipping issues are more prominent as the electrode gets retracted further into the chip. This is probably because of the reduction in the spring force (that acts in the direction of backward movement of the microelectrode) as the spring attached to the microelectrode begins to relax while the microelectrode is drawn into the chip, and the resistive forces due to friction become more dominant. The displacement error when the electrode is extended far out from the edge of the die is less variable.

The following reason can also potentially contribute to the slipping seen during backward microelectrode movement. In the current design, the reverse actuator pulls the microelectrode back while cooling down. As the actuator cools down it pulls back to its zero displacement position. Consequently, the actuator exerts force with a receding profile that diminishes to zero as the actuator reaches its zero displacement position. Therefore, in the presence of frictional forces, the actuator may not fully reach its zero displacement position. This can result in slipping as the actuator does not pull back far enough to ratchet over the next tooth and the microelectrode will not move at all.

In order to overcome slippage during backward movement, the reverse actuator could be operated at a higher voltage such that it extends out more than one peg each time and the actuator would have at least crossed one peg when resistive forces are present. Another way to avoid slippage during backward movement would be to operate the reverse actuator also in the hot condition similar to the forward actuator. However, this option is less desirable. There is approximately 20 MPa residual stresses in the polysilicon due to the fabrication process (Phinney, Spletzer, Baker, & Serrano, 2010). Packaging will add to the stresses in the die as well. Any stress in the actuator beams will cause the thermal actuators to have some small non-zero displacement even when unpowered. This might result in the reverse actuators and forward actuators becoming misaligned with respect to the pegs on the microelectrode resulting in failure of the MEMS device. Thus, arranging the actuators as in the current scheme, with one operating hot and the other cool fixes this problem and causes the actuators to be insensitive to stress

issues. An additional advantage of the symmetric placement of actuators is that it allows the device to function even when one of the drive actuator fails. When either one of the forward or reverse actuators fail, the waveforms for the disengage actuators can be switched and the functional actuator can be made to operate in the hot or cold condition, thus moving the microelectrode forward or backward.

The resistance measurements were sampled as the actuator was in dynamic state of transition from steady state displacement to zero displacement in the actuation cycle. The actuator resistance increases significantly with heating (because resistivity of poly is a strong function of temperature) and the variations seen during individual measurements may be attributed to this. But both the linear-ratcheting and the Chevron-latch actuators were sampled at various points of the heating and cooling cycle. The lower variations in resistance in the linearratcheting actuators could be due to better heat dissipation. Changing resistance implies change in the material properties of the legs of the actuator and mechanical deformations that affects it electrical functionality. The large variations in resistance measurements in the Chevron-latch microactuators could be due to two causes: i) the actuators are coupled to bulky release locks that could cause excessive strain and material degradation during displacement ii) a higher voltage is applied for actuation and the legs of the actuator are shorter, so the heat generation is more and the area available for heat dissipation is less. The SEM images of the Chevron-latch type of microactuators after four million cycles of actuation indicate wear in the actuator thermal strips, fracture at hinge joints and creep failure. It is known that creep rate in polysilicon increases as temperature increases (Tuck, Jungen, Geisberger, Ellis, & Skidmore, 2005). The higher heat dissipation in the Chevron-latch actuator can further accelerate plastic deformation. The resistance of the actuator beams in the linear ratcheting type actuator is lower than the Chevron latch type actuator which lowers heat dissipation and thus decreases the possibility of failure due to creep. After 4 million cycles of operation, the linear ratcheting actuator was still functional.

The mechanism for fracture due to wear is described by Spengen et. al. (van Spengen, 2003). Polysilicon in air is always covered by a thin native oxide layer. A small crack can develop

on the surface of this oxide layer which propagates by stress corrosion cracking mechanism. When the externally applied stress is large enough, crack growth is observed leading to oxidation of deeper polysilicon layers. This could be the possible mechanism of failure in Fig. 12A. In the linear ratcheting type of actuator such joints where there is high concentration of stresses is eliminated by constructing a simple pin like structure that tethers to the microelectrode.

Conclusions

We report here a new linear ratcheting microactuator that performs better than the Chevron-latch microactuator for potential implantable, long-term brain prosthetic applications. The optimal operating parameters for activating the linear ratcheting actuators were first determined through an exhaustive set of experiments with different operating parameters and subsequent statistical analysis. Optimal parameters set for the activation waveforms to move the microelectrode forward and backward were determined. The new MEMS devices with linear ratcheting microactuators generate twice as much force per heat strip compared to the Chevronlatch microactuator, appear to be mechanically robust due partly to less complexity in their mechanical structure, consume less power, and have finer movement resolution. Lifetime analysis using resistance measurements and SEM analysis after 4 million cycles indicate that the linear ratcheting microactuators may be less prone to failure than the earlier Chevron-latch microactuators. We therefore conclude that the MEMS movable microelectrodes with linear ratcheting microactuators are likely to perform better than the Chevron-latch microactuators in long-term brain implant applications.

CHAPTER 3

VARIABILITIES IN 24 HOUR EXTRA-CELLULAR NEURAL RECORDING

Introduction

As mentioned in Chapter 1, a prosthetic system has to be reliable in order to be clinically viable. Reliability implies that it can be used consistently without degradation of performance. Although the latest cutting edge microelectrode technologies and implantation techniques have increased the life span of neural recordings, it is still a safe assumption that the number of electrodes with good signal quality degrades over time. Signal sorting algorithms and decoding techniques are being designed to either counter or adapt with this signal loss (Schwartz et al. 2006, 205-220; Hatsopoulos and Donoghue 2009, 249; Gilja et al. 2011, 1-1; Ganguly and Carmena 2009, e1000153 EP -)(Andersen et al. 2004, 486-493; Lebedev and Nicolelis 2006, 536-546; Wahnoun, He, and Helms Tillery 2006, 162)(Moritz, Perlmutter, and Fetz 2008, 639-642). As other areas of neural prosthetics make rapid progress there is almost an urgency to develop reliable neural interface technology, else it risks becoming a serious bottleneck for the translation of human cortical prostheses. Movable electrode technology now gives us the opportunity to re-establish contact at failed interfaces by repositioning the electrodes (Jackson et al. 2010). Automation so that the electrodes can adaptively reposition themselves and seek functional interfaces when the quality of neural signal declines is the much needed next frontier in neural interface technology. Such microelectrodes that are not just passive sensors but can assess the recording environment and actuate to maintain a sustained signal output are the core of next generation of robotic neural interfaces.

The neural interface is highly dynamic. There are a host of physical changes and tissue response mechanisms that interact to affect the quality of recordings. Several reasons exist for non-stationarities in neural signal recordings such as brain micromotion, motion artifacts, physiological factors, brain tissue drifts and formation of glial sheath as a result of immune response to the implanted electrode etc. The above reasons for variability act over different time

scales ranging from seconds to weeks. In the following paragraphs we discuss in detail some of the observed non-stationarities and their reasons.

Variations in signal quality due to tissue response

Studies regarding the evolving interface have mostly focused on tissue response around the microimplants. Literature from the past few decades seem to suggest tissue response as the predominant factor for the lack of stability in neural recordings. Brain tissue reacts to implanted electrodes by mounting a glial cell mediated response. A thick continuous cellular sheath is formed that envelops the electrode. The response occurs in broadly two phases. The acute phase is dominated by reaction to injury caused by implantation of the electrodes. Initially electrode insertion compromises the blood brain barrier as capillaries in the electrode track are damaged. Blood borne macrophages enter the site of injury through severed vessels. The electrode damages the extracellular matrix, glial and neuronal cell processes as it penetrates deeper into tissue. Tissue that was initially in the region of the electrode track is pushed aside and tissue beneath the electrode tip is compressed leading to the build-up of a high stress region around the electrode. Edema is observed in most cases due to the injury caused. Microglia can become over-activated in response to the tissue damage and proliferate at the site after about a day of implantation. They have been shown to secrete reactive oxygen species as well as a number of inflammatory cytokines, which is toxic to neighboring neurons at high concentrations (Block, Zecca, and Hong 2007, 57-69). Inflammation, edema responses diminish after the first week and microglial activation is also reduced although some studies report the presence of macrophages even after 4 weeks (Biran, Martin, and Tresco 2005, 115-126). However, the extent of this injury is limited to only path of the implant and the initial reaction is not of major consequence to neural recording capabilities (Biran, Martin, and Tresco 2005, 115-126).

The second phase is a sustained chronic response to the continued presence of the probe that leads to the formation of a compact sheath that extends to a several tens of micrometers from the electrode (Turner et al. 1999, 33-49). Gliosis displaces neurons in the

volume surrounding the electrode and leads to neurons migrating away from the recording sites (Polikov, Tresco, and Reichert 2005, 1-18). In most studies of stability of neural recordings it is observed that there is significant change in neural activity over the first 4-6 weeks and the signal stabilizes after about 8 weeks (Nicolelis et al. 2003, 11041; Liu et al. 1999, 315-326). In a histology study, electrodes were explanted from feline cortex, the tissue was stained and it was found that those electrodes that had stable recordings in fact had a large neuron in the vicinity of the implant track (Liu et al. 1999, 315-326). And every electrode which was unable to record any action potential recordings had no neurons nearby. Most neural recordings decline over a period of weeks in a timeline that is similar to the formation of the glial sheath indicating that the gradual remodeling of the tissue architecture around the electrode as one of the primary reasons for recording failure (Griffith and Humphrey 2006, 81-86; Winslow and Tresco 2010, 1558-1567).

Local neurodegenaration

Neuronal cell loss of about 40% was found within a radius of 100µm in the cerebral cortex and there was a significant loss of neurofilaments as far as 230µm. It is hypothesized that persistence of microglia due to the continued presence of the implant leads to this cell loss (Biran, Martin, and Tresco 2005, 115-126; McConnell et al. 2009, 056003). Further evidence that the presence of the implant alters the tissue architecture surrounding it is that no electrode tracks were visible after several weeks when the electrode was inserted and immediately removed (Biran, Martin, and Tresco 2005, 115-126). This has strong implications for designing strategies with movable microelectrodes where the electrode can be retracted, the tissue allowed to heal and then reinserted to the recording region of interest.

Increase in impedance

The general increase in impedance observed in chronic neural implants is attributed to the formation of the compact and fibrous sheath around the electrode. The sheath is very dense and hence reduces the space for extracellular current flow. The largest contribution from reactive astrocytes on the impedance spectra is within a 100µm radius from the interface, the region from where electrodes are mostly likely to record electrical signals (Frampton et al. 2010, 1031-1047;

McConnell, Butera, and Bellamkonda 2009, 055005; Otto, Johnson, and Kipke 2006, 333-340)

Tissue response to movable microelectrodes

The tissue response elicited by a movable microelectrode in the brain is not well understood. More importantly, the extent and biology of the chronic tissue response is not known when the implant is non-stationary. To our knowledge this (Stice and Muthuswamy 2009, 046004) is the only study on tissue response due to repositioning of microelectrodes. The data from this study suggested that using a two-step implant procedure where the microelectrode will be moved to the final recording location in two stages could minimize the reactive astrocytic response to implanted microelectrode. Data from other groups also seems to suggest that a slow implantation procedure with movement steps spread over two weeks lead more stable recordings (Yamamoto and Wilson 2008a, 2430-2440). On the basis of these studies, it can be derived that allowing the microelectrode implants to remain stationary for the first two weeks allows the initial injury response to stabilize. Moving the microelectrode to the site of recording after this initial 'settling-in' period can potentially lead to good quality recordings. However, chronic tissue response to repeated movements of the microelectrode remains unexplored. Would such movements prevent the consolidation of reactive astrocytes into a dense sheath? The impact of continuous electrode motion on the presence of neurons around the electrode track is unknown. Also, the impact of parameters like the frequency and speed of movement of the microelectrode on chronic tissue response is also unexplored. Controlled experiments to answer these questions are required for long term strategies to cope with the tissue response.

From all the studies of tissue response a universally accepted view is that strategies to overcome foreign body response and glial scar formation are a critical part of any implant design. From the point of view of designing controls for movement of microelectrodes over a long term, four important messages emerge-

- The timelines of acute and chronic inflammation and consolidation of glial sheath should be considered while moving the electrodes to stabilize the neural recordings
- The distance the microelectrode has to move to seek out areas for the presence of neural

activity is dictated by the boundaries or physical extent of the chronic tissue response

- For chronic implants the injury response to initial implantation trauma stabilizes after two weeks, moving the microelectrodes after this period can be potentially beneficial to sustain the quality of neural recordings
- The fact that the tissue is restored to near pristine levels of neuronal densities when the implant is retracted could be used to design clever strategies of retraction and reimplantation to seek newer electrode-neuron interfaces.

Non-stationarities in signal recordings due to physical changes

Another source for variations in signal quality is the dynamic physical changes at the neural interface itself. During initial implantation it is often observed that the brain tissue can dimple over a millimeter as the tissue is pulled along with the microelectrode. An excellent illustration of the tissue strain caused during electrode penetration is given by the videos published by (Bjornsson et al. 2006, 196). Dynamic physical changes at the interface are caused when the microelectrode or tissue move relative to each other. The time course of the tissue response and how it returns to steady state is dependent on the mechanical properties of the material. The displacement of the tissue alters the relative position between the neuron and the recording site of the microelectrode. It is intuitive to expect that this change in relative distance causes changes in the signal amplitude and shape recorded by the microelectrode and in general accounts for differences seen in signal shapes due to physiological activity like breathing, blood pressure (Britt and Rossi 1982, 219-229; Fee 2000, 461-468), or physical movement of the animal and electrode drift.

Tissue micromotion

Brain tissue moves relative to the microelectrode mainly because of three reasons: (i) pulsations in the vasculature, (ii) propagating mechanical pressure waves due to breathing (iii) propagating mechanical pressure waves in freely behaving animals (vigorous movements of the head, sneezing etc.) The level of tissue micromotion is species dependent with large relative

displacements observed in non-human primates and humans, and less catastrophic displacements observed in rodent models (Muthuswamy et al. 2011, 3B. 2.1-3B. 2.4). Displacements of 100–250 µm due to vascular pulsations and 300–900 µm due to breathing were observed in the brainstem of cats using optical interferometry measurements (Britt and Rossi 1982, 219-229). In humans, the effect is more exaggerated; brains can displace and have torsional displacements over several millimeters from accelerated head movements. Anecdotal evidence from our own laboratory suggests approximately 20–80 µm of brain tissue displacement in the cortex during intra-cortical force measurements in an anesthetized adult rat.

Review of stability of neural recordings

Recent studies on stability of neural recordings have published results that indicate change in action potential shape and amplitude or changes in the population of units being recorded from freely behaving animals. The data for such stability analysis is typically recorded from three time scales: (i) day to day recording sessions which last from a duration of minutes to hours (ii) continuous recording systems that monitor and store data for over 24 hour (iii) long term recordings over a duration of several months to years.

Short term variations

Short recording sessions that last anywhere between 5 minutes to 20 minutes also show variabilities in spike amplitude (Fee, Mitra, and Kleinfeld 1996, 3823-3833; Williams, Rennaker, and Kipke 1999, 1069-1076). Variability in spike amplitude is usually modeled as a normal distribution in most spike sorting algorithms. Although some studies have shown that the *t*-distribution is a better fit to the variations seen in the spike recordings (Chah et al. 2011, 2558-2561). This implies that the standard deviation we observe in the data is only a conservative estimate and is much larger than what is normally expected. In these short timescales, changes in action potential amplitude can happen rather abruptly. Motion artifacts caused due to animal behavior that may lead to the electrode array shifting inside the brain is likely responsible for some of these changes. One such illustration of abrupt changes in action potentials due to

vigorous head movement (Santhanam et al. 2007, 2037-2050) where an accelerometer mounted on the monkey's head was used to measure the head accelerations.

The study of stability of neural recordings needs to be extended to longer time scales to track gradual changes in unit activity. Ideally in a cortical prosthetic application data would be recorded over 24 hour duration, 7 days a week. The variability in neural signals in such scenario is enormous and research groups are only now beginning to characterize the associated changes in neural waveforms aided by wireless signal acquisition systems (Chestek et al. 2011, 045005). Studying of neural activity at continuous time scales over 24 hours or days are imperative to provide insight into the mechanisms that may cause variability in neural data over the long term. Moreover such studies are rarely done in rodent models and data of neural signal variability over 24 hours in rodents is not available.

Long term variations

Most long-term studies of neural stability observe that waveform data was most variable in the first two weeks of implantation which might be directly correlated with the injury response. The electrodes that were selected and tracked for stability had varying spike shapes and amplitudes between different recording sessions. Although some attempts were made to track the neural population recorded in all the sessions using statistical approaches like Bayesian classifiers and ascertain whether they were from the same electrode. However, on an average a shifting population of multi-units was captured on the arrays (Nicolelis et al. 2003, 11041; Suner et al. 2005, 524-541; Liu et al. 2006, 91-100; Dickey et al. 2009, 1331-1339). In these long time scales, the variations seen in unit waveforms could be potentially attributed to a number of mechanisms such as neural plasticity, degradation of the electrode insulation, or chronic inflammation response. A common hypothesis for recording varying unit activity is that the electrode array sinks into the cortical tissue slowly over time, known as **electrode drift**.

One previous study examined day-to-day stability in recordings from the Utah array in a feline model and saw substantial inter-day changes in neural waveforms which correlated with measures of inflammation (Parker et al. 2011, 145-165). In all of these studies (Nicolelis et al.

