MICROSTIMULATION VIA CHRONIC INTRACORTICAL MICROELECTRODE ARRAYS: STUDIES OF FUNCTIONALITY

by

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ABSTRACT

The development of devices to electrically interact with the brain is a challenging task that could potentially restore motion to paralyzed patients and sight to those with profound blindness. Neural engineers have designed many types of microelectrode arrays (MEAs) with this challenge in mind. These MEAs can be implanted into brain tissue to both record neural signals and electrically stimulate neurons with high selectivity and spatial resolution.

Implanted MEAs have allowed patients to control of a variety of prosthetic devices in clinical trials, but the longevity of such motor prostheses is limited to a few years. Performance decreases over time as MEAs lose the ability to record neuronal signals, preventing their widespread clinical use. Microstimulation via intracortical MEAs has also not achieved broad clinical implementation. While microstimulation for the restoration of vision is promising, human clinical trials are needed. Chronic *in vivo* functionality assays in model systems will provide key insight to facilitate such trials.

There are three goals that may help address insufficient MEA longevity, as well as provide insight on microstimulation functionality. First, thorough characterizations of how performance decreases over time, both with and without stimulation, will be needed. Next, factors that affect the chronic performance of microstimulating MEAs must be further investigated. Finally, intervention strategies can be designed to mitigate these factors and improve long term MEA performance.

This dissertation takes steps towards meeting these goals by means of three studies. First, the chronic performance of intracortically implanted recording and stimulating MEAs is examined. It is found that while performance of implanted MEAs in feline cortex is dynamic, catastrophic device failure does not occur with microstimulation. Next, a variety of factors that affect microstimulation studies are investigated. It is found that many factors, including device

damage, anesthesia depth, the application of microstimulation, and the use of impedance as a reporter play a role in observations of performance variability. Finally, a promising intervention strategy, a carbon nanotube coating, is chronically tested *in vivo,* indicating that carbon nanotubes do not cause catastrophic device failure and may impart benefits to future generations of MEAs.

This work is dedicated to Mellow, Marsh, Ford, Robert, Max, Cole, and Spot.

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CHAPTER 1

INTRODUCTION

Summary of the introduction

Engineering an electrical interface with the brain is a complex but promising challenge. Communication with the cerebral cortex via an electrical interface could potentially treat a variety of conditions such as paralysis and profound blindness. In order to enable this communication, neural engineers have developed a variety of microelectrode arrays (MEAs), devices with many conductive contacts that can be directly inserted into brain tissue. These MEAs, including the Utah Electrode Array (UEA), allow for both recording of neuronal signals and the application of electricity to tissue with high selectivity and spatial resolution.

Human patients have been able to control a variety of effector devices, including prosthetic limbs, by means of chronic intracortical MEAs. These MEAs record neural signals, which are decoded by a computer. The computer then instructs an effector device, such as a prosthetic arm, to perform a desired action. While there have been continual improvements in the decode algorithms that allow for effector control, and while there have been many advances in effector design, the longevity of these motor prosthetic systems is limited due to a decline in neuronal recording performance of MEAs over time. This performance decline, often attributed to tissue response, has delayed the widespread clinical implementation of MEAs for motor prosthetic applications.

The MEA-based application of electricity directly to brain tissue (microstimulation), has also yet to be widely clinically implemented. It has been hypothesized that microstimulation via MEAs may be used to restore vision to blind patients, yet only a few human trials of microstimulation have been performed. The therapeutic utility of chronic neural stimulation has

been demonstrated in applications such as deep brain stimulation (DBS) and cochlear implants, but intracortical microstimulation has yet to show long-term functional success. In order to conduct human studies that will provide the best information on the functionality of microstimulation, nonhuman test evidence will be critical. *In vitro* and animal validation studies of microstimulation have reported that damage to both device and tissue occurs with chronic stimulation, and found that stimulation to effect can be difficult to perform for extended periods of time. Additional data on the chronic functionality of intracortical microstimulation via MEAs will provide key insight that may facilitate the performance of rigorous human microstimulation trials.

Neural engineers must overcome several challenges to transition intracortical MEAs, particularly microstimulating MEAs, into the clinical realm. This chapter will discuss not only the promise of MEAs for clinical use, but also these challenges. The need for a better understanding of chronic performance, investigations of the factors impacting performance, and intervention strategies to improve performance will be established. Finally, the work undertaken in Chapters 2-4 of this dissertation will be outlined.

MEAs for chronic recording

The human brain is a complex organ. With an estimated 100 billion neurons making an estimated 100 trillion connections (R. W. Williams & Herrup, 1988), it is not surprising that the computational power of the human cortex allows us to perform the broad array of functions associated with human life: sensing, moving, thinking, and even thinking about thinking (Kandel, Schwartz, & Jessell, 2000). Unfortunately, some humans do not get to experience the full range of these functions. For example, as of 2010, over 200,000 Americans suffered from paralysis as a result of spinal cord injury (NSCISC, 2011); some 20,000-30,000 suffered from paralysis as a result of amyotrophic lateral sclerosis (NINDS, 2010). Many of these patients have their mental faculties entirely intact, but cannot perform any voluntary motion. In many cases of amyotrophic lateral sclerosis, patients are unable even to speak. For such patients, connecting with the outside world by restoring their ability to communicate and move is a major priority (Anderson, 2009; Birbaumer & Cohen, 2007).

Restoring interaction with the outside world is one of the major objectives of neural prosthetics. Neurons within the brain communicate not only chemically, but via electrical potentials. For example, with sufficient depolarization of a postsynaptic neuron, a point-process action potential (AP) will be fired (Kandel et al., 2000). In addition to action potentials, summed local electrical activity of the cortex forms continuous, low-frequency local field potentials (LFPs) (Katzner et al., 2009). Using engineered electrodes, it is possible to record these APs and LFPs to "listen" to the brain. If these neural signals can be correctly interpreted, they can potentially be used to control an effector device, allowing a paralyzed patient to interact with their environment.

The use of electrical interfaces to interact with the outside world in this fashion is the premise of brain computer interfaces and associated motor prostheses. Many strategies are used to perform electrical recordings from the nervous system, including interfaces that do not require cortical access (Rutten, 2002), but for some patients, including those with amyotrophic lateral sclerosis, the brain may provide the only source of control signals. Electrodes can record electrical signals from the brain in a variety of ways, ranging from noninvasive EEG from the scalp to penetrating electrodes placed within actual brain tissue (Birbaumer & Cohen, 2007). A schematic for an intracortical electrode based motor prosthesis is shown in Figure 1.1a. The control signals recorded by intracortical electrodes can be decoded by a computer, with the decode depending on the application (Andersen, Musallam, & Pesaran, 2004). Decodes are often based on the interpretation of APs (S. Kim et al., 2008; S. Musallam, 2004; Santhanam, Ryu, Yu, Afshar, & Shenoy, 2006; Taylor, 2002; Velliste, Perel, Spalding, Whitford, & Schwartz, 2008; Wessberg, 2000), LFPs (Jun, Truccolo, Vargas-Irwin, & Donoghue, 2010; Markowitz, Wong, Gray, & Pesaran, 2011; Mehring et al., 2003; Pesaran, Pezaris, Sahani, Mitra, & Andersen, 2002), or a combination of all electrical activity (Bansal, Truccolo, Vargas-Irwin, & Donoghue, 2011; Fraser, Chase, Whitford, & Schwartz, 2009) to determine patient intention. The decoded control signals required to execute this intention are then transmitted to an effector device, such as a computer cursor or prosthetic limb (Hochberg et al., 2006). This effector device then executes the desired action, enabling the patient to engage in activities of daily living.

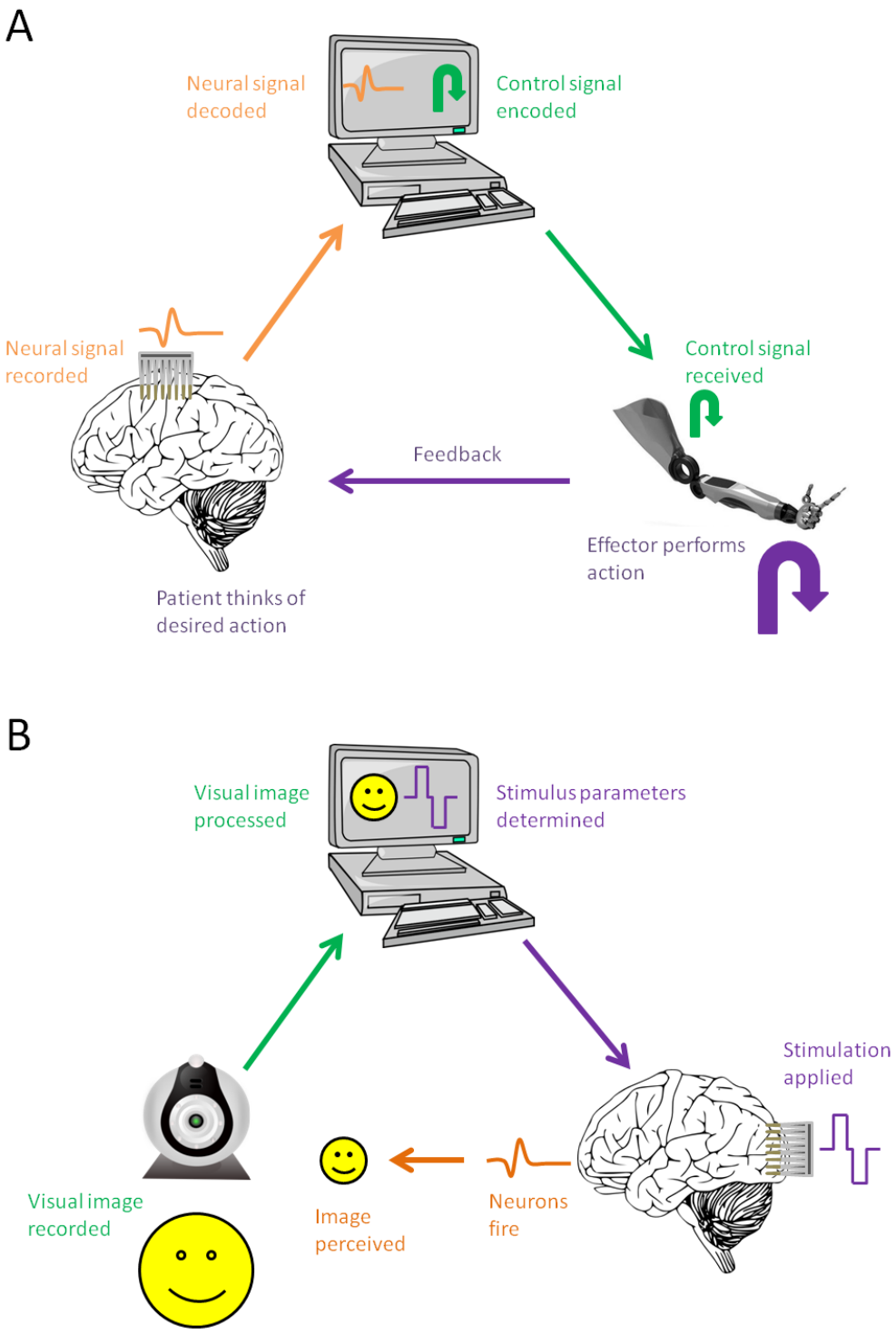

Figure 1.1. Schematic of motor and visual prostheses based on intracortically implanted MEAs. A. A possible motor prosthesis based on an MEA implanted in motor cortex. B. A possible visual prosthesis based on an MEA implanted in visual cortex.

There are many types of penetrating electrodes designed to obtain neuronal signal data from the brain. Multielectrode arrays (MEAs) currently being used for this purpose include the Moxon ceramic array (Moxon, Leiser, Gerhardt, Barbee, & Chapin, 2004), the Michigan/NeuroNexus probe (Hoogerwerf & Wise, 1994; Wise, Angell, & Starr, 1970), floating arrays by MicroProbes (Sam Musallam, Bak, Troyk, & Andersen, 2007), and the Utah Electrode Array (Campbell, Jones, Huber, Horch, & Normann, 1991; Normann, Maynard, Rousche, & Warren, 1999), among others. Penetrating MEAs have the important advantage of spatial resolution compared to nonpenetrating or macroelectrodes. The listening radius of a neuron is estimated to be between 100-200 microns (Henze & Buzsaki, 2000), though best signal quality is thought to be recorded at <50 microns (Moffitt & McIntyre, 2005). Penetrating MEAs, therefore, bring electrodes within recording proximity of many neurons in a cortical area of interest. Another important feature of MEAs is that the relative position of electrodes is known, making MEAs useful for mapping small areas of cortex (S.-J. Kim, Manyam, Warren, & Normann, 2006; Warren, Fernandez, & Normann, 2001).

The Utah Electrode Array (UEA) possesses both of these advantages (Maynard, Nordhausen, & Normann, 1997). The UEA is a monolithic silicon-based structure of 100 acid-etched silicon shanks, spaced 400 microns apart, laid out in a 10x10 grid. Four shanks are inactive. The conductive tips of 96 active electrode shanks range from 500 to 4000 μm^2 and are coated with a conductive metal, such as platinum (Pt) or iridium oxide. The array, aside from the tips, is insulated with Parylene-C for its electrical properties (Loeb, Bak, Salcman, & Schmidt, 1977) and biocompatibility (Winslow, Christensen, Yang, Solzbacher, & Tresco, 2010).

Wires for each of the shanks are bonded to the back of the array, and feed out to a percutaneous connector which is attached to the skull. Two fine reference wires are attached to the base of the percutaneous connector, and can be subdurally placed. The base of the percutaneous connector itself serves as an electrical ground. The specific geometry of the array is adjusted depending on the intended application. For example, in the peripheral nerve, the electrodes may range in depth in order to expose a cross-section to the conductive tips

(McDonnall, Clark, & Normann, 2004). In cortex, the length of the electrodes may be adjusted in order to take access specific cortical layers.

An advantage of rigid MEAs is the ability to access specific layers of cortex, which contain cells with certain functions and depth-dependent properties depending on cortical area (Figure 1.2). For example, in primary motor cortex (M1), layer V contains large pyramidal cells which fire APs encoding motor output (Kandel et al., 2000). The depth at which this layer occurs is dependent on species (Ghosh, 1997). The planar geometry of the UEA is well-suited to recording from many cells within a given layer, and the length of electrodes can be adjusted depending on the model system and desired target. For human motor prosthetic applications, M1 is generally the target for motor control signal recording MEAs. Cortical layers V and VI have been found to provide the best control signals for motor prostheses (Parikh, Marzullo, & Kipke, 2009).

Tests of intracortical, M1-implanted UEA-based motor prostheses have been performed in human patients. Particularly notable are the Brain Gate Clinical trials (Hochberg et al., 2006; S. P. Kim, Simeral, Hochberg, Donoghue, & Black, 2008; Malik, Truccolo, Brown, & Hochberg, 2011; Ojakangas et al., 2006; Truccolo, Friehs, Donoghue, & Hochberg, 2008). Signals obtained by implanted UEAs in these trials have been used to control communication effectors, such as computer cursors (S. P. Kim et al., 2008; Sung-Phil et al., 2011), as well as physical effector devices such as prosthetic limbs (Hochberg et al., 2006). While results from the Brain Gate studies have been promising, the use of UEAs to control prosthetic devices has not been widely clinically implemented.

One issue that has prevented more widespread clinical use of the UEA is the longevity of the control signals on which decoding algorithms rely. In order to be clinically useful, a motor prosthesis system of signal recording, decode, and execution by the effector must be stable and reliable, i.e., the effector must perform the desired action and only the desired action for the time span required by the patient. Some patients may require decades of performance. By implication, the control signals for the prosthetic device must also be stable and reliable. Reported performance of motor prosthetic control signals in human patients only lasts out to, at the most, a

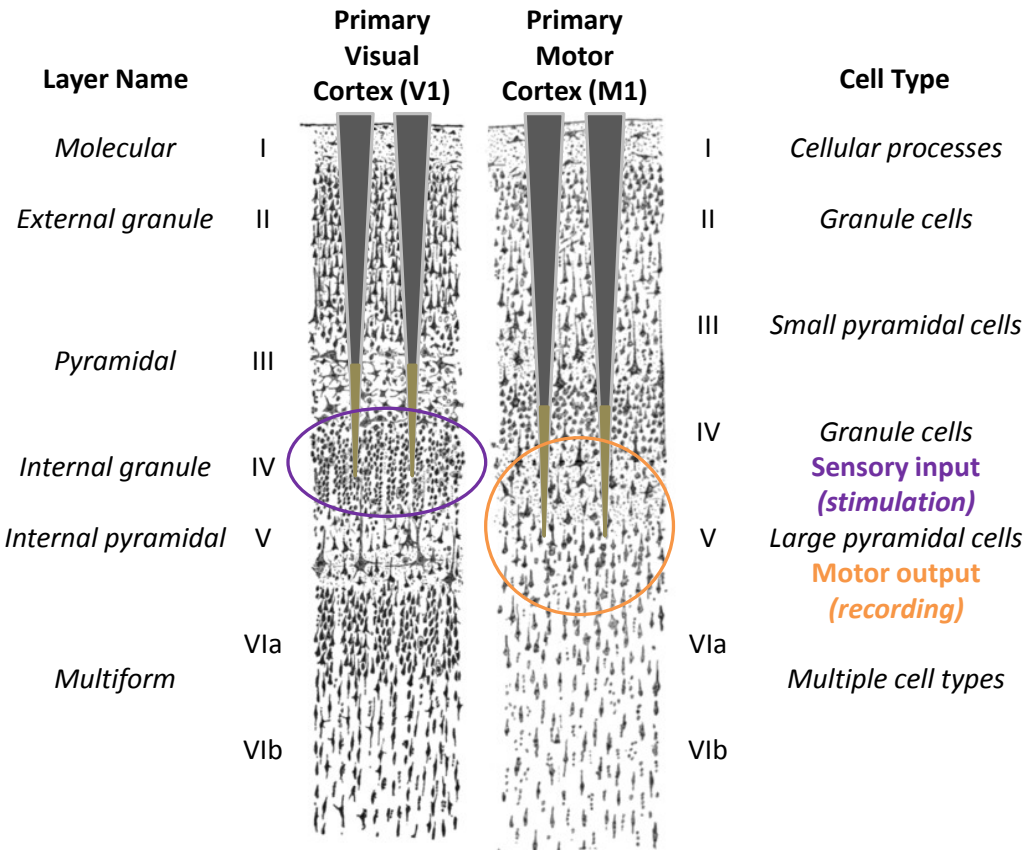

Figure 1.2: Layers of human cortex. Circled in **purple** is layer IV of visual cortex, a sample of a sensory input layer that may serve as the best target for stimulating MEAs to restore sensation. Circled in **orange** is layer V of motor cortex, a sample cortical output layer that may serve as the best target for a recording motor prosthetic. Adapted from drawings by Santiago Ramon y Cajal (1899).

few years (Simeral, Kim, Black, Donoghue, & Hochberg, 2011). Many studies have investigated the long-term ability of MEAs to record APs in model systems such as nonhuman primates (NHPs), felines, and rodents; these studies have found that the yield of APs is dynamic over the course of several months to a few years (Chestek et al., 2011; Dickey, Suminski, Amit, & Hatsopoulos, 2009; Krüger, 2010; M. A. L. Nicolelis, 2003; M.A.L. Nicolelis, Ghazantar, Faggin, Votaw, & Oliveira, 1997; Palmer, 1990; P.J. Rousche & Normann, 1998; Selim Suner, 2005; Super & Roelfsema, 2005; Szymusiak & Nitz, 2003; Vetter, 2004). In fact, it has been found that AP recordings are dynamic over time scales as short as days (Linderman et al., 2006). These results have established the generally accepted pattern of dynamic AP yield over time diagrammed in Figure 1.3: for the first few days to weeks following implantation, the number of APs recorded gradually increases or "fades in," reaching a plateau "high yield" level that can be maintained for a few months. AP yield then proceeds to "fade out" over months to years of recording.

Many factors have been implicated in these recording dynamics. For example, corrosion of microelectrodes, including insulation layers, may occur over time (Schmitt et al., 2000). Generally, however, recording dynamics are attributed to tissue response. Tissue response refers to a variety of processes occurring within the cortex, including the initial trauma of insertion, the formation of a glial sheath or scar, inflammation, and neuronal death (Polikov, Tresco, & Reichert, 2005). The initial trauma of device insertion is known to cause necrotic neuronal death and the disruption of local microvasculature (Bjornsson et al., 2006; Edell, 1992). Following this initial trauma, astrocytes in the area of the wound are activated and change conformation (Fitch & Silver, 2008; Silver & Miller, 2004), forming the barrier of astrocytes and other components (such as fibroblasts and extracellular matrix molecules) known as the glial scar (Szarowski, 2003; Turner et al., 1999). This sheath acts as a barrier around the electrode against the diffusion of molecules (Roitbak & Syková, 1999), and forms an electrically resistive layer (Grill & Mortimer, 1994). The barrier is fully formed at 4-6 weeks (Turner et al., 1999), and persists as long as the implant is in place (Griffith & Humphrey, 2006).

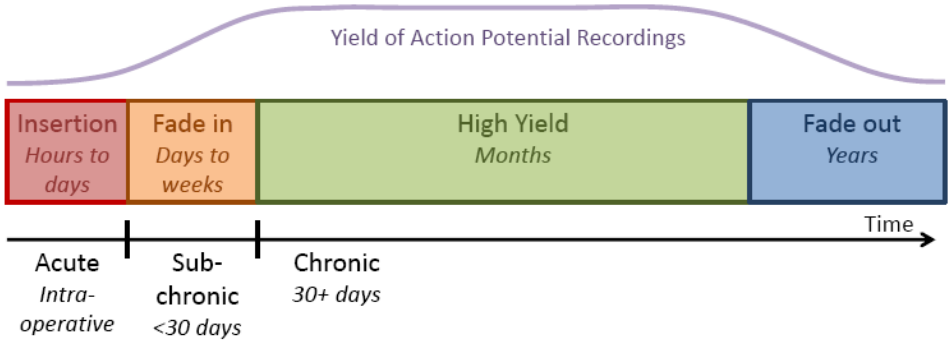

Figure 1.3. Time course of intracortical data recording. The generally accepted time course for recording follows a pattern of fade in, high yield, and fade out of action potential recordings (represented in purple) over time. For clinical trials, this recording pattern may impact results of studies that are made on acute, subchronic, and chronic timescales. For example, subchronic recording studies may observe more variable patterns of recording than studies conducted in the high-yield or acute period.

Inflammation occurs with both initial trauma and the continued presence of the MEA within the cortex. Inflammation is a complex network of signaling cascades and cellular reactivity (O'Callaghan, Sriram, & Miller, 2008); components relevant to MEA implants include the release of proinflammatory cytokines and the activation of both microglia native to the brain (Town, Nikolic, & Tan, 2005) and macrophages infiltrating cortex via a disrupted blood-brain barrier (Chen, 2008; Liu et al., 2009; Winslow, 2010). Areas that are inflamed and/or scarred are considered reactive tissue. The configuration of the MEA affects the radius of tissue reactivity (Seymour & Kipke, 2007), as does the size of the implant (Stice, Gilletti, Panitch, & Muthuswamy, 2007; Sugihara et al., 2011). Motion of a rigid device relative to cortical tissue also exacerbates reactivity; this exacerbation is particularly noted with MEAs tethered to the skull via wire bundles (Biran, Martin, & Tresco, 2007; Y. Kim, 2004).

This tissue response can adversely affect the AP recording ability of MEAs in multiple ways. Aside from necrotic neuronal death as a result of trauma, chronic inflammation corresponds to the apoptosis of neurons (Biran, Martin, & Tresco, 2005; George C. McConnell et al., 2009). The glial scar can physically push neurons outside the recording radius of electrodes, as well as maintain a resistive barrier preventing electrodes from recording actively firing neurons (Frampton, Hynd, Shuler, & Shain, 2010; Merrill & Tresco, 2004). Neuronal demyelination via loss of oligodendrocytes may also play a role in loss of signal (Winslow, 2010). Finally, recent evidence suggests that inflammation-mediated activity of both microglia and astrocytes can affect the firing of neurons (Pascual, Ben Achour, Rostaing, Triller, & Bessis, 2011).

In order to facilitate the widespread clinical implementation of chronically implanted MEAs for neural prosthesis device control, it will be important to better understand how and why recording performance changes over time. With an increased understanding of the factors affecting performance, such as device damage and tissue response, engineers will be better able to develop intervention strategies to improve recording MEA longevity. With the application of microstimulation, however, the challenge of improving chronic MEA performance becomes considerably more complicated.

Intracortical microstimulation

In addition to their use in chronic intracortical recording applications, MEAs can be used to stimulate the brain. By applying high selectivity microstimulation to the specific area of the brain that controls a certain sense, intracortical MEAs could serve as the foundation for sensory prostheses. An example of such a prosthesis is a visual implant to treat profound blindness (Normann et al., 1999; Schmidt et al., 1996), a problem affecting millions of Americans (Frick & Foster, 2003). A basic schematic for an intracortical MEA-based visual prosthesis is shown in Figure 1.1b. Such a prosthesis would entail an external sensor, such as a camera, which would then transmit visual information to a processor. This processor would encode sensory data as a stimulation paradigm. The stimulation would then be applied directly to the primary visual cortex (V1) via a chronically implanted MEA. While decades of research towards the clinical implementation of V1 stimulation to produce sensations for such a prosthesis has been performed (Brindley & Lewin, 1968; Dobelle, 1976; Dobelle & Mladejovsky, 1974), intracortical microstimulation for visual restoration has yet to undergo rigorous clinical trials.

