Novel In Vitro and In Vivo Neural Interfaces

By Kevin J. Otto and Justin C. Williams

Normal and Accelerated Failure Assessment

Neural implantation of devices and the subsequent tissue response are complex and cascading physical and biological phenomena. Creation of reliable neural interfaces remains a significant challenge. Penetrating central nervous system interfaces persist as the most challenging to realize but continue to be the most attractive because of the information bandwidth advantages they provide. This rich information source is essential for achieving next-generation prosthetic control. Specific challenges of penetrating central nervous system interfaces arise because of the reactive tissue response to the initial injury due to device insertion as well as the continued response due to device indwelling. These responses consist of biochemical signaling events, microglial activation, and astrogliotic cell reorganization that result in biophysical changes of the tissue near the implanted device and finally, electrophysiological neural cell/signal loss (Figure 1). The ultimate realization of reliable penetrating neural interfaces will require careful science and engineering approaches incorporating knowledge of relevant and critical biological, physical, and chemical factors, especially their interrelationship. In this article, we describe a comprehensive strategy to assess the reliability of penetrating central neural interfaces based on the biology and pathology of the injury and indwelling tissue responses. Our strategy involves a parallel, self-informing approach by simultaneous development of new in vitro and in vivo assessment techniques as well as using these state-of-the-art techniques to conduct accelerated lifetime assessments of neural interface degradation.

The in vitro acute brain slice test bed offers the advantages of natural configurations and proportions of cells and tissues.

As a function of time, electrode insertion activates signaling pathways in microglia, astrocytes, and neurons that result in biochemical changes that alter the biophysical characteristics of the tissue. The biophysical changes (e.g., decreased signal-to-noise ratio and increased tissue impedance) dramatically alter or abolish the ability to record from neurons. At each step in this cascade, we propose to correlate specific biochemical, optical, and electrical variables with electrode function. Also, biochemical stressors or biophysical manipulations will be applied to determine how these perturbations alter the magnitude and time course of the cascade of biochemical and biophysical effects on the decline of the interface reliability.

As part of the Reliable Neural Technology (RE-NET) Histology for Interface Stability over Time (HIST) program, our collaborative effort between Purdue University and the

Digital Object Identifier 10.1109/MPUL.2011.2175631
Date of publication: 6 February 2012

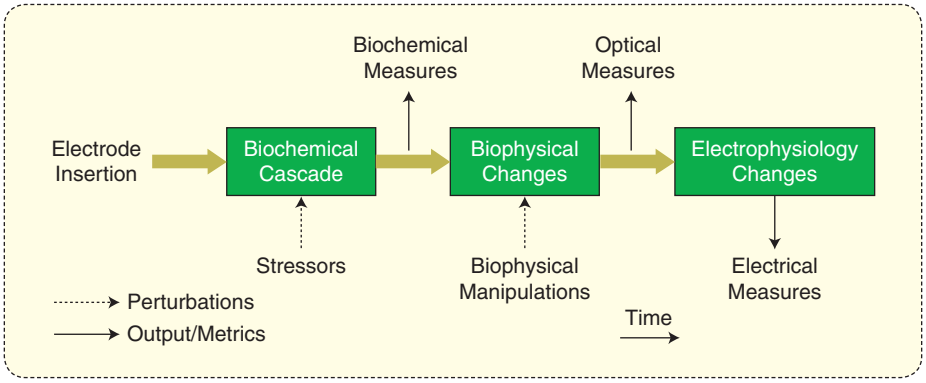

FIGURE 1 Electrode insertion initiates a deleterious cascade that decreases the ability to record or stimulate.