2003, 11041; Chestek et al. 2011, 045005; Suner et al. 2005, 524-541; Liu et al. 2006, 91-100; Williams, Rennaker, and Kipke 1999, 303-313; Tolias et al. 2007, 3780-3790; Taylor, Tillery, and Schwartz 2002, 1829; Schmitzer-Torbert and Redish 2004, 2259-2272; Rousche and Normann 1998, 1-15; Greenberg and Wilson 2004, 1042; Gray et al. 1995, 43-54; Krüger et al. 2010; Ryu and Shenoy 2009, 5; Vetter et al. 2004, 896-904), a slow declining trend in action potential voltage across many months is observed. This may be a result of several putative factors like tissue response, neuron migrating away from the site, accumulated tissue micromotion, physiological factors. Ultimately, all of the above factors appear to have a consolidated effect of the electrode not being in the vicinity of active neurons.

Freely behaving v/s head fixed experimental preparations

Most studies of stability analyze neural data from head fixed animals. The variations seen in such data may not truly represent the kind of signal variability inherent when the animal is freely behaving. Since microelectrode arrays are fixed to the skull it is expected that vigorous activity from the animal might translate to displacement in electrode position inside the brain. In head fixed animals, smaller variations in unit signal amplitudes were observed when compared to data from freely behaving animals (Santhanam et al. 2007, 2037-2050; Dickey et al. 2009, 1331-1339; Jackson, Mavoori, and Fetz 2007, 360-374; Zanos et al. 2011, 1-1; Wilson et al. 2003a, 49-61). Physiologically, the activity of the neurons when concentrating on a given task in a restrained experimental set-up versus when engaging in free behavior might be very different. The background noise which is a summation of the background activity of neurons may have different characteristics and vary much more in a freely behaving animal versus a head fixed animal. This in turn will influence the calculation of signal quality metrics.

24-hour neural recording from a freely behaving rodent implanted with a fully packaged MEMS microelectrode array

It is important to characterize the type, rate and reversibility of signal changes to be able to design effective adaptive control mechanisms. Neural data was observed over the course of 24 hours in a freely behaving rodent model to document the changes in neural activity. This experiment helped gain more insights into the dynamics of the plant we aim to control from a control systems perspective.

A fully packaged MEMS microelectrode array was implanted into an adult male Sprague Dawley rat using the standard surgical procedure described in the next chapter. The details of the packaging and device mounting procedure are also described in the following chapter. A single microelectrode was implanted into the somatosensory cortex and the microelectrode was not moved to obtain control recordings that were not confounded by variations that could possibly be caused by microelectrode movement. The MEMS device was mounted on a stereotactic frame and was advanced into the brain at a speed of 10 µm/second during implantation.

Methods

A 16 channel headstage was connected to TDT signal acquisition system (TDT Inc., Alachua, FL) using lead wires that were protected by a spring casing. Neural recordings were sampled at 25 kHz and bandpass filtered from 300 Hz to 3 kHz. Data was analyzed using Plexon offline sorter (Plexon Inc., Dallas, TX). The first hour of recording began about 1 hour after the initial implantation only after the anesthesia effects had worn off. Recordings were taken approximately every half hour and each recording session lasted for about 10 minutes for the first eight hours. There was an eight hour break in between as the animal recovered from a prolonged experimental session. Recording was resumed at the 16th hour and continued up to the 24th hour. Spikes were detected using appropriate user defined thresholds that were greater than three standard deviations above noise amplitude distribution. Spikes from each recording snippet were manually sorted. The interspike interval histograms were inspected to check for false positives. Overall about 22 units were identified from the spike sorting procedure. Since we did not have functional correlates it was hard to uniquely determine cell identity or track single units from the recordings.

Peak to peak amplitude was calculated for all detected spikes. The average peak-peak amplitude of every unit was also determined. Signal-to-noise ratio (SNR) was calculated from the

raw signal. The standard deviation of the entire recording snippet was determined. Any signal that was three times above this standard deviation was classified as spikes and the rest was considered as noise. Signal-to-noise ratio was defined as the average power in the signal divided by the average power in the noise. The amplitude of all data points that were classified as signal was squared, summed and normalized by the number of data points in the signal. Similarly the average squared amplitude of noise was determined. SNR was calculated as follows:

SNR = 10 log₁₀
$$\frac{\frac{\sum si^2}{S}}{\frac{\sum ni^2}{N}}$$

where *si* are the signal points and *S* is the total number of signal points, *ni* are the noise values and *N* is the total number of noise points. This equation gives the SNR in dB.

Results

The initial placement of the electrode next to an active neuron gave us recordings with distinguishable single unit. SNR values were approximately 30 dB. In the first hour, recordings were made every 15 minutes for 5 minutes and the peak-peak amplitudes of all the units were plotted as shown in Fig. 3. Though the waveform shape remained fairly stable in the first hour, huge changes in action potential amplitudes were observed. The highest recorded amplitude was 1.6 mV which indicates that the neuron was very close to the recording tip. The recording began approximately an hour after the initial implantation and perhaps captured a significant amount of signal variation due to tissue drift. The changes in waveform shapes in recordings in the first eight hours are shown in Figure 13.



Figure 13. Peak-peak amplitudes of all the units recorded by a single stationary polysilicon microelectrode in a freely behaving rodent over a continuous 7 hour recording session.

After the first hour, the amplitude suddenly decreased to 100-200 μ V. There could be at least two possible reasons for such a sudden change: the electrode could have impaled the neuron or the tissue relaxation could have led to the neuron moving away from the recording site. After approximately three hours of recording similar amplitudes, a sudden increase in amplitude was observed at the beginning of the 4.5 hours. However, these units did not sustain and the amplitudes decreased almost immediately within 15 minute. The animal was highly active and vigorously moving around during this period which might explain high variability in the quality of units recorded. The amplitudes came back up again towards the beginning of 6th hour. But the noise levels were very high at this period and the single units were difficult to sort from the background activity. The single unit action potentials were sometimes visible and sometimes not differentiable from the multi-unit hash, so very low number of spikes could be sorted.



Figure 14. Average signal to noise ratio (SNR) of all recorded units over 24 hrs with stationary microelectrode. SNR of 10dB corresponds to the level where action potential waveforms are barely discernible.

While individual unit amplitudes and waveforms did not show any systematic changes and had a high variability, the SNR was more consistent across time. SNR is determined from the power in the number of threshold crossing events divided by the power in the noise is plotted in Figure 14. The average peak-peak amplitudes of the signals at every hour are plotted in Figure 15. The SNR appears more consistent than the average peak-to-peak amplitude of the single units.



Figure 15. The average (±std. dev.) peak-peak amplitude of individual units over 24 hours.

The variations with average peak-to-peak amplitude are substantial with almost 50-100% signal changes per hour and the rate of changes is apparent from the abrupt changes between adjacent 1 hour intervals. For every individual unit in each recording snippet that lasted for about

6-10 minutes the peak-to-peak amplitude was normalized to the average amplitude for that unit so that the percentage deviations within a recording session could be examined. Peak-to-peak voltages of all units ranged from -80% to 100% of these mean values as shown in the Figure 16.



Figure 16. Percentage change in the peak-to-peak amplitudes in all the units. The change is calculated compared to the mean amplitude of the unit during each recording interval that lasted approximately 10 minutes.



Figure 17. The peak-peak amplitudes recorded from 19th hour to 24th hour and the corresponding waveform shapes.

When the recording was resumed after 8-hour break, only units of amplitude 100-150 μ V could be sorted from the multi-unit data as shown in Figure 17. However, the noise levels were significantly lower which may be a result of the settling down of tissue drift. The standard deviation of noise in the post 20 hour session was 10 μ V as compared to the average standard deviation 30 μ V in the first 8 hours session. The standard deviation of noise was plotted for the entire recording session as shown in Figure 18. The RMS value of noise is a measure of the background neural activity as sensed by the electrode (these spikes have too small amplitude to be detected above threshold because the neuron may be far away). This may reflect the effect of tissue relaxation and other immediate injury responses due to which there is shifting of active neurons in the recording sphere of the electrode tip.



Figure 18. RMS values of noise over the 24 hour recording duration. Huge variations in the RMS values of noise are seen in the first 1 hour. The first 8 hours also saw substantial variations in RMS values of noise. Post 19th hour the noise levels settle down to values less than 10 μ V.

Discussion

The results show that amplitude variation can be up to 100 %. This variation is large and has important implications for developing adaptive control mechanisms to maintain reliable recordings. Large amplitude variations observed may be due to the head movement and grooming of the rat which in turn could cause significant drift in electrode position. It was observed that certain mechanical shocks like the rat hitting against the cage led to sudden appreciable changes in the signal amplitude.

The changing amplitude and noise RMS levels also imply that the tissue compression due to initial electrode penetration can cause variabilities in the neuronal recordings over hours. Large variations in noise RMS values can possibly be indicative of massive tissue drifts. Stabilization of the RMS noise values is necessary to be able to accurately detect single units and compute SNR values.

Other studies of that monitored single unit recordings over continuous time durations show high variability in the order of 50% or more in unit amplitudes (Chah et al. 2011, 2558-2561; Santhanam et al. 2007, 2037-2050; Chestek et al. 2011, 045005)

Average SNR trend of the recording appears to be a more stable measure to regulate due to the large variations seen in amplitudes of single units over 24 hrs.

Further, a similar continuous recording session of 24 hours needs to be done after the electrode has been implanted for over two weeks and the initial injury response has stabilized to characterize signal variability under chronic implantation conditions.

All of these variabilities make the task of continuously maintaining single unit activity on an electrode extremely tedious. Experience from the above studies seems to suggest that the loss of quality of recordings from specific single neurons might have been mediated by a relative micromotion between the microelectrode and surrounding brain tissue.

CHAPTER 4

CHALLENGES FOR AUTONOMOUS NAVIGATION OF MICROELECTRODES IN THE BRAIN

Motivation for automated controls to position electrodes

"Machines could simultaneously control (movement of) more than one electrode and monitor multiple tips, tasks which are difficult or impossible for a single operator." (Scobey 1983, 3-9)The idea of automating electrode movement to improve positioning accuracies and reduce the tedium of manual effort has been articulated for over 30 years now. The earliest of screw based microdrives were devised with the conceptualization that motorization and computerized control would lead to smart recording systems.

It is already established that the signal recorded by the microelectrode at the region of implantation is highly variable. Moveable microelectrode arrays give us the flexibility of moving the microelectrodes to a new location when there is failure of recording at a particular site. In general, when the experimenter tunes the electrode position, by moving it up and down over intermittent intervals so as to obtain a well isolated unit, the unit shape and amplitudes remains stable over the session (Yamamoto and Wilson 2008a, 2430-2440; Jackson, Mavoori, and Fetz 2007, 360-374). Most neurophysiologists follow this classic approach to achieve stable units and fine tune single unit recordings at the beginning of an experimental session using microdrives to move electrodes. Most microdrives constructed till date have been used in experiments where the electrodes are adjusted manually over a period of hours. Positioning the electrode near well correlated neurons while doing real time analysis can significantly enhance the efficiency of basic neuroscience experiments and performance of neural prosthetic decoders. Research groups in the past have used microdrives to position electrodes before every recording session to obtain the best recording quality (Eliades and Wang 2008, 201-214; Battaglia et al. 2009, 291-300; Bilkey, Russell, and Colombo 2003, 152-158; Dobbins et al. 2007, 101-111; Gray, Goodell, and Lear 2007, 527-536; Haiss, Butovas, and Schwarz 2010, 67-72; Korshunov 2006, 179-185; Lansink et al. 2007, 129-138; Swadlow et al. 2005, 2959-2965; Sato, Suzuki, and Mabuchi 2007,

45-51; Park et al. 2008; Venkateswaran et al. 2005, 1035-1040; Wilson et al. 2003b, 49-61)The task of manually repositioning these electrodes, especially when scaled to higher density arrays can be very tedious and slow to serve any practical purpose in prostheses applications. Hence an adaptive algorithm to automate the process is proposed that is capable of coping with the variabilities inherent in a realistic recording environment. The automated controls shall provide a more reliable interface by ensuring high quality neural recordings all the time. Positioning electrodes to obtain good quality recordings in maximum number of channels would be the primary goal in order to establish a reliable interface. Typically, the measured variable is some metric of signal quality (SNR is a more stable measure as discussed before).

Strategies for control of microelectrode movement

Broadly, there have been three kinds of efforts so far to implement control algorithms to position microelectrodes:

(i) Manually operated or screw based microdrives where the experimenter adjusts the position of the microelectrode (Battaglia et al. 2009, 291-300; Bilkey, Russell, and Colombo 2003, 152-158; Haiss, Butovas, and Schwarz 2010, 67-72; Lansink et al. 2007, 129-138; Bragin et al. 2000, 77-82).

(ii) Semi-automated systems where the microelectrodes are driven by motors and the experimenter moves them to seek good quality recordings through external inputs (Gray, Goodell, and Lear 2007, 527-536; Venkateswaran et al. 2005, 1035-1040; Yamamoto and Wilson 2008b, 2430-2440; Fee and Leonardo 2001, 83-94).

(iii) Fully automated systems where the microelectrodes automatically position themselves and are programmed to provide high quality recordings or recordings from specific population of neurons without any external monitoring (Wolf et al. 2009, 1240-1256).

Manually operated microdrives

In the manual process for detecting neural activity electrodes are typically advanced slowly at the rate of 5 µm/s while monitoring the electrical signal. The summation of all activity

from the neurons in the region surrounding the electrode tip, beyond a radius of 100-150 µm, appears as background noise in neural recordings. Action potentials emerge initially as positive or negative impulse voltages that are larger than the noise levels. Electrodes are moved until the action potentials are clearly distinguishable from the background noise. Its position is then tuned to maximize the action potential. Classically, this process is followed by experienced neurophysiologists while listening to an audio monitor where action potentials sound like 'clicks'. At times it is possible to detect action potentials from two different neurons with similar amplitudes and this is termed multi-unit activity.

Semi-automated systems with long term control strategies for maintaining signal quality

Fee et. al., (Fee 2000, 461-8) proposed a method to overcome variations due to physiological signals and stabilize intracellular recordings by using closed loop feedback control to control the position of electrodes. Baker et. al., (Baker et al. 1999, 5-17) demonstrated a control paradigm to detect pyramidal tract neurons after anti-dromic stimulation. The algorithm controlled an acute microdrive that autonomously advanced electrodes until target cells were detected. A human operator then fine tuned the position of the electrode to optimize the recorded signal. Microelectrodes were advanced before every recording session in a similar manner. They also noted that if the quality of a given recording begins to deteriorate, small movements in the order of 10 µm was sufficient to improve the signal amplitudes. The rationale was that such small movements could help compensate for the tissue slippage relative to the electrode. Jackson and Fetz showed in elegant experiments using motorized drives that it is possible to record continuously from the same neuron with definite behavioral correlates over a period of 2 weeks in a freely behaving animal (Jackson and Fetz 2007, 3109-3118). The recordings were very stable as the electrodes were repositioned only 39 times to yield 119 units over 300 days of implantation in the above experiment. Yamamoto and Wilson employed a unique strategy to obtain stable recordings that involved slow movements of the electrode to compensate for tissue compression and injury response. They implanted tetrodes using motorized microdrives. The tetrodes were

advanced by about 50-100 μ m per day over a period of 3 weeks at very slow speeds of 2-5 μ m/s and at small steps of 4 μ m. The electrodes were left untouched for at least 2 days in the final position before they began recordings. In order to optimize the recordings, electrodes were moved by about 80 μ m from this site and then in small increments of 8 μ m to find discriminable units. They were able to obtain stable units for more than two weeks. Movement paradigms where the electrode was moved at faster speeds of 100 μ m/s and steps of 30 μ m yielded units that were stable only for a few hours (Yamamoto and Wilson 2008b, 2430-2440).

Fully automated systems with control strategies for short term experiments

Rigorous controls based on stochastic models of neuronal activity patterns have been used to position the microelectrodes at the start of each recording session to isolate and track specific units (Wolf et al. 2009, 1240-1256). The autonomous algorithm "SpikeTrack" builds a strong case that autonomous control could in fact isolate single units and maintain signal quality during recording sessions (Chakrabarti et al. 2012, 72-85). The algorithm is built on a framework of finite state machines that manages the electrode's movement to optimal depth (Cham et al. 2005, 570-579). Briefly, the electrode moves in step increments until sufficient spikes are detected using an unsupervised detection algorithm (Nenadic and Burdick 2006, 941-55). A Bayesian clustering approach is used to sort the spikes and signal quality metrics are computed on the sorted clusters. Additionally, a tracking feature is incorporated in the algorithm which keeps track of neurons from previous recording intervals and associates them with neurons found in the current recording interval using multiple-hypothesis tracking for clusters (Wolf and Burdick 2009, 2649-59). Signal guality metrics such as the Mahalanobis distance and SNR are calculated to ensure spike detectability and discriminability. The tracked clusters are used to build a history of quality metrics. A gradient curve of SNR v/s position of electrode is built using optimized regression analysis. Once a gradient is established, an optimization routine moves the electrode to the maximum of the curve (Wolf et al. 2009, 1240-1256).

In a recent study the "SpikeTrack" algorithm was tested on a five channel Eckhorn drive in macaques (Chakrabarti et al. 2012, 72-85). The algorithm's performance was compared with passive placement of electrodes and expert human experimenter's performance in positioning electrodes to obtain stable unit activity in semi-chronic experiments. The algorithm performed significantly better and enhanced signal quality when compared with passive placement of electrodes in recording sessions of 30 minute duration. The algorithm's performance was also comparable to expert human users in maintaining signal quality in experiments that lasted for at most 3 hours.

However, while the algorithm has been validated in semi-chronic experiments, its performance in a chronic implantation situation is not known. As a result of the control theory, the algorithm made several small movements, with less than 100 s intervals between movements on an average, of magnitude ~10 µm in order to maintain the quality of recordings. In an interesting comparison, expert human users employed assorted strategies ranging from frequent, large amplitude movements to rare adjustments with minimal directionality switches. However, no single strategy can be considered the most optimal to achieve the desired quality or stability. A control algorithm approach does remove individual biases.