Although microstimulation of the human brain to restore sensation has not been widely clinically implemented, macrostimulation of the human central nervous system has been used in a variety of applications, including diagnostics (Ronner, 1990), the treatment of psychiatric disorders such as depression and obsessive compulsive disorder (Goodman & Alterman, 2012), and a variety of motor conditions. The chronically implanted deep brain stimulator for the treatment of motor disorders, e.g., dystonia, essential tremor, and Parkinson's disease, has been particularly successful, having been clinically used for approximately two decades (Eller, 2011). DBS involves the chronic implantation of large electrodes in the basal ganglia. These electrodes apply stimulation which may serve to modulate neuronal activity, thereby restoring proper function to the basal ganglia (Mink, 1996, 2003). While changes in the environment of the DBS electrode, such as tissue reactivity and edema, do occur with DBS electrodes (Lempka, 2010; Moss, 2004), stimulation itself does not appear to cause tissue damage at levels that interfere with performance (Haberler & Budka, 2000). Furthermore, stimulation can be adjusted to overcome

changes in the tissue surrounding the electrode, thereby maintaining long-term performance (Deuschl et al., 2006). However, the deep brain stimulator is not a microstimulating implant.

Microstimulation of the nervous system has also demonstrated long-term functionality, particularly in the case of the cochlear implant (Tong et al., 1979). The cochlear implant involves a microelectrode array surgically inserted into the cochlea, which applies specific patterns of microstimulation to the auditory nerve, thereby treating deafness (Feigenbaum, 1987). Functionality studies show that the cochlear implant can be used without performance declines for over 20 years (Lenarz, Sonmez, Joseph, Buchner, & Lenarz, 2012). Tissue response to the cochlear implant does occur, including encapsulation and inflammation (Migirov, Kronenberg, & Volkov, 2011), but does not appear to interfere with device performance. However, the cochlear implant is not intracortical, and is not subject to both the immune system of the brain and the encroachment of neuroinflammatory cells and molecules from a broken blood-brain barrier.

Intracortically implanted MEAs combine the intracortical presence of DBS electrodes with the micro scale stimulation of cochlear implants. Only a few human trials of microstimulation to evoke percepts have been performed (Bak et al., 1990; Brindley & Lewin, 1968; Dobelle, 1976; Dobelle, Stensaas, Mladejovsky, & Smith, 1973; Girvin et al., 1979), and these have either been limited to intraoperative timescales, or involved only a single patient. In Schmidt's 1996 study (Schmidt et al., 1996), which spanned just four months, researchers were able to obtain a wealth of information about stimulus parameters and the characteristics of visual percepts (phosphenes) that were evoked via 38 microelectrodes. Stimulation parameters investigated included stimulus amplitude, frequency, and pulse duration. Characteristics of reported phosphenes included size, shape, color, and flicker. Importantly, phosphene characteristics were found to change over the course of the study. For many patients suffering from profound blindness, the need for a prosthesis will be lifelong. To clinically implement microstimulation of V1 for a visual prosthesis, long term data about stimulus parameters and percepts evoked will be critical. Much of this data about size and shape of percepts can be most efficiently obtained by means of verbal reporters of perception, which can only be acquired from humans. Demonstrating the safety and efficacy of

intracortical microstimulation over extended periods of time will be an important factor in enabling such human studies.

There have been many nonhuman studies examining the effects of microstimulation, both on the MEA itself and on tissue, to determine safe microstimulation parameters. *In vitro* studies have found that stimulation causes degradation of the metal coating of the conductive tips of MEA electrodes (S. Cogan, 2004). The amount of damage to the electrode depends on the parameters of stimulation, such as overall charge density applied, as well as charge per phase (D. McCreery, Agnew, Yuen, & Bullara, 1990). Microstimulating MEAs, including the UEA, often employ a sputtered iridium oxide film (SIROF) to inject charge, as this has been shown to have a high charge injection capacity (S. F. Cogan, 2008; S. F. Cogan, Plante, & Ehrlich, 2004). Despite this high charge injection capacity, however, iridium oxide films can be damaged at charge densities as low as 3 mC^cm^2 (Negi, Bhandari, Rieth, Van Wagenen, & Solzbacher, 2010).

In addition to electrode damage, microstimulation has been shown to damage tissue over time, beyond the damage caused by MEA implantation. Damage is thought to be dependent on the stimulus applied (Merrill, Bikson, & Jefferys, 2005). Excess stimulation may result in the production of free radicals, which can damage tissue (Halliwell, 1992). Animal histology studies have shown that the long term application of microstimulation at physiologically relevant levels leads to tissue damage, namely an increase in reactive tissue radius and increased neuronal loss when compared to nonstimulated controls (Douglas McCreery, Pikov, & Troyk, 2010). Furthermore, microstimulation causes changes in neuronal excitability (Goddard, McIntyre, & Leech, 1969; D. McCreery, W. Agnew, & L. Bullara, 2002; D. B. McCreery, W. F. Agnew, & L. A. Bullara, 2002). The limit of “safe” stimulation in some studies has been determined to be the water window, i.e., the potential range which does not result in the hydrolysis of water, -0.6 to 0.8 V (Troyk et al., 2004). Stimulating outside these limits may lead to changes in pH and the evolution of gas bubbles within the cortex. Other studies have investigated the amount of charge that can be applied without tissue or device damage, but no clear consensus as to safe limits exists (Negi et al., 2010). Questions remain as to whether stimulating outside of the water window impacts device functionality *in vivo.*

The ability to microstimulate to effect, i.e., the stimulation functionality, of MEAs is known to change over time. There have been many trials of intracortical microstimulation to evoke sensory percepts in rodent, feline, and nonhuman primate model systems, including auditory, somatosensory, and visual cortex (Sergejus Butovas & Schwarz, 2007; DeYoe, 2005; Dona K. Murphey & Maunsell, 2007; D. K. Murphey & Maunsell, 2008; Kevin J. Otto, Rousche, & Kipke, 2005a, 2005b; Romo, Hernandez, Zainos, Brody, & Lemus, 2000; P. J. Rousche & Normann, 1999). In contrast to DBS and cochlear implant electrodes, however, few have effectively stimulated at time points greater than 1 year (Bradley, 2004; Davis et al., 2012; P. J. Rousche & Normann, 1999); in these cases, the parameters for stimulation had to be changed in order to compensate for decreased stimulation efficacy. Importantly, the majority of microstimulation studies to perceptual effect have used behavioral reporters to indicate perception (E. J. Tehovnik, 1996). Behavioral reporters are subject to the attentional state of the animal (E. J. Tehovnik & Slocum, 2004), and such experiments require a time-consuming training period.

Some researchers have attempted to circumvent these difficulties by using physiological reporters to indicate microstimulation efficacy. While these results are not affected by the animal's attentional state, they generally require that the animal be anesthetized. Anesthesia is known to affect cortical activity and excitability (Alkire, Hudetz, & Tononi, 2008; Cimenser et al., 2011; Hanrahan et al., 2012), and may affect microstimulation. Other factors impacting ability to micro-stimulate to physiological effect may include the cortical layer of the implant (DeYoe, 2005; Edward J. Tehovnik & Slocum, 2009) and the distance from electrode to neuronal cell body (Torab et al., 2011). It is difficult to determine the spatial and temporal limits of electrical activation of nervous tissue, and reports of activation radius are varied between *in vivo, in vitro,* and modeling studies (S. Butovas, 2003; Histed, Bonin, & Reid, 2009; McIntyre & Grill, 2001; Tolias et al., 2005). Furthermore, the extent of neuronal activation is dependent on the animal and electrode type used, leading to additional variability.

The results of MEA-based microstimulation studies have varied widely in terms of reported safe and effective parameters. Furthermore, there are few long-term studies indicating that device functionality is preserved over time scales required for clinical applications of

microstimulation. Many factors may lead to this variability in chronic study results. In order to assist in the interpretation of results and clearly demonstrate functionality of microstimulating MEAs, engineers must be able to link long term *in vivo* efficacy data with *in vitro* and *in vivo* safety data in the context of chronic performance.

The use of impedance

Histology is traditionally used to assess the tissue response to implanted MEAs. While histology does provide a direct look at the tissue response to implantation and stimulation, it requires the sacrifice of many animals at several relevant time points to explore tissue response over time. Furthermore, *ex vivo* histology to evaluate the safety of microstimulation is generally not feasible in human subjects. However, there are electrical monitoring techniques that can be performed in order to evaluate the environment of microelectrodes.

One such technique is electrochemical impedance spectroscopy (EIS). EIS is frequently used in a variety of tissue monitoring applications (Dean, Ramanathan, Machado, & Sundararajan, 2008). EIS in brain tissue entails the application of a small electrical signal across a reference electrode and the measurement of the voltage drop on an electrode of interest, in order to report the resistivity of the electrode and its environment to the flow of current (Porter, Adey, & Kado, 1964). True impedance spectroscopy is performed over a range of frequencies. In neural engineering, impedance is often measured only at 1 kHz; this frequency reflects the major frequency component of APs.

1 kHz impedance values are used as a diagnostic for broken UEA electrodes, as well as a metric of the state of the electrode's environment. While impedance has been shown to reflect a variety of changes in the biological environment of an electrode, the noninvasive nature of EIS prevents engineers from knowing how much of measured impedance is due to the electrode itself and how much to the surrounding tissue over time. Modeling studies, however, generally attribute the major portion of measured impedance to the tissue surrounding the electrode (Butson, Maks, & McIntyre, 2006). By taking impedance measurements at regular intervals, neural engineers are

able to obtain a proxy measure of how the electrode environment is changing over the course of implantation.

Impedance is frequently used as a monitoring tool for chronic, active DBS implants (Butson & McIntyre, 2005; Hemm & Coubes, 2004; Lempka, Miocinovic, Johnson, Vitek, & McIntyre, 2009; Wei & Grill, 2009). In DBS studies, it has been found that both tissue and electrode play a role in changes in impedance over time. Studies over several months indicate that impedance is variable, following a pattern of initial increase, a high plateau for several months, and then a decline (Lempka, 2010). Edema, or the collection of fluid around the electrode, occurs when using DBS electrodes (Back, Alesch, & Lanmuller, 2003). It is possible that fluid surrounding the electrode could contribute to these impedance changes, as fluid is less resistive than tissue (Fujita, Ueda, & Yagi, 1972; Harting et al., 2010).

In the microelectrode array literature, these changes in impedance follow a pattern of increasing to plateau over time (Selim Suner, 2005; Ward, Rajdev, Ellison, & Irazoqui, 2009; J. C. Williams, Hippensteel, Dilgen, Shain, & Kipke, 2007), which is hypothesized to correspond to the tissue response (McConnell, Butera, & Bellamkonda, 2009; Merrill & Tresco, 2004), particularly to the formation of a glial scar over 4-6 weeks (Turner et al., 1999). Studies have correlated impedance measurements with histology (Grill & Mortimer, 1994; Mercanzini, Colin, Bensadoun, Bertsch, & Renaud, 2009), indicating that increased tissue density surrounding the electrode corresponds to increased impedance. However, these studies have not extended over multiple years, as have DBS studies. Evidence suggests that MEA impedance is related to AP recording ability (Prasad & Sanchez, 2012); a peak in recording ability was noted on electrodes in the 50-150 kOhm range. However, it has not been fully established that impedance changes measured by MEAs reflect tissue response, nor that decreased impedance corresponds to an increased ability to record; though it has been found that by applying acute “rejuvenating” pulses of electricity to electrodes, impedance decreases and signal quality temporarily increases (Johnson, Otto, Williams, & Kipke, 2004; K. J. Otto, Johnson, & Kipke, 2006). A better understanding of the phenomena underlying impedance changes would help researchers to interpret measured impedances over time and relate these results to other measures of device performance.

Using multiple metrics of performance over time, including impedance, ability to stimulate, and AP recordings, engineers can assess the chronic *functionality* of intracortical MEAs (Prasad & Sanchez, 2011). This functionality data can serve as valuable evidence for the clinical viability of MEAs in lieu of, or in addition to, traditional histology data. In order to transition MEAs into clinical applications, assessment of chronic functionality *in vivo* both with and without the application of microstimulation and identification of underlying issues affecting functionality will be important. Intervention strategies to prevent performance declines will also be useful. By characterizing chronic performance, identifying factors that impact performance, and designing intervention strategies, engineers can hasten the broad clinical use of neural prostheses for human patients with a variety of motor and sensory disorders.

Work to be described

The work described in this dissertation was performed to provide evidence for the long-term functionality of MEAs for microstimulation and recording; identify factors impacting functionality; and test a novel intervention strategy. In Chapter 2, the functionality of chronic intracortically implanted UEAs both with and without microstimulation was investigated in a feline model; it was determined that microstimulation via chronic UEAs at physiologically effective levels does not cause catastrophic changes in device performance as measured by stimulation ability, AP recordings, and impedance. In Chapter 3, a subset of the underlying issues which may affect the results of chronic *in vivo* microstimulation studies was examined via a combination of *in vitro* and *in vivo* experiments using UEAs; it was found that many processes likely contribute to noted performance variability, including the metrics used to assess that performance. Chapter 4 describes a chronic *in vivo* case study of a promising new intervention strategy, namely a carbon nanotube coating applied to a UEA; results indicate that carbon nanotube coated electrodes are as effective as current standards, and may help improve future generations of neural prosthetic devices. In Chapter 5, future developments in MEA technology in order to both broaden their utility and increase their longevity are discussed. It is hoped that the work detailed in this

dissertation will speed the clinical implementation of microstimulating MEAs for sensory prosthetic applications.

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CHAPTER 2

THE FUNCTIONAL CONSEQUENCES OF CHRONIC, PHYSIOLOGICALLY EFFECTIVE INTRACORTICAL MICROSTIMULATION

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CHAPTER 11

The functional consequences of chronic, physiologically effective intracortical microstimulation

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Abstract: Many studies have demonstrated the ability of chronically implanted multielectrode arrays (MEAs) to extract information from the motor cortex of both humans and nonhuman primates. Similarly, many studies have shown the ability of intracortical microstimulation to impart information to the brain via a single or a few electrodes acutely implanted in sensory cortex of nonhuman primates, but relatively few microstimulation studies characterizing chronically implanted MEAs have been performed. Additionally, device and tissue damage have been reported at the levels of microstimulation used in these studies. Whether the damage resulting from microstimulation impairs the ability of MEAs to chronically produce physiological effects, however, has not been directly tested. In this study, we examined the functional consequences of multiple months of periodic microstimulation via chronically implanted MEAs at levels capable of evoking physiological responses, that is, electromyogram (EMG) activity. The functionality of the MEA and neural tissue was determined by measuring impedances, the ability of microstimulation to evoke EMG responses, and the recording of action potentials. We found that impedances and the number of recorded action potentials followed the previously reported trend of decreasing over time in both animals that received microstimulation and those which did not receive microstimulation. Despite these trends, the ability to evoke EMG responses and record action potentials was retained throughout the study. The results of this study suggest that intracortical microstimulation via MEAs did not cause functional failure, suggesting that MEA-based microstimulation is ready to transition into subchronic (<30 days) human trials to determine whether complex spatiotemporal sensory percepts can be evoked by patterned microstimulation.

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Introduction

Multielectrode arrays (MEAs) are a promising technology for use in intracortical neuroprosthetics. For such devices to be clinically successful in recording applications, MEAs must be able to acquire sufficient neural data to control an effector device, such as a prosthetic arm, for the life span of the patient. While the action potential recordings currently obtained using MEAs are not stable over these timeframes (Dickey et al., 2009; Linderman et al., 2006; Suner et al., 2005), studies have shown that having well-isolated action potential recordings is not necessary for successfully decoding movements (Fraser et al., 2009; Rivera-Alvidrez et al., 2010). Numerous studies have also demonstrated the utility of MEAs for intracortical motor prosthetic applications in nonhuman primates (Aggarwal et al., 2009; Musallam, 2004; Nicolelis, 2003; Santhanam et al., 2006; Schwartz et al., 2006; Taylor, 2002; Wessberg, 2000). Further, chronically implanted MEAs have been used to control a variety of effectors in human patients (Hochberg and Donoghue, 2006; Hochberg et al., 2006; Serruya et al., 2002).

MEAs can also potentially be used as a high-resolution interface for the injection of charge directly into brain tissue. Such electrical microstimulation activates neurons, which can generate or modulate a neurophysiological process, such as sensory perception or movement. Numerous studies have evaluated the effects of acute microstimulation on different neural systems. These studies include experiments in the auditory system of rodents (Otto et al., 2005a; Rousche et al., 2003), and the somatosensory system (Romo et al., 1998), visual system (DeYoe, 2005; Murphey and Maunsell, 2007, 2008; Tehovnik, 2006; Tehovnik and Slocum, 2009), and motor system (Cooke, 2003; Fitzsimmons et al., 2007; Graziano, 2005; Graziano et al., 2002; Salzman et al., 1990; Schmidt and McIntosh, 1990) of nonhuman primates. Only a few studies, however, have investigated the consequences of chronic intracortical microstimulation to effect through a chronically implanted MEA (Bradley, 2004; Rousche and Normann, 1999). Human studies have demonstrated the ability to evoke percepts through intracortical microstimulation, but these experiments have been limited to intraoperative time frames (Bak et al., 1990) or to a single chronically implanted patient (Schmidt et al., 1996). Significant insight into the perceptual effects of microstimulation was obtained in these few human experiments, suggesting that further studies utilizing microstimulation via high-density MEAs in subchronic clinical trials will be valuable. Prior to transitioning such research into human patients, however, the functional effects of microstimulation must be evaluated in a nonhuman system to demonstrate both safety and efficacy over chronic timescales.

The consequences of chronic microstimulation are disputed. *In vitro* stimulation studies and investigations of the histological response of brain tissue to microstimulation have shown that damage to both device and tissue can arise from microstimulation (Cogan, 2004; McCreery et al., 2010; Merrill et al., 2005; Negi et al., 2010; Troyk et al., 2004). Intracortical stimulating macroelectrodes, such as deep brain stimulating electrodes, have also been shown to cause tissue reactivity with the application of stimulation (Moss, 2004). Despite the damage caused by stimulating macroelectrodes, however, such devices can deliver functional stimulation for years, especially when stimulation is titrated to effect (Deuschl et al., 2006). While histological markers, including antibodies for reactive astrocytes and neurons can indicate that tissue is damaged, histology cannot indicate whether or not stimulation was effective or whether tissue response would have had an effect on performance. Additionally, histology can only be collected at experimental end points, which means that tissue response cannot be tracked through the course of multiple months of

implantation and microstimulation without the sacrifice of many animals at several time points. In order to analyze the performance of microstimulation via microelectrodes over the course of a multiple-month implantation, electrophysiological markers of performance, rather than histological markers of safety, must be used. Electrophysiological markers, such as recorded action potentials, can demonstrate not only the viability of tissue in the vicinity of electrodes, but also that device integrity is maintained.

In this study, we investigate the *in vivo* performance of sputtered iridium oxide film (SIROF) metalized MEAs used to deliver chronic, physiologically effective intracortical microstimulation. Four felines were implanted with MEAs and two were stimulated to physiological effect, as measured by electromyogram (EMG). Functionality was evaluated using impedance measurements, electrophysiological recordings, and the ability of microstimulation to evoke EMG responses, to determine if device and/or tissue damage occurred with periodic microstimulation. Using these measures, we found that microstimulation efficacy could be maintained after several months of implantation and many microstimulation sessions. Further, we found that microstimulation did not appear to adversely impact either impedances or the ability to perform electrophysiological recordings over the course of the study.

Methods

Surgical procedures

Implantations and all other procedures were performed in accordance with protocols approved by the University of Utah Institutional Animal Care and Use Committee. Four felines (Felis catus) were used in this study. Felines each had an array implanted in motor cortex (Ghosh, 1997) so that applied stimulation would result in motor response measurable by EMGs recorded in either forelimb or hindlimb muscles. All implants were performed by the same clinical neurosurgeon to ensure consistency of implant technique and array placement. Anesthesia was induced using ketamine/xylazine and then continued using Isoflurane. Under sterile conditions, a midline incision was performed, and a craniotomy over the targeted area was made by means of a neurosurgical drill. The dura was reflected, and the array was pneumatically inserted into motor cortex (Rousche and Normann, 1992). Following implantation, the titanium percutaneous connector was attached to the skull using bone screws. A dural replacement (DuraGen, Integra Life Sciences, Plainsboro, NJ) was used to cover the array, and silicone polymer (Kwik-cast, World Precision Instruments, Sarasota, FL) was used to fill the craniotomy, if necessary. The scalp was sutured closed and the animal given at least 24 h to recover before data acquisition was attempted.

Electrode arrays

For Felines 1 and 2, arrays were obtained from Cyberkinetics, Inc. (Salt Lake City, UT) with electrode tips that had been coated by EIC Laboratories (Norwood, MA) with SIROFs. For Felines 3 and 4, 96-electrode SIROF Utah Electrode Arrays were commercially obtained from Blackrock Microsystems, Inc. (Salt Lake City, UT). Arrays were manufactured as described elsewhere (Jones et al., 1992) under Design Controls specified by the United States Food and Drug Administration. Electrodes were 1 mm long and spaced 400 μm apart. The SIROF used to coat the conductive electrode tips increased the charge injection capacity of the electrodes and reduced the possibility of electrode dissolution (Cogan, 2008; Cogan et al., 2009). Active electrode tips were ~40 μm in length, yielding ~4000 μm^2 of SIROF surface area per electrode (Negi et al., 2010; VanWagenen, 2004). The remainder of the array was coated with Parylene-C insulation for electrical isolation and biocompatibility. A summary of arrays used is presented in Table 1.

Table 1. Arrays used

Animal	Array material, manufacture date, location	Implant date	Preinsertion impedances (kΩ)
Feline 1	SIROF 2006, Cyberkinetics/EIC	January 2007	Mean 16, Median 11, Min 4, Max 110
Feline 2	SIROF 2006, Cyberkinetics/EIC	October 2008	Mean 23, Median 14, Min 5, Max 160
Feline 3	SIROF 2009, Blackrock Microsystems	July 2009	Mean 50.4, Median 50, Min 42, Max 74
Feline 4	SIROF 2010, Blackrock Microsystems	July 2010	Mean 46.9, Median 47, Min 40, Max 63

Four SIROF arrays were chronically implanted in feline motor cortex. Felines 3 and 4 were microstimulated, while Felines 1 and 2 were used to obtain comparison recording-only data for long-term patterns of 1 kHz impedance and electrophysiological measures of chronic performance.

Data acquisition

A 128-channel Cerebus data acquisition system (Blackrock Microsystems) was used to acquire neural data. The 96 channels of electrode data from the UEA were fed to a front-end amplifier using a Cereport patient cable.

Impedance measurements

One kilohertz (kHz) impedance measurements were made using a routine in the Cerebus data acquisition system. Briefly, a small sinusoidal current at 1 kHz was passed through a reference electrode, and impedance was simultaneously computed on all electrodes. Chronic impedance readings were taken throughout the multiple-month course of implantation in all four felines. Acute impedance readings were also taken pre- and post-stimulation for each microstimulation session in Felines 3 and 4.

Electrophysiological recordings

Neural recordings were obtained from awake felines to examine device performance over time both with and without the application of microstimulation. Felines were placed in a pet carrier inside an electrically shielded chamber to minimize noise, and connected to the Cerebus. Recordings were made at least weekly in all felines, as well as prior to and following every stimulation session in Felines 3 and 4. Recordings were made in several-minute sessions using band-pass filter settings of 0.3 Hz–7.5 kHz and sampled at 30 kHz in Felines 1–3, and band-pass filter settings of 0.3 Hz–2.5 kHz sampled at 10 kHz in Feline 4 to reduce file size.

Electromyography

EMGs were used periodically in Felines 3 and 4 to test the ability to evoke physiological responses via intracortical microstimulation. Following a control neural data recording, the animal was anesthetized with Telazol administered intramuscularly at 0.01 mg/kg. Sterile, clinical fine-wire electrodes were placed in the biceps femoris muscle of Feline 3 and either the triceps or extensor carpi muscle of Feline 4. Reference electrodes were placed subcutaneously near the intramuscular electrode. EMG activity in response to stimulation was recorded at 2 kHz using the Cerebus data acquisition system described above. For the EMG sessions performed prior to 29/Sep/2009, a MA300-18-002 commercial EMG system (Motion Lab Systems, Baton Rouge, LA) was used. For subsequent EMG sessions, a one-channel AC differential amplifier (DAM 80, World Precision

Instruments) was employed. Stimulus markers were output to Cerebus using in-house LabView code (National Instruments, Austin, TX).

Microstimulation

Six daisy-chained RX-7 stimulators (Tucker-Davis Technologies Inc., Alachua, FL) were used for microstimulation. These stimulators are capable of applying current-controlled waveforms with a voltage excursion of −24 to 24 V. Stimulation was controlled with in-house Matlab (The Mathworks, Natick, MA) and LabView code. Microstimulation was applied to Felines 3 and 4.

