Real-time Characterization of Neuronal Response for Selective Stimulation

Michelle L. Kuykendal[1], Gareth S. Guvanasen[1], Martha A. Grover[2], Steve M. Potter[1], Stephen P. DeWeerth[1*]

1 Laboratory for Neuroengineering, Coulter Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta, Georgia, USA
2 School of Chemical & Biomolecular Engineering, Georgia Institute of Technology, Atlanta, Georgia, USA
* Corresponding author. E-mail address: steve.deweerth@gatech.edu

Spatiotemporal selectivity of extracellular stimuli is a significant challenge in the development of neural interfacing devices. Although models have previously been used to investigate responses to various stimulus waveforms, it is of significant interest to experimentally validate the stimulus-evoked cellular response and use that information to optimize the stimulus patterns. To address these challenges, we have developed a high-throughput closed-loop system of multisite stimulation and recording that facilitates the characterization of the cellular activity evoked by extracellular stimulation. We explore excitatory waveforms applied to cortical networks at multiple sites in a multi-electrode array (MEA) to achieve increased precision and localization of extracellular stimuli. We measure the response of all individual neurons using fluorescent calcium-sensitive dyes in conjunction with novel real-time optical processing algorithms and an automated data acquisition platform. Using this system, we have characterized waveforms that excite neural activity within a 400µm X 400µm field of view, and in this work we investigate the use of such waveforms to explicitly direct the focus of an extracellular stimulus.

1 Introduction

Extracellular stimulation of neural tissue is essential for the enhancement of neuroprostheses, including retinal and cochlear implants, and the treatment of complex diseases, ranging from Parkinson's Disease to epilepsy and depression. If we could target our stimulation to only those cells and networks that are essential to the system, we could significantly increase our efficacy and eliminate side effects due to diffuse stimulation. Future clinical implementations of this technology will use systems that optimize their outputs based on real-time closed-loop processing of the evoked neuronal responses [1].

Our system enables the real-time processing of optical signals to measure the stimulus-evoked responses in the tissue so that we can automatically home in on the optimal stimulus. First we must study the excitatory aspects of neuronal tissue, and then we can explore the potential to inhibit certain cells while stimulating others, using the spatial precision that MEAs afford.

2 Methods

2.1 Cell Culture

Cortical neurons from embryonic day-18 rat were enzymatically and mechanically dissociated using papain and a vortex mixer, respectively. The neurons were then grown in DMEM with 10% horse serum on multi-electrode arrays (200/30-TiN, Multichannel Systems) coated in polyethylene amine and laminin to encourage cell adhesion [4].

2.2 Fluorescence Imaging

Cultures were bathed in an acetoxymethyl-ester calcium-sensitive fluorescent dye, Fluo-5F-AM [3], which was combined with pluronic F-127 and DMSO to enhance cell-loading. All synaptic communication in the network was then blocked using receptor antagonists APV (100 µM), bicuculine (50 µM) and CNQX (10 µM), which bind NMDA, GABAa and AMPA receptors, respectively [2]. All optical recording was performed using an electron multiplication CCD camera (QuantEM, Photometrics) while electrical stimulation was conducted with an STG2004 and electrical recording utilized the MCRack software (Multichannel Systems). All other software was developed in-house using MATLAB.

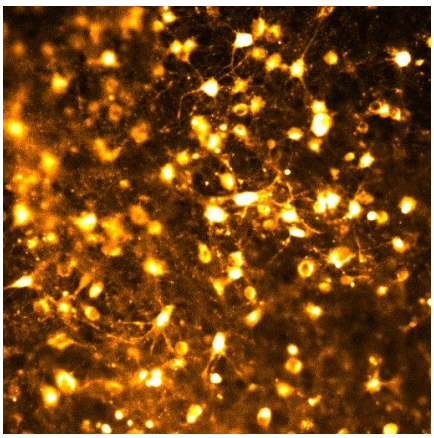

Fig. 1. Fluorescence image of integrated spontaneous activity across time (10 min) in a 2-D neuronal network. Post-processing includes frame averaging, median filter and coloring (ImageJ).

3 Results

Trains of five excitatory cathodic pulses were delivered 50ms apart to provide fluorescence summation improving signal-to-noise ratio in the optical recording. All stimulus trains were repeated three times in random order for averaging.

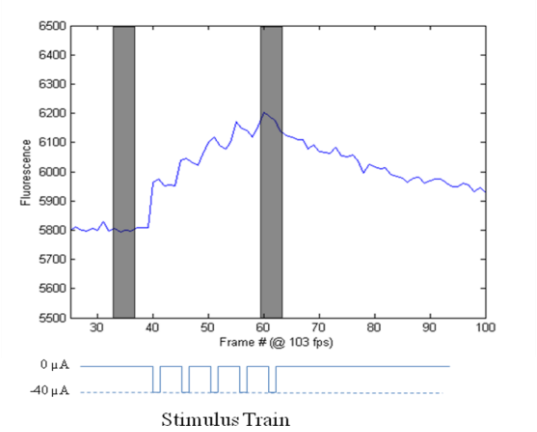

Fig. 2. Example of a stimulus train (bottom) applied to the culture via one electrode, and resulting fluorescence recording (top). Gray bars indicate regions of peak and baseline fluorescence for averaging.