University of Wisconsin, Madison, is using a comprehensive strategy to evaluate the reliability of neural interfaces based on the biology and pathology of the insertion injury and indwelling tissue responses. Specifically, our research strategy is designed to determine the failure modes of current and newly developed state-of-the-art neural interfaces. We approach this problem with a combination of novel assessment techniques, allowing us to manipulate and thoroughly evaluate the complex, dynamic tissue response both in vitro and in vivo, across multiple time points, from the molecular scale to the organ level. We are applying multiple evaluative methods to the same research subjects, strengthening the interpretation of our results.

We are using a multilevel approach involving two test beds: acute brain slice and in vivo animal preparations. This approach provides high spatial and temporal resolution that allows chronic interface assessment in molecular detail, while also allowing lower resolution but noninvasive data sets. The in vitro acute brain slice test bed offers the advantages of natural configurations and proportions of cells and tissues while simultaneously enabling higher experimental control than in vivo preparations. Conversely, the in vivo animal preparation provides high temporal resolution in an intact animal model. This multilevel approach provides a comprehensive assessment of the neural interface by leveraging various techniques in a single subject such as simultaneous electrical measurement, subcellular imaging, and state-of-the-art, noninvasive clinically relevant monitoring of the brain tissue response. Ultimately, all degradation and failure at the molecular and cellular levels will be corroborated by lower resolution, larger-scale techniques (impedance spectroscopy and noninvasive interface-assessment techniques, e.g., magnetic imaging, to be developed in the future).

We will implement and/or develop a number of advanced technologies for characterizing the intimate relationship between device performance and changes in the organization of surrounding cellular and biochemical elements. One of the open questions in the field is the temporal (and causal) relationship among biochemical signaling events, microglial activation, astrogliotic cell reorganization, and electrophysiological neural cell/signal loss. The current state of the art to address this challenge relies on the analysis of correlations between cellular events and measures of electrical signal patency at discrete points in time. Our approach is to use advanced multiparametric optical methods to longitudinally study changes in the biochemical and cellular state of the tissue surrounding implanted electrodes over the course of weeks to months. In this approach, one can take into consideration the key changes in electrophysiology and look back to analyze the time-varying changes that led to them. Simultaneously, we will use techniques to trigger specific biochemical and biophysical events and evaluate the resulting temporal changes in neural recording performance. Another challenge our approach will address is improving the throughput of experimentation by implementing microfluidic and robotic automation approaches. The rationale for our approach to this problem is underscored by our team's collective expertise in a number of key areas: 1) microfluidic approaches for improving in vitro models, 2) advanced models of in vitro brain slice physiology, and 3) particularly in developing advanced optical techniques for real-time imaging of in vivo living brain tissue. We feel that these new approaches will be instrumental in future efforts to develop and evaluate new approaches and devices that are designed to mitigate cellular reactions.

As a function of time, electrode insertion activates signaling pathways in microglia, astrocytes, and neurons that result in biochemical changes that alter the biophysical characteristics of the tissue.

Our approach using in vivo imaging is founded in the current lack of approaches for either characterizing or controlling cellular distributions around implantable neural devices with sufficient spatiotemporal resolution. This is primarily due to limitations imposed by traditional immunohistochemical techniques, all of which require sacrifice of the experimental animal and fixation of the brain tissue of interest. While these techniques have considerable merit and have led to our current understanding of brain reactions, advances in nonlinear multiphoton imaging techniques have opened up the possibility

of imaging gliotic reactions in vivo (while the implant is still resident in the living brain).

An important gap in neural interface degradation literature is the temporal relationship among biochemical events, microglial activation, astrogliotic cell reorganization, and neural cell/signal loss. Investigators who have performed neural implant studies can attest that failure often manifests itself in two forms: 1) a slow gradual loss of neural signals over weeks to months and, more often, 2) a sudden and catastrophic change in neural signals over hours to days. To add to the complexity of this issue, sometimes neural signals that were thought to be lost can suddenly and inexplicably return (likely to be lost again). To understand the relationship between biological reactions and neural cell degradation, one has to be able to simultaneously measure these biological stressor events over both short and long time scales. Additionally, the ability to induce cellular and biochemical stressors in tight synchronization with imaging and neurophysiology (so that relationships can be captured repeatedly) is crucial for establishing the causal relationship between the tissue response and reliability of neural signal recording.