Stimulus waveforms

Stimuli were applied in charge-balanced square waveforms to prevent charge buildup (Merrill et al., 2005). For each session, pulses of a square, charge-balanced, biphasic waveform at 0.2 ms per phase were applied in trains of 25, 50, or 100 pulses at 100 Hz, settings chosen for their efficacy at evoking responses in perceptual microstimulation studies (Table 5). Trains of pulses were applied in rounds such that every electrode was stimulated at a given amplitude before the current used to stimulate was increased.

All-channel stimulation

Stimulation was periodically applied to the feline on all 96 electrodes in sequence in rounds of each indicated amplitude in order to determine whether stimulation across all channels affected functionality. In none of these sessions did the feline respond in any manner (e.g., vocalizations or movements) that would indicate an adverse reaction to stimulation while awake (listed as all-channel sessions in Table 2). All-channel stimulation was also performed with the feline anesthetized in order to perform EMG (listed as all-channel EMG sessions in Table 2). Microstimulating current was increased in rounds over the course of the session to determine the threshold current required to evoke EMG responses on each electrode.

Test of parallel stimulation

To test the effects of synchronous stimulation on multiple electrodes, such as might be applied during a failure of patterned microstimulation, stimulation was applied simultaneously at 25 μA via 72 electrodes. The animal was disconnected from the experimental apparatus following five trains of stimulation.

Chronic stimulation

Chronic stimulation sessions were applied in two paradigms, low (15/20 μA) and high (20/25/30/25 μA), on 15 selected electrodes of the MEA in Felines 3 and 4 (Table 2). These specific 15 electrodes were chosen for microstimulation based on two factors: (1) Spatial distribution of charge application. (2) To stimulate on some electrodes which recorded action potentials and some electrodes which did not (Figs. 5a and 5c). Chronic microstimulation was applied at amplitudes ranging from 15 to 35 μA over the course of several months (Table 2). Stimulation levels were adjusted based on the results of EMG sessions.

Data analysis

All analyses and statistical tests were performed using custom Matlab code.

Impedance data

Electrodes which recorded 1 kHz impedance values over 2 MΩ on all days were considered

Table 2. Summary of microstimulation sessions

Feline	Figure label (Fig. 5a or c)	Date	Description	Stimulus parameters	Amplitudes	Electrodes
3	1	14/Aug/2009	All-electrode (EMG efficacy test)	5 trains, 50 pulses	5–50 μA in steps of 5 μA	All
3	2	29/Aug/2009	All-electrode (EMG efficacy test)	5 trains, 50 pulses	5–35 μA, steps of 1–μA	43,78,84
3	3	01/Sep/2009 02/Sep/2009	All-electrode stimulation	2 rounds, 5 trains, 25 pulses	20/30, 30/35 μA	All
3	4	07/Sep/2009	Test of parallel stimulation	5 trains, 25 pulses	25 μA	Distributed pattern of 72 electrodes
3	5	14/Sep/2009	All-electrode stimulation	5 trains, 25 pulses	20–35 μA in steps of 5 μA	All
3	6	30/Oct/2009	All-electrode (EMG efficacy test)	10 trains, 25 pulses	20–40 μA in steps of 5 μA	All
3	7	31/Oct/2009–09/Nov/ 2009	15/20 μA on seven electrodes	100 trains of 25 pulses	15/20 μA	88,89,90,91/ 79,80,81
3	15/20 μA (Teal bars)	11/Oct/2009–03/Dec/ 2009 (23 sessions)	15/20 μA paradigm	200 trains (two rounds), 25 pulses	15 or 20 μA	20 μA—Electrodes 79,80,81 15 μA—Electrodes 26,28,29,31,39, 40,41,43,89,90,91,92
3	8	04/Dec/2009	All-electrode (EMG efficacy test)	5 trains of 25 pulses	20–40 μA in steps of 5 μA	All
3	20/25/30/ 25 μA (Blue bars)	05/Dec/2009–13/Jan/ 2009 (20 sessions)	20/25/30/25 μA paradigm	200/electrode (two rounds), 25 pulses	20, 25, 30, or 35 μA	20 μA—Electrodes 39,40,41,43 25 μA—Electrodes 79,80,81 30 μA—Electrodes 89,90,91,92 35 μA—Electrodes 26,28,29,31
3	9	14/Jan/2010	All-electrode (EMG efficacy test)	5 trains of 25 pulses	100 μA	All
4	1	17/Sep/2010	All-electrode (EMG efficacy test)	5 trains of 25 pulses	5–50 μA in steps of 5 μA	All
4	2	28/Sep/2010	Test for feline response	5 trains of 25 pulses	5–50 μA in steps of 5 μA	All
4	3	01/Oct/2010	All-electrode (EMG efficacy test)	5 trains of 25 pulses	50–100 μA in steps of 10 μA	All

Table 2. Summary of microstimulation sessions (*Continued*)

Feline	Figure label (Fig. 5a or c)	Date	Description	Stimulus parameters	Amplitudes	Electrodes
4	4	28/Oct/2010	All-electrode (EMG efficacy test)	5 trains of 25 pulses	20–60 μA in steps of 10 μA	All
4	5–7	24/Nov/2010–30/Nov/2010	25 μA paradigm	100 trains of 25 pulses	25 μA	1,10,11,32,33,42,43, 66,67,75,76, 79,80,88,89
4	8	01/Dec/2010	All-electrode (EMG efficacy test)	5 trains of 25 pulses	40–100 μA in steps of 10 μA	All
4	9	22/Dec/2010	All-electrode (EMG efficacy test)	5 trains of 25 pulses	50–100 μA in steps of 10 μA	All

Microstimulation was applied to Felines 3 and 4 according to several different stimulus paradigms depending on the goals of microstimulation, for example, to evaluate efficacy or to determine the effects of periodic stimulation on device functionality.

to be out-of-specification and not included in any further analyses. Impedance values over 2 MΩ on a single day were replaced with a 2 MΩ ceiling value. *Z*-scored impedances were computed by electrode in each feline. The value for an impedance reading of a single electrode in a single dataset was compared to mean value for that electrode across all datasets, and then divided by the standard deviation of all values recorded on that particular electrode. *Z*-scores were averaged across all electrodes for each dataset. For pre- and poststimulation session impedance readings, median impedances and standard errors were computed. Wilcoxon's signed rank test was applied to demonstrate significant drops in median impedance values immediately pre- and post-all-channel-stimulation in Feline 3. The Komolgorov–Smirnov test was applied in Feline 4 due to the small number of pre/post-stimulation datasets. Wilcoxon's signed rank test was used to demonstrate the significance of acute impedance drops on 15 stimulated channels in both Felines 3 and 4. In Feline 3, the median impedance drops between the first and second chronic stimulation paradigms were compared using Wilcoxon's signed rank test on the last 20 datasets of the first paradigm and all 20 datasets of the second paradigm. Wilcoxon's signed rank test was also used to determine significance of drops in impedance over time. Impedances for the first and last 30 datasets were compared in Felines 1, 3, and 4, and the first and last 10 datasets were compared in Feline 2 due to the lower number of recording sessions.

Electrophysiological recording data

Action potential recordings were sorted using a PCA-based *t*-distribution algorithm (Shoham, 2003). A threshold for action potentials was subsequently imposed at 70 μV. *t*-tests were performed to quantify changes in the number of electrodes which recorded well-isolated action potentials over time (using the first and last 30 datasets in Felines 1, 3, and 4 and the first and last 10 datasets in Feline 2). Student's *t*-test was also applied to acute pre- and poststimulation number of electrodes which recorded action potentials. The distribution of number of electrodes which recorded action potentials across all microstimulation sessions during pre-stimulation recordings was compared to the immediate post-stimulation distribution for Felines 3 and 4.

Electromyographic data

EMG data was rectified, and a boxcar filter (size 50 ms, stepped per sample) was applied across all recorded twitches evoked by a given amplitude of stimulus via a given electrode on EMG response traces to demonstrate the population-level response to a train of stimulation. The Komolgorov–Smirnov test was applied to the rectified averaged EMG data at −400 to −100 ms prior, and the +100 to +400 ms following, the application of microstimulation.

Results

Chronic 1 kHz impedance

Impedances followed a pattern of increasing after implantation to a peak within the first month in all felines (Figs. 1 and 2). This increase was followed by a decrease over time. The first 30 impedance measurement datasets had higher mean impedance than the last 30 datasets in Felines 1, 3, and 4 ($p<0.001$, Wilcoxon's signed rank test). Due to the smaller number of datasets in Feline 2, only the 10 first and last datasets were compared, with the same result ($p<0.05$, Wilcoxon's signed rank test). A summary of impedances is included in Table 3. Impedances over 2 MΩ were considered to be out-of-specification. In Feline 1, three electrodes were out-of-specification. In Feline 4, seven electrodes were out-of-specification. Felines 2 and 3 did not have any out-of-specification electrodes.

Pre- and post-stimulation impedance

Median 1 kHz impedance decreased acutely on 19 of 23 days with the application of daily microstimulation (low paradigm) on 15 electrodes in Feline 3 ($p<0.01$, Wilcoxon's signed rank test, $n=23$ sessions; Fig. 3a), as well as acutely on 20 out of 20 days when stimulation (high paradigm) was not consistently applied on consecutive days ($p<0.01$, Wilcoxon's signed rank test, $n=20$ sessions; Fig. 3b). Median acute impedance drops were larger during the high chronic stimulation paradigm than the low paradigm ($p<0.01$, Wilcoxon's signed rank test). Parallel microstimulation on 72 channels of the MEA in Feline 3 also led to an acute decrease in median impedance ($p<0.001$, Wilcoxon's signed rank test, $n=96$ electrodes) on all electrodes of the MEA. Median impedance across the 96 electrodes of the MEA decreased when stimulation was applied simultaneously on 72 electrodes in Feline 3 (Feline 3, $p<0.05$, $n=8$ sessions, Wilcoxon's signed rank test). In Feline 4, only five impedance readings were made following all-electrode stimulation sessions. Median impedance decreased following all-electrode stimulation (Komolgorov–Smirnov test, $p<0.01$, $n=5$ sessions). Median impedance also decreased significantly on the 15 electrodes that passed current during the three applications of the 25 μA stimulation paradigm in Feline 4 ($p<0.001$, Wilcoxon's signed rank test, 15 electrodes over three sessions, $n=45$).

Action potential recordings

In both nonstimulated and microstimulated felines (Figs. 4 and 5), the number of action potentials recorded from motor cortex followed a previously observed pattern of initially increasing followed by a decrease in numbers over the course of months (Suner et al., 2005). This chronic pattern of action potential recordings was similar between microstimulated and non-microstimulated felines. In nonstimulated Feline 1, the number of action potentials recorded during the plateau period (sessions 30–60) was greater than that recorded during the fade-out period (last 30 datasets, $p<0.05$, Student's two-sample t-test). This same observation was made in the microstimulated Felines 3 and 4 ($p<0.05$, Student's two-sample t-test). In non-microstimulated Feline 2, in which only 39 data acquisition sessions were performed,

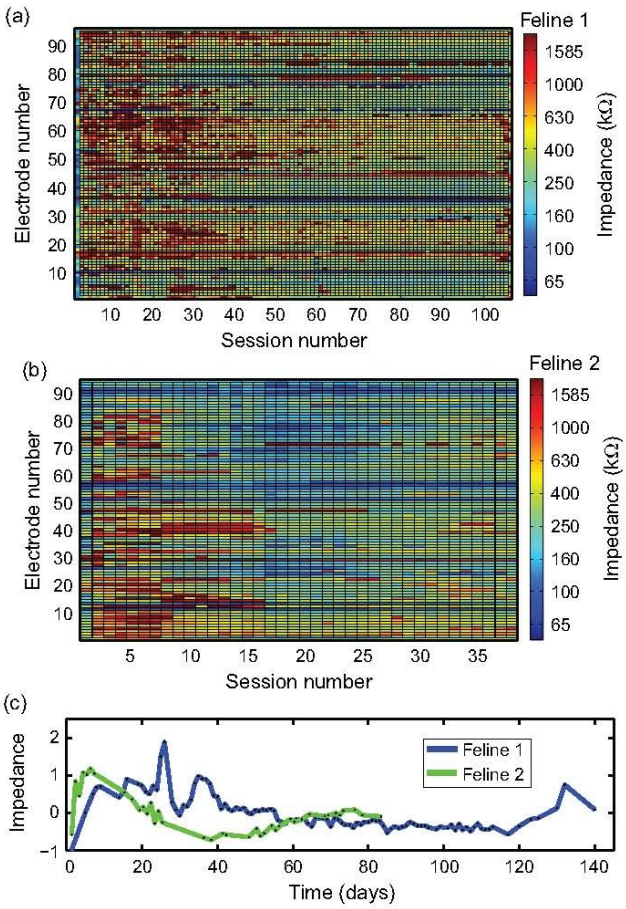

Fig. 1. 1 kHz impedance is variable over chronic timescales. (a, b) 1 kHz impedance measurements were made in the two nonstimulated felines over 8 and 3 months of implantation, respectively. Shown are impedance measurements colored by value. (c) Mean Z-scored impedance over time, in days, for both felines. Both felines exhibited a pattern of increase in impedance following implant that peaked within the first month, followed by a decrease toward preimplantation values ($p < 0.01$, Wilcoxon's signed rank test).

there was no significant change in the number of action potentials recorded between the first and last 10 datasets. All-electrode microstimulation, for example, for feline response tests and EMG sessions, did not lead to an acute decrease in the number of well-isolated action potentials recorded in either microstimulated feline ($p < 0.05$, one-tailed Student's t-test; Feline 3, prestimulation mean = 5 action potentials, poststimulation = 8 action potentials, $n = 8$ sessions; Feline 4, prestimulation mean = 60 action potentials, poststimulation = 70 action potentials, $n = 6$ sessions). The number of action potentials recorded prior to multielectrode synchronous stimulation in Feline

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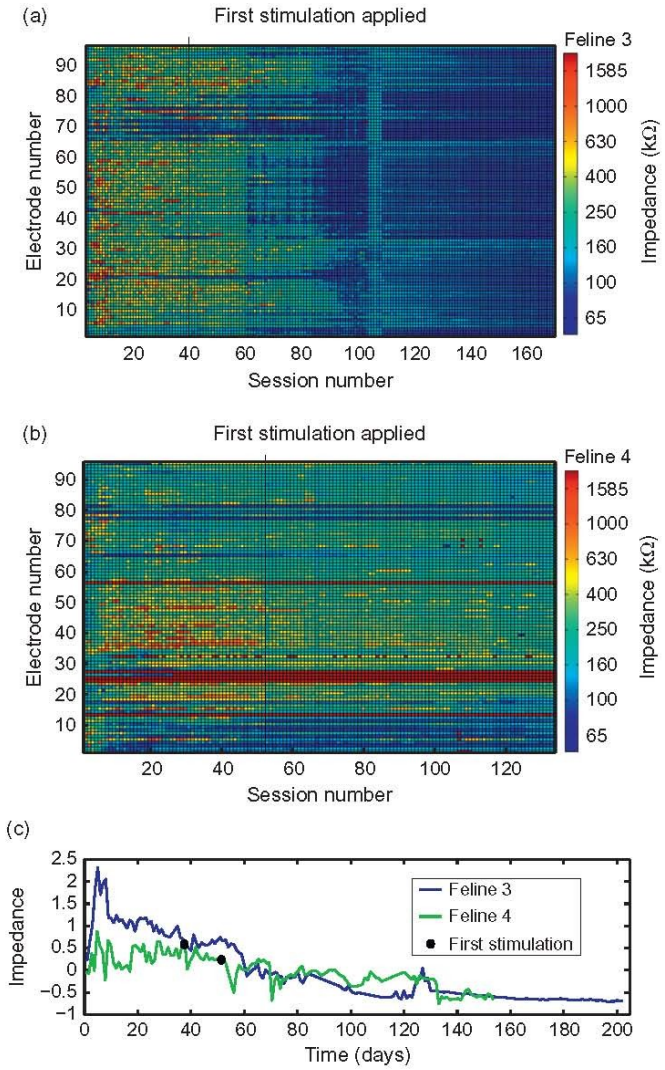

Fig. 2. Microstimulation did not change 1 kHz impedance patterns. (a, b) 1 kHz impedance measurements, colored by value, across all 96 electrodes of the MEA in Felines 3 and 4. Black lines and circles indicate the first application of stimulation. (c) Mean *Z*-scored impedance over time (prestimulation values shown) over time in days. In both Feline 3 (blue) and Feline 4 (green), impedances dropped toward baseline over time ($p < 0.01$, Wilcoxon's signed rank test).

Table 3. Summary of impedances during implantation

Feline	Mean (kΩ)	Median (kΩ)	Standard deviation (kΩ)	Recording sessions	Electrodes
1	462	353	144	107	93
2	358	285	161	39	96
3	157	91	78	168	96
4	244	99	73	134	89

Impedance values were used as a measure of device performance over the course of implantation and microstimulation. Shown here is summary data on impedances from all four MEAs used in this study.

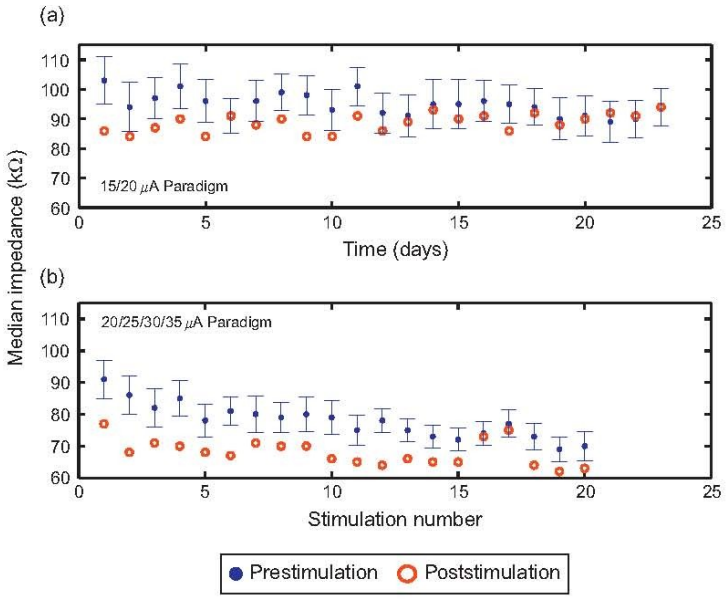

Fig. 3. Microstimulation leads to acute drops in 1 kHz impedance. Median impedance before (blue, with standard error bars) and after (red) microstimulation on the 15 electrodes that passed current into tissue is shown for (a) the low stimulation paradigm, applied daily for 23 days and (b) the high stimulation paradigm, applied 20 times over a 6-week course. Impedance decreased significantly following microstimulation for both stimulus paradigms ($p < 0.01$, Wilcoxon's signed rank test). Impedances drops were larger for the second paradigm ($p < 0.01$, Wilcoxon's signed rank test).

3 was 12, while the number recorded poststimulation was 13, demonstrating that acute parallel synchronous stimulation did not preclude recording ability. A summary of action potential recording data in all four felines is included in Table 4.

Microstimulation in awake felines

For awake stimulation sessions in both Felines 3 and 4, microstimulation was applied to one electrode at a time without anesthesia. No adverse

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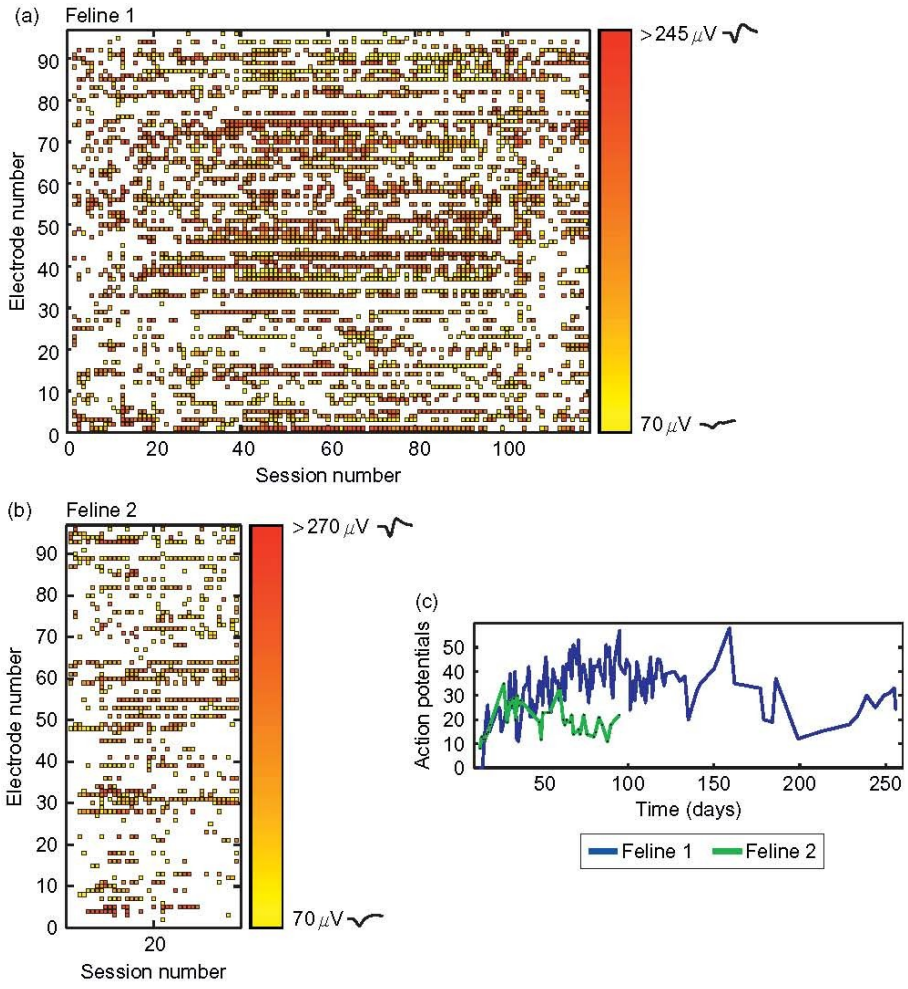

Fig. 4. Action potential amplitudes over chronic timescales. (a and b) Raster plots of thresholded, sorted action potential recordings across the array for the duration of the study for Felines 1 and 2. Each square represents the mean of the furthest cluster from noise as isolated by principle component analysis. Waveforms shown on color bar are samples of action potential shapes isolated at low and high amplitudes. (c) Number of isolated action potentials recorded over time in days. There was an initial increase in the number of action potentials recorded, followed by a significant decrease in Feline 1 by the end of the 8 months of implantation ($p < 0.01$, Student's t-test).

behavioral or physiological responses to such stimulation were observed. For multichannel parallel stimulation in Feline 3, stimulation was synchronously applied to 72 electrodes of the MEA (Table 2; 07/Sep/2009). A bilateral, tonic seizure of <1 min duration resulted from this microstimulation paradigm. Full ambulatory recovery occurred within 5 min of ictus. The animal exhibited neither

behavioral deficits nor spontaneous seizures in the 5 months between seizure induction and termination of the experiment.

