Use of Voltage Sensitive Dyes with Photoacoustic Brain Imaging

Project Proposal
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Topic and Goal

Current brain imaging techniques are insufficient to fully understand the action of neurons in the brain. Existing techniques suffer a steep tradeoff between temporal and spatial resolution. In 2014, The Johns Hopkins University and the University of Copenhagen won an NIH planning grant for "Imaging In Vivo Neurotransmitter Modulation of Brain Network Activity in Realtime." The goal of the project is to develop noninvasive techniques for human brain imaging that provide good results in the millisecond time scale (the speed range in which neurons "fire"). The initiative aims to use intravenously delivered voltage sensitive dyes and/or pH sensitive dyes that can indicate the firing of individual neurons in the brain, and to record the activity as signaled by the dyes.

The initiative involves a multidisciplinary effort to solve problems that include developing safe and effective dyes and delivery of the dyes across the blood brain barrier. One challenge is recording the signal produced by the dyes once they are in the brain. The skull is an obvious barrier to a fluorescent signal produced in the brain. Additionally, observability of fluorescence below the outer layers of brain tissue is difficult. The hope is that photoacoustic imaging will allow noninvasive, fast, and accurate observance of signal changes, through the skull and layers of tissue. This imaging sub-goal is the object of our CIS project. To further this goal, we will performing experiments and analysis to characterize various dye candidates, to assist in optimizing the signaling process.

Relevance and Importance:

Temporal resolution is the smallest time change at which an imaging modality can differentiate events. Action potentials (neural firings) usually take < 10 ms, so our imaging modality's resolution must be in the order of milliseconds. PET, one of the best available brain imaging modalities, is far too slow, imaging activity in the order of minutes. fMRI images more in real time, but only shows regions of activity that are nowhere close to the neuron level. While the scans provided by PET and fMRI are useful, they don't tell the whole picture of how the brain operates. Neurons fire in an asynchronous signal cascade. The signal begins at some neuron and propagates to different neurons as the event progresses. While these propagations often produce large amounts of activity in some region, the current regional imaging does not illustrate how the state of the response changes over time within the region. Improving the

spatial and temporal resolution of brain imaging is essential to progressing our understanding of brain function, and ultimately to developing and implementing therapies.

Voltage sensitive dyes have been proven in vivo to respond to voltage changes at the microsecond time scale. Eriksson, Tompa, and Roland showed that voltage sensitive dyes in a ferret brain can distinguish between an ON neuron firings and the complementary OFF firings, in response to a visual stimulus of less than 100 ms. Measuring the fluorescence change of voltage sensitive dyes, the experimenters were able to observe the lag between different layers of neurons involved in the response.

The results in the ferret experimenter are exactly the kinds of data brain researchers need. However, the technique used in the experiment is totally unsuitable for human use. The procedure was a grueling 36 hour ordeal, complete with craniotomy. With the skull physically out of the way, introducing dyes to the brain and imaging the fluorescent response is relatively easy. Retain the skull, however, and the fluorescence of the dyes cannot be directly measured with a photodiode array.

Photoacoustic imaging allows the fluorescence change of the dyes to be measured through the skull, and through layers of tissue. Although the response of the dyes is the same, the photoacoustic system adds focused energy to magnify the response and transform it to mechanical waves that process better through the solid tissue.

Technical Summary of Approach:

