Use of Voltage Sensitive and Other Dyes with Photoacoustic Brain Imaging

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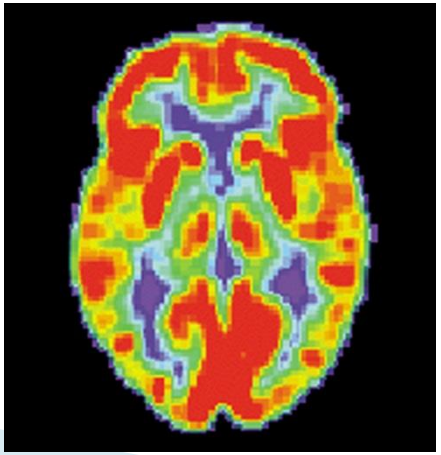
The Root Problem

- Current brain imaging modalities do not answer all questions about neuron action in the brain
  - Existing solutions have a steep trade-off between temporal and spatial resolution
  - For reference, the firing of a neuron (action potential) is in the order of ms (~ 5ms).
    - PET (okay spatial resolution, time sensitivity in minutes)
    - Magnetic helmet



http://en.wikipedia.org/wiki/Positron_emission_tomography

NIH Brain Initiative

- Kickoff meeting (start of collaboration) – January 29th 2015
- Currently received a grant to explore ideas
  - Explore feasibility of research in this area
- Next generation imaging: “Imaging In Vivo Neurotransmitter Modulation of Brain Network Activity in realtime”
- Emphasis on real-time

Potential Solution

- Voltage Sensitive Dyes (VSDs) can be designed that signal a change in cell voltage
  - Generally dyes are fluorescent, and voltage causes a change in the dye's fluorescence
  - Could be ideal for real time, spatially accurate observation of brain neurons "firing", which involves a change in electrical voltage
    - Would ideally like to discern individual neurons firing, in order
- pH sensitive dyes
  - Detect changes in hydrogen ion concentration
- Existing fluorescent dyes (such as cyanine family) may have some voltage sensitive characteristics

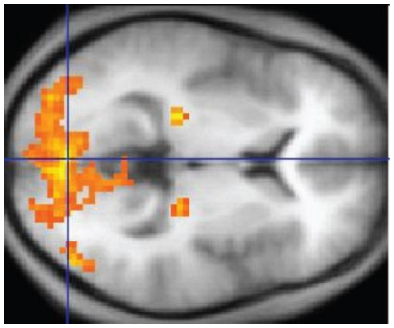
Potential Solution

- Photoacoustic imaging is a promising alternative for non-invasive observation of VSDs and other dyes in the brain
  - Photoacoustic imaging involves pulsing a target with focused laser light, which induces the target to produce pressure waves
  - The pressure waves can be recorded with a commercial ultrasound probe
  - Absorbance of target is well correlated to magnitude of response
    - And absorbance is highly correlated with fluorescence
  - Different materials have unique photoacoustic responses
  - Unique acoustic signatures can be used to identify change in a compound
  - Concentration, wavelength, and other factors influence the response

Previous Works in Brain Imaging

- Function Magnetic Resonance Imaging (fMRI) technique
  - Monitors changes in blood flow (Hemodynamic response) relate to energy use
  - Can be made to have good spatial resolution if signal from large veins suppressed
  - Most fMRI experiments monitor brain activity lasting in the order of seconds

http://en.wikipedia.org/wiki/Functional_magnetic_resonance_imaging



Previous Works in Brain Imaging (cont.)

- ***Non-Linear Population Firing Rates and Voltage Sensitive Dye Signals in Visual Areas 17 and 18 to Short Duration Stimuli***
  - Managed to observe voltage changes on small units of neurons by observing fluorescence of VSDs using photodiode array
  - Able to distinguish between “On” and “Off” neuron firings in response to millisecond introduction of stimulus
  - Skull was removed for duration of procedure and dyes introduced directly to cortex

Minimal Deliverables

- Heavily research topic
- Setup a system for characterizing dyes
- Research feasibility of maximum deliverables