Microstimulation to effect

Chronic stimulation was applied on the same 15 electrodes (shown in Fig. 5a) for a total of 43 days in Feline 3 (Table 2). It was possible to evoke EMG responses via an electrode that performed chronic microstimulation, both after it delivered 23 sessions of stimulation at 15 μA, and after 20 sessions at 30 μA (Fig. 6a), though this electrode did not record action potentials between EMG sessions. Of the 15 electrodes used for chronic stimulation in Feline 3, four recorded action potentials during the time that chronic stimulation was applied. EMG responses could also be evoked several months apart in time via electrodes that

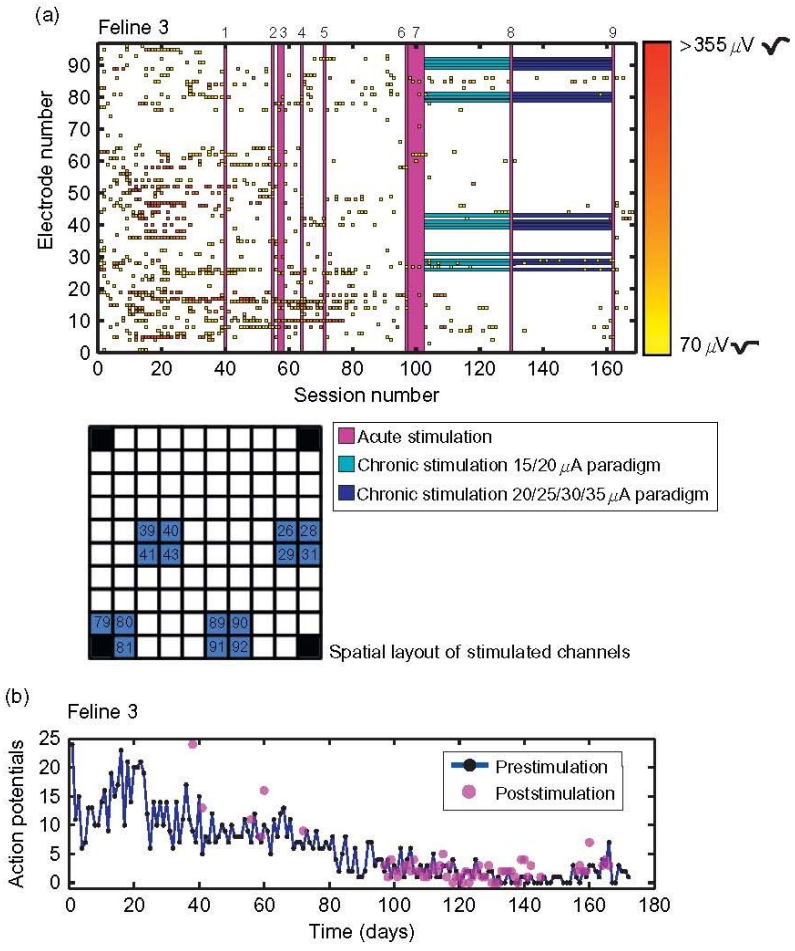

Fig. 5 (Continued)

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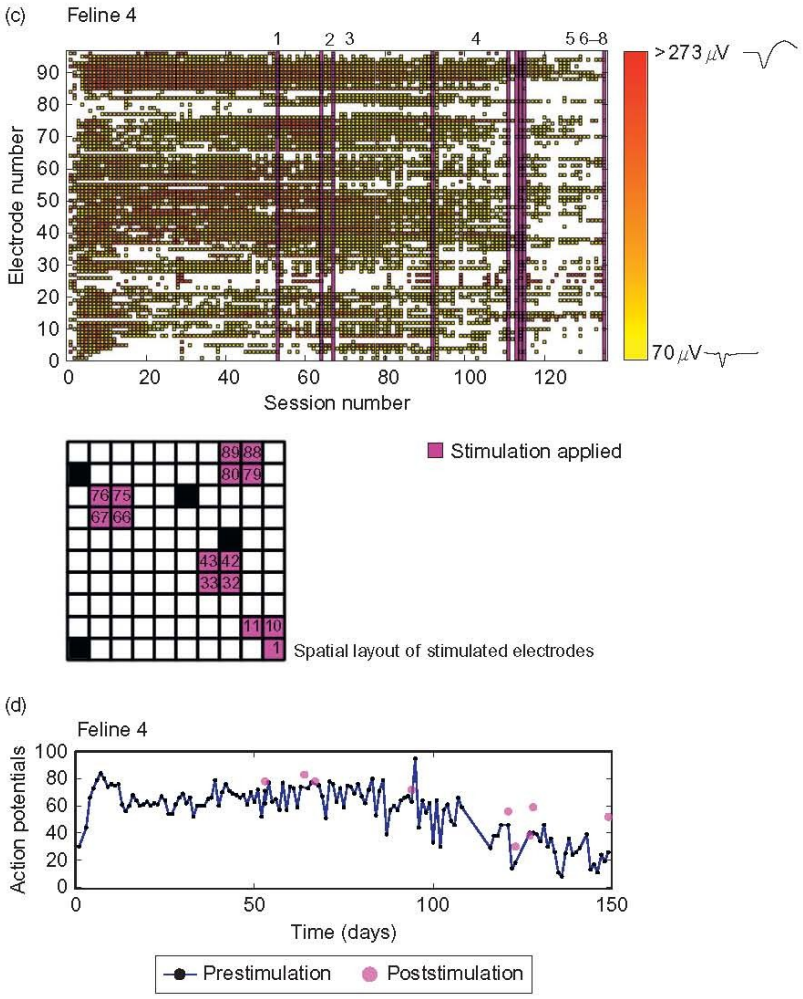

Fig. 5. Microstimulation did not have a clear effect on the number or distribution of action potentials across the array over chronic timescales. (a and c) Raster of action potentials in Felines 3 and 4, color coded by amplitude over recording sessions. Purple bars represent an acute stimulation session, detailed by number in Table 2. Teal bars in Feline 3 represent chronic stimulation at the low stimulation paradigm listed in Table 2, while blue bars represent the high stimulation paradigm applied to the 15 electrodes highlighted. Grids show the spatial layout of electrodes stimulated, blue in Feline 3 and purple in Feline 4. Waveforms shown on color bar are samples of action potential shapes isolated at low and high amplitudes. (b, d) Number of action potentials recorded both over time before (blue) and after (purple) microstimulation in Felines 3 and 4. While the number of action potentials overall did decrease significantly by the end of the experiment in both felines ($p < 0.01$, Student's t-test), the number of action potentials isolated acutely, that is, before and after individual stimulation sessions, did not decrease ($p < 0.05$, Student's t-test).

Table 4. Summary of thresholded action potential amplitudes

Feline	Number of recording sessions	Mean number of electrodes which recorded action potentials, all sessions	Mean number of electrodes which recorded action potentials, first 30 sessions	Mean number of electrodes which recorded action potentials, last 30 sessions
1	123	34	38	33
2	39	20	18 (first 10 sessions)	17 (last 10 sessions)
3	168	6	9	2
4	135	57	73	40

Electrophysiological data recorded from each MEA was used as a chronic measure of device performance. Shown are summary statistics for all four MEAs used in this study.

recorded action potentials in both microstimulated felines (Fig. 6b and c), though these electrodes did not deliver chronic microstimulation (three electrodes in Feline 3, two electrodes in Feline 4). In Feline 3, 13 electrodes evoked electromyographic responses at 10–35 μA on 14/Aug/2009, seven electrodes evoked responses between 20 and 40 μA on 04/Dec/2009, and 11 electrodes evoked responses at 100 μA on 14/Jan/2009 ($p < 0.05$, Komolgorov–Smirnov test). While responses were evoked at currents of < 40 μA during the second month of implantation in Feline 3, 100 μA current was required to evoke responses during the sixth month of implantation. Currents higher than 100 μA were not tested due to the voltage excursion limitation of the stimulator. Of the electrodes which evoked responses in Feline 3, two consistently evoked responses throughout all EMG sessions. In Feline 4, eight electrodes evoked EMG responses at 60–80 μA on 01/Oct/2010, two electrodes evoked responses at 60 μA on 28/Oct/2010, and eight electrodes evoked responses at 60–100 μA on 22/Dec/2010. Of the electrodes that evoked EMG responses in Feline 4, two retained the ability to evoke responses throughout the experiments performed.

Discussion

Many studies have shown that intracortical microstimulation to effect can be performed in nonhuman primate, feline, and other model systems for many sensory modalities (see Table 5). In most of these studies, stimulation was performed acutely on a single electrode in order to evaluate behavioral responses to stimulation. Few of these studies examined the chronic response to stimulation, and still fewer evaluated the long-term consequences of stimulation in a functional context. In this study, we found that chronic, intracortically implanted MEAs could stimulate to effect on multiple electrodes over the course of several months. By evaluating device performance using electrophysiological data, stimulation ability, and 1 kHz impedance, we found that effective stimulation via chronically implanted MEAs did not appear to destroy either the device or underlying cortical tissue.

The stimulation applied in this study was in the 5–100 μA range. While this exceeds the stimulus amplitudes that have been used in many *in vitro* studies that reported electrode damage with long-term pulsing, and several rodent studies that evoked behavioral response (Cogan, 2004; Houweling and Brecht, 2007; McCreery et al., 2010; Tehovnik, 1996), it is equivalent to the stimulus amplitudes that have been used to evoke perceptual or other effects in felines, macaques, and humans (see Table 5), as well as in other rodent studies (Otto et al., 2005b; Tehovnik, 1996). Some of these studies have noted damage to the tissue surrounding the electrodes using histological markers. While these histological markers indicate that tissue surrounding the electrodes reacted to stimulation, they cannot demonstrate whether or

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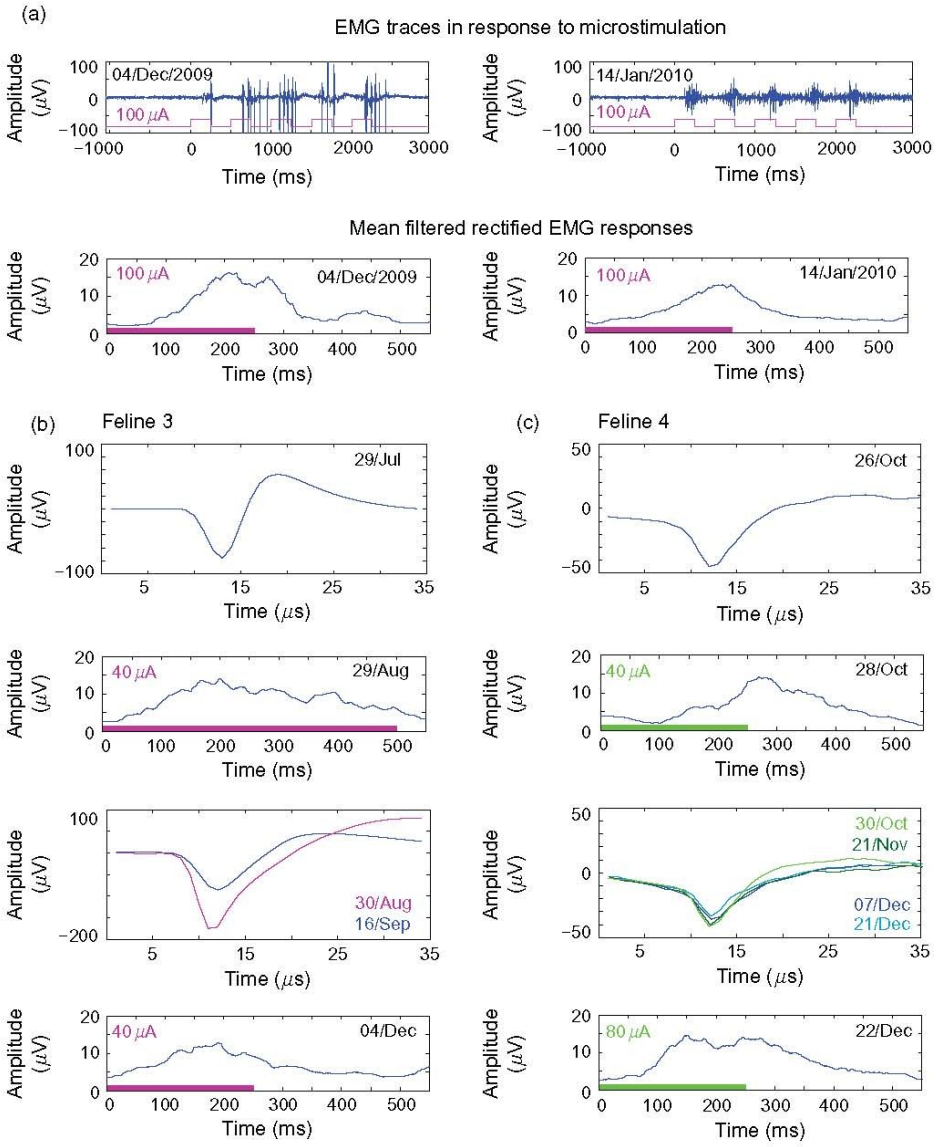

Fig. 6. Chronically stimulated electrodes maintained the ability to stimulate to effect for multiple months. (a) Sample EMG recordings from both 04/Dec/2009 and 14/Jan/2009 evoked by stimulation on and electrode which applied current at 15 and 30 μA, according to the chronic paradigms detailed in Table 2, with the respective filtered mean rectified EMG responses below. Blue represents the recorded EMG response, while purple represents the pulses applied. (b) Sample action potentials recorded before and between electromyographic sessions during which responses were evoked at 40 μA in Feline 3. Colored bars along the time axis mark the application of microstimulation. (c) Sample action potentials recorded before and between EMG sessions in Feline 4. Dates of action potential recordings or stimulation and amplitude of stimulation applied are noted in each panel.

Table 5. Summary of selected intracortical microstimulation studies

Citation	Model system	Electrode type	Reporter of efficacy	Current range applied	Frequency (Hz)	Impedance range 1 kHz	Damage?
Murphey and Maunsell (2008)	Macaque	Platinum/iridium, single electrode	Behavioral task (visual)	Up to 50 μA	200	0.2–1.5 MΩ	No
Murphey and Maunsell (2007)	Macaque	Platinum/iridium, single electrode	Behavioral task (visual)	3–12 μA	200	0.2–1.5 MΩ	No
McCreery et al. (2010)	Feline	Activated iridium, 16 microelectrode array	N/A	10–20 μA	50	Not reported	Yes
McCreery et al. (2002)	Feline	Activated iridium, 16 microelectrode array	Evoked potentials in medulla	0–32 μA	50	0.2–1.5 MΩ	No
Graziano (2005)	Macaque	Tungsten, single microelectrode	Arm movements	5–100 μA	100–250	1–2 MΩ	No
Graziano et al. (2002)	Macaque	Tungsten, single microelectrode	Arm movements	Up to 150 μA	200	0.5–2 MΩ	No
Tehovnik et al. (2003)	Macaque	Platinum/iridium, single microelectrodes	Behavioral task (visual)	Up to 30 μA	200	1–2 MΩ	No
Tehovnik et al. (2002)	Macaque	Platinum/iridium, single microelectrodes	Behavioral task (visual)	Up to 40 μA	200	1–1.5 MΩ	No
Romo et al. (2000)	Macaque	Platinum/tungsten, seven microelectrodes	Behavioral task (flutter discrimination)	65–100 μA	50–200	1–1.5 MΩ	No
Romo et al. (1998)	Macaque	Platinum/tungsten, seven microelectrodes	Behavioral task (flutter discrimination)	65–100 μA	50–200	1–1.5 MΩ	No
Rousche and Normann (1999)	Feline	Platinum, 96 electrode Utah Electrode Array	Behavioral task (auditory)	100 μA	25–2000	30–149 kΩ	No
Torab et al. (2011)	Macaque	SIROF, 96 electrode Utah Electrode Array	Behavioral task (visual)	0–96 μA	200	40 kΩ–2 MΩ	No
Bradley (2004)	Macaque	Iridium, 192 electrode array	Behavioral task (visual)	12–20 μA	200	80 kΩ–1.6 MΩ	No
Bak et al. (1990)	Human	Iridium, arrays of 1–3 electrodes	Verbal report (visual percepts)	20 μA–2 mA	100	Not reported	No
Schmidt et al. (1996)	Human	Iridium, 12 single electrodes, 13 paired electrodes	Verbal report (visual percepts)	1–80 μA	200	Not reported	No

The stimulus amplitudes and parameters applied in this study were similar to those used in other microstimulation experiments which evoked sensory percepts. Shown are sample studies for comparison.

not stimulation affected device performance. By using electrophysiological markers such as recorded action potentials and ability to evoke physiological responses, we were able to demonstrate sustained functionality, including action potential recording, which implies tissue viability in the recording radius of the microelectrode tips.

The chronic changes in impedance observed in our experiments followed a pattern which has been previously noted in the literature. Mean impedance of passively implanted microelectrodes tends to increase over the first weeks of implantation, followed by a decrease over time (Williams et al., 2007). The causes of this pattern remain unclear, though it could result from ongoing processes of the tissue response to implanted devices. We observed this trend during the first weeks of implantation, followed by a continued decrease in impedance throughout the duration of the experiments in both passively implanted felines. The same phenomenon has also been observed in deep brain stimulation studies, where current was applied via chronically implanted macroelectrodes over time (Lempka et al., 2009). Important to note is that the impedances of both microstimulated and nonstimulated felines followed the same general pattern over time. The application of microstimulation did not drive electrode impedances out-of-specification, that is, $>2\ \mathrm{M\Omega}$, as might be expected in the case of device failure or catastrophic tissue damage over time.

We also observed short-term decreases in impedance on stimulating electrodes with the application of microstimulation, which are reported to occur both *in vivo* and *in vitro* (Otto et al., 2006). These changes in impedance could reflect tissue response, for example, disruption of the glial scar by microstimulation. Impedance changes may also reflect processes of device damage known to occur with stimulation, such as dissolution of metallization or damage to electrode insulation. Finally, decreases could also indicate processes of electrochemical activation, which would change the valence state of the stimulating SIROF. The repeatability of the short-term impedance drops, with subsequent recovery, suggests that reversible electrochemical activation rather than cumulative damage may be reflected by these short-term changes in impedance values. The second chronic microstimulation paradigm in Feline 3 yielded larger acute impedance drops. This could be a result of either the increased current used to stimulate, or the increased time between stimulation sessions which would allow impedances to return to baseline. This further supports the idea that reversible electrochemical processes, rather than damage, contribute to observed impedance drops. Further, the maintained ability to stimulate and record indicates that any damage that may have occurred with microstimulation did not preclude device functionality. Importantly, catastrophic changes in impedance, which might indicate device damage or tissue death, did not occur with the application of stimulation.

The microstimulation amplitudes used in this study never evoked seizure-like or aberrant electrical activity when performed on a single electrode. Further, no adverse behavioral responses occurred with the application of microstimulation in awake animals at $50\ \mu\mathrm{A}$. However, a seizure did result from multielectrode simultaneous stimulation (Table 2; 07/Sep/2009) at $25\ \mu\mathrm{A}$. Though no long-term device performance or physiological deficits were noted following this simultaneous multielectrode microstimulation, clearly the induction of a seizure event is unacceptable for any neural prosthetic application. In order to evoke complex spatiotemporal sensory percepts, interleaved multielectrode stimulation will need to be performed; it remains unknown how many electrodes can be simultaneously used without adverse physiological consequences. Patterns of stimulation can be sparsely distributed in both space and time, but must also be able to convey useful sensory information. An acceptable, safe balance between spatiotemporal patterns of microstimulation which convey useful sensory information and those which result in seizure must be found. Additionally, mechanisms to

prevent unacceptably dense microstimulation will need to be implemented in the stimulation control electronics for human sensory prostheses to ensure that this failure mode does not occur.

It is unclear if complex spatiotemporal percepts can be evoked by patterned intracortical microstimulation. Testing patterned microstimulation in nonhuman primates is challenging (Bradley, 2004; Torab et al., 2011). It will be more efficiently addressed by means of psychophysical experiments conducted in human volunteers. Our ability to stimulate to effect and record electrophysiological data over multiple months demonstrated that tissue in the recording radius of the MEA remained viable after many months, and that the device maintained functionality over this time. These results suggest that microstimulation is ready for the next step in the development of sensory prosthetics, namely subchronic clinical trials in human subjects. Such trials will allow researchers to optimize stimulation parameters that are best at evoking sensory percepts, and will greatly speed the development of devices for the benefit of human patients.

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Abbreviations

EMG	electromyogram
MEA	multielectrode array
SIROF	sputtered iridium oxide film

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CHAPTER 3

FACTORS AFFECTING CHRONIC IN VIVO INTRACORTICAL MICROSTIMULATION STUDY RESULTS AND DEVICE PERFORMANCE

Abstract

Intracortical microstimulation via chronically implanted microelectrode arrays (MEAs) shows great promise for clinical applications, such as visual prostheses for patients with profound blindness. Long-term *in vivo* validation will provide key insight to facilitate the clinical implementation of such prostheses. Such *in vivo* validation has proven challenging, however, and few studies have demonstrated microstimulation to behavioral or physiological effect at time points greater than 1 year. In this study, Utah Electrode Arrays were used to investigate some of the underlying issues that may affect chronic *in vivo* studies of intracortical microstimulation, including mechanical damage, temperature, electrochemical effects of stimulation, and depth of test subject anesthesia. We also investigate the use of impedance as a metric of device functionality over time. This study reveals that mechanical damage as well as electrochemical effects may occur with implantation and stimulation. We confirm that temperature and microstimulation affect the electrical properties of MEAs. Additionally, we find that anesthesia impacts ability to stimulate to physiological effect, without affecting 1 kHz impedances. Finally, we find that lower impedances do not predict increased recording ability. This work should assist in the interpretation of results obtained in a variety of *in vivo* microstimulation studies.

Introduction

Microelectrode arrays (MEAs) are a promising electrical interface technology for use in a variety of intracortical prosthetic applications, including sensory and motor restoration (Schwartz, 2004). MEAs such as the Utah Electrode Array have already proven significant long-term clinical utility in human motor prosthetic applications (Hochberg et al., 2006), though the ability to record action potential data is generally limited to a few years at the most (Chestek et al., 2011; Selim Suner, 2005). However, due to the insight gained in such long-term human studies, improved ability to decode without sorted action potential data, (Fraser, Chase, Whitford, & Schwartz, 2009), and improvements in effector technology, MEA-based motor prostheses are rapidly advancing towards widespread clinical use.

MEA-based microstimulation of human cortex for vision restoration, unlike passive recording via MEAs, has yet to undergo rigorous trials. Human microstimulation experiments have been performed (Bak, 1990; Dobelle, 1976; Dobelle & Mladejovsky, 1974; Schmidt et al., 1996), but these experiments have been of short duration or involved only single patients. Long-term, detailed human trials will provide a wealth of information to accelerate the clinical implementation of MEAs for the microstimulation of V1 to restore sight. Animal validation work on the chronic safety and functionality of MEA-based intracortical microstimulation will provide valuable insights to facilitate such trials.

Towards this end, decades of microstimulation research has been performed in animal models, including rats (K. Otto, P. Rousche, & D. Kipke, 2005; K. J. Otto, P. J. Rousche, & D. R. Kipke, 2005), felines (P. J. Rousche & Normann, 1999), and nonhuman primates (Graziano, 2005; Murphey & Maunsell, 2007; Romo, Hernandez, Zainos, & Salinas, 1998; Edward J. Tehovnik, Slocum, & Schiller, 2003). Unfortunately, each of these model systems has its drawbacks, such as the small lissencephalic brain of rodents, the difficulty of training felines, and the expense of nonhuman primate studies. Furthermore, such studies generally require that the animal perform a behavioral task as a reporter of the efficacy of microstimulation, which is subject to the behavioral and attentional state of the animal (E. J. Tehovnik & Slocum, 2004). While such

studies have provided a great deal of information, very few have successfully stimulated to effect after more than a year of MEA implantation (Bradley, 2004).

To prevent the experimental confounds inherent in behavioral assays, stimulation can be performed in anesthetized animals. Such studies can use physiological reporters to report the efficacy of stimulation. Anecdotal reports suggest that anesthesia may impair microstimulation, however, requiring increased charge injection to achieve stimulation thresholds for responses comparable to those obtained in the awake and behaving animal. Furthermore, anesthesia is known to have an effect on overall cortical excitability, which may impair the ability to stimulate to effect. The relationship between anesthesia depth and ability to microstimulate to physiological effect has yet to be quantified.

There are many other issues, in addition to the difficulties inherent in model organism use and behavioral reporters, affecting *in vivo* microstimulation research. Microstimulation studies enter a large and complex parameter space. Mechanical damage, microstimulation parameters, the tissue response to implantation, and experimental procedures may all affect the results reported in nonhuman long-term MEA-based microstimulation studies.

Researchers have devised a variety of strategies for assessing or overcoming the impact of these factors. For example, in order to track tissue response long term without the need for histological evaluation of a large cohort of animals at regular intervals, 1 kHz impedance (Z) measurements are often used. The underlying causes of observed Z dynamics remain unclear, but are frequently hypothesized to include tissue response, electrochemical effects of microstimulation, and damage to electrodes. Tissue response is most frequently cited as the major contributing factor to Z dynamics, particularly the formation of a glial scar encapsulating the electrode. The glial scar forms a resistive barrier impeding the passage of current into excitable tissue (Ward, Rajdev, Ellison, & Irazoqui, 2009; Williams, Hippensteel, Dilgen, Shain, & Kipke, 2007). In addition to tissue response, temperature may be a factor affecting impedance (S. F. Cogan, 2008). Both acute electrode and tissue damage may occur with insertion of MEAs, and may play a role in impedance variability. Damage to both tissue and electrode is also known to occur with the long-term application of stimulation (S. Cogan, 2004; McCreery, Pikov, & Troyk,

2010), as are reversible decreases in impedance (Otto, Johnson, & Kipke, 2006). It is uncertain to what extent each of these processes plays a role in observed impedance variability. While the relationship between device functionality and impedance measurements has been recently examined (Prasad & Sanchez, 2012), the underlying factors affecting this relationship have not been fully investigated *in vivo.*

In this study, we use Utah Electrode Arrays to begin investigating how several of these factors affect the results obtained in long-term *in vivo* microstimulation studies. By performing rodent, feline, and *in vitro* experimentation, we show how various processes related to implantation and stimulation affect Z, and also clarify the relationship between chronic Z measurements and device functionality. In addition, we attempt to quantify the relationship between anesthesia depth and ability to stimulate to physiological effect. We find that implantation and stimulation affect impedance measurements via mechanisms of device damage, changes in electrode environment, and reversible electrochemical effects. Furthermore, we find that decreased 1 kHz impedance does not correspond to an increase in device performance over time, as we might expect if decreased impedance predicts increased recording radius. Finally, we find that anesthesia does affect *in vivo* microstimulation. Results from these studies suggest future work to be done on assessing the effects of anesthesia, microstimulation, and experimental protocols on *in vivo* microstimulation. It is hoped that these studies will assist in the development of more robust microstimulation assays, thereby facilitating the transition of microstimulating MEAs for sensory restoration into the clinical realm.

Methods

Electrode Arrays

Utah Electrode Cereport arrays were commercially obtained from Blackrock Microsystems, Inc. or Cyberkinetics, Inc. (Salt Lake City, UT). Arrays were manufactured as described in (Campbell, Jones, Huber, Horch, & Normann, 1991). Arrays were fixed-geometry, with 96 electrodes manufactured of silicon electrically isolated with glass and insulated with Parylene-C, aside from the conductive tips. Electrodes were 1 mm in length with 400 micron

spacing. Tips had an exposure of 60 ± 40 μm, yielding a geometric surface area of 500–4000 μm2 coated with either platinum (Pt) or sputtered iridium oxide film (SIROF). SIROF arrays were used in both *in vitro* and *in vivo* experiments. The Pt array was only used *in vitro* and in acute rat implantation. Arrays used are summarized in Table 3.1.