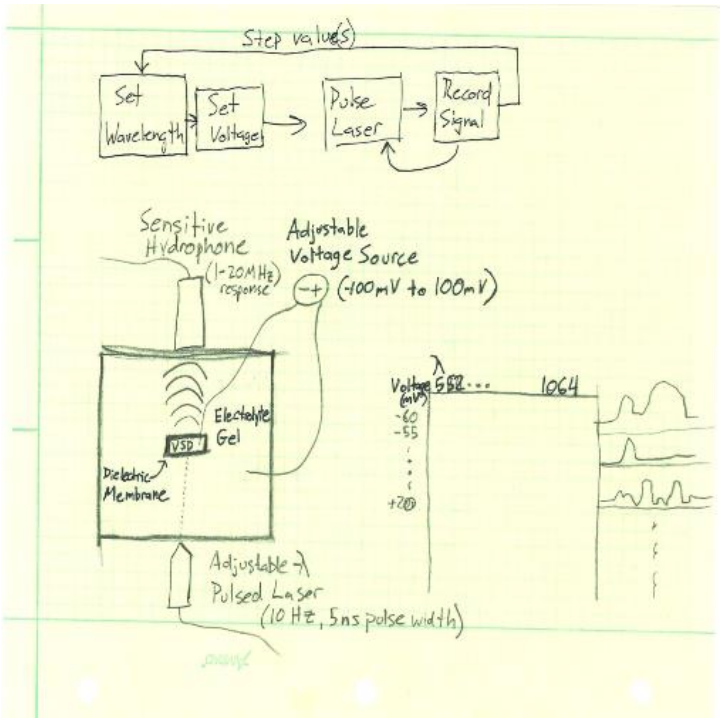
Photoacoustic imaging is broadly a two part system that involves an excitatory pulsed laser and a sensitive microphone to measure the acoustic output. The laser targets the desired region and pulses it with light of known wavelength. It is possible for the laser to shine through the skull and other tissue while remaining safe. The laser produces a thermomechanical response in the target. The target absorbs the energy from the light, causing thermal expansion and contraction. This movement produces pressure waves: an acoustic response. This pressure response can then propagate through tissue and even bone (for example from the brain through the skull) and can be recorded with an ultrasound pickup in contact with the exterior surface of the body.

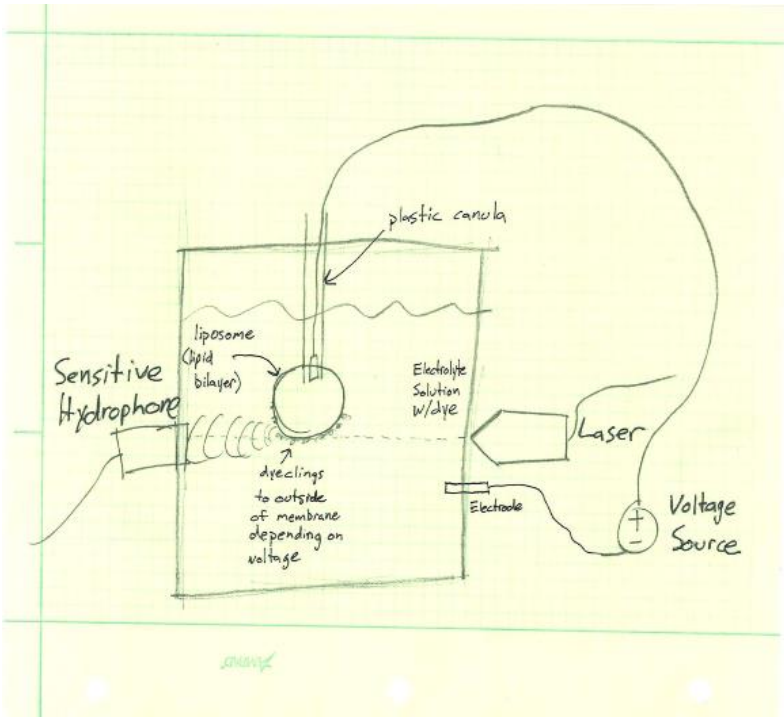
The light energy of the laser is used to induce the acoustic response in the laser's target. Because absorbance of light energy is an essential component of the process, the absorbance spectrum of the target is very strongly correlated to the output, an in fact different absorbance spectrums allow distinct constituents to be identified through the photoacoustic response. Since a change in fluorescence (such as that experienced by voltage sensitive dyes) is coupled with a change in absorbance, the potential for photoacoustic imaging being able to distinguish between changes in the dyes is very high.

In order to understand the photoacoustic response of the voltage sensitive dyes, we will be constructing test cells to characterize various dye's response at different wavelengths, at different voltage potentials. For a given dye, we want to determine a map of the photoacoustic pressure response across a excitation wavelength range of 562 to 1064 nanometers, for voltages between -100 and 100 mV. Two sketches of potential testing systems are shown below. One idea is to create a well inside of a polymer gel, line the well with dielectric material, and inject

dye into the well. The dielectric will allow a voltage to be applied between the inside and outside of the well. An adjustable wavelength laser will excite the response, and a sensitive hydrophone will measure the pressure output.