The fully automated systems with controls are desirable for cortical prosthetic applications that work in a closed-loop system along with neural signal decoders to operate a prosthetic device. Such prosthetic applications require on-demand, reliable neuronal signals typically from the motor and/or pre-motor cortical locations. An additional requirement of prosthetic application is that such systems operate reliably over the life-time of a patient. However, prosthetic systems do not necessarily require that a single unit be tracked over time. The decoders can be made to adapt to the best neuronal recording that is available at any given instant without significant loss of efficiency.

There is no clear consensus however, on a single control strategy to meet the requirements of establishing an autonomous, reliable neural interface. Rudimentary automation can be achieved by recording segments of neural signal, sorting the spike waveforms, identifying

well-isolated clusters, and maximizing the SNR of sorted units. Nevertheless, what constitutes the most optimal control strategy to maintain quality recordings over a long term remains an open question.

Challenges and goals for a fully automated system maintain reliable neural recordings

As summarized earlier, one of the main causes for signal non-stationarity is a change in the spatial distance between electrode tip and the neuron. Hence the primary goal of the control algorithm implicitly is to reposition the electrode to keep the relative position of the electrode with respect to the neuron of interest in the tissue constant or position the electrode at a new active neuronal interface. An ideal control algorithm designed for movable microelectrodes would be broadly required to meet the following end-goals:

(i) Automated/remote positioning of microelectrodes over a continuous time frame with minimal manual intervention and handling of awake animals.

(ii) Stability of recordings from single neurons of interest (in basic neurobiological studies on learning, memory, and plasticity). Detectability and consistently good quality of recorded units (particularly for prosthetic applications).

(iii) Increase in the yield of active electrodes.

The sources of variability in the recorded signal cannot be defined or modeled from a control systems perspective. Neural signal is the observable quantity and the metrics of signal quality can be evaluated. The control action is to move the electrode. However, the translation of this movement to a relative distance metric between the electrode and the neuron is the most significant challenge in defining a control strategy. In order to further elucidate this problem, let us consider an ideal recording environment. The electrode tip measures the change in the extracellular potential near the soma of a neuron. The amplitude of the measured signal is inversely proportional to the distance between the neuron and the electrode. We would ideally want to position the electrode as close to the neuron as possible but not too close in order to avoid impaling the neuron. In the brain due to shunting of currents in the extracellular matrix, it is theorized that the maximum distance from which an electrode can listen to a neuron is about 100-

150 µm before the neuronal signal becomes too weak to detect (Gold et al. 2006, 3113-3128). So when there is tissue slippage or movement of microelectrode due to disturbances we would need to reposition the electrode in this listening sphere. However, moving the electrode by a known distance does not always directly translate to an equivalent displacement in brain tissue. This is because of the mechanical properties of the brain tissue, which has a complex non-linear response to a step displacement. Hence in order to determine the actual displacement of the microelectrode in brain the mechanical properties of the brain have to be understood. In control systems terminology, we would need a model of the plant to translate the actuator signal to actual movement in the plant that in turn causes a change in the observed signal.

Let us assume that such a model of the plant (brain tissue and its constitutive mechanical properties) is available. One approach then will be to sense along with signal guality, forces acting on the microelectrode. The forces will be a direct measure of microelectrode movement or tissue micromotion. Using the mechanical model of the brain we could deconvolve the resultant displacement between the recorded neuron and electrode tip. The accuracy of such deconvolution will depend on the accuracy of the constitutive properties of brain tissue used. The problem is further compounded by the fact that moving the electrode in order to correct the change in relative distance also causes tissue compression or relaxation. Hence the control algorithm has to estimate and compensate for this tissue compression or relaxation using the constitutive model of brain tissue. Then the algorithm inputs the final control signal to the actuators to move the electrode to the estimated position. Adding to this complexity is the fact that the brain-electrode interface undergoes significant changes (in its composition, texture, and function) in the weeks following the implantation of the microelectrode (Polikov, Tresco, and Reichert 2005, 1-18; Winslow and Tresco 2010, 1558-1567). Further, a range of different constitutive properties of the brain have been used in mechanical models of brain tissue in prior studies indicating significant dependencies on experimental conditions, species etc.

Mechanical properties of brain tissue

Brain tissue is best modeled as a hyperelastic viscoelastic medium (Miller et al. 2000, 1369-1376). The viscoelastic properties of the brain tissue are not well understood (McConnell et al. 2007, 1097-107; Elkin, Ilankovan, and Morrison lii 2011; Clayton, Garbow, and Bayly 2011, 2391-2406). Viscoelasticity is a type of a constitutive model used to explain time dependent behavior of materials to a history of stress or applied strain patterns. The main characteristic of viscoelastic materials is that they dissipate energy under loading conditions and this dissipation is known as hysteresis. This implies that the loading portion of the stress-strain curve would be higher than the unloading portion. In a purely elastic material, there is no energy dissipation and hence the loading portion (when strain is applied) and unloading portion (when strain in removed) is the same. Hence the relationship between stress and strain for a spring is linear. In a movable electrode context, loading of brain tissue can be considered as forward movement of the electrode and unloading as the backward movement of electrode. Experimental results on brain tissue show that there is a non-linear relationship between stress and strain rates. A linear viscoelastic model thus does not fully describe the material characteristics (Miller 2000). Hence a hyperelastic viscoelastic model is used to describe brain tissue. Hyperelasticity is a type of a constitutive model used to explain non-linear stress-strain relationship in biological tissues. The response of such materials is dependent on temperature, strain history and strain rate.

Two main characteristics of viscoelastic materials are that of creep and relaxation. These two characteristics shall determine the response of the brain tissue to step perturbations of stress or strain. Creep is the characteristic of viscoelastic material to undergo deformation under constant stress until an asymptotic level of strain is reached. Relaxation is the behavior of the brain tissue where stress levels reach its peak and then relaxes over time under constant strain. The primary assumptions underlying the model are as follows:

 The properties of the brain tissue are assumed to be isotropic which means that the mechanical properties remain the same in all directions. Considering the microscale movements made by the electrodes this may not be a very accurate assumption and the

properties could be in fact anisotropic due to the presence of microvasculature and tissue heterogeneity.

 Brain tissue is assumed to be incompressible. The property of incompressibility implies that when the electrode penetrates through the tissue, it displaces a volume of tissue along the electrode track. This leads to deformation of tissue around the electrode.
The implications of the mechanical characteristics of brain tissue on the movement of microelectrodes in the brain are as follows:

Prediction of any change in the electrical recording from single neurons due to the relative micromotion between the brain tissue and the recording microelectrode becomes complicated due to the viscoelastic (and sometimes hyperelastic) nature of the surrounding brain tissue. The real distance between the recording site of the microelectrode and the neuron that is being recorded will follow a trend as a function of time due to the movement of the electrode. A single step movement of the electrode can be modeled as an induced strain. Brain tissue compresses under forward movement of the electrode. Tensile stress acts on the tissue during the reverse movement of the electrode. The forward movement of the electrode causes a constant strain, the stress levels in the surrounding brain tissue reaches a peak value and then relaxes over time. The relaxation time constant R is defined here as the time taken by the stress levels to reach one-third of its initial value under constant strain. It depicts the time taken by the tissue compressed by the electrode to relax to a steady state configuration. Hence when the electrode moves by a step displacement, the real distance between the electrode and a neuron in the surrounding tissue evolves as a function of time and reaches steady state only after a time interval of at least 4R has elapsed.

This has an important consequence while mapping SNR as a function of electrode position in the brain tissue to find an optimal site where the SNR is above a certain threshold. If the relaxation time constants are not taken into account, the relative distance between the electrode and a neuron and hence the SNR changes with time. The electrode would have to be repositioned after the tissue relaxes to adjust for the change in SNR, in a cyclic effect, where the neuron becomes a moving target. To find the relaxation time constants, the strain values are input into the mechanical model of the brain tissue, the stress response is calculated and the time taken to reach steady state is hence found. However, the exact viscoelastic constants of brain tissue in vivo under small levels of strain are not yet determined.

Impact of brain viscoelasticity on autonomous navigation of microelectrodes

The SpikeTrack algorithm is an example of a very rigorous stochastic approach that is also based on statistical modeling but incorporates a clustering and signal tracking framework to isolate neurons and maximize SNR. The algorithm does not factor in the mechanical properties of the brain tissue and the related tissue drift. Essentially, the electrode is blind to the medium it operates in. The algorithm builds an estimate of the SNR as a function of electrode depth using optimal regression analysis and then positions the electrode at the maximum of this function.

However, in a regression model the prediction depends on the history of the previously sampled number of points. Since the algorithm does not take into account the viscoelastic nature of the tissue the SNR at these previously sampled points would change with time. Not accounting for such delays can cause instability in control systems (Levine 1996). It is compensated for by not moving the electrode backwards and by quickly estimating the maximum of the neuron isolation curve with small steps of forward movements (Branchaud 2007) Nevertheless, the compensation mechanism is not a suitable strategy to maintain signal quality over a long term. Since it is a statistical based approach at its core, sufficient sampling of points is needed to perform regression analysis. A similar statistical approach is proposed by Natora et. al., (Natora 2011). Frequent adjustments of electrode in the order of once every 100 s as reported in the study may be undesirable in chronic implantation situation (Chakrabarti et al. 2012, 72-85). Further, directionality switches of the electrode indicate a lack of knowledge of direction of movement of neuron relative to the electrode. Even with prior histories of SNR, every time the electrode needs to be moved, the experimenter or a statistical based approach has to 'guess' the direction of movement. This in turn increases the number of movements required to optimally position the electrode. Ideally, in a chronic implantation situation we would want to minimize the

number of movements of the electrode. Thus, it can be concluded that incorporating the mechanical properties of tissue in the control algorithm is extremely critical to a long term strategy of autonomously maintaining neural signal quality. Our aim is to incorporate a sense of the constitutive properties of the brain tissue in the algorithm so that the movements respect the viscoelastic time response of the brain tissue.



Figure 19. Sorted units amplitudes before and after movement of MEMS microelectrode during the first two weeks of implantation. A transient increase in signal amplitude due to downward movement of microelectrode is observed in the top panel. However, signal amplitude remains steady with time after moving the microelectrode upward shown in the bottom panel.

The change in signal amplitudes as a result of the tissue relaxation after microelectrode movement was also observed experimentally in a different set of long-term experiments. The MEMS microelectrodes were moved downward in the first week of implantation by 100 µm when the SNR decreased below 10 dB. The signal amplitude increased by an order of magnitude in response to microelectrode movement at day 3 and day 6 in both the cases shown above. There is hysteresis or a lag in time between electrode movements and changes in signal quality probably due to the relaxation of the tissue. An example can be seen in Figure 19, in which a transient increase in signal amplitude is observed in response to electrode movement of 100 µm.
It takes more than one minute to see a change in the quality of the signal and about 3 minutes for the amplitude to reach steady state. However, the identity of the units before and after movement could not be verified. So we could not confirm if the signal seen after movement was from the same neuron. The electrical changes observed here, where the neural recording activity 'settles down' over a transient time frame of 100-120 s is consistent with the viscoelastic relaxation time constants observed in the force – displacement curve. It is reasonable to assume that four times the relaxation time constant is the approximate time taken for the tissue to reach steady state. From the table, during the first week of implantation it can be seen that the relaxation time constants are 60s and hence the time taken to reach steady state can be estimated as 240 s. However, transient changes in signal amplitude is not observed during upward movement of microelectrode in contrast to the changes seen during downward movement of microelectrode. The stress-strain response curve for a viscoelastic medium under unloading conditions is different from that of loading conditions. The force-displacement data for unloading conditions is currently unavailable. From the above discussion it follows that the SNR curve, as a function of depth, estimated under downward movement (under loading conditions) may be different or shifted when the electrode is retracted back (under unloading conditions).

Uncertainty in relative position between electrode and neuron

Searching for a neuron along one dimensional track of the electrode is achieved in principle by finding a gradient of SNR and positioning the electrode at the maximum of this gradient. However, the relative position of the neuron in the space surrounding the electrode track is highly variable. Apart from tissue micromotion, neuronal drift, perturbations of tissue due to mechanical shocks, the movement of the electrode in the one dimensional space also causes the neurons to shift positions. The causes for such uncertainty in relative position from a mechanical perspective are:

• Tissue deformation due to the continued presence of the electrode i.e. a constant stress in the tissue, a phenomenon known as 'creep'

 As the viscoelastic material is subjected to loading and unloading cycle i.e. forward and backward movements, due to energy dissipation in each cycle the tissue surrounding the electrode is permanently deformed. So it could be hypothesized that due to every forward and backward movement the relative position of the neurons in the grid surrounding the electrode is shifted. Though the tissue relaxes to steady state it may never returns to its original position.

Acute in vivo experiments to search to optimally position the electrode in the brain tissue to obtain high SNR. The gradient of the SNR is estimated using a linear search optimization technique. The problem of finding the microelectrode position for optimal SNR is essentially a one dimensional linear search problem. The SNR gradient as a function of depth is known to have valleys and peaks corresponding to positions where the electrode is closest to neurons along the track. There is evidence from experimental data to show that these peaks are typically at distances of 50 -100 μ m apart in the macaque cortex (Branchaud 2007). So the aim of the one-dimensional algorithm would be to find a local maximum. Several candidate linear search algorithms are listed here:

- Interval bounding algorithm
- Uniform search
- Bisection method
- Quadratic fit line search
- Piecewise linear approximation
- Inexact line search methods: quasi newton method, bisection method, backtracking algorithm

The most appropriate line search algorithm is chosen based on the data from the above three experiments to best suit the optimal movement speeds. The chosen algorithm should be able to achieve convergence with minimum number of iterations and should be implementable with the movement parameters obtained from the optimization experiments above. The objective is to obtain a stable SNR curve as a function of depth of the electrode in acute experiments. The

physiological variations notwithstanding, the experiment aims to minimize signal non-stationarities due to tissue motion from microelectrode movement and compensate for hysteresis during backward movement of electrode. The electrode would be optimally positioned in such a procedure so that the SNR observed, as a function of time would remain fairly stable with nonstationarities from tissue drift minimized. Hence the neuron would no longer be a moving target.

CHAPTER 5

ADAPTIVE MOVEMENT STRATEGY FOR PRECISE POSITIONING OF MICROELECTRODES IN BRAIN TISSUE

Introduction

Robotization in the field of neurotechnology can bring about automation to the fine art of recording neural activity in the brain. Advances in this field opens up exciting possibilities of micro-robots performing standard electrophysiological techniques that would previously take researchers several hundreds of hours to train and achieve the desired skill level. Automation would enable researchers to devote more time to science and design more complex experimental paradigms as they are freed from the tedium of managing the recording set up. Innovation in robotic neural interfaces would result in high fidelity signals, high yield, increased throughput, and adaptability to higher level integrated systems, decreased manual intervention and lowers barriers for adoption of neurophysiological techniques.

The field of robotic neural interfaces is still nascent. Burdick et al developed one of the first robotic systems that autonomously positions electrodes to record extracellular action potentials. The SpikeTrack algorithm controls electrode position based on stochastic models of neuronal activity patterns to isolate and track specific neuronal targets (Cham et al., 2005; Wolf & Burdick, 2009). Others have developed semi-automated systems with closed loop control paradigms for recording of neural activity (Fee & Leonardo, 2001a). Recently, Kodandaramaiah 2012 et. al., (Kodandaramaiah, Franzesi, Chow, Boyden, & Forest, 2012) successfully demonstrated an automated in vivo patch clamping system. The robot detects a neuron by tracking temporal sequence of impedance changes.

SpikeTrack algorithm's performance was comparable to expert human experimenter's performance in maintaining signal quality in experiments that lasted for at most 3 hours. (Chakrabarti et al., 2012). The algorithm made several small movements, with less than 100 s intervals between movements on an average, of magnitude ~10 µm in order to maintain the

quality of recordings. It is likely that one of the putative causes for such short-term variation in signal activity is tissue drift requiring re-adjustment of the electrode. (Ainsworth & Okeefe, 1977; Battaglia et al., 2009b; Bilkey & Muir, 1999; Bilkey, Russell, & Colombo, 2003; deCharms, Blake, & Merzenich, 1999; Gray, Goodell, & Lear, 2007b; Haiss, Butovas, & Schwarz, 2010b; A. Jackson & Fetz, 2007; Kern, Rorup, Werthschutzky, & Tammer, 2008; Lansink et al., 2007; S. Park et al., 2008; Sato, Suzuki, & Mabuchi, 2007; Seidl et al., 2010; Venkatachalam, Fee, & Kleinfeld, 1999; Venkateswaran, Boldt, Ziaie, Erdman, & Redish, 2005; Wilson, Ma, Greenberg, Ryou, & Kim, 2003; Wolf et al., 2009). In an interesting comparison, expert human users employed assorted strategies ranging from frequent, large amplitude movements to rare adjustments with minimal directionality switches. However, there is no single strategy that is considered the most optimal to achieve the desired quality or stability.

In order to further elucidate this problem of tissue drift, let us consider an ideal recording environment. The electrode tip measures the change in the extracellular potential near the soma of a neuron. The amplitude of the measured signal is inversely proportional to the distance between the neuron and the electrode. We would ideally want to position the electrode as close to the neuron as possible but not too close in order to avoid impaling the neuron. In the brain due to shunting of currents in the extracellular matrix, it is theorized that the maximum distance from which an electrode can listen to a neuron is about 100-150 µm before the neuronal signal becomes too weak to detect (Gold, Henze, Koch, & Buzsaki, 2006). So when there is tissue slippage or movement of microelectrode due to disturbances we would need to reposition the electrode in this listening sphere. However, moving the electrode by a known distance does not always directly translate to an equivalent displacement in brain tissue. This is because brain tissue has biomechanical properties that are non-linear (hyperelastic) and time varying (viscoelastic) (Karol Miller, Chinzei, Orssengo, & Bednarz, 2000). A step movement of the electrode towards a specific neuronal target exerts forces on the surrounding brain tissue causing it to deform and displace. The compressed brain tissue relaxes over time causing the neuronal target to move away. The electrode would have to be repositioned to chase the neuron that has moved away resulting in a cyclic effect. Thus the neuron becomes a moving target.