Data acquisition

Data was acquired at 30k samples/second at 0.3 Hz – 7.5 kHz using a 128-channel Cerebus system (Blackrock Microsystems, Inc.). Z was measured at 1 kHz using a 10 nA constant current sine wave signal. The impedance tester was built into the patient cable supplied with Cerebus. Tests for intercable reliability of impedance readings were performed; no significant differences were found between five tested cables.

In vitro experiments

In vitro experiments were performed at room temperature unless otherwise stated. Phosphate buffered saline (PBS, Sigma-Aldrich, St. Louis MO) was used in the preparation of agarose, to saturate arrays prior to experimentation, to store arrays, and to stimulate arrays in an ionic environment with the same osmolarity and ionic concentrations as human tissue. Saline rinses and the collection of saline baseline data were obtained prior to and following each experiment listed below. Figure 3.1 shows the order of *in vitro* experiments.

Saline saturation: Arrays were placed in a 1% PBS solution. Arrays were given 24 hours to saturate such that impedances reached a baseline value at room temperature (22 degrees Celsius). Z measurements were made at regular intervals to ensure that no changes in impedance occurred prior to the performance of other procedures.

Agarose implants: 1% agarose gel (Sigma-Aldrich) was poured into petri dishes to serve as a brain chimera (Chen et al., 2004); 2% agarose was used as a more rigorous test of the mechanical stability of array metallization. A thin plastic membrane was also used to simulate pia mater. Arrays were pneumatically inserted into the agarose, and impedance measurements were taken following insertion. Following four implantations (1%, 1% with pia, 2%, 2% with pia), the

Table 3.1. Arrays used in this study.

Array Number/Model System	Metal	# of in-spec electrodes	Preexperiment Impedances (kΩ)
1290-5/Feline 1	SIROF	93	Mean 16, Median 11, Min 4, Max 110
1290-6/Feline 2	SIROF	96	Mean 23, Median 14, Min 5, Max 160
0386/Feline 3	SIROF	96	Mean 50.4, Median 50, Min 42, Max 74
0511/Feline 4	SIROF	86	Mean 46.9, Median 47, Min 40, Max 63
0021/In vitro and rat	Pt	91	Mean 352, Median 263, Min 94, Max 1017
0449/In vitro and rat	SIROF	83	Mean 62.2, Median 60, Min 39, Max 98

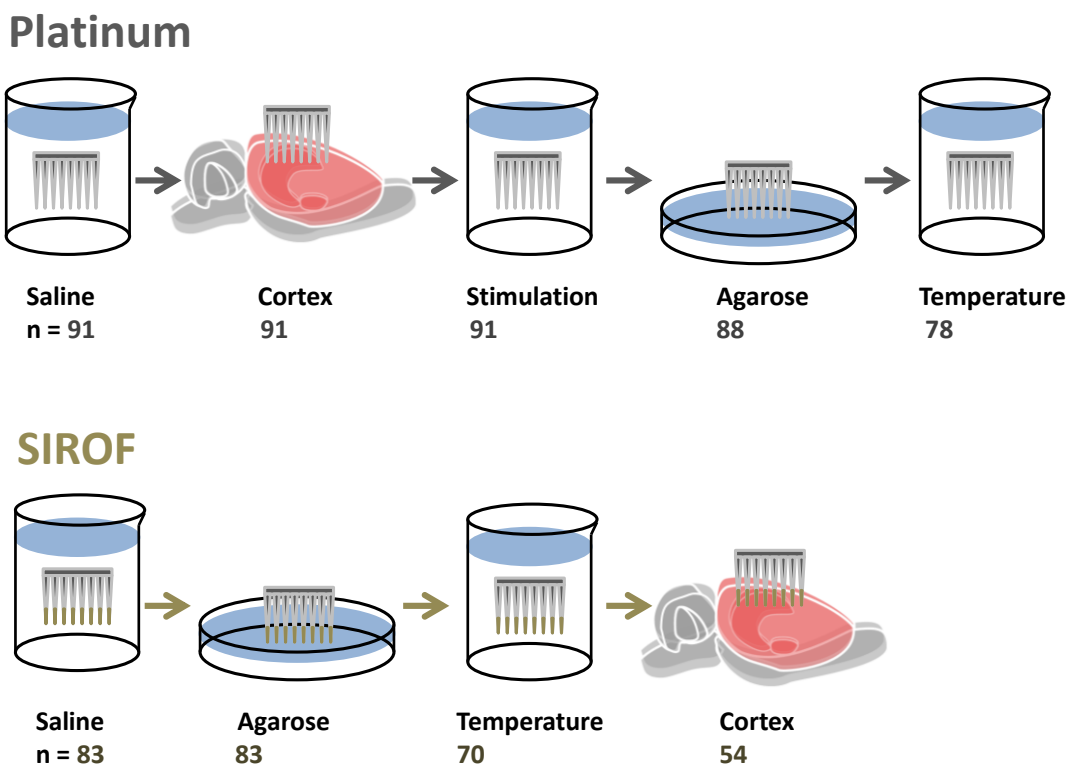


Figure 3.1. Sequence of *in vitro* experiments. Impedances were first equilibrated in saline, then either cortical (Pt) or agarose (SIROF) damage tests were performed. Next, arrays were enzymatically cleaned and placed back into saline for further tests, followed by a second round of damage testing. Following cortical testing, the SIROF array was no longer suitable for further tests, but temperature tests were performed on the Pt array. The number of in-specification electrodes used to analyze the results of each test are indicated.

agarose was examined under light microscope to determine if metallization had been left in the implant site. Arrays were then rinsed in saline and cleansed with Enzol enzymatic detergent (Advanced Sterilization Products Inc., Irvine CA) to remove debris.

Temperature variation: Arrays were placed in a refrigerated beaker of PBS. A thermometer was placed in the beaker to monitor temperature. This beaker was then placed on a hot plate with stir bar, and periodic readings were made of impedance as temperature increased. Once a peak temperature was reached by the hot plate, the beaker was allowed to cool and impedances were taken as temperature decreased. No significant difference was found between the heating and cooling traces.

Cortical implants: All implants were performed in accordance with protocols approved by the University of Utah Institutional Animal Care and Use Committee (IACUC). Rodents were induced with 5% isoflurane, which was maintained throughout the procedure by a vaporizer at 1-5%. Rodents were placed in a stereotaxic apparatus. An incision was made in the scalp, and the skull was removed over the right hemisphere of the brain using handheld surgical tools. The dura was resected. 10x10 Pt or SIROF arrays were pneumatically inserted into the cortex (P.J. Rousche & Normann, 1992). The percutaneous connector was temporarily fastened to the skull using two titanium bone screws. The brain was periodically irrigated with PBS. Impedance measurements were made until a plateau value was reached. Once plateau was reached, the rodent was sacrificed with pentobarbital solution and the array was removed, rinsed in saline, and cleaned using Enzol.

Saline microstimulation: Microstimulation was performed in PBS for *in vitro* stimulation experiments. Stimulation parameters are detailed in Table 3.2.

Feline experiments

All feline surgical and experimental procedures were performed in accordance with United States Department of Agriculture guidelines and were approved by the University of Utah's Institutional Animal Care and Use Committee. Four chronic feline (Felis catus) implants were performed as described in (Parker, Davis, House, Normann, & Greger, 2011). Arrays were placed

Table 3.2. Stimulation applied with pre- and poststimulation impedance readings.

Session	Description	Stimulus Parameters	Amplitudes	Electrodes
Pt in vitro	Saline test of stimulation	10 trains, 25 pulses of 100 Hz, 0.2 msec/phase	5, 10, 15 and 20 μA	47 out of 91 electrodes stimulated
Feline 4 202 days post-implant	Microstimulation without EMG under Telazol	5 trains 25 pulses 100 Hz, 0.2 msec/phase	100 μA	All electrodes (1 at a time)
Feline 4 209 days post-implant	Microstimulation with EMG under Telazol	5 trains 25 pulses 100 Hz, 0.2 msec/phase	100 μA	All electrodes (1 at a time)
Feline 4 230 days post-implant	Microstimulation with EMG under Telazol	5 trains 25 pulses 100 Hz 0.2, 0.4, and 0.6 msec/phase	100 μA	All electrodes (1 at a time)
Feline 4 274 days post-implant	Microstimulation with EMG under Telazol	5 trains 25 pulses 100 Hz, 0.6 msec/phase	100, 150, 200 μA	All electrodes (1 at a time)

Table 3.2 continued

Feline 4 279 days post-implant	Microstimulation with EMG under Telazol	5 trains 25 pulses 100 Hz, 0.2-0.6 msec/phase	200, 250, 300 µA	All electrodes (1 at a time)
Feline 4 286 days post-implant	Test of Blackrock micro-stimulator; TDT stimulator validation	Single trains 25 pulses 100 Hz;200 Hz	150 and 215 µA; 300 µA	18 electrodes (2 simultaneous sets of 9)
Feline 4 307 days post-implant	Single-electrode stimulation efficacy test under Telazol	30 trains 25 pulses 100 Hz, 0.6 msec/phase	200 µA	2 electrodes (11 and 90)
Feline 4 428 days post-implant	Multichannel stimulation under Telazol	5 trains 25 pulses 100 Hz, 0.2 msec/phase	50, 100, 150, 200 µA	72 electrodes in distributed pattern of 9 electrodes simultaneously

in motor cortex (Ghosh, 1997). All feline implants were performed by a clinical neurosurgeon to ensure consistency. Animals were given at least 24 hours to recover prior to data acquisition.

Manipulation of anesthesia depth: Initial anesthesia depth results were noted across time when Feline 4 was anesthetized with a bolus injection of Telazol. Further stimulation efficacy tests were performed in Feline 4 using both Telazol bolus injections and a propofol infusion system. Propofol was controlled as described in (Hanrahan et al., 2012). Propofol, a sedative hypnotic GABA-a antagonist, was administered via a Harvard perfusion pump controlled with STANPUMP (Stanford University) software. Plasma concentration level (PCL) of propofol was determined using a target-controlled infusion model (Egan, 2003) based on canine data (Lee et al., 2009). The model was adapted for felines using feline-specific propofol metabolic data (Bester, 2009). Eye blink, ear twitch, and toe pinch withdrawal reflexes were monitored, as were heart rate, respiratory rate, and blood oxygenation levels. Impedance measurements and neural recordings were taken under various levels of propofol; however, the window for evoking EMG responses under propofol was too narrow to form myometric curves.

Electromyogram (EMG): Feline 4 was anesthetized with either Telazol or Propofol. Sterile, clinical fine-wire electrodes were placed in neck and forelimb muscles which had been observed to twitch in previous experiments. Large, low-impedance surface reference electrodes were placed proximal to recording electrodes, and a large ground electrode was placed on the stomach. EMG activity in response to stimulation was recorded at 25k samples/sec using an Intan RHA-2000 EVAL amplifier board (Intan Technologies, Los Angeles CA).

Data analysis

Spike sorting was performed using a t-dist EM algorithm (Shoham, 2003) built into the commercially obtained Offline Sorter (Plexon, Inc. Dallas TX). All other data analysis was performed using in-house Matlab (The Mathworks, Inc., Natick MA) code or Excel (Microsoft, Inc., Redmond WA).

Microstimulation

Six daisy-chained RX-7 stimulators (Tucker-Davis Technologies Inc, Alachua FL) were used for stimulation *in vitro*. Stimulation in Feline 4 was applied via an IZ2 stimulator (Tucker-Davis Technologies), capable of delivering 300 µA of current across 50 kOhms on all 96 electrodes of the array simultaneously. Stimulation was controlled by in-house Matlab and LabView (National Instruments, Austin, TX) code. Constant-current stimuli were applied in charge-balanced square waveforms to prevent charge build-up (Merrill, Bikson, & Jefferys, 2005). Stimulation parameters are detailed in Table 3.2.

Results

Impedance increased significantly for SIROF electrodes following pneumatic insertion into agarose and cleaning (Figure 3.2), suggesting that device damage occurred. Furthermore, the number of electrodes remaining in-specification (<2 MOhm) after agarose insertion tests decreased by 13 electrodes, again suggestive of device damage. Pt impedances, however, were unaffected by agarose insertion once cleansed, though three electrodes were out of specification following implantation. No traces of metallization or electrode tips were found in the implant site under light microscopy. Impedance also increased while in agarose and in cortex (Figure 3.2), decreasing to saline baseline when removed and cleansed for Pt, indicating that the major portion of increased impedance readings can be attributed to the environment of the electrode. Impedances were 387+/- 222 kOhm in cortex compared to 236/- 186 kOhm for Pt in agarose; 96 +/- 56 kOhm in cortex and 73 +/- 61 kOhm in agarose for SIROF, indicative of the increased resistivity of the tissue environment. Finally, impedance decreased with increased temperature in saline (Figure 3.2), which is expected as conductivity increases with increased energy in the system. Changes in impedance were greater for SIROF than Pt, indicating that porosity of metallization likely has an effect on impedance.

Impedance is known to decrease with the acute application of stimulation *in vitro*, which has been linked to electrochemical activation via changes in the valence state of metallization. It has also been shown that impedance decreases acutely *in vivo*, which has been attributed to the

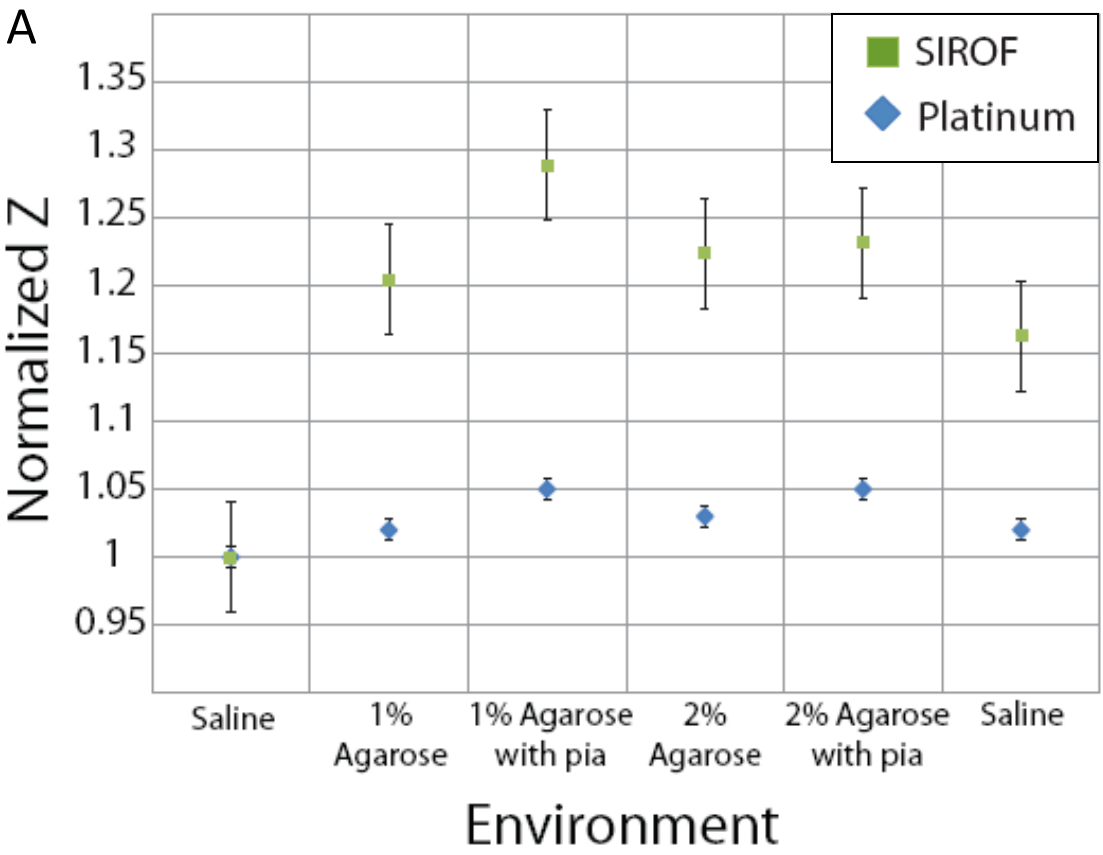
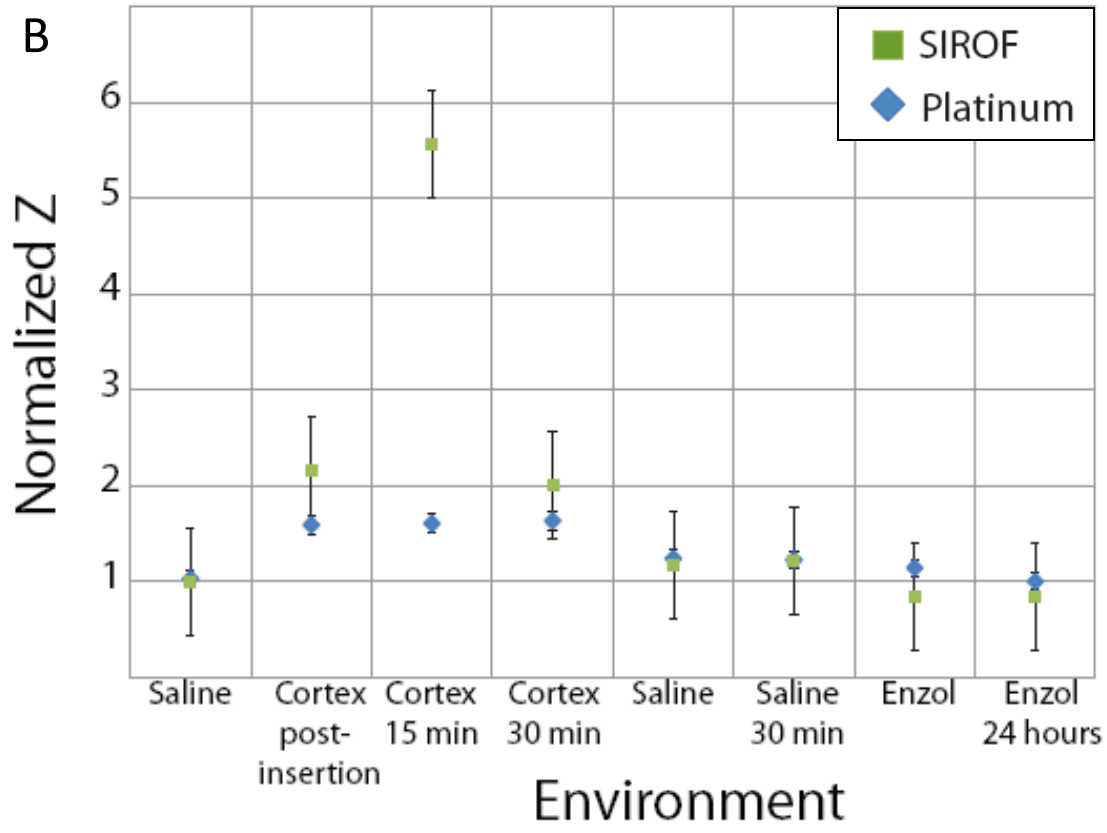
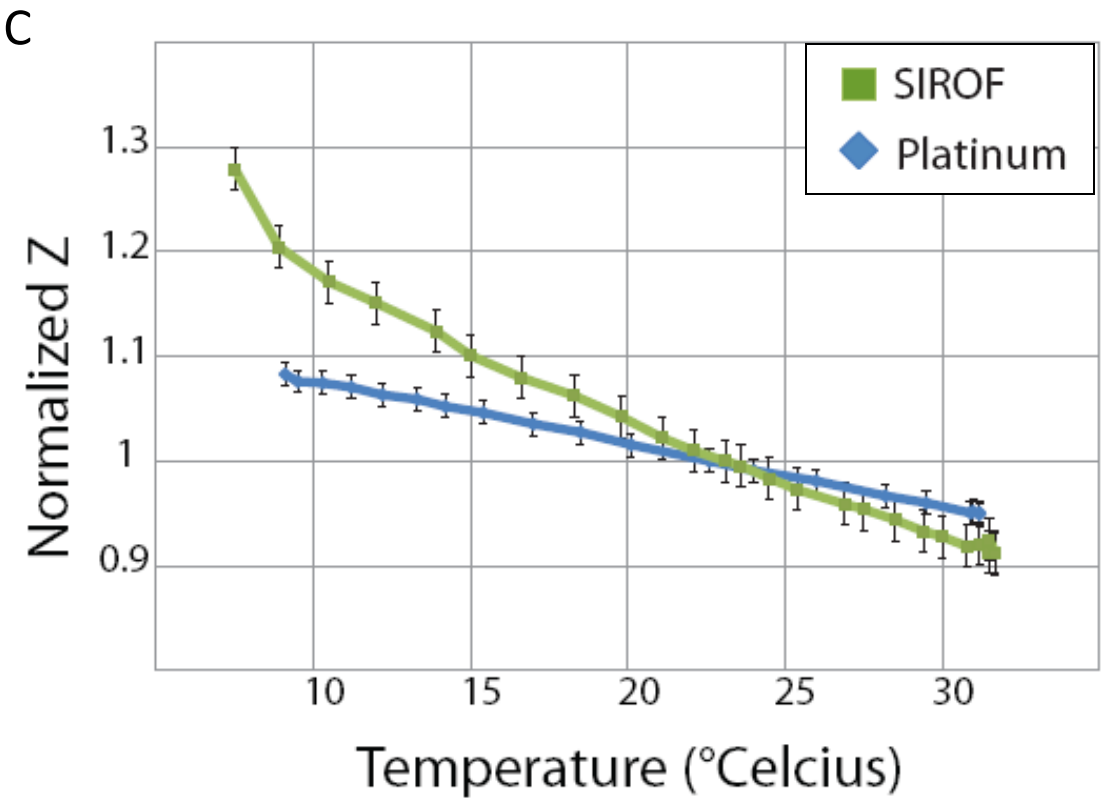


Figure 3.2. Impedance measurements reflect both acute device damage and electrode environment. A. Impedance normalized to saline baseline. Impedances taken in successive pneumatic insertions into 1%, 1% with artificial pia, 2%, and 2% with artificial pia agarose. Arrays were subsequently rinsed in saline. Pt impedances returned to baseline following enzymatic cleaning, but SIROF did not. B. Impedances in cortex, normalized to a preimplant saline baseline. Impedances were read over the course of 30 minutes in cortex, followed by a 30 minute saline rinse and a 24 hour Enzol soak. C. Impedances in a warmed beaker of saline.

Figure 3.2 continued





disruption of scar tissue surrounding electrodes. We confirmed drops in impedance both *in vivo* and *in vitro* (Figure 3.3). Impedances on the Pt array dropped in saline not only on electrodes that were stimulated, but also on electrodes that were not stimulated. Z increased towards baseline over time, but did not reach baseline at 72 hours. A test of stimulation on one SIROF array with impedance measurements on a passive array placed proximal in a petri dish of agarose was performed. However, the number of electrodes in-specification on the stimulating array at the time of the test was inadequate (<20) to draw statistically significant conclusions about the field effects of electrochemical activation, and inconclusive results were obtained on the recording array.

In vivo impedance drops were observed on all electrodes following each stimulation session (Figure 3.3), despite the variable number of electrodes stimulated and the variation in stimulation parameters noted in Table 2. In some cases, very few electrodes were stimulated yet mean Z dropped significantly across the array. The baseline mean Z was also variable across the stimulation time frame, in keeping with results from our previous work in which Feline 4 was used (Parker et al., 2011). Additional stimulation sessions were performed in Feline 4 in between reported sessions; only sessions in which pre- and poststimulation impedance readings were taken are shown.