Expected Deliverables

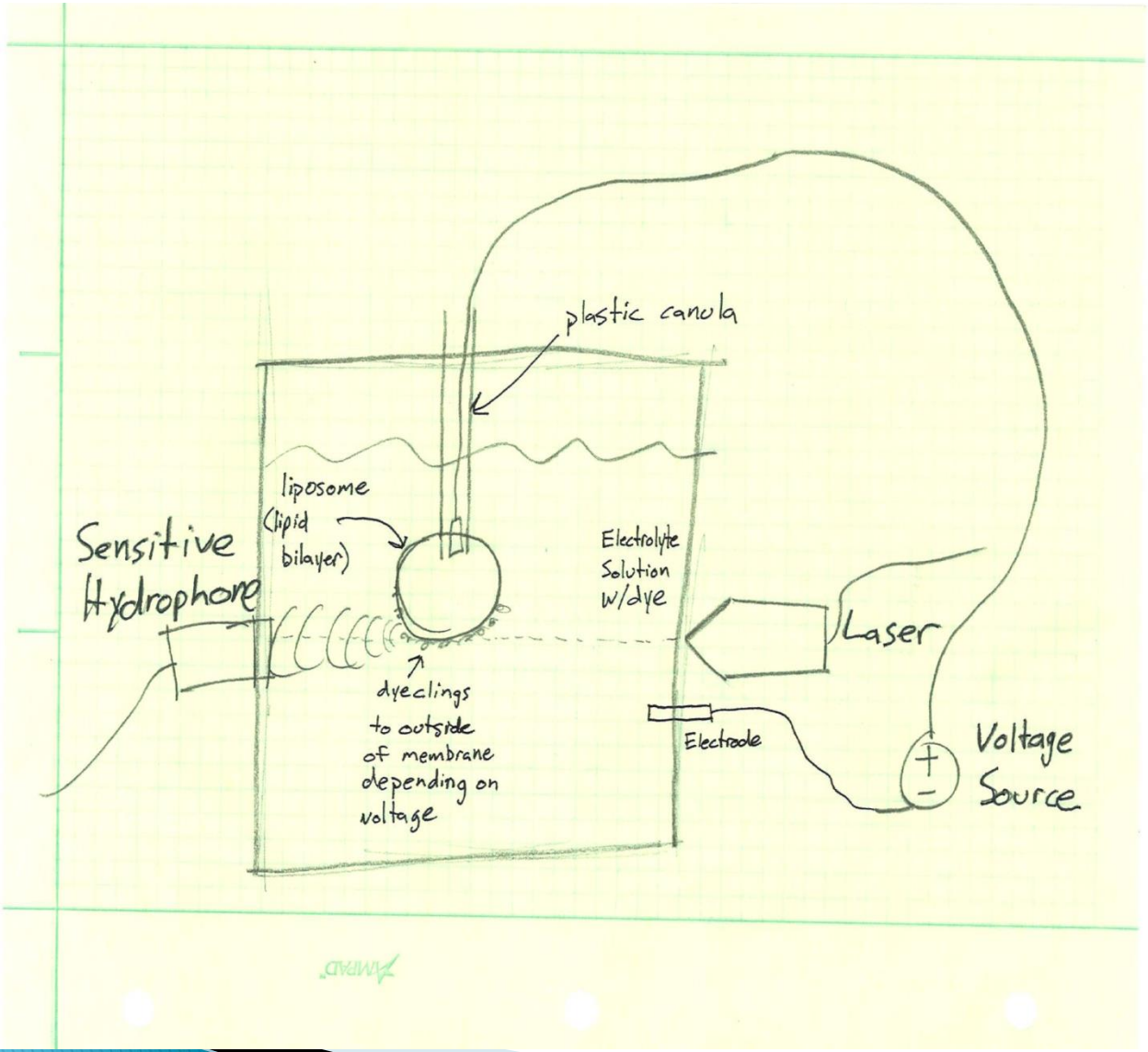
- Create test cell to simulate action potential in order to photoacoustically observe various dyes (pH sensitive, VSD, infrared)
  - Vary the voltage and pulsed laser wavelength
- Accrue data on promising dye candidates

Maximum Deliverables

- Observe the output of characterized VSDs when bombarding and recording through a skull-like barrier, at different distances from the barrier

- Multi-wavelength laser encoding
- Photoacoustic imaging of VSDs in live animal subject
- Use 3D beamforming to minimize sensors in acoustic pickup array