In the field of surgical robotics, the problem of controlling needle insertion in soft tissue to reach a specific target like a tumor or blood clot has similar challenges of tissue deformation and target relocation. One of the approaches used for robotic manipulation of the needle is to simulate the position of the target due to the tissue deformation caused by the movement of the needle. The system will iterate until the needle path and target converge. Visual data from intraoperative imaging and haptic feedback from force sensors are used to update the model parameters for needle deflection and tissue deformation (Elgezua, Kobayashi, & Fujie, 2013).

Accurate positioning of an electrode in brain differs from the above problem in the following ways - a) does not involve targeting a specific anatomical region and the electrode has to be positioned in a region of high neural activity along a linear track b) absence of visual feedback and force feedback with the positioning mechanism guided only by electrical activity c) micro scale movements of the order of a few hundred microns are needed to find a region of acceptable neural activity as compared to millimeter scale movements made during surgical interventions.

Mechanical models of tissue deformation using FEM are more accurate but they are computationally expensive and have limited validity range. Hence many of these models are not used in automating interactions with soft tissue and are used only for pre-operative planning. Mass-spring-damper models are not as accurate but are computationally less intense and are used for intra-operative needle guidance. Applicability of generic tissue parameters (Karol Miller & Lu, 2013; K Miller et al., 2010) to compute computational models for tissue displacement during electrode navigation is limited because biological tissue is known to have large inter as well as intra subject variability. Moreover, there is limited knowledge about translating these tissue parameters to micro scale movements in vivo (Sharp, Ortega, Restrepo, Curran-Everett, & Gall, 2009b), as there is a lot more inhomogeneity in the tissue at the micro scale with blood vessels, variations in tissue layers due to the organization of cell bodies and processes.

Several techniques for needle steering are used (DiMaio & Salcudean, 2005) and it is shown that by reducing needle-tissue interaction forces decreases compression of soft tissue and potentially results in accurate positioning. Interestingly, it has been shown that rotational motion of the needle along its translational axis reduces insertion forces and hence tissue deformation (Meltsner, Ferrier, & Thomadsen, 2007). Therefore we hypothesize that minimizing the microelectrode tissue interaction forces minimizes the internal tissue stresses that would reduce tissue compression and consequently tissue drift once the electrode is positioned in a region of high neural activity. Potentially, this would give more stable recordings once the electrode is positioned by eliminating one of the putative causes of non-stationarity in neural signal recordings.

A systematic study of the movement parameters of a recording electrode and its impact on tissue drift and hence recording stability has not been done. Our objective here is to define the most optimal strategy for microelectrode movement in brain tissue such that tissue deformation is minimized. We define a quasi steady state of stresses on surrounding tissue where the stresses remain at a constant level both i) in between step movements of the microelectrode and ii) after the final step movement on reaching the target location. Traditional approaches to microelectrode movement intuitively recognize that long settling times for tissue relaxation, small step sizes of movement, slow speed will minimize tissue drift and allow more reliable and stable recording (Fee & Leonardo, 2001b; A. Jackson & Fetz, 2007; Tolias et al., 2007; Yamamoto & Wilson, 2008). The following study investigates the rationale from a mechanics perspective to such intuitive strategies and aims to achieve engineering precision in developing a movement strategy for accurate placement of micromechanical structures in brain tissue.

We measure the forces acting on the microelectrode as it is moved through brain tissue using a precision load cell. The microelectrode is moved at varying step sizes and wait times between movements in a series of experiments. A stress relaxation model of brain tissue is fit to the experimental data. Further analysis of experimental data reveals that an inchworm type

movement protocol achieves quasi-steady state level of stresses. The parameters for such a movement protocol is recommended based on the modeling results.

Methods

Microelectrode-tissue interaction forces during micro scale navigation inside the brain were measured in vivo experiments in rodents. Stresses on the surrounding brain tissue were estimated from these force measurements. The experiments were performed in vivo in adult Sprague Dawley male rats of weight 300-500g. A total of n=13 animals were used in this study. All animal procedures were carried out with the approval of the Institute of Animal Care and Use Committee (IACUC) of Arizona State University, Tempe. The experiments were performed in accordance with the National Institute of Health (NIH) guide for the care and use of laboratory animals (1996).

Surgical procedure

The animals were induced using a mixture of (50 mg/ml) ketamine, (5 mg/ml) xylazine, and (1 mg/ml) acepromazine administered intramuscularly with an initial dosage of 0.1 ml/100 g body weight. The anesthesia state of the animals was monitored closely throughout the procedure using the toe-pinch test. Updates containing a mixture of 50 mg/ml ketamine and 5 mg/ml xylazine were given at a dose of 0.05 ml/100 g body. The rat was attached to a stereotaxic frame (Kopf Instruments, Tujunga, CA, USA). The scalp was reflected by a mid-line incision. A small hole was drilled in the skull with a trephine drill of 3 mm diameter with the center point being 2 mm posterior to bregma and 3 mm lateral of the sagittal suture exposing the cerebral cortex in the barrel cortex area. The dura was then carefully removed with a pair of micro scissors. The craniotomy was drained with physiological saline to clean the area of any blood clots and bone debris. In all experiments the pia was also removed from the surface of the brain. It was found that there was significant increase in compressive forces recorded when the brain surface became dry. In order to avoid confounding the measured data with this effect, care was

taken to always keep the exposed brain surface moist by applying physiological saline on the craniotomy. Insertion sites were chosen such that surface blood vessels were avoided.

Experimental set-up for force measurements

Polysilicon microelectrodes of dimensions 50 µm width and 4 µm thickness that tapers to a tip over 150 µm were used. The microelectrodes were dip coated with epoxylite (48-1696 P.D. George Company, St. Louis, MO, USA and the coating added about 20 µm of thickness overall. The final dimensions of the probe were approximately 90 µm width and 44 µm thickness as shown in Figure 20(a). The polysilicon microelectrodes were fabricated at Sandia National Laboratories, Albuquerque, NM. The experimental set-up is as shown in Figure 20(b). A single microelectrode was attached to a connecting screw post and mounted on a precision 10 g load cell (Futek, LSB210, Irvine, CA). The load cell with the microelectrode set-up was held on a hydraulic micromanipulator (FHC#50-12-1C, Bowdoin, ME). Contact with the brain surface was confirmed by increase in force readings and the microelectrode was lowered into the brain at the rate of 10 µm /s and implanted to a depth of 1 mm below the cortical surface. Once implanted, it was left in place for about 90 minutes to allow the forces to settle to steady state and stabilization of the tissue around the microelectrode. The microelectrode was moved according to pre-defined movement patterns in each experiment and the resulting forces recorded. In order to drive the microelectrode in specific step sizes with precise intervals between steps, defined henceforth as the inter-movement interval (IMI) and control the direction of movement, it was necessary to automate the movement of the microelectrode. A hardware hack on the FHC hydraulic manipulator that was manually controlled was performed by interfacing the remote control pin in the front panel with the TTL ports of a computer that generates voltage pulses according to a programmed movement pattern. Specifically, a LabView based platform generates TTL pulses that control the step size, IMI and the direction of movement of the microelectrode. Forces were recorded at the rate of 32 samples per second.



Figure 20. Experimental setup for measuring forces in vivo during microelectrode navigation inside brain tissue a) The load cell with microelectrode attached mounted on a motorized microdrive in a stereotactic frame. b) Microelectrode graphic showing the shape, dimensions and coated with epoxylite insulation.

Representative stress measurements in response to microelectrode movement

In order to establish that stress relaxation measurements are indeed reliable measure of tissue drift and characterize the stresses resulting from micro scale movement of the microelctrode, a bi-directional movement pattern is executed as shown in Figure 21(a). Consecutive downward and upward movements of increasing step sizes are executed with random wait times between movements where the tissue is allowed to relax. This sort of movement pattern is typical of the "search" phase where the microelectrode is moved seeking a region of interest where neural activity is of acceptable quality. The forces measured are converted to stress values and the characteristic stress recorded for such a movement pattern is shown in Figure 21(b). Data recorded is smoothed using a 32-point averaging window. The microelectrode is moved in increasing step sizes, and held in position until steady forces are recorded. Time needed to achieve steady state forces varies with step size and residual stresses

due to prior movements in the surrounding tissue - a phenomenon known as hysteresis due to the time dependent mechanics of brain tissue. Forces acting on the microelectrode represent both shear and compressive forces during downward movement. The compressive forces register as negative force increments in the load cell. During upward movement of the microelectrode we possibly measure tensile forces with the combined effect of tissue slippage. These forces register as positive increments in force in the load cell. It is observed that the resulting stresses for both movement directions are proportional to the step size of movement.



Figure 21. Stress measurement in brain tissue is a measure of tissue drift. (a) Displacement of microelectrode in an alternate downward and upward movement pattern with increasing step sizes at the rate of 100 μ m/s. The microelectrode is held in place for random intervals of time for the tissue to relax before making the next step movement. (b) Stress measurements show tissue relaxation patterns in response to microelectrode movement in both downward and upward movement directions. (c) Stresses due to unidirectional downward and subsequent upward movement of microelectrode in 30 μ m steps and 1 minute IMI. The microelectrode is held in place after 10 steps of 30 μ m movements and tissue relaxation due to the cumulative stress build up is seen. Similarly when the microelectrode is held in place after 10 steps of upward 30 μ m movement, there is tissue relaxation due to tensile stresses in the tissue.

Further, the stress relaxation response for downward movement has slow dynamics

varying over a time scale of minutes whereas the upward movement has a fast exponential

decrease to steady state. Figure 21(c) represents the stresses measured in brain tissue for

unidirectional movement of microelectrode in steps of 30 µm and IMI of 1 minute. 10 steps of 30 µm downward movement are executed. This type of movement pattern is typical of the 'seek' phase of fine-tuning neural recordings, where once a region of interest for recording neural activity is reached, the microelectrode is positioned as close to the target neuron as possible to achieve signal quality above an acceptable threshold. Such movements are typically slow and the step sizes are comparable to neuron soma diameters (~ 10 - 20 µm). We choose a step size of 30 µm as a standard for movements during the 'seek' phase. Each step movement is characterized by an instantaneous peak stress value that decreases with time to an asymptote for the duration of the IMI producing a time-dependent relaxation. At consecutive step movements, there is a build up of stress possibly because the tissue is not given enough time to relax between steps to a final cumulative stress level of 4kPa. The microelectrode is left in place for about 10 minutes and the brain tissue relaxes in a time-dependent, viscoelastic manner. The stresses relax to only about 50% of its maximum value in 10 minutes. A series of 10 upward movements with the same movement parameters are executed and there is a positive increment in stress levels and when the microelectrode movement is stopped, the tissue similarly settles to steady stress values. It can be deduced from these measurements that if the target area for neuronal activity were reached after 10 downward movements of 30 µm with 1 minute IMI, then the recorded activity would vary after 10 minutes due to tissue drift that would move the target away. Figure 22 provides an illustration of neural activity varying due to tissue drift.

Estimating stress

The load cell records the force acting on the microelectrode during navigation inside the brain and as the brain tissue relaxes around it. Effective stresses on the microelectrode are estimated as force per contact area. At each time point in the force curve, the total surface area of the microelectrode in contact with the brain tissue is calculated in accordance with the loading rate. The measured force was divided by the calculated contact area to get the total stresses acting on the microelectrode at all time points.



Figure 22. Variation in signal recorded due to tissue drift. Neural signal recorded after downward movement of microelectrode from a depth of 1000 μ m to 1090 μ m. Varying signal quality is observed when SNR is sampled 10 minutes after movement with the microelectrode held stationary. The waveform templates at these time points differ in amplitude and shape from one another with the appearance and disappearance of an unit. The microelectrode was implanted in the somatosensory cortex of rodents.

Estimation of viscoelastic parameters

The estimated shear stresses relax in a time-dependent manner once movement is stopped and is characterized using conventional viscoelastic models. The generalized Maxwell model is the most popular in estimating the stress relaxation behavior of viscoelastic materials. The relaxation function G(t) is defined in terms of Prony series parameters. Microscale movements exert small strains, so the stress relaxation response described by a viscoelastic model with a 2nd order Prony series expansion was found to be the best fit ($r^2 > 0.90$):

$$G(t) = G(0) + \sum_{k=1}^{N} (1 - G_k e^{-\frac{t}{\tau_k}})$$

where, N=2, G(t) is the relaxation response as a function of time, G(0) is the instantaneous shear modulus which is the maximum peak stress value measured immediately when a microelectrode is moved. G_1 and G_2 are the short-term and long-term shear moduli that characterize the relaxation response of the brain tissue. τ_1 and τ_2 are the corresponding short-term and long-term relaxation time constants. This model to describe stress relaxation is extensively described in previous brain tissue modeling studies (Gefen, Gefen, Zhu, Raghupathi, & Margulies, 2003; Gefen & Margulies, 2004). Prior studies that fit a non-linear viscoelastic model use the first 90 s of the stress relaxation response of brain tissue. Similar convention was used in this study, however it was observed that in most cases the model fit the entire duration of the IMI.



Figure 23. Modeling the viscoelastic relaxation response due to downward and upward movements of microelectrode. a) Stress response for 9 steps of 30 µm downward movement with 3 min IMI followed by 10 steps of 30 µm upward movement with 3 min Data is smoothed using 32 point window averaging. b) First 90 s of individual stress relaxation curves for every 30 µm downward (left panel) and upward (right panel) movement. c) Average of the curves taken after normalizing all the curves in (b). Normalization was by subtracting each curve by the mean of the curve. Viscoelastic stress relaxation model fit the the average curve.

Figure 23(a) shows the stresses in brain tissue when the microelectrode is moved

downward and upward in 10 steps of 30 µm each with 3 min IMI. In order to characterize the individual relaxation curves after each step, the curves were split from the instance of movement for the duration of IMI as shown in Figure 23(b). The 10 individual stress relaxation curves in response to both downward and upward movements were normalized by subtracting the average value of the steady state response beyond the first 90 s. The normalized downward and upward movement relaxation curves are averaged. The short-term and long-term relaxation parameters of the non-linear viscoelastic model were estimated as shown in Figure 23(c). The model fitting exercise and simulations were run in OriginLab 8.1[®]. The instantaneous shear modulus was calculated as the magnitude of the step change in stress value at each instant of movement. The same analysis procedure was followed for other trials of step sizes and IMIs. While retracting the

microelectrode, tensile forces develop as the forces acting on the tip of the probe are released. The forces registered by the load cell during upward movement are largely frictional forces (Sharp et al., 2009b). It was found that a single exponential relaxation model provides best fit (r^2 >0.9) to characterize the stress relaxation response to a step upward movement:

$$G(t) = G(0) + G_1 e^{-\frac{t}{\tau_1}}$$

where G(0) is the instantaneous shear modulus, G_1 is the short-term shear modulus and τ_1 is the relaxation time constant.



Figure 24. Intuitive strategies to overcome tissue drift effects result in steady stress levels. Stress measurements resulting from 10 movements, each with step size of 3 μ m (<neuron diameter) with inter movement intervals of 3 min in two separate animals. Step size of 30 μ m with long wait times of 20 min between movements also results in minimal stress levels with the peak value of stress at about -1.5 kPa.

Modeling stress response for a given movement pattern

The next objective was to simulate the stress response to consecutive step movements. The asymptotic stress value at the end of the IMI from the previous step was added to the instantaneous shear modulus at the instant of movement of the next step. The same stress relaxation parameters are used to predict the time-course of relaxation at every step. The instantaneous shear modulus had a negative sign for downward movements to denote compression and positive sign for upward movements to denote tension. These simulations produced a stress profile versus time in response to a movement pattern and were run in MATLAB (Mathworks®). In order to study the difference in time course of relaxation G(t) for different step sizes and IMI, the relaxation coefficients in equation 1 and 2 for all trials of step sizes and IMIs were evaluated in a 2-factor ANOVA. Inter-animal variability for the viscoelastic parameters in equation 1 and 2 was evaluated for a particular step size (30 µm) using 1-factor ANOVA.

Experimental design

A range of microelectrode micro scale movements of random step sizes from 3 μ m to 150 μ m were made and the forces recorded from the instant of movement till they reached steady state. In order to test the effect of IMI, 30 μ m step size was chosen as this is about the step size we move to search for neurons in the cortical region that were about 10 μ m in diameter. IMI is varied in the following sequence, 30 s (approximately the secondary relaxation time constant τ_2 for the stress response curve derived from moving 30 μ m and waiting for the stresses to reach steady state), 1 min (two times the secondary relaxation time constant τ_2), 3 min (six times the secondary relaxation time constant τ_2) and 20 min (forty times the secondary relaxation time constant τ_2). Ten steps of 30 μ m movements were made for each IMI and forces measured during movement and tissue relaxation after all movements. Stresses were estimated from these force curves and the estimate for model parameters derived. Step size of 3 μ m, 3 min IMI was

chosen to test forces exerted while moving in small steps less than the diameter of a neuron, movement typically executed while optimizing neural recordings.

