Darian Hadjiabadi - Group 5
600.446 Computer Integrated Surgery II
Paper Seminar Report

Paper Citation:

Visualizing the Cortical Representation of Whisker Touch: Voltage-Sensitive Dye Imaging in Freely Moving Mice. *Ferezou*, Isabelle; *Bolea*, Sonia; *Peterson*, Carl. Ecole Polytechnique Federale de Lausanne. Neuron *50* 617-629. Elsevier, 2006.

Project Recap

Suppose you wanted to observe neuronal signal cascades in a cubic millimeter of brain space. With current imaging modalities, there is a steep trade off between spatial and temporal resolution. We define temporal resolution as the smallest time change at which an imaging modality can differentiate events. Neural spikes occur on the order of 3-8 ms, thus imaging these energy propagations require very high temporal resolution. Voltage senstive dyes (VSDs) are one such way of resolving the spatiotemporal dynamics needed. Detecting the signals produced by the VSDs can be done by taking advantage of the photoacoustic output of a material bombarded by light energy, and can be easily picked up through a sensitive hydrophone. Our work is a small part of a larger project headed by the NIH to observe neurotransmitter movement through the brain in real time. For our project in particular, we aim to acquire the photoacoustic output of a large variety of dyes, with the eventual aim of obtaining a photoacoustic spectrum of several dyes over a number of voltages found in living cells. These spectrums will provide a roadmap for those scientists and engineers who continue on to experiments with live models.

Paper Relevance

This paper benefits our work directly and, additionally, yields interseting insight on research in live animal studies. For our project, the paper serves as a proof of concept - that VSDs are capable of providing the spatial and temporal resolution needed to detect neuronal transmission. However, this is not to assume that this paper was the first to use VSDs. Emperically obtained figures, which can be found in the presentation document, depict the mathematical basis for the physical phenomenon observed in the experiments. The authors provided valuable detail on reducing experimental error through pre-testing calibration, leading to informative results. In addition, the authors posted interesting questions that have the opportunity to lead to more fundamental understands of signal processing in the brain.

Goal

This paper focuses on the processing of sensory input from mystacical vibrissae (whisker), which map to the primary somatosensory barrel cortex. Neocortical sensory processing can be imaged with VSDs to high spatial and millisecond temporal resolution in head-fixed mice. The authors believe that brain state and behavior affect sensory processing, thus their ex-

periments are performed on anesthetized and awake mice. In addition, the paper provides a new technique for imaging cortical spatiotemporal dynamics in mice.

The specific objectives of this research were to (1) observe somatosensory response in anesthesized mice, (2) observe somatosensory response in anesthesized mice vs awake mice, (3) observe sensory reponses during Quiet and Active Whisker Behavior, and (4) image cortical representation during active touch

Animal Model Setup and Determining Spatial Resolution

It is necessary with animal models to produce as controlled an environment as possible. VSD RH1691 was introduced in the rat cortex by staining for one hour. Excitation light of 630 nm was reflected using a dichoric mirror and focused onto the end of a fiber optic array. The fiber optic cable is placed above the head fixed mouse cortex, and emitted fluorescence was focused into a high speed camera sensor.

Excitation light penetration was measured via photomicrograph calculating contours of the normalized light intensity; light penetration does not go past layers 2/3 (the supragranular layer). The depth of penetration will effect the spatial lateral resolution. The region, fluoresced by RH1691, was imaged and observed to have equivilant spatial resolution when compared to standard green light excitation.

Lastly, it was necessary to ask where imaging should take place. In particular, which layer in the barrel cortex are the RH1691 VSDs most likely to be found. Staining the rat cortex with VSDs and observing fluorescence, it was determined that neocortical layer 2/3 contained the highest population of dye. Given the depth of layer 2/3 and the results above, the fiber would image VSD fluorescence with excellent spatial resolution.

Summary of Findings

Effort was put into determining the correlation between RH1691 VSD signals and membrane potential of layer 2/3 neurons. Results have shown that after observing whole cell membrane potentials, VSDs signals were correlated to sponteneous subthreshold memrane potential changes. In addition, there was no correlation between APs of these neurons and VSD signal. Furthermore, observation of the temporal changes in the VSD signal showed that the subthreshold potential changes correlated with propogating waves of excitation.

Experiments were conducted on the signaling pathway of the right C2 whisker. Whisker deflection is performed under computer control. Results show that under anesthesia, earliest sensory response was observed in the C2 barrel column of the somatosensory cortex. Over the next 20ms, response spread over the entire barrel cortex.