To investigate the correlates of this impedance variability, datasets from Felines 1-4 of our previous work were investigated. It has been demonstrated that acute impedance drops correspond with an increase in recording performance, attributed to increased listening radius. To test the hypothesis that decreased impedance predicts increased recording ability, histograms were made of impedance in all 4 animals dependent on whether or not an action potential was recorded on that electrode (Figure 3.4a). It was found that there was no difference in the distribution of recording and nonrecording electrode impedance in Felines 2 and 3, though these felines had a particularly low action potential yield. In Felines 1 and 4, both of which demonstrated high AP yields, electrodes that successfully recorded action potentials exhibited *higher* impedances than electrodes which did not ($p < 0.05$, Student's 1-tailed t-test). This relationship was explored over time using mean Z on all electrodes in conjunction with the

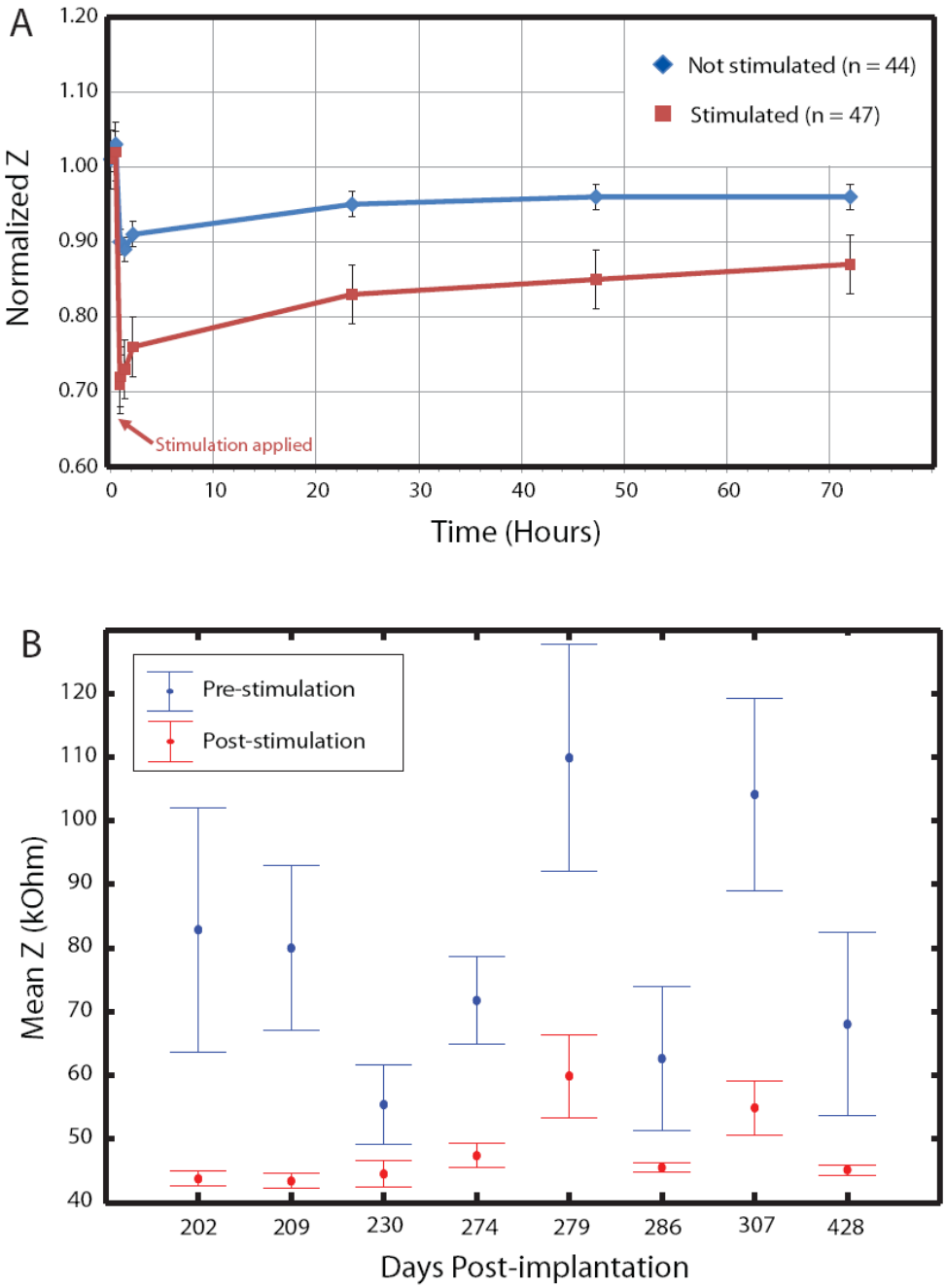


Figure 3.3. Impedance drops both *in vitro* and *in vivo* with acute microstimulation. Stimulation was applied according to Table 2. A. Stimulation was applied to half of the Pt array in a beaker saline. Impedances were measured at intervals for 72 hours. Impedances decreased on both stimulated and nonstimulated electrodes. Mean impedance and standard errors are shown. B. Pre- and poststimulation impedance measurements were taken from 8 stimulation sessions in Feline 4 at >6 months postimplantation. Mean impedance values and standard deviations are shown. Variability in prestimulation impedance values may be due to intervening microstimulation sessions and variable number of days between stimulations.

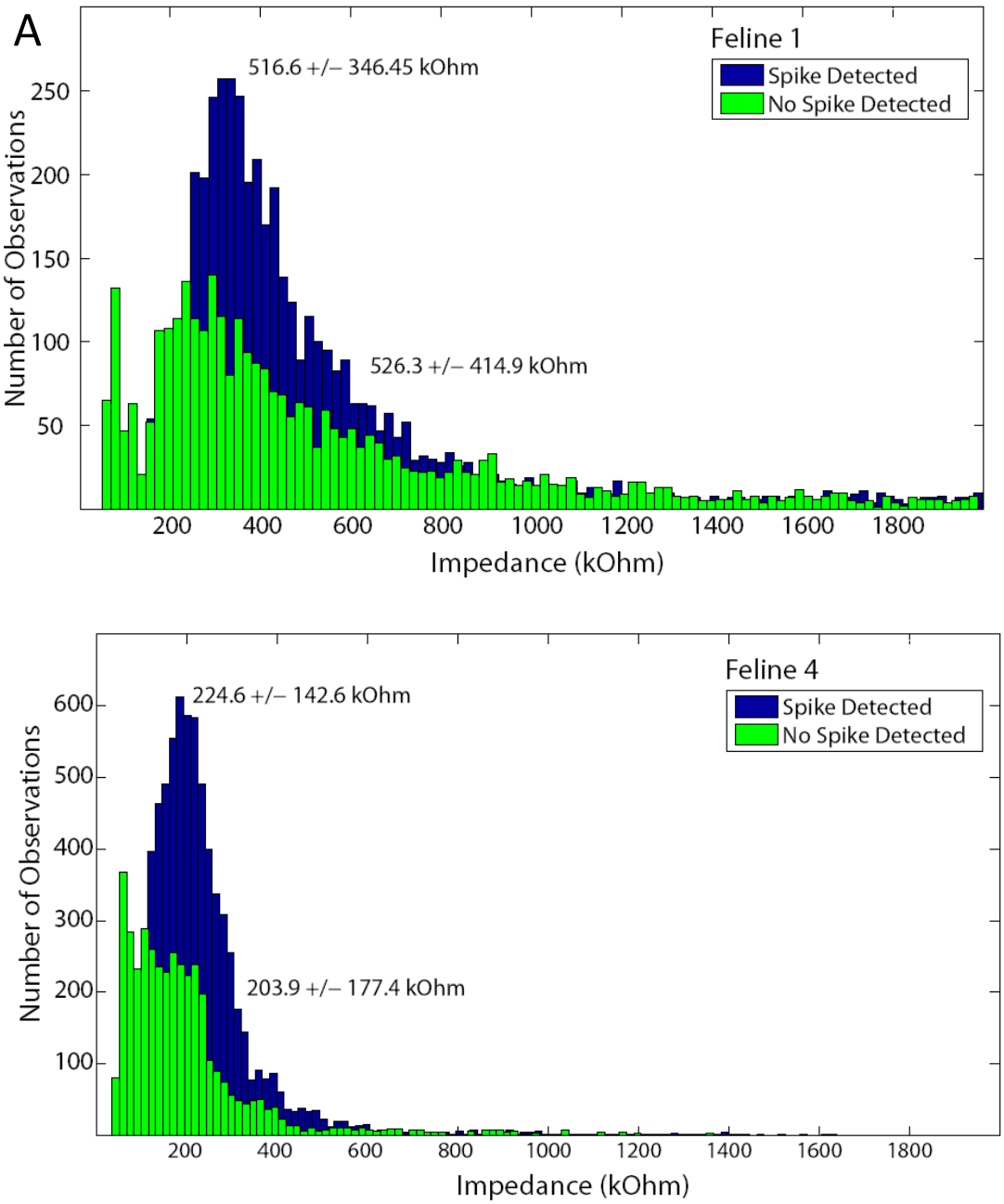
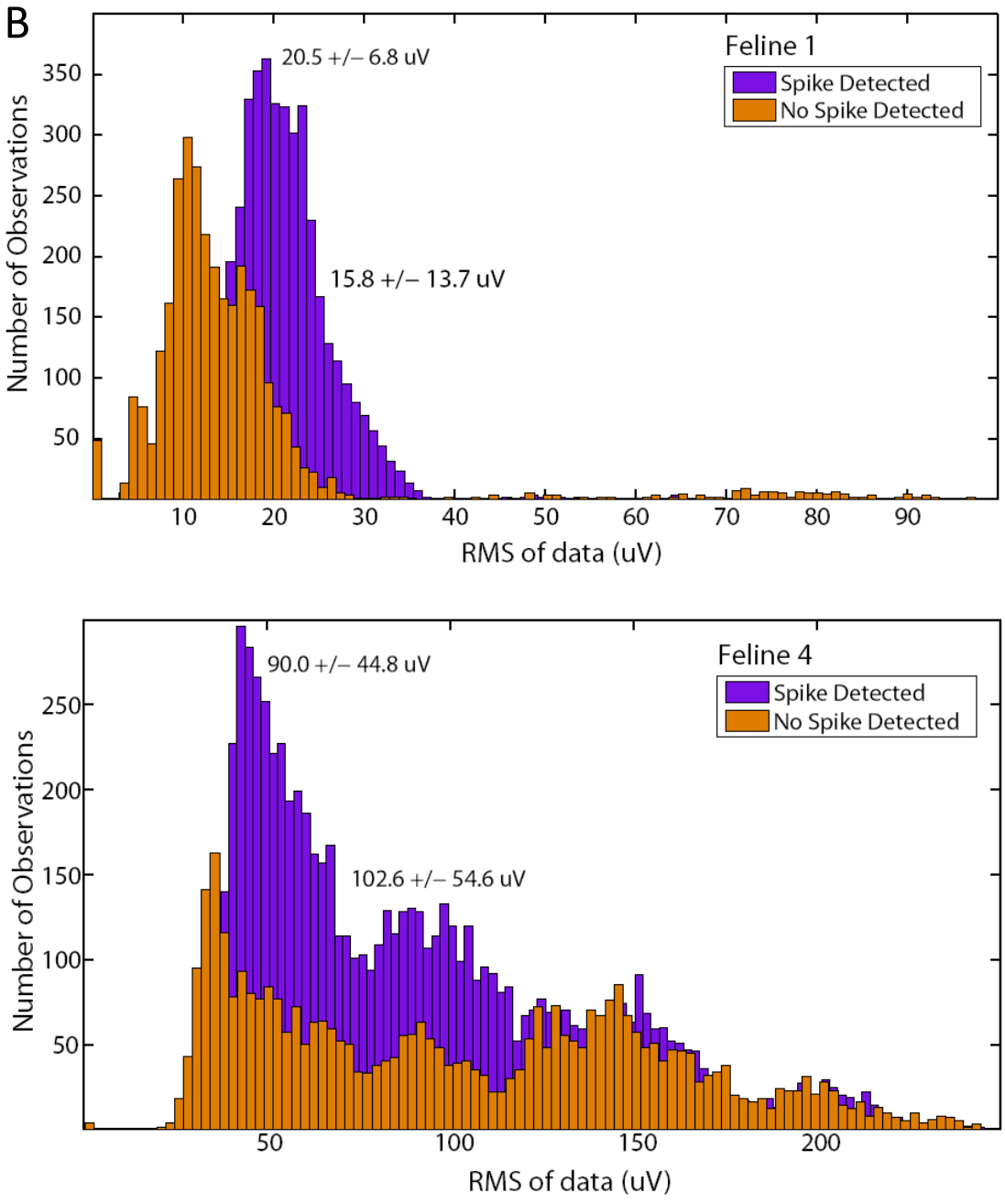


Figure 3.4. Decreased impedance does not predict increased recording ability. A. Histograms of impedances measured based on whether or not action potentials were recorded in Felines 1 and 4. Impedances were significantly higher on AP-recording electrodes in Felines 1 and 4 (one-tailed t-test, $p< 0.05$). There was no significant difference in the distributions on Felines 2 and 3, likely due to the low number of action potential recordings (<33% of in-specification electrodes) in these animals. B. Histograms of root-mean-square (RMS) of voltage data (first 60 seconds of each recording session) amplitude on electrodes which did and not record spikes (same electrodes as in 4a). RMS distributions were significantly different ($p < 0.01$) in both Felines 1 and 4 (Komolgorov-Smirnov test).

Figure 3.4 continued



probability of spike detection in Felines 1 and 4. The relationship between impedance over time and spike detection, however, is less clear than the individual electrode comparisons.

In addition to investigations of device damage and the use of impedance as a reporter, tests of microstimulation efficacy based on anesthesia depth were performed. Multiple experiments were performed using bolus injections of Telazol. Once the feline was anesthetized, stimulation was performed in rounds spaced 10 minutes apart and EMG was recorded during each round. The findings from one such stimulation session are shown in Figure 3.5. Stimulation at the same stimulus parameters yielded a higher response to stimulation at 15 minutes post-injection compared to 5 minutes postinjection on all EMG electrodes. Visual observation of twitches confirmed these results. In fact, on all Telazol-bolus EMG experimental sessions, visible twitches appeared more robust as the animal's reflexes indicated a decreased depth of anesthesia. To test this effect, stimulation was performed under varied PCLs of propofol. Unfortunately, Feline 4 did not exhibit motor responses to stimulation under PCLs (PCLs) of 8-12 ug/dL; nor was voluntary motion inhibited at PCLs less than 8. This narrow window for anesthesia prevented correlating motor responsiveness with anesthesia depth. However, impedance measurements were taken at each PCL to determine if depth of anesthesia, and by extrapolation cortical excitability, had an effect on impedance (Fig. 5b). It does not appear that anesthesia affects measured Z.

Discussion

Many studies have used Z as a noninvasive measure of the state of the tissue-electrode interface. Results in other studies have shown that Z increases over the course of a month, reaching a plateau level at a few weeks (Ward et al., 2009; Williams et al., 2007). While this increase was observed in our studies, mean impedance was variable with a tendency to decrease over the course of several months (Parker et al., 2011) . It is expected that if impedance does reflect tissue response, we would see the plateau value maintained over several months, as the glial scar has completely formed over the course of 4-6 weeks and is a resistive barrier. We would also predict that the impedance of electrodes surrounded by a thick glial sheath would be

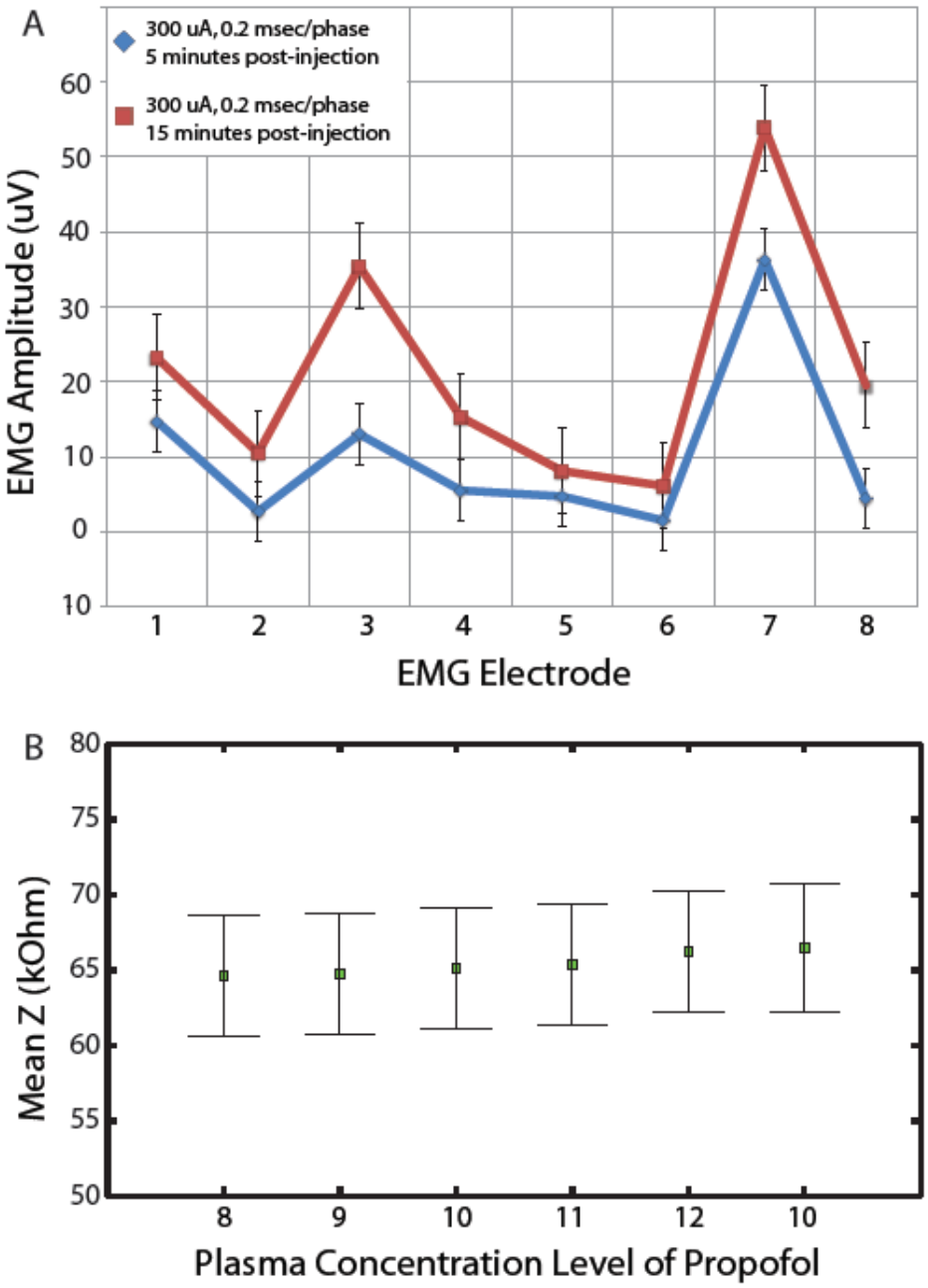


Figure 3.5. Anesthesia depth affects stimulation ability, but not impedance. A. Mean rectified electromyographic response to stimulation at 5 minutes after Telazol and 15 minutes after Telazol. Eight electrodes were placed in the neck and shoulder muscles of Feline 4. Stimulation is listed in Table 2 under Feline 4, 279 days postimplantation. EMG responses were significantly larger 15-minutes poststimulation ($p < 0.01$, one-tailed t-test) B. Mean impedance values on 86 electrodes across various concentrations of propofol. No twitches were noted in response to test stimulation over the course of this experiment. No significant change in impedance occurred with an increasing plasma concentration of propofol.

higher than that of less encapsulated electrodes, and that less encapsulated electrodes would be more likely to record an action potential. However, we found that this was not the case. In fact, electrodes with higher impedances appeared more likely to record action potentials than those with lower impedances.

One possible explanation for decreasing impedances over time is that the array could be gradually working its way out of cortical tissue. If this were the case, however, we would not expect to record viable action potentials on any channels. The continued presence of action potentials suggests that array is still within the recording radius of neuronal tissue. Another possible explanation is the development of edema surrounding the electrode. This effect has been observed with deep brain stimulation electrodes. Fluid, such as cerebrospinal fluid, has lower impedance than cortical tissue, as impedance mainly reflects the physical structure of the environment surrounding the electrode. If a fluid layer formed, it would account for both decreased impedance on electrodes that did not record action potentials, and for the increased number of electrodes required to stimulate to effect observed in both Feline 4 and a nonhuman primate (Davis et al., 2012); fluid would provide a path of least resistance for stimulating current, thereby creating a “bioshunt”. Such shunting is also consistent with the decreased RMS noise recorded on electrodes that did not record action potentials.

In addition to impedance decreases over time, impedance decreased acutely with the application of stimulation both *in vitro* and *in vivo*. Other groups have observed decreases in impedance with stimulation; some have noted a corresponding increase in signal to noise ratio of neural data recorded on an electrode “rejuvenated” using this technique. However, it has been observed that the ability to effectively rejuvenate is temporary. Microstimulation *in vitro* may be decreasing impedance by electrochemically activating the Pt metallization. The decrease in Z on both stimulated and nonstimulated Pt electrodes *in vitro* suggests that some form of field effect or interelectrode shunting may occur. This emphasizes the importance of measuring shunting in addition to Z, which may be possible with alternative measurement techniques (Gunalan, Warren, Perry, Normann, & Clark, 2009).

It appears that the microstimulation induced decrease in Z in saline may result from electrochemical activation effects, and it is possible that some portion of rejuvenation is due to this mechanism *in vivo.* An acute decrease in impedance may serve to temporarily increase the listening radius of the electrode via electrochemical mechanisms, rather than disrupting the glial scar surrounding the electrode as has been hypothesized. If this is the case, the overriding trend towards loss of signaling and decreased impedance, possibly due to the development of a fluid layer, would preclude the temporary increased recording ability imparted by stimulation. Histological examination, particularly using advanced microscopy techniques in glial scar models (Polikov, Block, Fellous, Hong, & Reichert, 2006) or in chronically implanted tissue, will provide valuable insight on the biological processes contributing to observed impedance and recording trends. Further tests of the effects of microstimulation are also warranted. Such tests may serve to elucidate the mechanisms by which impedance decreases over time and with stimulation, concurrent with decreased ability to record action potentials.

In addition to the relationship between impedance and recording ability, it was found that impedance decreased with increases in temperature. The effect was greater for SIROF electrodes than Pt. While this makes sense in the context of increased conductivity at higher temperatures, it should be noted that the difference is significant enough to have an impact on results reported *in vivo,* which are taken at body temperature, as compared to typical *in vitro* experiments performed at room temperature. In fact, this effect has been noted with other forms of microelectrode technology, but should be considered when testing electrodes prior to implantation. Interestingly, temperature has been implicated in cortical excitability (Kowski, Kanaan, Schmitt, & Holtkamp, 2012), further emphasizing the importance of considering temperature when reporting results in chronic studies.

Another factor found to impact impedance was acute implantation. While Pt electrode impedances recovered following pneumatic insertion into agarose, SIROF impedances did not recover completely to baseline. This suggests that SIROF may have been damaged with insertion, though no metallization was observed in the implant site. Of course, it must be noted that the agarose used is more rigid than cortical tissue; SIROF may not be damaged during

typical cortical implantations. If the initial insertion causes changes in impedance, however, this would cause an initial change in impedance independent of the environment surrounding the electrode. Furthermore, if the integrity of the metal coating is damaged on insertion, damage to the electrodes may be exacerbated with microstimulation. Mechanisms of dissolution are known to occur with microstimulation via Pt electrodes, and delamination has been observed with SIROF (S. F. Cogan, 2008). Z did not recover completely to baseline in either SIROF or Pt electrodes following stimulation, suggesting that the effects of stimulation could be cumulative and could hasten performance declines. It is possible that stimulation causes electrostatic repulsion between the electrode and the insulating Parylene-C surrounding it, allowing the ingress of fluids and tissue constituents. This could lead to permanent decreases in electrode impedance by effectively increasing the surface area of the electrode, in addition to reversible drops in impedance as a result of electrochemical activation effects. While it would take advanced techniques to visualize this process, confirmation of such electrostatic effects would be valuable. Both processes of initial damage and damage with stimulation require further research, but the results of this study should be considered when interpreting impedance measurements in chronic studies.

Finally, increased anesthesia depth has been linked to decreased cortical excitability (Hanrahan et al., 2012). Corresponding to these results, we found that it required more charge to stimulate to effect when the animal was deeply anesthetized than when lightly anesthetized. While attempts to quantify this relationship under propofol anesthesia were unsuccessful due to the narrow window between wakefulness and sleep, Telazol anesthetic results indicated that anesthesia depth does in fact play a role in ability to stimulate to effect. As microstimulation is often performed in anesthetized preparations, it is important to consider the effects of anesthetic depth on ability to stimulate. Interestingly, impedance was not related to anesthesia depth or, by extrapolation, cortical excitability. Similar results of anesthesia on impedance have been obtained in a study using multiple anesthetics (Golovchinskii & Rapoport, 1971), and have been hypothesized to result from the small effect of neuronal membranes on total cerebral impedance. Further investigations to quantify the effects of anesthesia depth on stimulation ability are

warranted, particularly using alternative anesthetics for which models of anesthesia depth have been developed.

The results of this study have indicated that many factors affect the results for chronic intracortical MEA studies. Furthermore, device performance may be impacted by these factors, including device damage, electrochemical effects of microstimulation, and depth of test subject anesthesia. Future experiments will help tease out the contributions of these processes to the long-term performance of microstimulating MEAs, and may assist in the development of more robust assays for performance.

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CHAPTER 4

THE USE OF A NOVEL CARBON NANOTUBE COATED MICROELECTRODE ARRAY FOR CHRONIC INTRACORTICAL RECORDING AND MICROSTIMULATION

Abstract

Microelectrode arrays (MEAs) have been used in a variety of intracortical neural prostheses. While intracortical MEAs have demonstrated their utility in neural prostheses, in many cases MEA performance declines after several months to years of *in vivo* implantation. The application of carbon nanotubes (CNTs) may increase the functional longevity of intracortical MEAs through enhanced biocompatibility and charge injection properties. An MEA metalized with platinum (Pt) on all electrodes had a CNT coating applied to the electrodes on half of the array. This Pt/Pt-CNT MEA was implanted into feline motor cortex for >1 year. Recordings of action potentials and local field potentials, and 1 kHz impedance measurements, were made on all electrodes to evaluate device functionality. Additionally, EMG was evoked using microstimulation via the MEA to measure device performance. These metrics were compared between Pt and Pt-CNT electrodes. There was no significant difference in the data acquisition or microstimulation performance of Pt and the Pt-CNT electrodes, however, impedances were lower on the Pt-CNT electrodes. These results demonstrate the functionality of CNT coatings during chronic *in vivo* implantation. The lower impedances suggest that for microstimulation applications CNT coatings may impart enhanced interface properties.