Results

Movement parameters commonly used to achieve stable neuronal recordings based on the experimenter's skill, experience and intuition are generally small step movements in the order of 3 - 6 µm and long wait times between movements as discussed before. The stress patterns in brain tissue while moving the microelectrode using such intuitive movement strategies were measured as shown in Figure 24. Small step sizes of 3 µm movements with 3 minute IMI were tested in two different animals and the stress levels are represented as mean and SD of the stress values of the 3-minute interval after each instance of movement occurred in Figure 24. Similarly, 10 movements with step sizes of 30 µm movements with long wait times of 20 minutes were tested. The resulting stresses as shown in Figure 24 were fairly constant for both movement paradigms with long wait times and short IMIs and the maximum stress exerted by the brain tissue was 1kPa. Hence these strategies intuitively minimize the stress exerted on the surrounding tissue and thereby reduce the relaxation effects/tissue drift effects that lead to more stable neuronal recordings. The stress relaxation curves at the end of these experiments were fit to the 2nd order Prony series and the long-term relaxation constants were 11 s and 7 s for the 30 μm step size - 20 min IMI and 3 μm step size - 3 min IMI movements respectively. This further confirms that there is minimal stress build up due to these movement paradigms, as the tissue relaxes zero stress levels in less than a minute. However, the time taken to move 100 µm is 70 min (30 µm step size - 20 min IMI) and 96 min (3 µm step size - 3 min IMI) in each of these cases. For autonomous control applications, it is desirable to optimize the "search/seek" time to find stable and high fidelity neural signals and improve the efficiency of the process.



Figure 25. Instantaneous shear modulus for downward movement of microelectrode. a) Linear dependence of G(0) with step size b) Variation of G(0) with In to exponential base of IMI. G(0) thus has non-linear dependencies with IMI c) Box plot of mean G(0) measured for 30 μ m step size across n=7 animals tested d) Tukey test results for multiple comparisons of the mean G(0) for pairs between all animals tested. If the result of Tukey test is significant then the means of G(0) for 30 μ m step size for the pair of animals tested is significantly different.

Instantaneous shear modulus

Instantaneous shear modulus is an indicator of the stiffness of the brain tissue, higher the modulus, stiffer the tissue. Experimentally derived instantaneous shear modulus for micro scale downward movements of the microelectrode show a linear relationship (r^2 =0.82) the step size of movement as shown in Figure 25(a). The slope of the fit was 33.4 +/- 1.8Pa and the y-intercept was 371.8 +/- 88.7 Pa. Ideally, the intercept for this linear fit should be zero. However, since this data is pooled across different animals and movements are recorded at different depths deviations from ideal values are acceptable.

A 95% prediction interval that predicts a range of G(0) for a given step size is also plotted to account for these variations. The instantaneous shear modulus has a non-linear dependence on the IMI, which is expected due to the viscoelastic (time-dependent stress response) nature of brain tissue as shown in Figure 25(b).



Figure 26. Instantaneous shear modulus for upward movement of microelectrode. a) Linear dependence of G(0) with step size b) Variation of G(0) for 30 μ m step size with the depth in the cortex at which the movement occurred. Pearson's correlation coefficient of -0.187 indicates that there is no correlation between G(0) and depth of microelectrode in cortical brain tissue. c) Box plot of mean G(0) measured for 30 μ m step size across n=10 animals tested d) Tukey test results for multiple comparisons of the mean G(0) for 30 μ m step size for the pair of animals tested is significant then the means of G(0) for 30 μ m step size for the pair of animals tested is significantly different.

Similarly, the instantaneous shear modulus for upward movement has a linear

relationship with step size as in Figure 26(a). Modeling results show that the modulus values for

downward and upward movement for the same step size are of the same order as the shear

modulus for downward movement. For example, for 30 μ m downward and upward step, the predicted G(0) from the linear model is 1.3 kPa and 1.1 kPa respectively. It is important to note that this linearity holds true only for micro scale movements up to 100 μ m tested in this study. For a step movement of 100 μ m the forces registered are around 300 μ N. For displacements in this range, when the microelectrode is moved downward it is compressing the tissue beneath it and thus there is a linear relationship between force and displacement. Sharp et. al., report that for a critical force that microelectrode penetrates the tissue resulting in a decrease in forces. This force is 328 +/- 68 μ m for a flat punch probe of 100 μ m diameter with a loading rate of 11 μ m/s. We observe instantaneous increase in forces > 300 μ N for displacements above 100 μ m and hence limit the linearity regime to this step size. The maximum instantaneous shear modulus observed for downward displacements was 3 kPa. We estimate the elastic modulus of brain tissue as per equation 3 assuming a linear viscoelastic model, with Poisson's ratio of 0.5 (ref) with 3 kPa as shear modulus. The elastic modulus of 4.5 kPa matches well with the elastic modulus of brain reported widely in literature. This is significant, as it indicates that for micro indentations within the brain, the tissue responds linearly and acts as a homogenous isotropic medium.

Although we however know brain cortical tissue is an inhomogeneous structure at the micro scale with laminar organization, blood vessels, cell bodies and process with different mechanical properties. Therefore, we investigated if the values of the instantaneous shear modulus would vary with the location of the microelectrode in the cortex. However, as shown in Figure 26(b) there seems to be no correlation between depth of microelectrode and instantaneous shear modulus for upward movement (Pearson's r=-0.187). A Tukey test comparing the mean of experimentally determined instantaneous shear modulus parameters for the same step size (30 μ m) across pairs of all animals used in this study was conducted. The test revealed significant difference (p<0.05) in mean instantaneous shear modulus in 9 out of 21 pairs of animals for downward movement and in 23 out of 45 pairs for upward movement as shown in Figures 25(d) – 25(e) and 26(d) – 26(e). It can only be concluded from these results that we could

expect inter-animal variability in the estimation of mechanical parameters in vivo, however, the degree of variability varies across the population.

Stress relaxation model

The values of the long-term and short-term shear modulus G_1 and G_2 and the respective relaxation time constants τ_1 and τ_2 were determined using the bi-exponential model described in equation 1. The sample size consisted of a total of 108 stress relaxation curves for downward movements from all the individual trials that converged to a good fit (r^2 >0.9) with the model. For upward movements, the stress relaxation curve is fitted to single exponential model and hence has only two fitted parameters G_1 and τ_1 were estimated. The sample size consisted of a total of 60 upward stress retraction curves from all the individual trials. Two-factor ANOVA was conducted for testing the effect of factors step size and inter-movement interval on the model fitted parameters for both downward and upward movements. Results showed that the shear modulus G_1 and the short-term relaxation time constant τ_1 for the downward model are not dependent on both these factors (p>0.4).



Figure 27. Histogram of short-term and long-term viscoelastic shear moduli and relaxation time constants. a) The short-term and long-term viscoelastic shear moduli from fitting the stress relaxation response to a 2nd order Prony series model. Model parameters were obtained after fitting108 stress relaxation curves in response to downward movement with different step sizes across n=14 animals. The values of relaxation time constants are consistent across step sizes. The variations of shear moduli are within the ranges of short-term and long-term shear moduli for brain tissue reported in literature. b) Histogram of model parameters obtained after fitting 60 stress relaxation curves in response to upward movement with different step sizes across n=14 animals to a single exponential model. The viscoelastic shear modulus for upward movements is consistent and falls in the range of 200-400 Pa across all step sizes.

The relaxation constant τ_2 showed a dependency on step size (p<0.05) and intermovement interval (p<0.05). The shear modulus G₂ also showed a dependency on step size of movement (p<0.05). The shear modulus G₁ and the relaxation time constant τ_1 do not vary with either step size or inter-movement interval. Statistical power in each of these tests was >0.9. One-way ANOVA was performed for each of the model fitted parameters to test if there was variation across animals. However, this test was not significant (p>0.3) indicating that the model generalizes well across all animals (n=14). Figure 27 shows the spread of the model fitted parameters across all step sizes and IMIs. The variations seen are largely due to the anisotropic and heterogeneous nature of brain tissue as also the variability inherent to in vivo experimentation. Since these parameters define the constitutive material properties of the brain tissue, average values for all the coefficients are taken and the mean, standard deviation, lower and upper 95% confidence intervals are reported in Table 2.

Table 2. Model Fitting parameters for (a) downward and (b) upward movements

(a)	Parameters	Mean(Pa)	Standard Deviation	Lower 95% Cl of Mean	Upper 95% CI of Mean
	G1	468.78	185.82	433.34	504.23
	G2	559.87	456.27	472.83	646.90
	t1	1.55	1.22	1.31	1.78
	<u>t2</u>	33.64	34.48	26.65	40.62
(b)	Parameters N	Aean (Pa) St	andard Deviation Lo	wer 95% Cl of Mean U	pper 95% Cl of Mean
	G1	324.17	208.87	270.21	378.13
	t1	2.32	1.96	1.81	2.83

The values of the viscoelastic shear moduli fall within these ranges but as discussed earlier they trend with step size as well. It was observed that the overall stress response prediction model for a given movement pattern however, was not sensitive to variations in parameters with IMIs. For a 30 μ m step size, stress response model was set up with the following parameters - G1=460 Pa, G2=560 Pa, τ_1 =1.5 s, τ_1 =30 s and G(0)= -1300 Pa for downward movement. The IMI was varied as 30s, 1 min, 3 min and 20 min. Figure 28 shows that the simulations of the stress response model closely follows experimental data with 30 µm step size and the above IMIs respectively. It is observed that the model simulations fit well with experimental data and are robust at predicting stress values in wide range of time scales ranging from 300s for 30s IMI to 6000s for 20 min.



Model parameters:

Unidirectional 30 um steps downward movement go=-1300 Pa G1=460 Pa G2=560 Pa t1=1.5 s t2=30 s

Figure 28. Stress measurements showing tissue relaxation patterns for uni-directional microelectrode movement at the rate of 100 μ m/s. The electrode was moved in steps of a) 30 μ m with 30 s minute inter-movement interval (at relaxation time constant) b) 30 μ m with 30 s minute inter-movement interval (2 times relaxation time constant) c) 30 μ m with 3 minute inter-movement interval (6 times the relaxation time constant) d) 30 μ m with 20 minute inter-movement interval (40 times the relaxation time constant). Stress response due to these movements measured experimentally were compared with simulations of the stress relaxation model for the same movement parameters.

While 30s is approximately the long-term relaxation time constant for this step size, the

other IMIs are twice, 6 times and 40 times the relaxation time constant respectively. Modeling

results indicate that for moving a total distance of 300 µm (10 steps) at the above movement

specifications, the asymptotic value of stress at the end of the 10th IMI were -4.8 kPa, -3.5 kPa, -2.78 kPa and -2.71 kPa respectively for the above IMIs. Comparing the first 5 movements experimentally it is seen that the asymptotic value of stress at the end of the 5th IMI were approximately -1.3 kPa for both 3 min and 20 min IMIs. Model simulations predict the same stress values as well. Interestingly, increasing the IMI to 20 min did not reduce the stress build up significantly at -2.71 kPa. Hence waiting for long durations between step movements while moving towards a target in a viscoelastic soft tissue does not prevent residual stress build up and eventual relaxation on reaching the target beyond 6 times the relaxation time constant. Our goal was to achieve quasi-steady state stresses i.e. zero or minimal residual stresses build up between steps and a final steady state stress value with no further tissue relaxation at end of all step movements. Therefore, the stress response model was further explored to predict a movement pattern that could result in quasi-steady state stresses.

Bidirectional inchworm type movement results in quasi-steady state stresses

Model simulations indicated that a bidirectional inchworm type movement pattern is successful in achieving quasi-steady state stress conditions in brain tissue during microelectrode navigation. For unidirectional movement parameters described above with 30 μ m step size and 3 min IMI, we simulated a bidirectional movement pattern that effectively moves the same step size in the same IMI. An inchworm type movement pattern with 60 μ m downward movement, 1 min IMI and subsequent 30 μ m upward movement, 2 min IMI resulted in an effective movement of 30 μ m in 3 min.



Model parameters:

Unidirectional 30 um steps downward movement go=-1300 Pa G1=460 Pa G2=560 Pa t1=1.5 s t2=30 s Bi-directional cumulative 30um downward movement 60 um upward movementgo=2000 Pa G1=650 Pa G2= 1000 Pa t1=1.5 s t2=30 s 30 um upward movementgo=800 Pa G1=324 Pa t1=2.5 s G2=124 Pa t2=80 s Inter-movement interval between 60 um down and 30 um up movements = 60s Inter-movement interval between 60 um down and 30 um up movements = 120s

Figure 29. Quasi-steady state stresses i.e. stresses close to zero distinctly visible during bidirectional movement as compared to build-up of stress with every step in unidirectional movement. a) Model simulations of stress response to bidirectional movements resulting in effectively moving 30 μ m in 3 min compared with unidirectional step movements of 30 μ m in 3 min. b) Bidirectional movement pattern of 60 μ m downward movement, IMI1 of 1 min and 30 μ m upward movement, IMI2 of 2 min. c) In vivo measurements of stress response to bidirectional step movements resulting in effectively moving 30 μ m in 3 min compared with unidirectional step movements of 30 μ m in 3 min in n=2 animals. Quasi-steady state stresses are observed throughout the bidirectional movement sequence and at the end of all movements. d) In vivo measurements of stress response to four movements of bidirectional and unidirectional patterns described above.

The simulation results shown in Figure 29 (a) show that the bidirectional movement pattern (inset) produces near zero stress levels after every step and at end of all step movements. Experimental results as shown in Figure 29(b) and (c) in n=3 animals confirm near zero stress levels for bidirectional inchworm type movement compared to unidirectional movement pattern with same movement parameters. In all the trials of bidirectional movement it is seen that even after termination of movement, the stresses remain at steady state levels with no significant tissue relaxation. We investigated further into what ratio of step sizes for a bidirectional movement pattern would result in steady state stresses as shown above in the least interval of time.



Figure 30. Bidirectional movements with optimal and non-optimal IMIs. a) Stress response measured experimentally for 30 μ m downward and 20 μ m upward movements with IMI1=15s and IMI2=15s compared with model simulations for the same movement pattern. The IMIs were optimized through modeling and validated experimentally. n=75 movements are made and hence the microelectrode moves a total of 750 μ m. There is some compression of brain tissue due to insertion of the microelectrode deeper into the brain however, the stresses after all movements remain at steady state. b) Stress response measured experimentally for 10 μ m downward and 5 μ m upward movements with IMI1=15s and IMI2=15s compared with model simulations for the

same movement pattern. Model simulations with optimized IMIs (IMI1=50 s and IMI2=10s) and the resulting steady state stresses are also shown.

Optimizing IMI

With the inchworm type movement mechanism, it is possible to have multiple ratios and combinations of downward and upward steps that result in a net downward movement. Three ratios of step sizes were explored to minimize the IMI such that microelectrode movement is executed in the shortest possible time while still maintaining steady state stresses. The aim of this optimization exercise is to find an IMI that is less than 4 times the long term relaxation time constant (τ_2) corresponding to the step movement we execute. The optimization of IMI is essential as it increases the speed of movement of the microelectrode while maintaining steady stresses. Ratios of 2:1, 3:2 and 3:1 where the microelectrode was moved two steps downward and one step upward, three steps downward and two steps upward and finally three steps downward and one step upward were simulated. The step sizes chosen for executing this ratio of movements and the simulation results of IMI are shown in Table 2. IMI1 is the inter-movement interval between the downward and upward movement.

Ratio of steps	Step sizes (μm)	Effective downward movement (μm)	IMI1 (s)	IMI2 (s)	Time taken to move 120 μm (s)
2:1	10:5	5	50	10	1440
2:1	60:30	30	60	20	320
2:1	120:60	60	>180	-	-
3:2	30:20	10	15	15	360
3:2	60:40	20	20	20	240
3:1	30:10	20	120	15	810

Table 3. Simulation results for bidirectional inchworm type movement for different ratio of step sizes and the resulting optimal IMIs

Results indicate 3:2 is the most optimal ratio of downward and upward steps for an inchworm type movement pattern that minimizes IMI. A total IMI (IMI1+IMI2) between two effective steps of 10 µm and 20 µm is 25s and 40s respectively for the 3:2 ratio, whereas the total

IMI in all the other cases is greater than 1 min. The optimization routine is meaningful only for step sizes below 100 µm. For example, when we tested the 120 µm : 60 µm step sizes for 2:1 ratio of movements, we found that the iterations did not converge to steady state stresses even after IMI1 was increased to 4 times the relaxation time constant ($\tau_2 = 56s$) for 120 µm step downward movement. 3:1 ratio of movements with large differences in the downward and upward step sizes also do not optimize the IMI effectively and result in total IMI>2 min. 3:2 ratio with IMI1=15s and IMI2=15s is validated experimentally with the following step sizes: 30 µm downward movement and 20 µm upward movement. Figure 30(a) shows experimental data with the stress response to the above movement pattern and the simulated results follow closely with experimental data for n=75 movements. The microelectrode moves a total of 750 µm and the final stress levels at the end of all movements are about -1 kPa. There is some tissue compression as the microelectrode is inserted deeper into the cortical tissue but the resulting stresses are at steady state at the end of all movements. When the IMIs are not optimized for the bidirectional movement pattern it is possible that the resulting stresses are not at steady state and there is significant residual stresses and tissue compression as shown in Figure 30(b). Experimental data shows that the movement pattern with non-optimal IMIs results in almost -10 kPa of stresses and eventual relaxation of brain tissue was observed

Bidirectional movement leads to steady state stresses in a 16 week chronically implanted microelectrode

Bidirectional movement strategy to obtain steady state stresses was also tested in a 16 week chronically implanted microelectrode in rat brain tissue. The microelectrode was implanted to a depth of 1 mm in the cortex and left in place for a period of 16 weeks. Arati et al., 2013 provide a detailed report of the implantation procedure and maintenance of the implant over 16 weeks. The microelectrode was moved initially over a distance of 500 µm to move through the scar tissue zone seen around chronic implants (Sridharan, Rajan, & Muthuswamy, 2013). Bidirectional movement pattern in the most optimal ratio of 3:2 was to be tested. The mechanical

properties of brain tissue in chronic implants will change due to the remodeling of tissue around the implant over time (Sridharan et al., 2013). A step movement of 60 µm downward (n=3) was made and the tissue was allowed to relax to steady state. Similarly, 40 µm upward movement (n=3) was made and the tissue was allowed to relax to steady state. The modulus parameters in the viscoelastic stress relaxation model were derived as described above. A bi-exponential model was fit for both downward and upward movements and the following modulus values were determined for 60 µm downward movement, G(0) = -1280 Pa, G₁ = 459 Pa G₂ = 239 Pa T₁ = 5.7 s and T₂ = 70 s. And for 40 µm upward movement, G(0)= 518 Pa, G₁ = 691 Pa G₂ = 961 Pa T₁ = 1.8 s and T₂ = 35 s. The stress relaxation model was simulated to find the most optimal IMIs for the above step sizes, IMI1 = 60 s and IMI2 = 60s.