Cortical responses were observed under awake and anesthetized mice. For the awoken mouse, whisker deflection was performed using magnetic pulses. Results for this experiment show that primary reponse takes place in the C2 barrel column, however whisker deflection on the

awoken mouse evoked a longer response over a greater area. This has suggested that mice that are awake perform more complex processing of the sensory signals.

Quiet and active whisker sensory responses were further recorded. When the mouse was still and not whisking, passive magnetic deflection of the C2 whisker evoked large sensory responses, which propogated across the barrel cortex. At 70ms after the peak of sensory response, the mouse will make an active whisker movement. Spontaneous waves of activity were also observed when deflection was not occuring and the mouse was not actively whisking. However, while actively whisking, passive whisker deflection produced small sensory response.

Sensory responses were recorded while the mouse actively sensed objects in its environment, causing the C2 whisker to bend upon contact. Results showed sensory responses similiar to quiet behavior, with initial output at the C2 barrel cortex and propagating over time.

To highlight: Processing of single whisker related information occurs through a similiar spatiotemporal dynamic in anesthesized and awake animals. The earliest cortical supragranular reponses were localized to the C2 whisker barrel column. Depolarization spread to cover the entire barrel cortex. The authors believe that the spread spread of sensory response to neighboring columns may help to integrate multiwhisker sensory input. Passive deflection of mice under active whiskering did not evoke strong sensory responses whereas whiskers actively making contact with an environment produced responses similiar to those obtained from passive deflection in a quiet sate.

Evaluation

The paper provides a novel way for testing head-set mice in a controlled environment. Mice that are awake have attached to their whisker a small wire with metal particle. The system causes deflection of the whisker when a magnetic pulse is introduced. The fiber based imaging with live rat is relatively simple and easy to use, allowing for repeatibility in the scientific community. This setup could potentially be useful for those who continue our project due to its simplicity. In addition, the authors were very careful in their data collection through their preliminary tests. Acquiring light penetration depth, dye penetration depth, and ultimately spatial resolution of their system allowed for reliable data to be acquired. Researchers will have to make certain that they perform these preliminary studies in order to convince readers their data is valid.

Temporal resolution was not tested prior to experiment. It should be noticed in the paper that the authors spend quite a bit of time analyzing the spatial resolution before any experimenting, which is mentioned in the setup discussion. However, temporal resolution is not analyzed until after running tests on anesthetized mice (observing correlation between membrane potential and VSD signal). Surely if effort was put pre-experiment to find the spatial resolution, than effort could have been put in to confirm the temporal resolution pre-experiment. However, one flaw I see to my argument is that measuring temporal

resolution may not be as easy as it seems, and that doing so can quite literally determine the merit of an experiment.

While the results are a step away from group 5's project in particular, they are nonetheless interesting. Even more interesting are the questions asked, and the understanding (or lack thereof) of the scientific community on this topic in 2006. It is quite appearent that the authors did not understand the underlying mechanisms behind the data they collected. This is not to discredit their work in any way; truly if there had been any information on this topic they would have found and cited it. The results represent a very high level of the signaling process in the somatosensory cortex, and the authors are very well aware of this. Regardless, the work presented serves as a useful application of VSDs for modeling physiological phenomenon. Even more importantly, the work presented serves as a guide for future work on this topic; including but not limited to imaging of the visual cortex and motor cortex. I will conclude this report by listing the questions posed by the authors in their discussion.

Questions to Consider

One big issue that the authors brought up was why smaller sensory responses to passive magnetic whisker stimulation are evoked during active whisker behavior.

- What role does the trigeminal nerve (serves as a connection between follicle and cortex) play in active whisking vs quiet behavior
- Is the suppression of whisking mediated by processes downstream of the whisker follicle?
- Are thalamaic synapses weakened during whisking by short-term synaptic depresion inducing by increased thamalic "background" firing rates
- Is the cortical brain state different between active and quiet whisking behavior?

Why is it that strong responses were recorded during active touch?

- Trigeminal sensory neurons may be engaged in a different manner during active touch
- During active touch the whisker is consciously accelerated into an object. Is trigeminal activity amplified by this conscious act?
- Is there a "top-down" influence on sensory activity in the barrel cortex. That is, when a mouse explores its environment it has a mental image. Is passive deflection just regarded as noise?