Temperature-modulated acceleration, based on the Arrhenius equation, is a common method to predict the lifetime of samples.

Accelerated lifetime testing is a stress testing methodology for assessing product reliability in the engineering sciences. Within the neuroengineering community, there are no current methods for accelerating implant failure; on the contrary, most attempts have been to try to slow or stop these failures. Anecdotally, most researchers have seen the result of a reaction that has progressed at an abnormal, even catastrophic rate and a corresponding rapidly failing implant; this provides an interesting perspective that the concept of accelerated failure may indeed be plausible. Outside the neuroengineering community, this idea is not nearly as novel. Temperature-modulated acceleration, based on the Arrhenius equation, is a common method to predict the lifetime of samples. In the biological community, investigators routinely attempt to modulate biological processes to investigate the effects of progressive neurological diseases and provide both positive and negative controls for evaluating therapeutic interventional approaches. The primary tools they utilize are rooted in basic biochemistry approaches, environmental control, and more advanced genetic manipulations. To this end, our project uses methods for both accelerating and decelerating cellular and biochemical responses to implanted neural devices. The envisioned overall goal for this area is to produce animal models and/or experimental protocols that can be shared with the neural interface community as common accelerated testing protocols.

One mechanism for performing accelerated testing is through the delivery of chemical stressors. By activating putative reactive or inflammatory pathways biochemically, we aim to quantify the relationship between microglial or astrocytic responses and neurophysiology signal decline. Through these studies, we will develop methods for exacerbating the foreign body response in a controlled manner. This will provide a key test bed for evaluating the foreign body response, identifying early mechanisms that lead to this response, and recognizing key check points to target therapeutics to prevent the initiation or progression of the biological cascade that leads to encapsulation of the neural implant and ultimate device failure.

A number of our experimental approaches leverage microfluidic systems for spatiotemporal manipulation of biochemistry. Microfluidic technology affords a number of advantages in this type of application due to factors such as laminar flow, surface tension, small solution volumes, and increased surface to volume ratios. In particular, our approaches will use these fluid properties to allow us to achieve a number of specific functionalities: 1) laminar spatial flow control will be utilized to spatially confine flows to produce gradients or step functions in biochemistry, 2) microfluidic approaches will be applied to directly deliver chemicals in slice and in vivo preparations or thermally control the local environmental conditions, 3) techniques such as convective enhanced diffusion by which microfluidic counterflow can be used to enhance the diffusion into a slice, thereby increasing slice viability and improving the time constants of biochemical delivery, and 4) microfluidic systems lend themselves to parallel experimentation that can be used to increase the throughput.

To sum up, our proposed complementary, progressive, and multilevel approach will provide deliverables that address critical gaps in the knowledge of the mechanisms underlying neural-recording interface performance and stability. We hope to provide important contributions to the work of the RE-NET HIST program.

Acknowledgments

This work was sponsored by the Defense Advanced Research Projects Agency Microsystems Technology Office under the auspices of Dr. Jack W. Judy (jack.judy@darpa.mil) as part of the RE-NET program through the Space and Naval Warfare Systems Command Systems Center Pacific grant N66001-11-1-4013.

The views, opinions, and/or findings contained in this article are those of the author and should not be interpreted as representing the official views or policies, either expressed or implied, of the Defense Advanced Research Projects Agency or the Department of Defense.

Kevin J. Otto (kotto@purdue.edu) *is with the Department of Biological Sciences and Weldon School of Biomedical Engineering, Purdue University, West Lafayette, Indiana.* ***Justin C. Williams (jwilliams@cae.wisc.edu)*** *is with the Department of Biomedical Engineering, University of Wisconsin-Madison, Wisconsin.*