Figure 31. Stress response to bidirectional movement in 16 week chronic implant. Stress response measured experimentally for optimized movement parameters - 60 μ m downward and 40 μ m upward movements with IMI1 = 60 s and IMI2 = 60 s. The IMIs were optimized through modeling and validated experimentally. n=7 movements were made and hence the microelectrode moves a total of 140 μ m. The stresses remain at steady state after all movements validating the bidirectional inchworm type movement strategy in brain tissue with chronic implants.

Therefore, the microelectrode was moved in vivo with the above optimized bidirectional

movement parameters and the resulting stresses were as shown in Figure 31. The

microelectrode was moved a total of 140 µm and steady state stresses are maintained at the end

of all movements validating the bidirectional movement strategy in brain tissue with chronic microscale implants. However, the modulus parameters need to be adaptive to the change in mechanical properties of the brain tissue around chronic implants and were re-derived. Variations were observed in between the consecutive step movements and these could be due to inhomogeneity in brain tissue. The overall magnitude of the stresses seen during bidirectional movements was also significantly larger than that seen during navigation in acute experiments.



Figure 32. Stress response to bidirectional movement in 4-microelectrode and 16-microelectrode multi-electrode array. 4-microelectrode and 16-microelectrode stress response was measured experimentally for the following bidirectional movement parameters - $60 \mu m$ downward and 30 μm upward movements with IMI1 = 60 s and IMI2 = 120 s. Not that the entire array was moved and not individual electrodes in the array. The stress response for these multi-electrode arrays was compared with the stress response from moving a single microelectrode with the same parameters. For both the single microelectrode case and the 4-microelectrode array with 1 mm spacing there are no residual stresses and the stress remain at steady state after all movements. However, for the 16-microelectrode array with 500 μm spacing there is build up of residual stresses with final relaxation of tissue at end of all movements.

Discussion

For small micro scale displacements compressive forces are more dominant than shear. However, the modulus values derived from micro scale movements in brain tissue in this study compare well with values derived from large-scale indentation studies in in vivo rat and porcine models (Karol Miller & Chinzei, 2002; Karol Miller et al., 2000; Sridharan et al., 2013). The shear modulus values are also similar to previous studies of microelectrode penetration in millimeter scales in rat and mouse brain tissue (Sharp et al., 2009b; Sridharan et al., 2013). We defined quasi-steady state of stresses where the stresses are almost constant during movement. The rationale for achieving such quasi-steady state stresses is multifold. Primarily, it achieves precise positioning in a non-visual environment with viscoelastic mechanical properties by overcoming tissue relaxation effects. As the target is approached, by compensating for deformation properties of the soft tissue, the relative distance between the probe and the target is always maintained and targeting accuracy increased.

Another important consideration for minimizing the stresses created during micro scale navigation inside brain tissue is to keep the stresses well within the tissue thresholds for mild traumatic brain injury (El Sayed, Mota, Fraternali, & Ortiz, 2008; L. Zhang, Yang, & King, 2004) and minimize inflammation response (Bjornsson et al., 2006; Jensen, Yoshida, & Hofmann, 2006; Werner & Engelhard, 2007). Limited data is available on the stress magnitudes and durations that cause tissue injury (De et al., 2007). McConnell et. al., (McConnell, Schneider, Owens, & Bellamkonda, 2007) showed that increased peak extraction forces of microelectrodes correlated with GFAP expression in astrocytes. Zhang et. al., (L. Zhang et al., 2004) state that the threshold levels of shear stresses at 6 kPa could indicate mild traumatic brain injury with 25% probability. These limits for injury are only representative thresholds, as they may not translate accurately to the case of micro scale movements with very low strain rates. Therefore, by maintaining quasi-steady state stresses close to zero with consecutive movements we would minimize the possibilities of inducing tissue damage and associated cellular inflammation response.

Measurements of forces required to move micro scale displacements in brain tissue are in the order of a few 100 uN. If the tissue is not allowed to relax between consecutive movements, these forces increase to the order of mN. For bidirectional movements, the maximum force registered is in the range of 300 uN - 500 uN. Therefore, force requirements reduce by orders of magnitude for penetrating soft viscoelastic materials using optimal movement strategies. Particularly, for applications using MEMS based micro devices used for navigation in soft tissue, optimal movement parameters would lower the design requirements for force generated by the device.

The stress relaxation model accurately quantifies the effect of the movement parameters such as step size and IMI on the stresses developed in brain tissue. Thus, it is possible to predict the stresses developed given a random sequence of movements. Adhoc strategies of wait times ranging from 30 min to 24 hours to allow the tissue to relax before recording neural activity could thus be eliminated (Battaglia et al., 2009a; Eliades & Wang, 2008; Gray et al., 2007a; A. Jackson & Fetz, 2007; Yamamoto & Wilson, 2008). The precise quantification of factors such as how much to move and how long to wait to overcome tissue drift effects shall further the development of adaptive automated neural recording systems.

While recording extracellular activity in the brain multi-electrode movable array it is observed that moving one electrode affects the activity recorded in the neighboring electrodes. When guasi-steady state stresses are maintained, there is minimal compression and the stresses do not propagate to the neighboring electrodes. Thus this technique could possibly ensure that with multiple electrodes, movement of one electrode does not affect the signal quality at the other electrodes. Further extensions to this study with multiple electrodes could investigate the optimal spacing to mechanically isolate electrodes in a multi-electrode movable array. Preliminary data to support this hypothesis is presented in Figure 32. Bidirectional movements of 60 µm downward movement and 30 µm upward movement were made with a 16-channel array with an interelectrode spacing of 500 µm and a 4-channel array with an inter-electrode spacing of 1 mm. The entire array was mounted on the micromanipulator and moved inside the brain tissue in an experimental setup as described above. It is observed that quasi-steady state stresses are maintained during movement of the 4-channel array with 1 mm electrode spacing similar to the single electrode case. However, movement of the 16-channel array shows a build-up of stress due to tissue compression indicating that in closely spaced arrays there might be second order effects due to overlapping mechanical spheres of influence around individual electrodes. A mechanical model that incorporates the effect of the spacing between the electrodes on the resulting stresses in tissue during movement is necessary. Such a model could be used to design for optimal spacing between electrodes in a multi-electrode array.

The force curves measured at different regions and depths of the brain had some minor and localized variations but the overall trend is predictable and consistent. In some trials, the measured curves would not fit the viscoelastic model because of noisy data that could be a result of the microelectrode severing a blood vessel, changes in intra-cranial pressure or tissue drying effects due to an open craniotomy. The probe used in the experiments here is a planar structure but it is coated with brain epoxy and hence can be considered as an incompressible material that does not deflect on insertion into the brain. Hence the modeling analysis applies only for rigid, incompressible probes and does not take into account the effects of deflection. The data presented here was measured from the cortical regions of adult rats; thus, mechanical properties of the deeper structures of the brain and differences in gray matter and white matter tracts are not studied. The response of the stress relaxation model for consecutive microelectrode movements is sensitive to the viscoelastic parameters chosen. Although the model has greater tolerance to G_1 and τ_1 values, the values of G_2 and τ_2 are critical to determine response. Thus in order to improve model predictions in micro scale navigation and targeting in a viscoelastic medium, subject-specific characterization of the viscoelastic parameters in vivo would enhance the accuracy of the model (Henken, Van Gerwen, Dankelman, & Van Den Dobbelsteen, 2012; Karol Miller & Lu, 2013; van Gerwen, Dankelman, & van den Dobbelsteen, 2012). This technique also has applications in navigation and placement of micromanipulation tools during robotic surgeries. For example, robotic micro scale needle manipulation in clinical procedures like targeting tumors for ablation and biosensing where it is critical to reach the specified anatomical target with minimal error margins.

Conclusion

The goal of the study was firstly, to evaluate the magnitude of stresses that would result in the surrounding brain tissue and tissue relaxation dynamics given a microelectrode movement pattern. Secondly, to optimize the movement pattern, step size and IMI such that movement of the microelectrode results in quasi-steady state stresses in the tissue. A viscoelastic stress relaxation model was developed that predicts the stress response in brain tissue to a microscale

movement pattern. An optimal bidirectional movement strategy was proposed that would maintain stress levels close to zero and was validated in vivo. This results in detailed quantification of the parameters for navigation inside soft viscoelastic brain tissue that would induce minimal stresses in brain tissue and minimize the time taken for movement. Finally, the bidirectional movement pattern was also validated in a 16 week chronically implanted animal and steady state stresses were obtained.

In conclusion, the bidirectional inchworm type movement technique results in precise targeting and positioning of microelectrode in brain tissue while overcoming tissue drift effects.

CHAPTER 6

CLOSED-LOOP CONTROL FOR ADAPTIVE MICRO-SCALE ROBOTIC INTERFACES

Introduction

This chapter describes the closed loop control algorithm for autonomously positioning a microelectrode to monitor neural activity. Signal quality of the neural activity is monitored and the microelectrode position is adjusted based while optimizing the signal quality.

Signal quality metrics

The question of how to achieve high quality recordings through such adaptive controls is dependent on the signal quality metrics that are to be optimized. The signal quality metrics chosen should be objective measures of robust/reliable control. In general, three global signal properties are recognized: i) SNR and baseline noise level ii) spike waveform statistics such as peak-peak amplitude, waveform statistics and spike train statistics iii) impedance measurements.

Changes in electrode impedance give us a clue to the underlying causes that result in a changing interface. Impedance spectroscopy measures the flow of current between electrodes in a electrolytic medium, allowing the resistive and capacitive circuit elements of the electrodes and the medium between the electrodes to be characterized. But studies have not been able to provide a definite explanation linking changes in waveform shapes to observed electrode impedances (Suner et al. 2005, 524-41; Williams et al. 2007, 410). Hence although electrode impedance does provide an observable measure of the microenvironment around electrodes it cannot predict signal quality. Also, in a BMI paradigm, there is now an emerging consensus among researchers that spike sorting leads to very little improvement in the performance of the decoding algorithm (Fraser et al. 2009, 055004; Herzfeld and Beardsley 2010, 046012). In fact, high performance studies recently reported have used only 'threshold-crossing' events, in which the firing rate of one unit per electrode is determined regardless of whether this activity results from more than one neuron (Chestek et al. 2011, 045005). The signal to noise ratio (SNR) is

taken as the objective measure that is controlled in the proposed algorithm. Apart from the above, one of the other reasons for choosing SNR was already described above, as it proved to be a more stable measure of neural activity when compared to the spike shapes or amplitudes.

SNR will be computed from the time-continuous data and independent of any spike sorting. The time-continuous data will be full-wave rectified (by squaring the signal to enhance the signal and reduce the noise floor), and data exceeding mean plus three times the standard deviation will be classified as signal and the rest as noise. The threshold for signal and noise detection will be computed in a time-resolved fashion to account for temporal changes in noise levels and signal variance, which could be expected. SNR will be computed using the following formula:

$$SNR = 10 \log_{10} \frac{(signal)^2}{(noise)^2}$$

where, the signal and noise are the average amplitude of signal and noise taken over the length of each time window.

Modes for control algorithm

A conceptual diagram of the proposed control algorithm is shown below in Figure 33. The proposed closed loop control algorithm has four modes:

- Maintain The microelectrode is held stationary at the site of interest as long as the neural activity recorded is above the set threshold value.
- 2. Linear Search If neural activity is below the set threshold the control algorithm is in the linear search phase. A uniform linear search routine searches for neural activity above a set threshold. The microelectrode moves along a linear track with 30 µm step size with the optimal movement strategy described above such that quasi steady state stresses are maintained in brain tissue. Once the microelectrode is positioned at a location where the SNR is above the threshold value the linear search routine is terminated and the microelectrode moves to the optimization phase.
- 3. Optimization The SNR is optimized by moving the microelectrode along the gradient of
the SNR curve in increments of 10 µm. Here as well, quasi steady state is maintained by determining an apriori optimal movement pattern. The SNR as well as peak-peak amplitude of the maximum spike detected is monitored and the microelectrode position is optimized to maximize the amplitude of the maximum unit detected.

4. Wait - In order to account for non-linearities such as when a neuron stops firing or when the signal amplitudes diminish suddenly due to possible mechanical shocks, the control algorithm is put on a wait cycle to allow the system to regain equilibrium.



Figure 33. Schematic representing an overview of the proposed control system

Closed-loop control algorithm proposed by Burdick's group referenced before is as shown in Figure 35 (b) where the feedback element is only the signal quality metric. The control scheme has an outer feedback loop that continuously monitors action potential data and optimizes the SNR as shown in Figure 34. The inner feedback loop translates the microelectrode displacement commands from the controller to stress values in the brain and provides an optimal inter-movement interval that would keep the stresses at quasi steady state. In the previous chapter, we determined the stress values and thereby the optimal movement pattern for a range of step sizes of movement of the microelectrode. In this chapter, a predetermined bidirectional inchworm type movement pattern with the inter-movement interval and step sizes optimized to produce quasi-steady state stress was employed. Hence, the mechanical model introduces a delay before moving the microelectrode to the next step to minimize the residual stresses in the brain. In the degenerate case, the mechanical model simply reduces to a delay element, where an extended period of wait time between movement steps of microelectrode shall also maintain steady state stresses in the brain as shown in Figure 35 (a).



Measured SNR

Figure 34. Block diagram of control scheme with the viscoelastic stress relaxation model of brain tissue incorporated for optimal navigation of microelectrode in brain tissue.



Figure 35. a) Block diagram of the control scheme with a simple delay element incorporated for navigation of microelectrode in brain tissue. b) Block diagram of the control scheme with no regulator and only SNR as feedback for microelectrode movement.

Estimating the direction of movement

Every time there is a decrease in the signal amplitude, there is an ambiguity about the direction of movement of the neuron with respect to the electrode. Although repositioning of the electrode by a small distance may correct the problem, since the direction of movement is unknown we may end up moving the electrode away from the neuron. After passing a set threshold, a gradient can be determined by a uniform linear search method. When an increasing gradient is detected the electrode is positioned at a sub-optimal spot that is not necessarily at the

peak of the SNR curve. When tissue relaxation occurs or the neuron moves away from the electrode the direction of movement of the neuron is known by its movement along the gradient denoted by a drop or rise in SNR. This helps in resolving the direction of movement while trying to maintain neural signal quality and the decision to move forward or backward can be taken. However, the map of SNR vs. depth is transient. So if the microelectrode is moved back to the same spot the SNR value may not be the same as before due to the rearrangement of the neuronal matrix in the tissue around the electrode.

Software implementation of algorithm

This part of the specific aim involves the real time implementation of the control algorithm. The neural signals will be acquired through the Tucker Davis Technologies® (TDT) signal acquisition system. The raw signal will be sampled at 25 kHz and filtered from 500 Hz to 3 kHz. A MATLAB routine is called from the TDT interface that does the signal detection and computes the signal-to-noise ratio (SNR). The methodology to do this is already described in the previous chapter. The SNR is then used as input to the control algorithm. The algorithm will be implemented in MATLAB. A graphical user interface will be built that gives the user control over the algorithm parameters and positioning of the microelectrode. The movement commands will be sent from MATLAB to TDT, which would then initiate the waveforms necessary for the electrode to move. A block diagram of the full system in which the microelectrodes are positioned autonomously is shown in Figure 36. It is a feedback control system with the components identified as follows. The plant includes the microelectrode and the neural tissue interface. The microelectrodes are the sensors. The controller is the software implementation of the algorithm of the previous chapter. The recorded neural signal activity is the sensor inputs to the signal processing block. The signal-to-noise ratio is the input to the controller. The output (actuating signal) of this controller is an electrode movement command for each electrode being controlled. These commands are typically sent to actuation signal generation circuit which translates the movement commands into the proper voltages to move the electrodes, closing the feedback loop.



Figure 36. Block diagram showing the implementation of the feedback control system that autonomously positions microelectrodes in the brain.

Potential pitfalls: When controlling multiple channels the analysis of the computational times required for each of the processing blocks is critical to prevent overlap in real time implementation of the algorithm. The programs could be multi-threaded so that different programs run simultaneously in the background and some of the data processing can be pipelined.

In vivo experiments were conducted to quantify how the signal improves due to such movements, which are based on tissue properties and the characterization of the signal quality from such adaptive controls is shown below.

Results

The microelectrode was implanted at 1 μ m/s speed to a depth of 560 μ m in animal A and 1 mm in animal B to examine the effect of tissue drift post implantation on neural activity. A single unit was isolated in both the cases and the variation in amplitude of both the units is distinctly observed in Figure 37. Depending on the relative position of the neuron around the microelectrode listening sphere, the activity modulates with tissue drift. Unit B shows a steady decrease with time over the span of 6-7 minutes completely disappearing at about 12 minutes. The microelectrode was implanted to a depth of 590 μ m with the optimal movement strategy determined in the previous chapter using a bidirectional 60 μ m downward and 30 μ m upward inchworm type movement pattern. A single unit was isolated at this depth and the microelectrode was left in place and neural activity recorded. It was observed that the unit remained stable for more than an hour before the amplitude began to decrease. The change in amplitude due to tissue drift in the time scale of 10 minutes as observed in the 1 μ m/s was not seen indicating that tissue might have indeed been at steady state stresses. The causes for change in activity beyond could be due to a number of putative causes outlined in Chapter 3.