Introduction

Microelectrode arrays (MEAs) are promising devices that enable bidirectional communication with the central nervous system. MEAs provide a high-selectivity method for recording information from, and imparting information to, neural tissue. While MEAs have already proven their utility for clinical neural prosthetic applications (Hochberg et al., 2006), it has been noted that over time, the ability of MEAs to obtain high-yield, stable action potential recordings decreases (Linderman et al., 2006; Parker, Davis, House, Normann, & Greger, 2011; Suner, Fellows, Vargas-Irwin, Nakata, & Donoghue, 2005). Many underlying causes have been hypothesized to account for these dynamics in performance, including device damage, cortical plasticity, and the tissue response to the implant. Tissue response is a particularly complicated problem that is known to occur with any type of injury to the cortex. It comprises a complex, interconnected network of mechanisms, including macrophage activation, disruption of the blood brain barrier, and glial scarring, all of which can cause both neuronal death and changes in neuronal signaling (Polikov, Tresco, & Reichert, 2005; Turner et al., 1999). Many surface modification intervention strategies have been tested in order to attenuate the tissue response, and thus, improve long-term MEA performance in cortical applications (Cui, Wiler, Dzaman, Altschuler, & Martin, 2003; Wadhwa, Lagenaur, & Cui, 2006). One such promising modification is the use of carbon nanotubes (CNTs) (Pancrazio, 2008). CNTs have a variety of beneficial electrical properties, such as high capacitive charge injection limits and high conductivity which make them ideal for use in electronic interfaces with the brain. Furthermore, CNTs offer a variety of methods for the attachment of biomolecules, including covalent modification, noncovalent attachment, and biomolecule wrapping (Voge & Stegemann, 2011). CNT coatings have been shown to improve neuronal recordings (Keefer, Botterman, Romero, Rossi, & Gross, 2008). In this study, we further characterized the long-term performance of a CNT coating applied to a chronic intracortical MEA used for both microstimulation and recording, in order to determine if the beneficial electrical properties imparted enhanced stimulation ability, and to ensure that CNTs do not adversely affect long-term MEA performance.

Methods

Microelectrode array

The MEA used in this study was a commercially obtained 96-electrode platinum (Pt) Utah Electrode Array (Blackrock Microsystems, Inc.). Forty eight of the 96 electrodes were coated with commercially obtained multiwall CNTs (CheapTubes, Inc.) by Plexon, Inc. as described in [11]. Prior to implantation, the array was imaged under scanning electron microscope, and electrochemical impedance spectroscopy and cyclic voltammetry were performed to confirm the presence of the CNT coating.

Feline implant

Implantation was conducted in January of 2011 by a clinical neurosurgeon. Implantation procedures followed those described in [4]. Briefly, the cortex was exposed over the right motor cortical region, and the array was pneumatically inserted to minimize tissue disruption. The array was then covered with a silicon polymer, the percutaneous connector was attached to the skull using bone screws, and the scalp was sutured shut. The animal was given 48 hours to recover prior to attempting data acquisition.

Neural data acquisition

Neural recordings were obtained daily for the first 90 days following implantation, and at least weekly for the subsequent 3 months. Recordings were made using a patient cable, amplifier, and Cerebus system from Blackrock Microsystems, Inc. Sample data is shown in Figure 4.1. During data acquisition, the awake animal was placed in a standard pet carrier and the patient cable was attached to the percutaneous connector. The animal was allowed to behave as normal during two 5-minute recordings, band-pass filtered at 0.3Hz – 7.5 kHz and acquired at 30 kS/sec. Following neural data acquisition, 1 kHz impedance measurements were made using a proprietary mechanism built into the Blackrock patient cable.

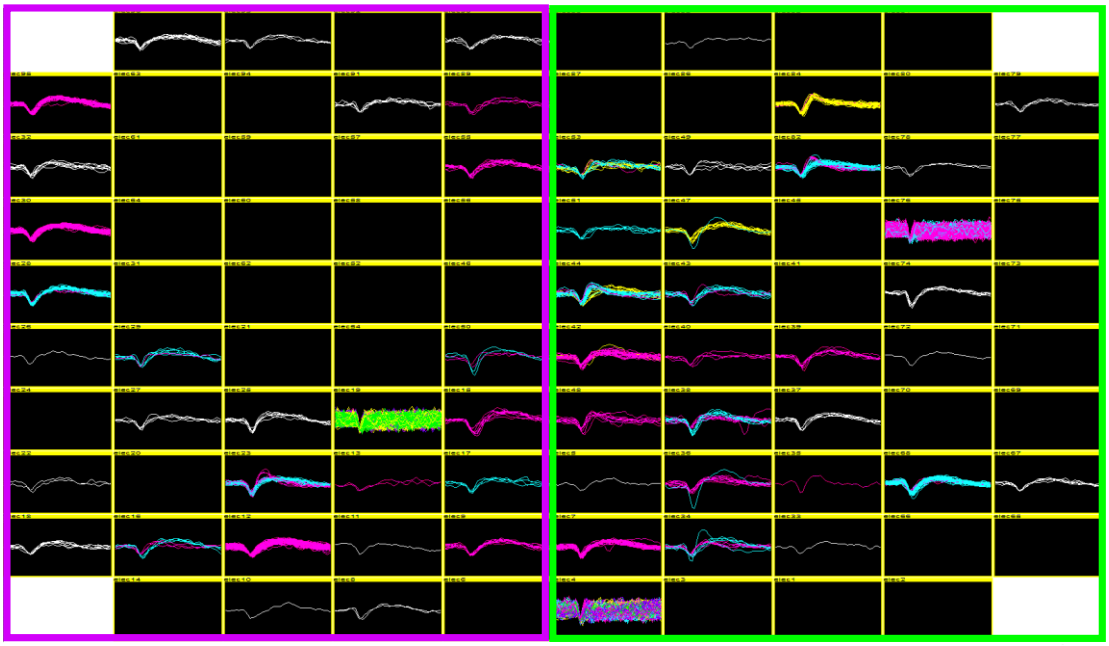

Figure 4.1. Action potential data recorded at 1 month postimplantation. Purple box denotes Pt-only portion of the array; green indicates CNT coated electrodes. Note similar numbers of well-formed action potentials on both sides of the array, as well as noise recordings on both portions.

Microstimulation and electromyogram

During microstimulation sessions, the feline was anesthetized with Telazol in order to prevent spontaneous movement. Microstimulation was performed under current control using an IZ2 system fed through the feline's percutaneous connector (Tucker-Davis Technologies, Inc.) The voltage excursion of this system is ±15V, allowing for microstimulation currents of up to 300 μA across a 50 kOhm load. Stimulation amplitude was varied from 50-300 μA in intervals of 50 μA. Trains of 25 biphasic pulses, at pulse durations of 0.2-1 msec, were applied one electrode at a time to all electrodes of the array on two occasions in order to determine the amplitude of current required to evoke a motor response. Motor responses were recorded at 25 kS/sec using an Intan amplifier board (Intan Technologies, Inc.) and bipolar fine-wire electrodes placed in the hind limb, trunk, and neck muscles of the feline. Data acquired using the Intan board, recording of stimulation parameters through the Cerebus system, and information from the stimulator was synchronized using in-house Matlab (The Mathworks, Inc.) code.

Data analysis

Action potentials were extracted from the 0.3Hz – 7.5 kHz data using a modified t-dist EM principal component analysis algorithm (Shoham, 2003). Following spike sorting, a 90 μA threshold was applied to sorted action potential data in order to remove noise clusters using in-house Matlab code. All local field potential, impedance, and statistical analysis was also performed using in-house Matlab code.

Results

Results of recordings, stimulation, and impedance measurements were compared between the Pt-only and CNT portions of the array as an internal control. Further comparisons were made between the long-term performance of the novel array and sputtered iridium oxide film arrays previously implanted in the same intracortical feline preparation by our group (Parker et al., 2011).

Pt and CNT show similar performance

Counts of well-isolated action potentials were used as a measure of performance over time. As seen in Figure 4.2, the number and amplitude of recorded well-isolated action potentials changed over time, but there was not a significant difference in the number of action potentials recorded between Pt and CNT portions of the array. The yield over time of action potentials recorded was similar to the fade-in, high yield, fade-out pattern observed in SIROF array animals (Parker et al., 2011).

CNT coated electrodes retain stimulation ability

Stimulation sessions were performed on all electrodes of the array, one at a time, on two separate occasions, with EMG recordings as a measure of efficacy. Shown in Figure 4.3 is a sample mean binned rectified EMG response across 5 100 Hz trains of 25 biphasic pulses, 0.4 msec/phase and 100 μA current on a stimulated CNT channel. Few such response were evoked on either side of the array, but were evoked via microstimulation of both Pt and CNT channels.

CNT electrodes have low impedances

One kHz impedance measurements were used to evaluate the state of the electrode-tissue interface over time (Figure 4.4). It was found that CNT electrodes exhibited lower impedances over time (Wilcoxon's signed rank test, $p < 0.05$). Furthermore, the pattern of mean impedance followed a similar pattern to that previously observed in animals implanted with SIROF arrays for both Pt and CNT electrodes, namely, an initial increase to a plateau value followed by fade out over time. Impedances did decrease acutely with the application of microstimulation on both Pt and CNT electrodes, but recovered towards baseline after 24 hours.

Discussion

Chronic recording performance

Action potential yield followed a fade-in (low yield during the first days), high-yield (plateau over several weeks), fade-out (gradual decline in yield) pattern similar to that described

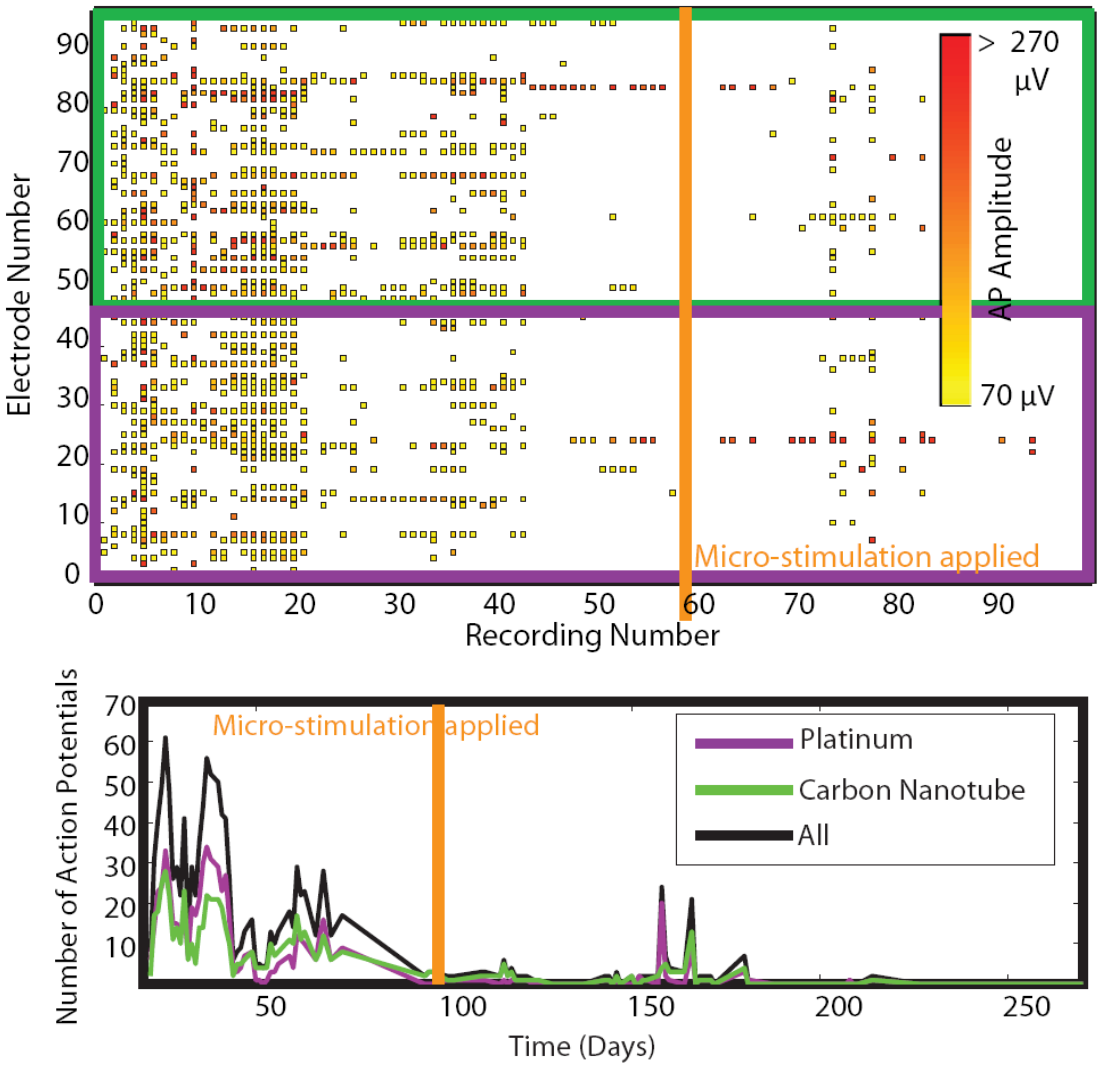

Figure 4.2. Action potential recordings over time. Top: each square represents a well-isolated action potential, with amplitude indicated by the color of the square. Purple box denotes Pt electrodes, while green box denotes CNT. The orange line represents the first application of microstimulation. The ability to record action potentials was maintained following microstimulation. Bottom: yield of action potentials, Pt yield, and CNT yield.

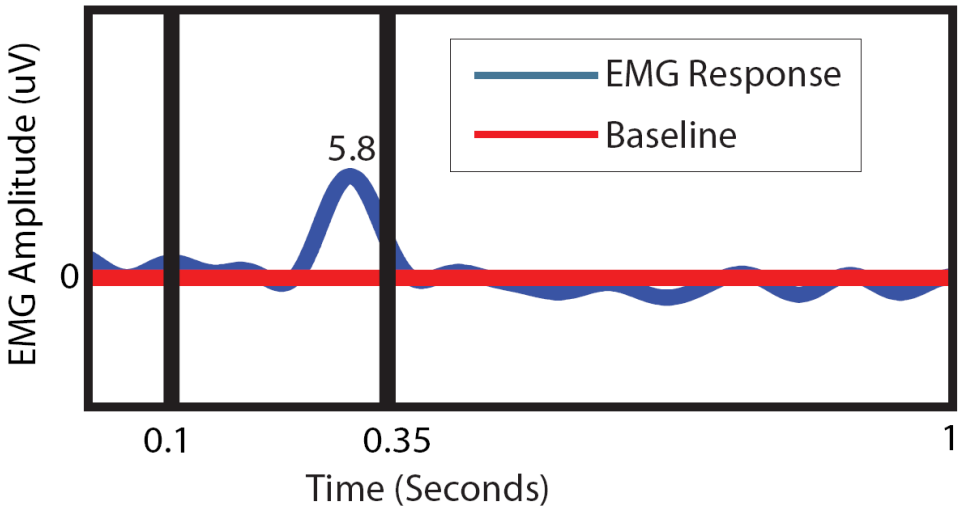

Figure 4.3. Example of mean binned rectified electromyographic response to stimulation applied. Stimulation was applied in 25 biphasic pulses at 100 Hz between 0.1 and 0.35 seconds on a CNT electrode following >3 months of implantation. Electromyogram recorded muscle twitches, such as this one, were used to indicate efficacy.

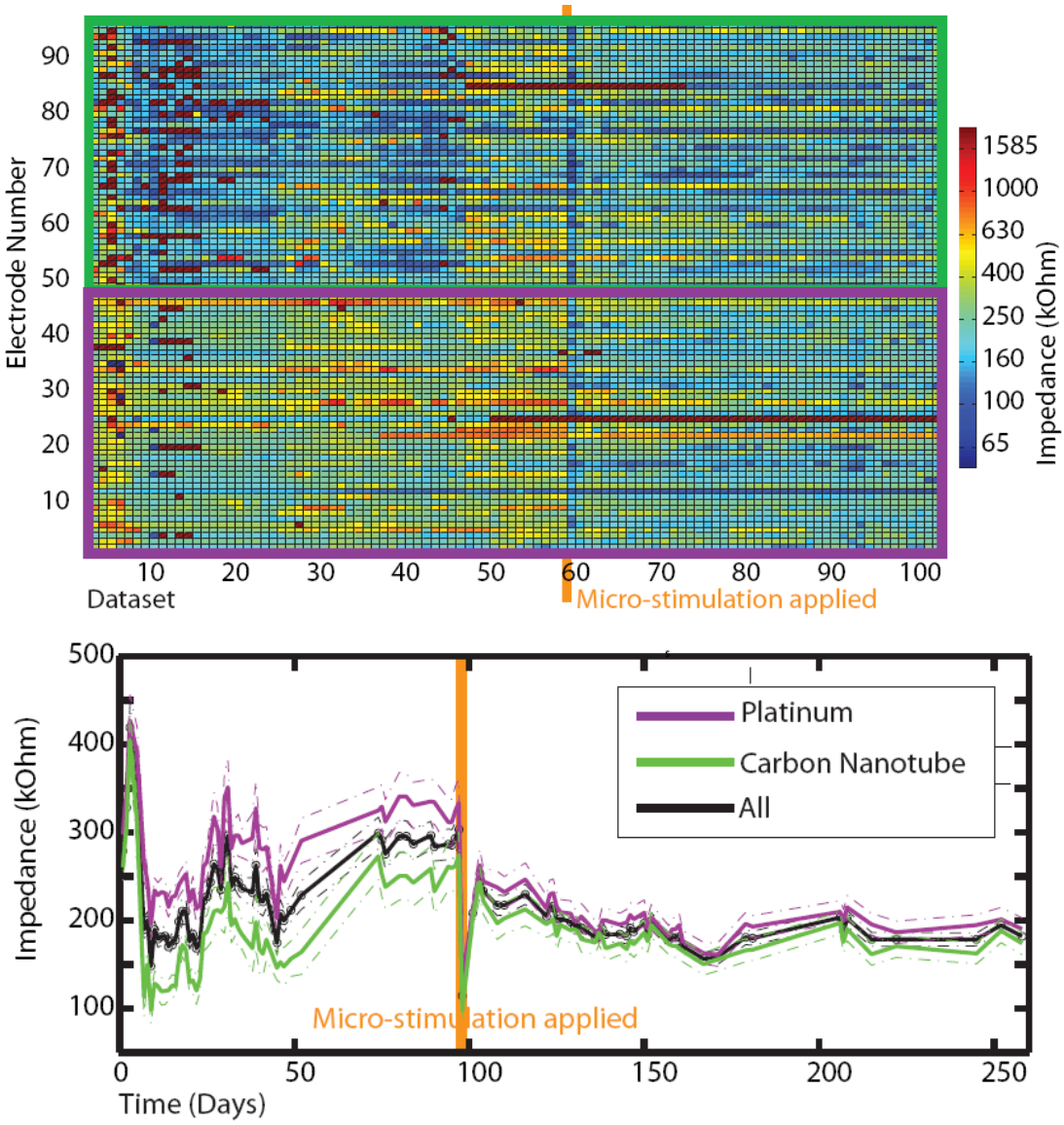

Figure 4.4. One kHz impedance measurements over time. Top: each box represents the impedance on one electrode during one data acquisition session. The value of the impedance is denoted by the color of the box. CNT electrodes are outlined in green, Pt electrodes in purple. The impedances for the CNT portion of the array were lower than that of the Pt. Impedance decreased abruptly following the initial microstimulation session, shown by the orange bar. Bottom: mean impedances across days.

in Linderman et. al., (2006) and Parker et. al., (2011), which is generally accepted as the time course of recording performance for most MEAs. This pattern may be a result of the tissue response to the implant. The initial trauma of implantation is known to disrupt the local vasculature, which may cause the initial low yield of action potentials. Over the first few days of implantation this response clears and gives way to the chronic response, during which time the yield of action potentials increases. Over time, however, the glial scar may push neurons outside the recording radius of electrodes, lead to neuronal death, or cause neurons to cease signaling, which may result in the observed fade-out of action potential yield. As no significant differences were observed between CNT and Pt portions of the array, it seems that CNTs do not exacerbate this tissue response. Furthermore, 9 months of action potential recordings demonstrate that tissue is still viable in the 100-200 microns surrounding the electrode tips during this time.

Impedance measurements

Impedance measurements on both Pt and CNT electrodes also followed patterns previously observed in the literature, namely, impedances gradually increased over the first few weeks of implantation, consistent with the formation of a glial scar (Ward, Rajdev, Ellison, & Irazoqui, 2009). Impedances decreased after reaching this high point for the duration of the implant, which may reflect changes in the ionic environment surrounding the electrodes.

Mean impedance of the CNT electrodes was significantly lower than the Pt electrodes. This is consistent with preimplantation impedance spectroscopy (mean 1 kHz impedance for Pt = 231.98±107.8, CNT = 49.71±5.07) and is expected, both due to the electrical properties of CNTs as well as the increased geometric surface area resultant from the application of a coating. This lower impedance was maintained for the duration of the implant, suggesting that the CNT coating was retained throughout processes of implantation and tissue response. Furthermore, it suggests that CNTs may retain an increased conductivity when compared to Pt even following multiple months of implantation.

Impedances dropped acutely with the application of stimulation on both Pt and CNT, but recovered towards prestimulation values. This is consistent with findings in the literature (Otto,

Johnson, & Kipke, 2006). Such acute drops in impedance suggest that acute electrochemical changes occur in the electrode, device damage occurs, or that some disruption of the tissue response surrounding the electrode results from stimulation. While it is unclear to what extent each of these processes plays a role in the observed phenomenon, the recovery of impedances towards baseline suggests that the effect is not only reversible, but that no permanent damage to the electrodes or CNT coating occurred.

Conclusions and future directions

Performance of CNT electrodes over several months of intracortical implantation and microstimulation was similar to both internal Pt controls and previously observed SIROF MEAs in many respects, including yield of action potential recordings, impedance dynamics, and ability to stimulate to effect. While CNTs did not show enhanced action potential recording ability, as their electrical properties might suggest, it is hoped that their ability to scaffold biomolecules will prove useful in attenuating tissue response and thus enhancing performance. Work is underway to determine if the lower impedances of CNT electrodes correspond with an enhancement of stimulation ability, i.e., an ability to stimulate to effect on more electrodes with lower currents than Pt counterparts.

Histology on the implant is also underway, in order to determine the effect of CNTs on tissue. Finally, local field potentials were also recorded during neural data acquisition, and an analysis of the LFP recording performance of CNT electrodes compared to Pt is also being performed. It is expected that CNTs will show an enhanced recording ability in some frequency bands of neural data, which could potentially be used for neural decodes.

Overall, the results of this pilot study have been promising. CNTs are as safe and effective for use as FDA-approved Pt in chronically implanted devices, and have great potential for biomolecule modification. It is hoped that these results will encourage the use of CNTs in future generations of neural prosthetic devices.

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CHAPTER 5

CONCLUSIONS

Summary of the conclusions

In order to utilize microstimulation via intracortical microelectrode arrays (MEAs) for clinical sensory prostheses, it is important to validate and improve long-term functionality in model systems. Such validation includes the investigation of the long-term performance of MEAs both with and without the application of microstimulation, the identification of factors that may adversely affect long-term performance, and the development of intervention strategies to address these factors.

The first study detailed in this dissertation investigated the chronic performance of intracortically implanted Utah Electrode Arrays (UEAs) both with and without the application of microstimulation, finding that stimulation did not preclude functionality. Next, this dissertation described work investigating how factors such as device damage, stimulation, anesthesia depth, and the use of impedance measures impact the results of chronic *in vivo* studies of microstimulation; finding that many factors affect long-term device performance. Finally, a case study using a novel carbon nanotube (CNT) coated array was reported, finding that CNTs are promising for future generations of neural prostheses. These studies are a first step towards addressing the requirements facing neural engineers in their endeavor to transition UEA-based microstimulation into clinical applications such as visual prostheses. While the work in this dissertation has provided evidence that microstimulation via intracortical MEAs may be ready for subchronic human trials, much work remains to be done.

In this chapter, strategies for improving long term device performance will be discussed. Such strategies include interventions for tissue response, changes in device design, surface

modifications, improved decode algorithms, and the development of wireless technology. Alternatives to chronic intracortical MEAs will be mentioned. Additional work based on this dissertation will also be described.

Strategies for improving chronic performance

Improved decode algorithms

One method for improving the long term performance of MEAs used to extract information from the cortex, such as MEAs that extract control signals for motor prostheses, is to improve neural signal decode algorithms. As shown in Chapter 2 of this dissertation, the yield of action potential (AP) recordings obtained by arrays of microelectrodes drops markedly over the course of multiple months. Other groups have observed this same effect (Selim Suner, 2005). To compensate for the decrease in action potential yield over time, some groups have developed decodes that do not require AP sorting (Bansal, Truccolo, Vargas-Irwin, & Donoghue, 2011; Fraser, Chase, Whitford, & Schwartz, 2009). These decodes can be stable over many months. Over very long (multiple year) time frames, however, the loss of action potentials is complete. Even in the absence of viable AP data, though, there is a high yield of recorded local field potentials (LFPs). Decodes based on LFPs have been performed (Jun, Truccolo, Vargas-Irwin, & Donoghue, 2010a, 2010b; Kennedy, Kirby, Moore, King, & Mallory, 2004; Markowitz, Wong, Gray, & Pesaran, 2011; Mehring et al., 2003; Pesaran, Pezaris, Sahani, Mitra, & Andersen, 2002; Saleh, Reimer, Penn, Ojakangas, & Hatsopoulos, 2010), and may be more stable over extended time frames than AP-based decodes.