3.1 Current Sweep

While holding the stimulus duration constant, we varied the current amplitude and analyzed the resulting neuronal response. Increasing current activated more cells within any given radius, and this activation falls off with distance from the electrode similarly in each stimulus condition. Radii that produced the same incremental area were used to divide the cells into quartiles moving away from the center electrode.

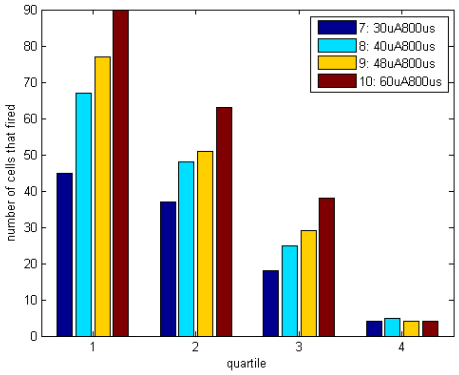

Fig. 3. Activation of neurons versus distance from the stimulating electrode under four stimulus amplitude conditions. Quartile radii are as follows: 181.0µm, 256.0µm, 313.5µm and 362.0µm.

3.2 Equal Charge Delivery

The same total charge was delivered in each stimulus; however, the amplitude and duration were varied. From these data we see that at the lowest stimulus amplitude we may be approaching the limits defined in a chronaxie curve because the evoked response is consistently less than that of the others. However, all other stimuli produce similar responses. This is consistent with the theory that the total stimulus charge dictates the cellular response, independent of amplitude and duration, once the total stimulus charge is above threshold.

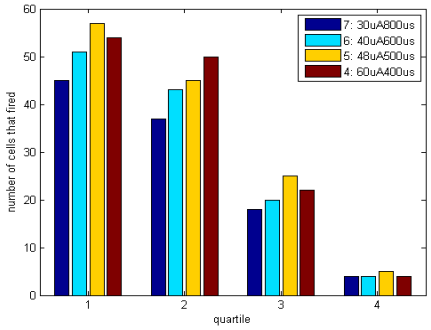

Fig. 4. Activation of neurons versus distance from the stimulating electrode for four stimulus waveforms of equal charge delivery. Quartile radii are as follows: 181.0µm, 256.0µm, 313.5µm and 362.0µm.

4 Conclusions

Experimentation has previously demonstrated the potentially inhibitory effects of below-threshold excitatory stimulation [5]. If we simultaneously combine these waveforms on multiple electrodes with our excitatory stimuli, we may be able to further localize our stimuli in both space and time. Because data collection and analysis are fully automated in our system, future work will use feedback of neuronal activation to optimize our stimuli and spatiotemporally target a population of cells. Furthermore, the use of optical imaging provides us with a ground truth of the activity both at and in between electrodes so that we can calibrate our simultaneous electrical recording to what we know is actually happening in the network. This could ultimately allow us to electrically stimulate *in vivo*, without the need for optics, while still maintaining the ability to focus our stimulus.

The ability to selectively stimulate neurons will help us to improve the development of neural interfacing devices, including motor prostheses and deep brain stimulators that are free from side effects.

Acknowledgement

We thank the NSF for supporting our work through NSF EFRI grant #0836017 and the NSF Graduate Research Fellowship Program.

References

[1] M. Arsiero, H. Luscher, et al. (2007). "Real-time closed-loop electrophysiology: towards new frontiers in in vitro investigations in the neurosciences." Arch Ital Biol 145(3-4): 193-209.

[2] D. J. Bakkum, Z. C. Chao, S. M. Potter (2008). "Long-Term Activity-Dependent Plasticity of Action Potential Propagation Delay and Amplitude in Cortical Networks." PLoS ONE 3(5): e2088.

[3] A. Minta, J. P. Y. Kao, and R. Y. Tsien (1989). "Fluorescent Indicators for Cytosolic Calcium Based on Rhodamine and Fluorescein Chromophores." Journal of Biological Chemistry, vol. 264, pp. 8171-8178.

[4] S. M. Potter and T. B. DeMarse (2001). "A new approach to neural cell culture for long-term studies." Journal of Neuroscience Methods, vol. 110, pp. 17-24.

[5] J. D. Ross, N. E. Reddy, D. J. Bakkum, S. M. Potter, and S. P. DeWeerth (2007). "Experimental Platform for the Study of Region Specific Excitation and Inhibition in Neural Tissue" Proceedings of the 29th Annual International Conference of the IEEE EMBS. pg:4759.