Figure 37. Average peak-peak amplitude variation of unit isolated at 10 μ m/s implantation. Activity of unit A changes on isolation after the first 2 min and there is change in activity subsequently as well. A single unit was isolated in animals A and B respectively.



Figure 38. Average peak-peak amplitude variation of unit isolated with bidirectional inchworm type movement pattern. The unit remains stable for more than an hour.

During the optimization phase, if the microelectrode moves past a point of local maxima of the SNR, it can retrace its step back. However, as shown in Figure, the SNR values may not be the same when the microelectrode retracts back to the same depth. The same observation was made while estimating the direction of movement of the microelectrode above. An illustration of this case is shown in Figure 39. The microelectrode is moved forward in the optimal bidirectional movement pattern to depth of 1600 µm and retracted back.



Figure 39. SNR variation when microelectrode is traced back to the same depths. SNR values as the microelectrode is advanced forward (shown in blue) and retraced back through the same depths (shown in red) using a bidirectional inchworm type movement pattern. The SNR values do not exactly match up when the microelectrode re-visits the same recording sites.

Further, long periods of silence are observed in the activity of a single neuron being recorded. The neuron maybe absent in consecutive observation windows of 1 minute and then reappears again in the next window. In Figure 40, two single units were simultaneously recorded from the microelectrode. The raw extracellular recording is shown on the left and the sorted units are shown on the right. The neurons isolated had peak-to-peak voltage of about 3.5 mV with a long latency period of about 3 min. During such a period if the controller tries to optimize the position of the microelectrode there is a possibility of damaging the neuron and losing the signal altogether. Therefore, a trade-off exists while designing a control scheme for an automated system between consistently good quality recordings versus tolerance for periods of inactivity. In our control scheme we define a wait time of 3 min before the controller considers the activity lost and switches to the linear search mode.



Figure 40. Wait time before responding to decrease in SNR. (a) Sorted units of amplitude 3.5 mV were isolated (b) Illustration of intermittent neural activity of the isolated units observed with a period of latency lasting for about 3 min. A trade-off exists while designing a control scheme for an automated system between consistently good quality recordings versus tolerance for periods of inactivity.

The closed loop control algorithm was tested for with the non-optimal control scheme with the control being blind to mechanics of brain tissue. The results of autonomous control of neural activity are as shown in Figure 41. The controller isolates three single units during the duration of the experiment; however, each unit remains stable only for a span of 6-10 minutes. The controller attempts to re-isolate the unit by finding local maxima of the peak of the SNR/peak-peak amplitude curve. Upon examination of extracelluar activity and the units isolated in this experiment, evidence of tissue drift as observed by the changing signal activity was clearly visible as shown in Figure 42. The microelectrode chases a neuron that is a moving target as the microelectrode tries to position close to the neuron. Tissue relaxation over a period of 6-7 minutes post microelectrode positioning drives the neuron away.



Figure 41. Algorithm performance with a conventional regulator with only SNR as feedback. There was no wait time between movement except to estimate SNR over a 9s window and mechanics of brain tissue was not taken into account. The microelectrode responds to every change in SNR. From the SNR and peak-peak amplitude plots it is seen that three units were isolated and lost during a 35-minute duration experiment.



Figure 42. Variation of extracellular activity with tissue drift during non-optimal autonomous control of microelectrode movement. A unit is isolated at depth 1252 um and activity is lost within minutes possibly due to tissue drift. Microelectrode moves forward and regains the unit at depth 1342 um. However, activity is again lost and the microelectrode tracks the unit at depth 1372 um. The target neuron moves away from this site as well and the microelectrode is unable to track the neuron again through subsequent movements.

The microelectrode is able to track the same neuron over a distance of 120 μ m. This gives us an estimate of the range of change in relative distance between microelectrode and neuron during and the magnitude of tissue drift due to relaxation of brain tissue around the microelectrode. The results of algorithm performance with the knowledge of tissue mechanics incorporated in the regulator to control the positioning of the microelectrode is shown in Figure 43. The algorithm isolates a single unit once through the duration of the experiment and maintains a stable unit for nearly 90 minutes.



Figure 43. Algorithm performance by closed loop control with an optimal regulator with SNR as feedback and a predetermined optimal movement strategy that maintains quasi steady state stresses. The microelectrode was moved with the optimal bidirectional movement strategy determined in the previous chapter with step size of 30 µm and IMI of 3min. The microelectrode isolates a single unit, from the SNR and peak-peak amplitude plots it is seen that it attempts to maximize both these signal quality measures and holds a steady unit for approximately 90 minutes.

A step-by-step illustration of the isolation of the unit during closed loop control adaptive to tissue mechanics is shown in Figures 44, 45 and 46. In Figure 44, the microelectrode moves bidirectionally in the linear search phase



Figure 44. Isolation of single unit activity during linear search phase of control with regulator adaptive to tissue mechanics. A single unit is isolated in three effective step movements of 30 µm each as the microelectrode is moved bidirectionally over a total distance of 90 µm. The threshold for SNR was set to 17 in this experiment to isolate large single unit activity.

Once the microelectrode locates unit activity and the SNR is above the set threshold the

algorithm switches to the optimization phase. In the optimization phase the microelectrode is

moved bidrectionally with 10 µm effective step size (steps of 20 µm forward and 10 µm backward)

and IMI optimized as described earlier.



Figure 45. Optimization of single unit activity as the microelectrode is moved in steps of 10 μ m bidirectionally around the site of interest found during linear search. The microelectrode is moved both in forward and backward directions to determine the direction of increase in gradient of peak-peak amplitude. The amplitude of unit activity is optimized and long as there is an increasing gradient in peak-peak amplitude.

The algorithm switches to maintain phase once unit activity is optimized and the

microelectrode remains stationary. SNR is monitored in windows of 1 minute continuously to

ensure that the signal quality remains above the desired threshold. The algorithm was able to

maintain a steady unit for more than 90 minutes after isolation in the maintain mode as shown in

Figure 46.



Figure 46. Steady unit post isolation in maintain mode of control with adaptive regulator. The unit remains stable for about 90 minutes before losing the unit altogether.

It could be concluded that by optimally positioning the microelectrode one of the putative causes of change in signal quality i.e. tissue drift is eliminated. Tissue drift as seen from results above causes changes within the first 10 minutes of positioning of the microelectrode. Thus, the mechanical properties of the tissue will help in determining a more optimal strategy for long-term placement of electrodes. However, non-stationarities that cause signal changes over long term still do exist and the control algorithm has to counter for that variability by repositioning the microelectrode. In Figure 47, the peak-peak amplitudes of unit activity in 6 different trials (n=3 animals) of non-optimal autonomous control with conventional regulator and 1 trial (n=1 animal) optimal control with regulator adaptive to tissue mechanics is presented.



Figure 47. Variation of peak-peak amplitude with non-optimal control and modes of the control algorithm in 6 different trials (n=3 animals). Total time of control from all 6 trials = 216 minutes. Below panel - Variation of peak-peak amplitude with non-optimal control and modes of the control algorithm in 1 trial (n=1 animal). Total time of control = 180 minutes.

The modes of the control algorithm during the duration of the experiment are also presented. During non-optimal control the algorithm is mostly in the search phase or waiting to recover activity due to instability in the unit isolated. The main reason for this instability is tissue drift due to non-optimal microelectrode positioning post the linear search/optimization phase. The algorithm continuously isolates several units over the duration of the experiment in an effort to keep the SNR above threshold value. As seen in Figure 48, the microelectrode traces three neuron isolation curves during duration of 35-minute experiment. In the optimal control routine where quasi-steady state stresses are maintained and hence there is no tissue relaxation. Thus the units remain steady post isolation as shown in the bottom panel of Figure 47 with the control in maintain mode for the most duration of the experiment. Only a single unit isolation curve of SNR is traced by the microelectrode as shown in Figure 49. However, the maximum peak-peak

amplitude curve for the same experiment shows three peaks. The control is blind to this metric in the linear search phase and perhaps does not detect the large unit activity that shows up in the peak-peak amplitude curve but is missing in the SNR curve possibly due to lower firing rate or long latency periods. Thus the control based only on SNR as a measure of signal quality is biased to high firing rate neurons with large amplitudes. Maximum peak-peak amplitude could be used as an alternate or additional metric for control. In some cases spikes were spotted when the microelectrode was moved 60 μ m down but did not appear after consecutive 60 μ m -30 μ m movements.



Figure 48. SNR versus depth curve showing the neuron isolation curve during the non-optimal control routine. Multiple isolation curves are traced as the controller attempts to re-isolate lost neurons due to tissue drift for the duration of the experiment. Maximum peak-peak amplitude versus depth curve showing multiple neuron isolation peaks. The SNR and maximum peak-peak amplitude were calculated over a 1 minute window.



Figure 49. SNR versus depth curve showing the neuron isolation curve during the optimal control routine. A single isolation curve is traced and the neuron is held stable for the duration of the experiment. Maximum peak-peak amplitude versus depth curve showing multiple neuron isolation peaks that were missed in the SNR curve possibly due to low firing rate neurons that do not increase the SNR substantially in a 1 minute window.



Figure 50. Histogram of inter-movement intervals between microelectrode movements in nonoptimal and optimal control strategies. With optimal control adaptive to mechanics the number of movements are much lesser. The two peaks seen at 60 s and 120 are the IMIs of the bidirectional movement pattern during the linear search phase. In non-optimal control large number is IMIs less than 50s are seen as the controller is mostly in linear search phase attempting to isolate the neuron. The duration of experiments in both cases are comparable at 216 min and 180 min with non-optimal and optimal control experiments respectively.

	Conventional Regulator	Regulator adaptive to mechanics	
Total microelectrode movement during control	7570 μm	2100 μm	
Frequency of movement	1.48	0.3	
Average Peak-Peak amplitude	190.11 μV	258.24 μV	
Average SNR	14.56 dB	17.77 dB	
%Time in Linear Search	25.25	27.4	
%Time Optimization	30.1	8.9	
%Time Waiting to recover	21.9	0	
%Time Maintain	22.74	55.5	
%Total Time spent in search	77.27208781	36.22809542	
% Time unit isolated	22.73565501	55.28804184	

Table 4. Summary of algorithm performance for non-optimal and optimal control strategies

In order to evaluate the performance of a controller that is blind to tissue mechanics and a controller that adapts to tissue mechanics we compare the following performance metrics as shown in Table 4. The IMIs are compared in Figure 50 and it can be concluded that with the adaptive control strategy the frequency of interventions needed to restore activity are less indicating more stable isolation of unit activity. This figure presents strong evidence that for comparable experiment times, the optimal controller by adapting to the viscoelastic behavior of brain tissue leads to more stable unit activity by eliminating tissue drift as one of the putative causes of non-stationarity.

CHAPTER 7

PACKAGING AND IMPLANTATION TECHNIQUES FOR INCREASING LONGEVITY OF MEMS IMPLANTS

The goal is to assemble an autonomous robotic implant that is capable of maintaining neural signal quality. Specifically we would want to implement the control strategy developed in the previous aim on a MEMS microelectrode array platform.

The first part of the aim deals with challenges in chronically implanting MEMS microelectrode arrays in vivo. The challenges involved could be split into the following subcategories:

- i) packaging of MEMS devices for chronic implantable application
- ii) mounting techniques that provides an interface for the electrodes to actuate over a long term,
 is of small form factor and ensures that the integrity of the packaged device is maintained
- iii) surgical techniques for MEMS devices with movable parts

Packaging MEMS implantable devices

The following section gives a brief description of the steps followed to package the MEMS die into a fully implantable package. The MEMS devices are fabricated at Sandia National Laboratories using the 5-layer SUMMIT-VTM process.

 Testing-The MEMS dies are checked for functionality before packaging. The microelectrodes are actuated forward and back on an electrical test bench. Testing has to be done with care so that the electrical bond pads are not damaged. Damaged bond pads can cause the silver epoxy to flow down to the active areas during the reflow process in the packaging process that follows.
 Insulation - The MEMS microelectrode array is insulated with brain probe electrical insulator (Epoxylite 6001, P.D. George Company, St. Louis, MO). The microelectrode are extended off the edge of the die by 3.5 mm. Care is taken that all the three microelectrodes are actuated by the same distance. The brain probe epoxy is taken in a container and the meniscus is visualized under a microscope to ensure that it is uniform to avoid coating the microelectrodes at different lengths. The die is mounted on a post fastened to a z-axis manipulator and slowly lowered. The microelectrodes are dipped into the epoxy while observing under the microscope. The meniscus can be pulled up under surface tension and contaminate the die area. This is catastrophic for the functioning of the actuators. Hence the microelectrodes are dipped only to about 2.5 mm with about 1 mm spacing from the die edge. The tips of the microelectrode are etched using dicholoromethane using the same dip technique. The microelectrodes are soft baked at 99°C for 30 minutes and then hard baked at 199°C for 30 min. The microelectrodes are tested by actuating them forward and backward again after insulation.

3. Fabrication of interconnects - The second level interconnects that has leads running from the bond pads to the Omnetics® connector are fabricated on a glass die. Glass is used as the substrate to allow for visualization of the microactuators in function and the microelectrode movement. 1mm thick, 4 inch, glass wafer is used as the substrate. The interconnect leads are patterned in gold on the glass substrate using standard micro-fabrication procedures.

4. Electrical testing and resistance measurements of interconnects- The width and thickness of interconnects were optimized so that the resistance of the leads is minimal to keep the power consumption low. The traces are of width 80-100 μ m and thickness 500 μ m. The average resistance is about 18 Ω which does not add significantly to the actuator resistance. The spacing between interconnects is large enough to prevent crosstalk between the electrical lines. 5.Packaging- Packaging of MEMS devices is done by using a novel flip-chip technique using silver epoxy (Sutanto, Anand, Patel, & Muthuswamy, 2012).The devices are non-hermetically sealed using silicone gel to keep contaminants away from the device surface (Sutanto, Anand, Sridharan, et al., 2012).

6. Final package testing – The Omnetics® connector serves as the final interconnect that interfaces with the actuation signal generation circuitry and the entire package is covered with epoxy for protection. The package is tested for functionality. During the development of the packaging process tests were done to ensure that the silicone seal prevents fluid contamination of the package at intra-cranial pressure levels.

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Design of mount and surgical techniques for MEMS implantable devices

The package will be implanted in Sprague Dawley rats. In a chronic experiment, the device has to be securely mounted on the skull. Further, it has to be protected against physical damage and mechanical shocks in awake, behaving rats. The mount has to be light and small for the animal to wear comfortably. The mount is made using plexiglass/ABS plastic as it is easy to machine and widely used in 3-D printing technology. Two rails or placeholders are designed inside the mount to form a snug fit with the packaged device. A CAD diagram of the mount and the fully packaged device are shown in Figure 51 and Figure 52.



Figure 51. CAD diagram of the mount and the package.



Figure 52. Fully flip-chip packaged implantable device.

A critical requirement is that the mount has to allow for the microelectrode to move in and out of the package while preventing fluid entry from the craniotomy. For this requirement, we cast microchannels in the craniotomy through which the microelectrodes can move. Standard surgical procedure up to the creation of craniotomy is followed as described before (Jackson et al. 2010). The microelectrodes are implanted in the somatosensory cortex and a craniotomy is made using trephine drill bit of 2.7 mm diameter. In order to cast the microchannels, three microneedles of 200 µm diameter are perfectly aligned as the microelectrode array and fixed onto a glass die. The dura is knicked and excised. The craniotomy is covered with silicone gel and is not disturbed for a few hours. This is to allow all the residual bleeding from the skull and the dura excision to subside. The silicone gel is removed and the microneedles are positioned in the craniotomy with the tip of the needles touching the brain. The edges of the craniotomy are completely lined with gel foam to absorb any bleeding and CSF during microelectrode penetration. Kwik-cast® (World Precision Instruments, Inc., Sarasota, FL) is poured into the craniotomy around the microneedles. This has to be done quickly, seeing that no lumps are formed as the curing time of Kwik-cast is only 1 minute. The edges of the Kwik-cast are sealed to the skull using cyanoacrylate adhesive. The microneedles are removed after the cast is fully cured to form three channels.

Potential pitfalls: Spacers made of silicone gel and of thickness 0.5 mm are glued on to either side of the craniotomy. The spacers serve a functional purpose. When there is blood or CSF exuding from the channels, if the device is held flush with the craniotomy the pressure built up could be large and the fluids can get into the packaged device by capillary force. The spacers prevent this by opening up the channels to ambient atmospheric pressure. The gel foam also prevents drying up of blood in the craniotomy which could potentially block the channels. The mount needs continuous maintenance and cleaning to avoid fluid contamination and blocking of the channels. An illustration of the mount on the skull is shown below in Fig. 53. The animal is allowed to recover from surgery for about two days.

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Figure 53. An illustration of the mounting of the device on the skull and the interface with the brain.

CHAPTER 8

CONCLUSION AND FUTURE WORK

Neural interface technology has tremendous potential to create brain-machine connections that enable functions such as control limb prosthetics or computer cursors using thoughts or brain signals. For patients with ALS, paralytic stroke, limb amputees, such technology can enhance their quality of life significantly. Aforementioned application demands that sensors that interface with the brain perform reliably over the lifetime of a patient, typically 70 years. Currently sensors are fixed electrodes implanted in the brain and have serious issues with reliability and often fail over a period of a few months.

Implantable microelectrodes that are currently used to monitor neuronal activity in the brain *in vivo* have serious limitations both in acute and chronic experiments. Movable microelectrodes that adapt their position in the brain to maximize the quality of neuronal recording have been suggested and tried as a potential solution to overcome the challenges with the current fixed implantable microelectrodes. While the results so far suggest that movable microelectrodes improve the quality and stability of neuronal recordings from the brain *in vivo*, the bulky nature of the technologies involved in making these movable microelectrodes limits the throughput (number of neurons that can be recorded from at any given time) of these implantable devices. Emerging technologies involving the use of microscale motors and electrodes promise to overcome this limitation.