Another advantage of using LFP-based decodes is that recording individual APs is not critical. This means that electrodes with only population recording ability can be used. As there is evidence to suggest that LFPs are generated within a 250 μM radius (Katzner et al., 2009), the spatial resolution of intracortical MEAs is still useful even if AP recording ability is not required; however, for some applications, penetrating technology may not be necessary. For example, studies have shown that it is possible to perform motor and speech decodes using non-penetrating, subdural electrocorticographic grids (ECoGs) (Acharya, Fifer, Benz, Crone, &

Thakor, 2010; Leuthardt et al., 2011; Pei, Barbour, Leuthardt, & Schalk, 2011; Schalk & Leuthardt, 2011; Wu et al., 2010). In order to improve the spatial resolution of ECoG, micro-ECOGs have been designed and preliminary studies on the ability to decode population data obtained from them have been performed (Kellis, House, Thomson, Brown, & Greger, 2009). They also show promise for the ability to microstimulate (Wilks, 2009). In applications where recording from, or stimulation of, a particular layer of cortex is not required, these devices may prevent much of the trauma resulting from implantation of intracortical MEAs. Another promising type of surface electrodes currently in development are conformal electrodes placed on the cortical surface using dissolvable layers of silk fibroin, though this work is still in preliminary stages (D. H. Kim et al., 2012). Of course, the recording target for control signals should be determined based on the application (Andersen, Musallam, & Pesaran, 2004).

Reducing tethering forces

The work done in this dissertation focused entirely on the UEA, a fixed-geometry MEA of 96 electrodes (Campbell, Jones, Huber, Horch, & Normann, 1991; Jones, Campbell, & Normann, 1992). The benefits of such an array include structural integrity, known spatial relationship between electrodes, and high spatial resolution and selectivity in a given area of cortex. Although these devices have already shown great clinical promise (Hochberg et al., 2006), further engineering may improve their long-term performance. It has been shown in the literature that the rigidity of the device may cause chronic problems in soft tissue as a result of device micro-motion (Biran, Martin, & Tresco, 2007; Gilletti & Muthuswamy, 2006). It is worth noting that many of the problems with a rigid array could be lessened if the long-term forces on the array were decreased; i.e., if the use of wires to attaching the array to the skull could be eliminated. Furthermore, the elimination of a percutaneous connector could lessen the risk of infection as there would no longer be a pathway from the external world into cortex. While no infection was observed with the devices used in this study, infections can cause serious problems for chronic studies (Bradley, 2004). The use of wireless arrays for stimulation and recording, therefore, is desirable for multiple reasons.

The development of wireless

Steps are underway to transition wireless technology to the field of neural prosthetics (Kamboh, 2010; S. Kim et al., 2008; Rizk et al., 2009; Wise, 2004). In addition to the work already described in this dissertation, a novel multiplexer version of the UEA was tested in a feline for 30 days. This array was capable of recording APs and LFPs via a greatly reduced wire bundle (7 wires, as compared to 96 in the standard UEA). This not only decreases the tethering forces of the array, but is also a first step towards being able to extract large amounts of neuronal data in a less-bit-intensive manner. APs were recorded on multiple channels of this array. However, it was determined that the insulation was not yet adequate for chronic applications. Also performed, but not reported elsewhere in this dissertation, were further tests of wireless devices that attached to the percutaneous connector and transmitted high-resolution data wirelessly to a router in the room (Figure 5.1). While these trials are promising, yielding APs on many channels, a considerable amount of noise was recorded. It will be necessary to improve data transmission ability and decrease the size of the onboard wireless electronics on the UEA before these devices can be clinically implemented and fully implantable. Furthermore, there is some question as to the effects of heat on brain tissue. While research has been done on the thermal impact of wireless electronics in the brain (Ibrahim, Abraham, & Rennaker, 2007; S. Kim, Tathireddy, Normann, & Solzbacher, 2007), it is unknown if the long-term heating of MEAs as a result of wireless electronics will be problematic . Finally, the issue of powering a completely implanted MEA is still under investigation, but it is likely that wireless devices will greatly assist in improving the longevity of neural prostheses.

Beyond the implementation of wireless, there are other changes to array geometry that are promising. While the tip geometry of the electrode itself does not have a clear effect on tissue, interelectrode spacing and the overall volume of tissue displaced by the device do affect tissue reactivity (Jeyakumar Subbaroyan, Martin, & Kipke, 2005; Sugihara et al., 2011). For example, work using Michigan probes has revealed that by reducing the overall volume of material implanted in the brain by making “lattice” probes, the radius of reactivity to implantation can be reduced (Seymour & Kipke, 2007). This reduction in volume often comes at the cost of device

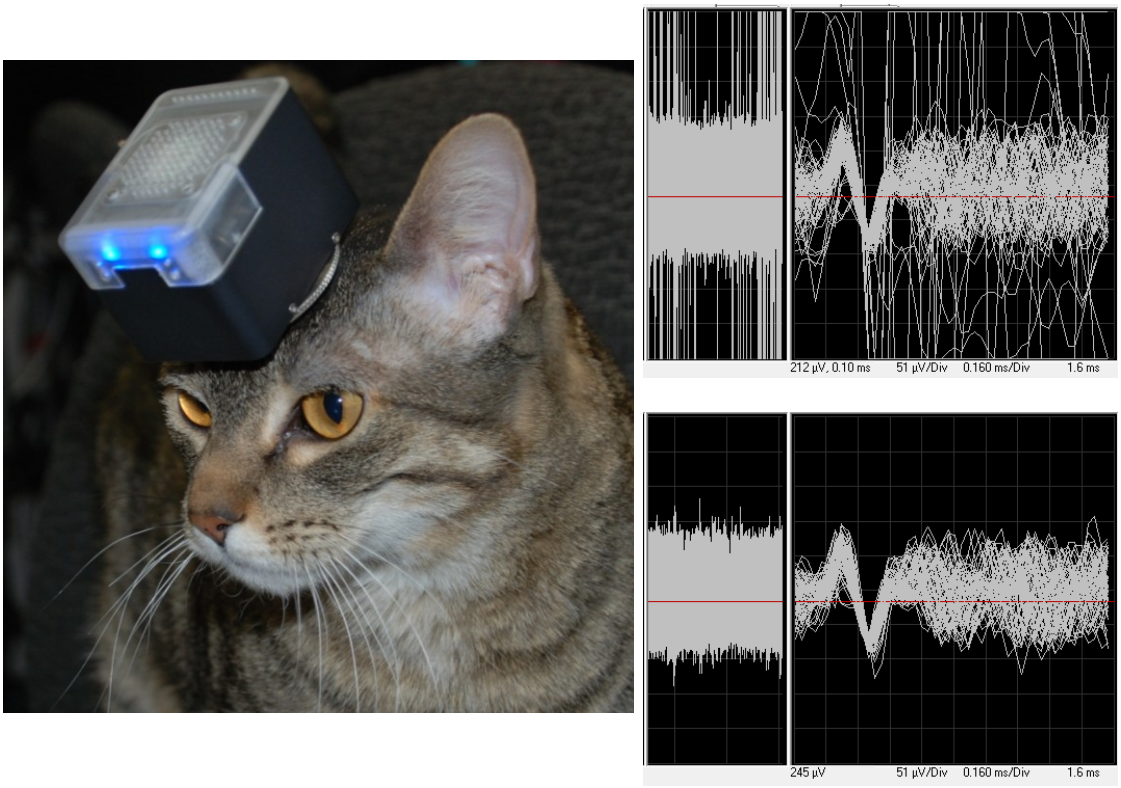

Figure 5.1. *In vivo* testing of an external wireless transmitter. Feline 4 is shown with the wireless transmitter attached to the percutaneous connector. The feline did not exhibit any behavior consistent with discomfort as a result of testing the device. Examples of neural data recorded before and after applying a -6.250*RMS noise multiplier is shown. The upper non-thresholded trace shows signs of noise that may have been due to the wireless router.

robustness, however, and such devices are prone to breakage on insertion. As micromachining techniques improve, more electrodes can be placed on smaller shanks, thereby improving the spatial resolution of MEAs as well as encouraging cortical integration.

Changes in implantation technique

As discussed in Chapters 1 and 3 of this dissertation, the initial trauma of insertion is a factor affecting device function. Trauma sets in motion the signaling cascades that lead to chronic inflammation (Bjornsson et al., 2006). Multiple techniques have been developed to decrease this trauma, including the use of collagenase (Paralikar & Clement, 2008) and mechanical inserters, which may lead to better performance than hand insertion (Rennaker, Street, Ruyle, & Sloan, 2005; Rousche & Normann, 1992). Preventing the attachment of dura to arrays is also known to improve performance (Maynard, Fernandez, & Normann, 2000). Clinical neurosurgeons are reporting their experiences implanting MEAs in human patients (House, Macdonald, Tresco, & Normann, 2006; Waziri et al., 2009); such reports will provide valuable information for future device improvements. This in turn may lead to decreases in the initial trauma of insertion.

Understanding tissue response

In addition to structural changes and improved techniques, understanding tissue response is necessary in order to develop intervention strategies. Many groups have investigated the response of brain tissue to indwelling microelectrodes (Biran, Martin, & Tresco, 2005; Dymond, Kaechele, Jurist, & Crandall, 1970; Edell, 1992; Grand et al., 2010; M.M. Holecko, 2004; X. Liu, McCreery, Bullara, & Agnew, 2006; McConnell et al., 2009; Stensaas & Stensaas, 1978), demonstrating that glial scarring, chronic macrophage activation, and neuronal death all occur with chronic implantation. What is less clear is to what extent other tissue response processes, such as complement activation and coagulation cascades (Hulka, Mullins, & Frank, 1996), play a role in AP recording dynamics. It is also unclear to what extent distress signals sent from damaged neurons and other cells may lead to changes in neuronal firing patterns. The chronic effects of injury include not only the response of brain tissue, but also the

circulatory system. While it is known that MEAs may disrupt the blood-brain barrier long term, allowing for infiltration of brain tissue by a variety of circulating immune cells and molecules (H.-L. Liu et al., 2009), the effects of these cells and molecules is less well-understood. Finally, the effect of chronic MEA implantation on other cell types present in the brain, such as oligodendrocytes, is poorly understood. It is known that inflammation and damage can lead to an oligodendrocyte injury response (Rhodes, Raivich, & Fawcett, 2006), but the involvement of oligodendrocytes in neuronal signaling loss on MEAs has only recently been suggested (Winslow & Tresco, 2010). Oligodendrocyte malfunction may have profound consequences for neuronal signaling, especially as one oligodendrocyte may myelinate as many as 50 neurons (Baumann & Pham-Dinh, 2001).

The fields of implant biology and immunology are both actively investigating processes of tissue response, but it remains unclear why the pattern of AP recording discussed in Chapter 2 of this dissertation occurs. While the glial scar is frequently implicated in performance dynamics, it is thought to be fully formed after 6 weeks of implantation (Szarowski et al., 2003; Turner et al., 1999). The scar may actually serve to separate inflamed tissue from viable neurons that remain in the recording radius or electrodes. It is likely that chronic blood-brain barrier disruption and the mechanical mismatch between rigid device and soft tissue, lead to chronic proinflammatory signaling that exacerbates signaling loss (Biran et al., 2007; Gilletti & Muthuswamy, 2006). In fact, it is known that macrophages stay active within the site of an implant for extended periods of time (B. K. Leung, Biran, Underwood, & Tresco, 2008). It is possible that the many cytokines involved in neuroinflammation (John, Lee, & Brosnan, 2003; Merrill & Benveniste, 1996) could be doing more than inducing apoptosis. They could, for example, be changing neuronal firing patterns. Furthermore, recent evidence suggests that microglia and astrocytes may play a larger role than previously thought on neuronal signaling (Pascual, Ben Achour, Rostaing, Triller, & Bessis, 2011), which could play a role in AP recording fade out. As the processes of neuroinflammation are complex, however, much research remains to be done in this field.

Expanding on the work in Chapter 2 of this dissertation, immunohistochemical staining of tissue from Felines 1 and 2 was performed by collaborator Dr. M.B. Christensen. While statistical

significance of these results was not sufficient, a weak inverse correlation between the luminance intensity of a marker of generalized inflammation (isolectin B4) and the recording of action potentials was noted in Feline 1. Also in Feline 1, an inverse correlation between the number of cell nuclei (marked by DAPI) within 100 microns of the electrode implant site and the recording of action potentials was observed. There was no correlation in either feline between the number of neuronal nuclei (as marked by NeuN) and whether or not an action potential was recorded. While this was only a preliminary study, future work correlating electrophysiology with histology could tease out these relationships with better statistical power. This may become easier as more advanced techniques, such as histology with the device in place or *in vitro* tissue response models become more common (Holecko, Williams, & Massia, 2005; Koeneman et al., 2004; Polikov, Block, Fellous, Hong, & Reichert, 2006; Polikov, Su, Ball, Hong, & Reichert, 2009; Woolley, Desai, Steckbeck, Patel, & Otto, 2011).

Attenuating tissue response

Although engineers lack a full understanding of tissue response, many groups are testing methods for attenuating known tissue reactivity targets. By attenuating tissue response, neural engineers will likely improve tissue viability and neuronal longevity. Methods currently being tested include drug administration and surface modifications.

Drugs to attenuate tissue response

Anti-inflammatory drugs can be administered on either a global or local scale. Two compounds in particular have shown great promise for decreasing tissue response to implantation: dexamethasone (DexM) and minocycline. Minocycline is known to decrease inflammation levels in traumatic brain injury (Yrjänheikki, 1999), and has been shown to improve MEA performance when administered systemically (Rennaker, Miller, Tang, & Wilson, 2007). It is known for decreasing microglial activation in particular (Abraham, Fox, Condello, Bartolini, & Koh, 2012). DexM has also yielded promising anti-inflammatory results in other studies (S. Zhong, 2005; Y. Zhong & Bellamkonda, 2007), and methods for controlled release of DexM over time

have been promising (Wadhwa, Lagenaur, & Cui, 2006). Unfortunately, it is known that non-neural inflammation, such as the inflammation that may be induced by frequent injections, can lead to increased neuroinflammation (Riazi et al., 2008). Chronic global drug administration may also lead to tolerance and unwanted somatic side effects. Engineering local delivery methods for these drugs, therefore, is of great importance. Several prototype devices have been manufactured for drug delivery directly to the implant site, including MEAs with onboard microfluidics (Berdichevsky, Sabolek, Levine, Staley, & Yarmush, 2009; Papageorgiou, Shore, Bledsoe, & Wise, 2006; Retterer et al., 2004). Long-term-release hydrogels are being tested for chronic drug delivery to the brain (Cadotte & DeMarse, 2005; S. B. Jun et al., 2008; Lu et al., 2009; Rao, Zhou, Li, Li, & Duan, 2012; Winter, 2007), as are nanoparticles for targeted delivery (Bondi, Di Gesu, & Craparo, 2012; McCabe et al., 2012; Wei et al., 2012). It is likely that novel anti-inflammatory drugs and improved drug delivery methods will yield more stable neuronal signaling, both by decreasing neuronal death in response to implantation as well as attenuating chronic inflammatory signaling.

Surface modifications

In addition to attenuating tissue response by drug administration, nanoscale surface modifications are being actively investigated (B. K. F. Leung, 2007; Manwaring, Biran, & Tresco, 2001; Massia, Holecko, & Ehteshami, 2004). For example, it has been found that texturing the surface of MEAs improves neuronal adhesion and fosters neurite outgrowth (Khan, Auner, & Newaz, 2005; Moxon, Hallman, Aslani, Kalkhoran, & Lelkes, 2007). Encouraging the growth of neurites, particularly axons, close to the electrode may increase the chances of recording action potentials. Neurite ingrowth into electrodes actually provides the foundation for a particularly stable form of microelectrode, the neurotrophic electrode (Bartels et al., 2008; Kennedy & Bakay, 1998). These electrodes consist of a glass cone with microwires placed inside the cone along with neurotrophic factors to encourage neuronal processes to enter the electrode. These devices have shown great recording promise, including obtaining data in human patients for decodes (Brumberg, 2011; Kennedy, Bakay, Moore, Adams, & Goldwaithe, 2000), providing evidence that

the use of neural growth promoting molecules may be a wise strategy for improving chronic performance.

Another method of surface modification that may improve device performance and allow the long-term use of neurotrophic molecules is the use of surface coatings (Pancrazio, 2008; A. Wang et al., 2007; Y. Zhong, 2006). For example, laminin and alpha-MSH coatings have demonstrated beneficial effects for chronic implants (He & Bellamkonda, 2005; He, McConnell, & Bellamkonda, 2006; Y. Zhong & Bellamkonda, 2005). Many groups have tested a variety of other surface coatings, including polypyrrole (Cui, Wiler, Dzaman, Altschuler, & Martin, 2003; Yang & Martin, 2004), PEDOT [Poly(3,4-ethylenedioxythiophene] (Abidian, Ludwig, Marzullo, Martin, & Kipke, 2009; Ludwig, Uram, Yang, Martin, & Kipke, 2006; S. J. Wilks, 2011), stem cells (Richter, Kruse, Moser, Hofmann, & Danner, 2011), silica sol gel (Pierce, 2009), PEDOT doped with CNTs (Luo, Weaver, Zhou, Greenberg, & Cui, 2011), and CNT coatings such as the one presented in Chapter 4 of this dissertation. CNTs have beneficial charge injection properties, and can be modified by a variety of biomolecules (Ansaldo, Castagnola, Maggiolini, Fadagia, & Ricci, 2011; Voge & Stegemann, 2011). In fact, it has been found that a CNT coating such as the one applied in Chapter 4 yields good neural recordings (Keefer, Botterman, Romero, Rossi, & Gross, 2008), and may enhance stimulation ability. The results described in Chapter 4 of this dissertation confirm the promise of CNTs, though histology has yet to be performed. Given the long-term recording ability of the CNT coated side of the array, however, it is unlikely that an adverse reaction of CNT implanted tissue compared to Pt implanted tissue will be observed. Further study on the chronic performance of CNT-coated MEAs for chronic recording and microstimulation are warranted.

Improving the materials that make up electrodes may also decrease tissue response and enhance performance. For example, some groups have tested electrodes made entirely of CNTs, with promising results (Ben-Jacob & Hanein, 2008; Gabay et al., 2007; Shoval, 2009; K. Wang, 2006; K. Wang, Fishman, Dai, & Harris, 2006). Still others are working on making flexible probes (Mercanzini et al., 2008; J. Subbaroyan & Kipke, 2006; Wester, Lee, & LaPlaca, 2009), as these may reduce the mechanical mismatch between probe and tissue. Organic electrodes are also

promising (Zhou et al., 2012). Research on the properties and biocompatibility of novel insulations (Hsu & Solzbacher, 2007; J. M. Hsu, Tathireddy, Rieth, Normann, & Solzbacher, 2007; Winslow, Christensen, Yang, Solzbacher, & Tresco, 2010) and metallization may also improve long-term electrode performance in both recording and stimulating applications.

MEAs for chronic microstimulation

Work performed on Feline 4, in conjunction with experiments detailed in this dissertation, has demonstrated that the number of electrodes that must simultaneously stimulate in order to evoke responses increases with the duration of implantation. For example, it was possible to evoke motor responses in Feline 4 on single electrodes at 100 μA after 6 months of implantation, while at 505 days postimplantation, it required 9 electrodes stimulating at 100 μA in order to observe EMG responses. Similar results were observed in the production of behavioral responses in a nonhuman primate. After more than 1 year of implantation in both Feline 4 and a nonhuman primate, impedances had decreased to near preimplantation values (Davis et al., 2012). One possible explanation for these results is that edema, or a fluid layer, has developed around the microelectrodes over time. Fluid could serve as a shunt for applied current, thus interfering with the ability to record. Edema has been observed with traumatic brain injury (Donkin, Nimmo, Cernak, Blumbergs, & Vink, 2009; Unterberg, Stover, Kress, & Kiening, 2004), and would be expected to lower impedance as fluid is less resistive than tissue. The results of this multichannel stimulation study, as well as the results described in Chapter 3 of this dissertation, warrant the further investigation of the role of edema in chronic microstimulating MEA performance.

This study demonstrated that while it may have required multichannel stimulation to evoke physiological responses after more than 1 year of implantation, the MEA retained the ability to stimulate to effect. Careful consideration of the selectivity required for sensory prostheses should be made. In the case of the visual system, MEAs can stimulate at more than 1 year postimplantation, using simultaneous multichannel stimulation rather than single electrodes (Davis et al., 2012). With human trials, it could be determined how this decrease in selectivity

corresponds to patient needs. Such trials would yield a better understanding of the electrode spacing required for intracortical visual prostheses.

There is an important caveat, however, concerning the use of simultaneous multi-electrode stimulation. It is unclear what stimulation parameters lead to seizure induction. In Chapter 2 of this dissertation, a bilateral seizure was reported when 72 electrodes were simultaneously stimulated at 25 μA; however, simultaneous stimulation at 250 μA on 9 electrodes did not lead to the induction of a seizure. There have been anecdotal reports of seizure induction in many model systems, including felines and nonhuman primates, and electrical stimulation with the purpose of inducing seizures (kindling) is routinely performed in rodents (McNamara, 1986). One impediment to seizure prevention is that it remains unclear how far stimulation spreads within cortical tissue. Studies have investigated charge spread and neuronal activation using a variety of techniques, including modeling and *in vivo* studies (Butovas, 2003; Ezure, 1985; McIntyre & Grill, 2001; Nathan, Sinha, Gordon, Lesser, & Thakor, 1993; Rattay & Wenger, 2010). Unfortunately, spread appears to vary by model system, method of stimulation, and electrode type. Rigorous studies of both synchronous and interleaved stimulation will better establish safe spatiotemporal limits for the multielectrode stimulation required for visual prostheses.

Alternative stimulation methods

In addition to work being done to determine safe and effective parameters for electrical intracortical microstimulation, alternative methods of cortical stimulation are being developed. For example, transcranial magnetic stimulation (TMS) has long been used to stimulate large populations of neurons (Bolognini & Ro, 2010). While the precision required for a visual prosthesis has yet to be obtained, work is underway to decrease the stimulating radius of TMS technology (Bolognini & Ro, 2010). Another promising method of stimulation is the use of optogenetics (Kravitz et al., 2010). While optogenetics is quite precise, using brief pulses of light delivered at a specific wavelength, it requires that the model system in which it is used be genetically altered. While this is currently feasible only in a few model systems, mainly rodents,

optogenetics research is moving towards other model organisms at an aggressive pace (Han, 2012).

Contributions of this dissertation

In addition to its use described in this dissertation, the dataset collected from Felines 1-4 may be used for a variety of other studies. For example, it has already been used to analyze the underlying differences between sleep and wakefulness (Dehghani et al., 2012). Work is currently being performed on the dataset to analyze the long-term changes in LFPs. It may also be useful for other novel analyses, as it provides multiple months of LFP and AP recordings in multiple felines, as well as 1 kHz impedance measurements. It is my sincere hope that further insight into the performance dynamics of chronic intracortical MEAs will be gained from this data.

The work described in Chapters 2-4 of this dissertation, as well as the companion work described in this chapter, is aimed at understanding and improving the long-term performance of intracortical MEAs for both recording and microstimulation. As set forth in Chapter 1, such work will be valuable for transitioning microstimulating MEAs, such as the UEA, into chronic human clinical applications. In this dissertation, it was established that physiologically relevant microstimulation can be performed without interfering with device functionality. Furthermore, the stimulation applied routinely exceeded theoretical “water window” and device damage limits, reaching several volts (Negi, Bhandari, Rieth, Van Wagenen, & Solzbacher, 2010). While this does not directly demonstrate device safety, it does indicate that high amplitude microstimulation does not catastrophically interfere with performance. The use of functionality metrics such as recording ability and impedance may help substitute for or supplement chronic safety data, such as histology, though such chronic metrics must be correctly interpreted.

While this dissertation is only a step in the direction of clinical microstimulating MEA implementation, Chapter 2 established that the *functionality* of stimulating intracortical UEAs is sufficient to warrant subchronic human clinical trials of microstimulation for visual restoration. Chapter 3 of this dissertation has discussed the impact of device insertion, microstimulation, electrode environment, and anesthesia depth on the functionality of MEAs, both by measuring

impedances and testing the response of an anesthetized animal to stimulation. It was established that lower impedances do not correspond to an increase in the probability of recording an action potentials. Furthermore, it was revealed that many processes affect the results of chronic intracortical microstimulation studies. Chapter 4 investigated the use of a promising intervention strategy, a CNT coating, and found that the coating did not impair device functionality *in vivo.* Additional work described in this chapter has discussed the need for improved decodes, wireless devices, and tissue response interventions in order to increase the longevity of MEAs in intracortical applications.

Hopefully, these studies will lead to not only further trials of intracortical microstimulation for sensory restoration, but also assist in making these trials more robust. Using multiple measures of device functionality in a single study will provide additional insight into the factors impacting long-term intracortical MEA performance. It is my sincere hope that this insight will facilitate the transition of stimulating MEAs into chronic clinical applications, which may improve the quality of life for millions of patients worldwide.

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