Diagrams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liposome
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Sensitive
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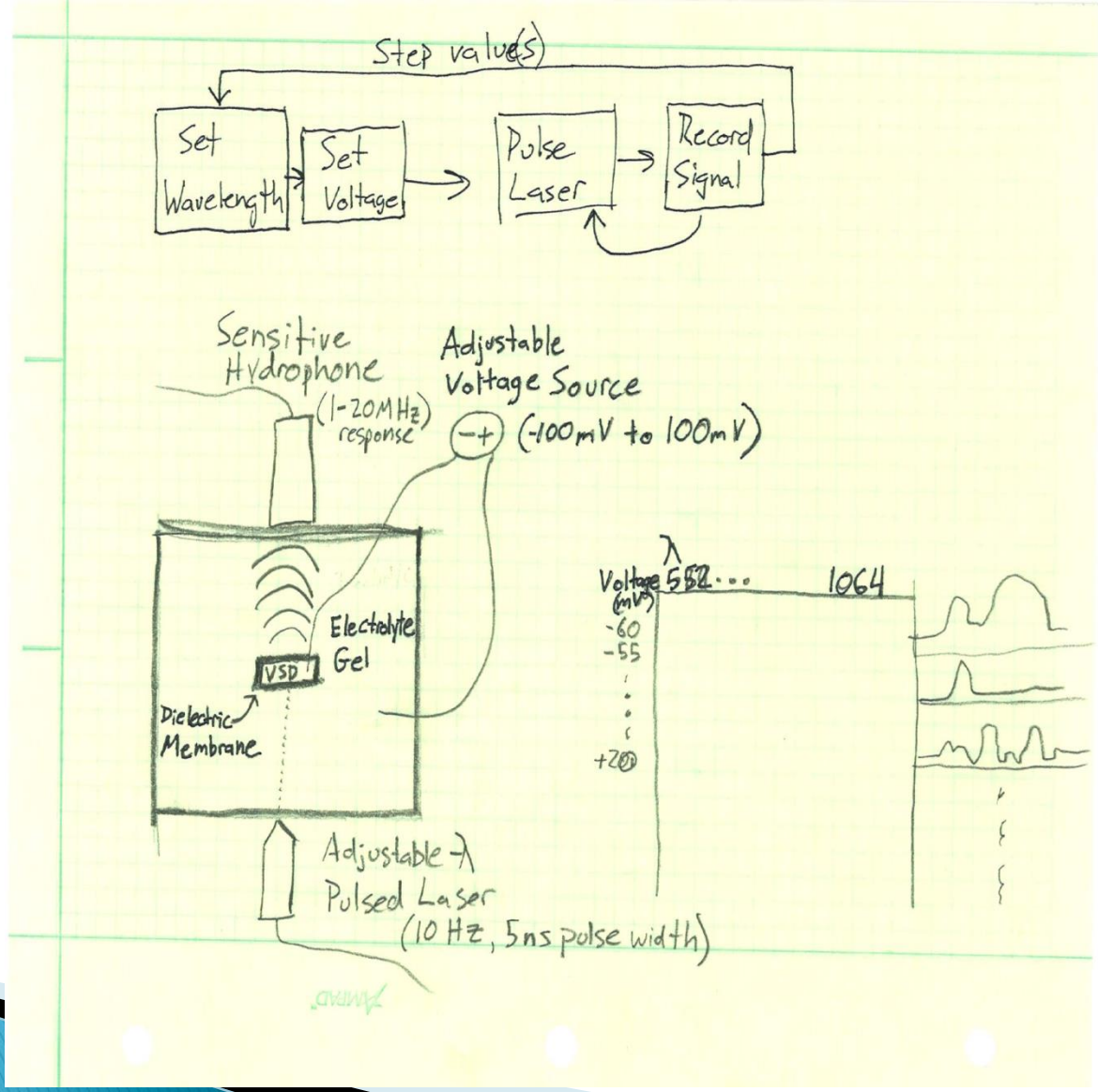
Diagrams (cont.)



Dependencies

- Mentors: Dr's. Boctor and Rahmim
- Access to experts in the field: Dr Les Leow, Dr Per Roland, etc
- Laboratory to preform experiments
  - Necessary equipment includes pulsed laser emitter, laser diodes, automated testing battery software, hydrophones, phantom skull model
- Access to Dyes
  - Available commercially and custom synthesized work from lab of Dr. Loew
- Synthesized liposomes from med campus lab
- Funds to purchase consumables (dyes, gels, etc)
  - Rough estimate $400 – $500
- Knowledge of Matlab and/or R to interpret data

Timeline

- **Weeks 1- 4:**
  - Training (working with lasers and in-house test battery software)
  - Acquisition of materials
  - Designing cell
    - Determining dielectrics/gel interface, generating potential across liposomes, finding suitable voltage supply
  - If stalled out: Methylene Blue, other members of cyanine dye family
  - Milestone: Test cell ready to go
- **Week 5:**
  - Start experiment and collecting results
- **Weeks 6-7:**
  - Start interpretation and analysis of results
  - Sketch out final paper and presentation
  - Start planning for next experiment: using phantom skull model
- **Weeks 8:**
  - Running phantom model experiment. If not possible:
    - Run experiments for off the shelf dyes
- **Weeks 9 - 10:**
  - Finish final experiments and analysis
  - Wrap up – produce final paper and presentation
- **Ongoing throughout semester**
  - Literature research of existing dyes looking for candidates with good photoacoustic properties
    - Also researching voltage/current clamping of biological models

Preliminary 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*