Control algorithms that search and optimize for maximum cluster separation and units in all recording channels can probabilistically increase the neuronal yield manifold in an implanted array. Thus along with miniature movable microelec-trode array technology, intelligent adaptive controls would enhance the reliability of the neural recording system and is a promising approach to ensure the stability, consistency, yield, and longevity of neural recordings from implanted micro-electrodes. With simultaneous developments in novel packaging and interconnect microtechnologies that will allow these microtechnologies to perform in challenging *in vivo*

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behavioral contexts in long-term experiments, the movable micro-electrode approach will dramatically enhance the information throughput from single neurons in the brain. Achieving the elusive goal of stable long-term interfaces with single neurons will also facilitate discoveries in neuronal plasticity, learning, and memory studies. In the longer term, the tunable nature of these microelectrodes will allow the possibility of tracking specific neuronal circuits of interest and changes in their connectivity and synaptic strengths with time as shown in Figure 51.



Figure 54. Conceptual diagram on adaptive autonomous control of movable microelectrodes where the controller could seek for specific neuronal activity that correlates with function for neuroprosthetic applications or alter neural circuits through neuromodulation to provide a specific response.

The control algorithm detailed here is simple SNR feedback based and nevertheless shows tremendous improvement in signal stability due to the adaptive nature to tissue mechanics. Further, this adaptive positioning scheme could be applied for positioning any microscale probes and sensors in soft viscoelastic tissue. The optimal navigation strategy of microscale probes could find medical applications like tissue biopsy and in microscale surgery. Further improvement of the algorithm to track individual signal units by using rigorous statistics and regression based algorithms as detailed by Wolf et. al., could be incorporated. Currently, the optimal movement strategy is predetermined, but with the integration of a force sensor with microscale probes could potentially lead to online computations of stresses in surrounding brain tissue and bidirectional navigation to minimize those stresses. The mechanical model parameters could be determined for each individual subject leading to greater prediction accuracy and more accurate positioning.

Packaging of MEMS devices and surgical implantation and maintenance are areas on which considerable effort has been done during the course of this thesis work. Further development of high-density array of moveable microelectrode systems depends on the ability to reliably package and implant these devices over long durations of time. Tissue response to movement of microelectrodes over chronic implantation is currently unknown. The performance of the optimal control algorithm in chronic implantation conditions is also not evaluated here. These are some of the key areas that would drive the development of high throughput micro-scale robotic interfaces.

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APPENDIX A

MOVABLE MICROELECTRODE TECHNOLOGIES

Author/Vendor	Size (L × W × H) (mm)	Weight (g)	Number of Channels	Full stroke	Motion System			
	Manual mayah	la miaraala		length				
Manual movable microelectrode systems								
Deadwyler 1979	20 (H)	2	1	5	Screw driven			
Bilkey 2003	22 (H)	0.39	1	4	Screw driven			
Swadlow 2005	35 (H)	0.5	7	5	Screw driven			
Dobbins 2007	53 (H)	12	12	10	Screw driven			
Battaglia 2009	23 (H)	1.8	6	5	Screw driven			
Thomas Recording Systems		1.5	1	5	Screw driven			
deCharms 1999	26 × 22 × 16	20	49	5	Pin push, Hydraulic			
Venkatachalam 1999	5.85 × 14.5	1.4-3.3	6	3	Gear			
Eliades 2008	17 × 12 × 8	0.8	16	2	Removable electrode pusher			
Neuralynx	37.5 × 52.8	16.9	28	10.5	Gear			
Automated movable microelectrode systems								
Fee and Leonardo 2001	6 × 17	1.5	3	3.5	Synchronous motor			
Venkateshwaran 2005	30 × 50	30	4	5	DC motor			
Charm 2005	35 × 16	40	4	5	Piezoelectric linear actuator			
Sato 2007	23.5 × 37	15	22	4	Hydraulic			
Gray 2007	143 (H)	129.5	8	11	Stepper motor and gear			
Park 2008	9 × 7 × 13	5	3	5	PZT (lead zirconate titanate) actuators			
Yamamoto 2008	30 (H)	29	21	7	Servo motor			
Wolf 2009	71 (H)	26.1	3	5.6	Piezo-electric linear actuators			
Yang 2010	24 (H)	1.82	4		Piezo motor			

APPENDIX B

CONTROL ALGORITHM PARTIAL CODE
Main Control

```
%Electrode position
low=500;%um
up=3000; %um
np=10;
eta=30;
Iteration=0;
oldSNR=1;
%elecpos=low;
elecpos=564;
failed=0;
thrs=17;
while TDT.GetSysMode ~= 0
while (oldSNR>0)
disp('In Maintain mode');
[SNR,Iteration,StartIndex]=maintain(TDT,StartIndex,BufSize,Iteration,elecpos,oldSNR);
```

oldSNR=SNR;

```
while oldSNR<thrs
  disp('Waiting to recover SNR');
 % Before going to linear search mode wait and record SNR for
 % three more cycles
 % Cycle 1
  [SNR,Iteration,StartIndex,ppmax]=snr(TDT,StartIndex,BufSize,Iteration,elecpos);
  oldSNR=SNR;
  if oldSNR>thrs
     break;
  end
  %Cycle 2
  [SNR, Iteration, StartIndex, ppmax]=snr(TDT, StartIndex, BufSize, Iteration, elecpos);
  oldSNR=SNR;
  if oldSNR>thrs
     break
  end
  %Cycle 3
  [SNR,Iteration,StartIndex,ppmax]=snr(TDT,StartIndex,BufSize,Iteration,elecpos);
  oldSNR=SNR;
  if oldSNR>thrs
     break:
  end
```

disp('In Linear Search mode');

[StartIndex,trackSNR,Iteration,elecpos,failed,up,Iow]=linearsearch(TDT,StartIndex,BufSize,Iteration,elecpos,up,Iow,np,eta,thrs);

```
oldSNR=trackSNR;

if failed==1

break

end

disp('In Optimization mode');

[Iteration,elecpos,newSNR]=optimization(TDT,StartIndex,BufSize,Iteration,elecpos,oldSNR);
```

Maintain

function [SNR,Iteration,StartIndex]=maintain(TDT,StartIndex,BufSize,Iteration,elecpos,oldSNR) %%%%%Monitor phase %Monitor SNR before beginning control%%%%%%

for i=1:6 pause(9); %Grab and plot data every half a second. CurrentIndex = TDT.GetTargetVal('Sindhu3c.sWave');

if CurrentIndex > StartIndex %Check to make sure that the buffer hasn't rolled over StringLength = CurrentIndex-StartIndex; else %If it has we have to use the size of the buffer to determine how many samples to grab StringLength = BufSize - StartIndex + CurrentIndex; end;

data = TDT.ReadTargetVEX('Sindhu3c.dWave~1',StartIndex,StringLength,'F32','F32');

StartIndex = CurrentIndex; %Next loop start where we left off

%plot(data);

Iteration = Iteration + 1;

%Calculation of SNR rawdata=data; %Std=std(rawdata) %takes the standard deviation Std=10e-6; avg=mean(rawdata); posthreshold=avg+(3*Std); negthreshold=avg-(3*Std); signaldata=(rawdata>posthreshold | rawdata<negthreshold); sigdata_ts=find(signaldata==1); sigdata=rawdata(signaldata); g=size(sigdata); h=max(g);

noisedata=(rawdata<posthreshold & rawdata>negthreshold); %cutoff for what is noise noidata=rawdata(noisedata); e=size(noidata); f=max(e); spower=sum(sigdata.^2)/h; %takes the signal data squares it and sums it %up then divides by the number of signal data points npower=sum(noidata.^2)/f; %same as above except it does it for noise ratio=spower/npower; SNRiter(i)=10*LOG10(ratio); %this calculates the signal to noise ratio

%Find peak-peak amplitude sigbegin=sigdata_ts-25; sigbegin(sigbegin<26)=26; sigend=sigdata_ts+25; for j=1:size(sigdata_ts) p_p(j)=max(rawdata(sigbegin:sigend))-min(rawdata(sigbegin:sigend)); end ppmaxiter(i)=max(p_p);

```
end
```

```
%Max SNR over 54 second duration
SNR=max(SNRiter(i));
clk=clock;
```

%Average peak-peak amplitude ppmax=mean(ppmaxiter);

```
Linear Search
function
```

```
[StartIndex,trackSNR,Iteration,elecpos,failed,up,low]=linearsearch(TDT,StartIndex,BufSize,Iterati
on,elecpos,up,low,np,eta,thrs)
failed=0;
cnt=0;
x=zeros(np,1);
f=zeros(np,1);
```

for c1=1:np/2 mp=(up+low)/2; b=mp-eta;

%Setting direction of movement

if elecpos<b

movedist=b-elecpos;

d=1; %move forward else

movedist=elecpos-b; d=0; %move backward

d=0; %

end clk=clock;

disp(clk(4:6));

```
disp('Upper and lower limits');
disp([up low]);
```

disp('Midpoint');

```
disp(mp);
```

```
%Moving electrode in 30 micrometer steps at 3 min intervals
while (abs(elecpos-b)>30)
k = 1;n=6; %Micromotor set at 10 um/s speed
moveelectrode(d,k,n,TDT);
pause(60);
if d==1
    d=0;
else
    d=1;
end
k = 1;n=3; %Micromotor set at 10 um/s speed
moveelectrode(d,k,n,TDT);
if d==1
    d=0;
```

d=0; <mark>else</mark>

```
d=1;
```

end

```
%Updating electrode position
if d==1
elecpos=elecpos+30;
else
elecpos=elecpos-30;
end
```

pause(60);%Waiting only 1 min instead of 3 as SNR recording takes about 1 min.

```
%Calculate SNR after every movement
[SNRb,Iteration,StartIndex,ppmax]=snr(TDT,StartIndex,BufSize,Iteration,elecpos);
clk=clock;
disp(clk(4:6));
disp('Calculated SNR after movement'); disp(SNRb);
trackSNR=SNRb;
if SNRb>=thrs
disp('SNR>thrs achieved');
return
end
```

end

```
<u>Optimization</u>
function
[Iteration,elecpos,newSNR]=optimization(TDT,StartIndex,BufSize,Iteration,elecpos,oldSNR)
```

```
maxSNR=0;
[oldSNR,Iteration,StartIndex,oldppmax]=snr(TDT,StartIndex,BufSize,Iteration,elecpos);
```

```
%Move electrode 30um step forward
d=1;
k = 1;n=6; %Micromotor set at 10 um/s speed
moveelectrode(d,k,n,TDT);
pause(60);
d=0;
k = 1;n=3; %Micromotor set at 10 um/s speed
moveelectrode(d,k,n,TDT);
clk=clock;
disp(clk(4:6));
```

```
%Update electrode position
elecpos=elecpos+30;
disp('Electrode moved fwd by 30um to position-');disp(elecpos);
fid = fopen('movement_log.txt','at');
fprintf(fid,'%d\t %d\n',clk(4:6),elecpos);
fclose(fid);
```

pause(60);

[newSNR,Iteration,StartIndex,newppmax]=snr(TDT,StartIndex,BufSize,Iteration,elecpos);

```
%Direction of movement of electrode
 %Optimization in forward direction
 if (newSNR>1.1*oldSNR && newppmax>1.2*oldppmax)
    oldSNR=newSNR:
    d=1; %Continue moving forward
    disp('Optimize moving forward');
    disp ('New and old SNR');
    disp ([newSNR oldSNR]);
 else
    pause(60); %Wait for a minute
    %Move electrode 30um step forward
    d=1;
    k = 1;n=6; %Micromotor set at 10 um/s speed
    moveelectrode(d,k,n,TDT);
    pause(60);
    d=0;
    k = 1;n=3; %Micromotor set at 10 um/s speed
    moveelectrode(d,k,n,TDT);
    clk=clock;
    disp(clk(4:6));
    %Update electrode position
    elecpos=elecpos+30;
    disp('Electrode moved fwd by 30um to position-');disp(elecpos);
    fid = fopen('movement log.txt','at');
    fprintf(fid,'%d\t %d\n',clk(4:6),elecpos);
    fclose(fid);
    pause(60);
    [newSNR,Iteration,StartIndex,newppmax]=snr(TDT,StartIndex,BufSize,Iteration,elecpos);
 end
 if (newSNR>1.1*oldSNR && newppmax>1.2*oldppmax)
    oldSNR=newSNR;
    d=1: %Continue moving forward
    disp('Optimize moving forward');
```

disp ([newSNR oldSNR]);

disp ('New and old SNR');

else

d=0; %Move backward by two steps disp('Optimization in forward direction failed - move back to original position');

[newSNR,Iteration,StartIndex,newppmax]=snr(TDT,StartIndex,BufSize,Iteration,elecpos); clk=clock; disp(clk(4:6));

```
disp ('New and old SNR'); %SNR at same position as where oldSNR was calculated
   disp ([newSNR oldSNR]);
   d=0; disp('Optimize moving backward');
 end
while(maxSNR==0)
k = 1;n=2; %Micromotor set at 10 um/s speed
moveelectrode(d,k,n,TDT);
pause(60);
if d==1
   d=0;
else
  d=1:
end
k = 1;n=1; %Micromotor set at 10 um/s speed
moveelectrode(d,k,n,TDT);
if d==1
  d=0;
else
   d=1;
end
%Update electrode position
if d==1
  elecpos=elecpos+10;
else
   elecpos=elecpos-10;
end
clk=clock;
disp(clk(4:6));
disp('Electrode moved by 10um to position-');
disp(elecpos);
fid = fopen('movement log.txt','at');
fprintf(fid,'%d\t %d\n',clk(4:6),elecpos);
fclose(fid);
pause(60);
[newSNR,Iteration,StartIndex,ppmax]=snr(TDT,StartIndex,BufSize,Iteration,elecpos);
if newSNR>oldSNR
   oldSNR=newSNR;
   clk=clock;
   disp(clk(4:6));
   disp('Moving along the optimization curve');
```

disp ('New and old SNR'); disp ([newSNR oldSNR]);

else

```
maxSNR=1;
clk=clock;
disp(clk(4:6));
```

```
disp('Probably at optimal SNR - move to previous elecpos');
disp ('New and old SNR');
disp ([newSNR oldSNR]);
end
```

....

end

```
%Recalculate SNR
```

[newSNR,Iteration,StartIndex,ppmax]=snr(TDT,StartIndex,BufSize,Iteration,elecpos); clk=clock; disp(clk(4:6)); disp('Recalculating SNR at optimal location'); disp(newSNR); disp('Electrode position'); disp(elecpos);

<u>SNR</u>

function [SNR,Iteration,StartIndex,ppmax]=snr(TDT,StartIndex,BufSize,Iteration,elecpos) %%%%%Monitor phase %Monitor SNR before beginning control%%%%%

for i=1:6 pause(9); %Grab and plot data every half a second. CurrentIndex = TDT.GetTargetVal('Sindhu3c.sWave');

if CurrentIndex > StartIndex %Check to make sure that the buffer hasn't rolled over StringLength = CurrentIndex-StartIndex; else %If it has we have to use the size of the buffer to determine how many samples to grab StringLength = BufSize - StartIndex + CurrentIndex; end;

data = TDT.ReadTargetVEX('Sindhu3c.dWave~1',StartIndex,StringLength,'F32','F32');

StartIndex = CurrentIndex; %Next loop start where we left off

%plot(data);

Iteration = Iteration + 1;

```
%Calculation of SNR
rawdata=data;
%Std=std(rawdata) %takes the standard deviation
Std=10e-6;
avg=mean(rawdata);
posthreshold=avg+(3*Std);
negthreshold=avg-(3*Std);
signaldata=(rawdata>posthreshold | rawdata<negthreshold);
sigdata_ts=find(signaldata==1);
sigdata=rawdata(signaldata);
g=size(sigdata);
h=max(g);
```

noisedata=(rawdata<posthreshold & rawdata>negthreshold); %cutoff for what is noise

noidata=rawdata(noisedata); e=size(noidata); f=max(e); spower=sum(sigdata.^2)/h; %takes the signal data squares it and sums it %up then divides by the number of signal data points npower=sum(noidata.^2)/f; %same as above except it does it for noise ratio=spower/npower; SNRiter(i)=10*LOG10(ratio); %this calculates the signal to noise ratio

%Find peak-peak amplitude

sigbegin=sigdata_ts-25; sigend=sigdata_ts+25; sigbegin(sigbegin<26)=26; for j=1:size(sigdata_ts) p_p=max(rawdata(sigbegin:sigend))-min(rawdata(sigbegin:sigend)); end ppmaxiter(i)=max(p_p);

end

%Max SNR over 54 second duration SNR=max(SNRiter(i)); clk=clock;

%Average peak-peak amplitude ppmax=mean(ppmaxiter);

APPENDIX C

PRIOR PUBLISHED WORK

Chapter 2 in this dissertation corresponds to previous published work in the Journal of MEMS in which I am the first listed author. I hereby confirm that all listed co-authors in the publication have granted permission to the incorporation of contents of the published journal article as a chapter in the dissertation. My contributions in any other prior published work in which I am a co-author has also been reproduced in parts of the dissertation with full permission from the co-authors.

BIOGRAPHICAL SKETCH

Sindhu Anand graduated with Bachelor's degrees in Electrical Engineering and Biological Sciences from Birla Institute of Technology & Sciences, Pilani in India. Since entering the PhD program in Biomedical Engineering at Arizona State University she has worked in the Neural Microsystems Laboratory under the guidance of Dr. Jit Muthuswamy. Her research focus has been on engineering microscale robotic neural interface technology. Her expertise is in MEMS microsensors and microactuators, clean-room fabrication, packaging and reliability analysis, mechanical models for microscale navigation in brain tissue, in vivo electrophysiology and computational analysis of neural signals.