A second design is a more realistic model of the human body. A liposome bubble will be produced, into which dye will be injected. Again, a voltage will be applied across the membrane, and the response map recorded. The goal of these experiments is to determine, for a given dye, what wavelengths should be monitored. In a real world implementation, a fixed wavelength laser with improved speed would be used to perform the imaging. The wavelength must be chosen from good experimental data.





Deliverables

Minimum:

Design document for analyzing photoacoustic output across several applied voltages that are characteristic of the voltage gradient across a cell membrane. This design will include all necessary materials, procedures, and analysis techniques.

-Construction of test apparatus and test battery procedures

- Short paper summarizing existing fluorescent dyes and research regarding potential as photoacoustic VSD and pH dye candidates

Expected:

A complete map of photoacoustic output across multiple voltages and wavelengths. This map will inform us for any given voltage the strongest acoustic response and the input that gives us such a response.

Maximum:

Apply photoacoustic imaging technique with fluorescent dye with skull phantom in between laser source and dye. This test will aim to see if a strong photoacoustic response is still present through any interference provided by the skull.

In the Works:

3D beam forming using 1.5 D array

In vivo test on a mouse

Dependencies and Resolutions

Dependency	Resolution
Access to mentors	Aim to schedule at least 1 meeting / 1.5 weeks
Access to laboratory. Sub-dependencies are necessary training and equipment	Training provided via Dr Boctor's masters student. Dr Boctor will allow us to use his lab, which contains all equipment needed, to perform experiments
Access to dyes	Order far enough in advance via commercial source or via an outside lab so that dyes are in possession when experimentation begins
Synthesized liposomes from JHMI	Established contact with Dr Thorek. Once our system is defined (solute and phospholipid choice, concentrations, etc), a batch of liposomes can be physically produced in < 1 hour
Photoacoustic test software	Dr. Boctor's lab has some software for automating the photoacoustic measurement process. During our first meeting with our contact in Dr. Boctor's lab, we will discuss how to tie into this software for our own testing procedures.

Timeline with Milestones

February 20 – March 13

- Training (working with lasers and in-house test battery software)
  - ❖ **Milestone**: March 5, Training Complete
- Ordering materials

- Designing test apparatus
- Determining suitable dielectric to mimic cell membrane, generating potential across liposomes, finding suitable voltage supply

March 20 – March 27

- Construct test apparatus
- Perform dry runs of test procedure (sample titration, wavelength stepping)
  - ❖ **Milestone:** March 27, Test Apparatus Complete, Test Procedures Verified

March 27 – April 3

- Start experiment for mapping photoacoustic response over multiple voltages
- If custom VSDs not acquired: Methylene Blue, members of cyanine dye family
  - ❖ **Milestone:** April 3, Characterization Data for At Least One VSD Acquired

April 3 – April 10

- Begin analyzing data from characterization
- Run tests on a pH sensitive dye
  - ❖ **Milestone:** April 10, Data from VSD analyzed

April 10 – April 17

- Have analyzed data and have created photacoustic response map

April 17 – May 1

- Sketch out final paper and presentation
- Start experiment for next experiment using phantom skull model

May 1

- Finish final experiments and all data analysis
- Start the process of creating final report and presentation

Management Plan

We plan to meet with Dr. Boctor at least every week and a half. We will also maintain contact with Dr Thorek, to discuss the literature and catalogue search for dyes, and so that liposomes will be ready when we need them. All code for data analysis will be stored in a shared Dropbox folder, alongside written work and copies of informative research paper's we wish to share with each other and reference.

Reading List

- *Real-Time Imaging of Electrical Signals with an Infrared FDA-Approved Dye.* Treger, Jeremy; Priest, Michael.
- *Non-Linear Population Firing Rates and Voltage Sensitive Dye Signals in Visual Areas 17 and 18 to Short Duration Stimuli*. Roland, Per E; Eriksson, David; Tompa, Tamas
- *Photoacoustic imaging of prostate brachytherapy seeds in ex vivo prostate*. Boctor, Emad; Kuo, Nathanael
- *Biomedical Photoacoustic Imaging.* Beard, Paul
- *The challenges for quantitative photoacoustic imaging.* Cox B.T., Laufer J.G., Bear P.C.