Crown 85: Visual Perception: A Window to Brain and Behavior

Lecture 3: Techniques for Studying Brain and Behavior

Reading:
- Amherst College Course
- Stanford College Course

Looking:
- Optogenetics
- VGL

Understand the basic principles upon which the following techniques and the kinds of information about neural processing which their application can provide:

1. Anatomical
   a. Neuron staining
   b. Electron microscopy
   c. Pathway tracing

Anatomy: Golgi, Nissl staining of neural substrate

Golgi staining of entire neuron with silver chromate. Only stains a subset of cells.

Camillo Golgi
1843-1926

Franz Nissl
1860-1919

Nissl staining of cell body with dyes (e.g. cresyl violet) that interact with RNA (and DNA).
Santiago Ramón y Cajal—arguably the most accomplished anatomist in the history of neuroscience—became recognized as such not only because of his incredible anatomical skills and his indefatigable working habits, but also because of his uncanny sense of the functional implications of his work, a sense that made him a true genius in the field of biology.

Anatomy: electron microscopy


Figure 32.20 The Rod Cell
(L) Left: Scanning electron micrograph of retinal rod cells. (Right) Schematic representation of a rod cell. [Photograph courtesy of Dr. Deric Bownds.]

Pathway Tracing:
Injecting, via fine needle, a ‘tracer substance’ into or near a neuron which is then transported down the axon (anterograde: soma → axon terminal) or up the axon (retrograde: axon terminal → soma). The pathway is then visualized by the color or radiographic ‘footprint’ of the tracer. The color may come from a tracer that is itself a dye or one that is produced by a subsequent ‘developing’ reaction.

anatomy: tract tracing (e.g. HRP, 3H proline, etc)

http://www.ncbi.nlm.nih.gov/books/NBK22541

http://journal.frontiersin.org/article/10.3389/neuro.01.032.2009/full

http://www.nature.com/nrn/journal/v9/n6/full/nrn2391.html

http://vision.ucsf.edu/hortonlab/ResearchProgram.html

got ‘em

1. Anatomical
   a. Neuron staining
   b. Electron microscopy
   c. Pathway tracing

2. Electrophysiological recording of neural activity
   a. Single cell recording in neurons
   b. Electroencephalography (EEG)
   c. Magnetoencephalography (MEG)
   d. Electrocorticography (ECoG)
electrophysiology: single cell recordings of neuronal activity

Place an electrode on a single neuron and measure the frequency of firing.

UCSC multielectrode array

electrophysiology (neural recordings): electrocorticography (ECoG)

Subdural electrodes implanted inside the cranium. For purely clinical reasons, patients with epilepsy are sometimes implanted with such electrodes to localize their seizure onset prior to surgical therapy.

electrophysiological recording: electroencephalography (EEG)

EEG: measures electric fields produced by neural electrical activity

The time course of shape discrimination in the human brain. Ales JM, Appelbaum LG, Cottereau BR, Norcia AM.
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**Lecture 3**  

**electrophysiological recording: magnetoencephalography (MEG)**

**MEG:** measures the magnetic fields generated by neural activity

- [SQUID SENSOR ARRAY](http://www.washington.edu/news/author/mollywmc/)

**got ’em**

- Electrophysiological recording of neural activity
  - a. Single cell recording in neurons
  - b. Electroencephalography (EEG)
  - c. Magnetoencephalography (MEG)
  - d. Electrooculography (EOG)

**imaging: tomography (CT, PET, OCT)**

- Collection of absorbed or emitted radiation (or electrons/positrons) from multiple detectors distributed in space; allows one to localize and image the source of the absorption or emission

**imaging (metabolic): positron emission tomography (PET)**

- Insert radioactive tracer into blood stream
  - e.g. 2-deoxy-2-[18F]fluoro-D-glucose (FDG)
- FDG ([a-2] glucose) and metabolism concentrates in areas of high neural activity = high metabolism
- [18F] isotope emits positrons (electrons with + charge)
- Detectors map areas of positron emission

**imaging (metabolic): functional Magnetic Resonance Imaging (fMRI)**

- Magnetic Resonance Imaging involves putting the sample (i.e. your head!!) in a large external magnet
- Brain imaging is most often done with a spectrometer that measures the magnetic properties of hydrogen (in water) in various parts of the brain
- Hydrogen nucleus behaves like a tiny magnet with different energies when aligned with or against an external magnet. However the exact energies also depend on nearby molecules that provide "local" magnetic fields. The "flip" energies are measured using radio wave pulses.
- Deoxygenated blood is magnetic while oxygenated blood is not magnetic ([TAKE CHEM 1](http://www.drugabuse.gov/publications/teaching-packets/understanding-drug-abuse-addiction/section-ii/2-positron-emission-tomography-pet-scan-person-us)).

**THE BOLD SIGNAL:** Blood Oxygen Level Dependent fMRI

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- Deoxygenated blood is magnetic while oxygenated blood is not magnetic (TCA: 80-90).

Also See: What is Functional Magnetic Resonance Imaging (fMRI)? Hannah Devlin, FMRI an Introduction: Michael Esterk
THE BOLD SIGNAL: **Blood Oxygen Level Dependent fMRI** (cont.)

- Near inactive neurons there is both oxygenated and deoxygenated blood due to normal metabolic activity.
- When a neuron fires there is a momentary increase in the nearby deoxygenated (magnetic) blood but then a rush of blood flow brings fresh a great concentration of oxygenated (nonmagnetic) blood in nearby capillaries.
- fMRI monitors the BOLD signal for parts of the brain with active vs inactive neurons.

**THE BOLD SIGNAL:**

- Blood Oxygen Level Dependent fMRI
- Monitoring brain activity through changes in blood flow and oxygenation
- Used to study brain function during tasks

**THE NEURAL CORRELATES OF MATERNAL AND ROMANTIC LOVE**

- Fig. 3. Overlap between activity of maternal love and romantic love.
- Activity obtained in this study (contrast: cO vs. cA) was colored in yellow and overlaid on sections through a template brain, along with activity obtained in our previous study on romantic love.

**The neural correlates of maternal and romantic love**

- Andreas Bartels, Semir Zeki

**imaging (binding agents): Ca\(^{2+}\) imaging**

- The flow of Ca\(^{2+}\) ions is associated with neuronal activity (e.g. synaptic vesicles, remember!!)
- Certain molecules that change their fluorescence ("glow") when attached to Ca\(^{2+}\)
- These molecules are inserted into neurons
- The brain preparation is excited with UV light and the neurons fluorescence ("glow") with an intensity depending on concentration of Ca\(^{2+}\)
- Cameras used to follow the action potentials fluorescence as Ca\(^{2+}\) "flows".

**functional imaging: voltage sensitive dyes**

- Certain dye molecules change their optical properties [for example the color that they 'glow/fluoresce]] when in the strong electric field of an action potential.
- These dyes are painted on the surface of the cortex.
- Cortical areas with neurons responding to a specific stimulus fluoresce with different wavelengths (colors) than do inactive areas.

**done with 3**

3. Imaging
   - a. Positron emission tomography (PET)
   - b. Functional magnetic resonance imaging (fMRI)
   - c. Calcium dyes
   - d. Voltage sensitive dyes

4. Neural activation by external stimulus
   - a. Optogenetics
   - b. Intracranial electrical stimulation
   - c. Transcranial magnetic stimulation (TMS)
neural excitation by external stimulus

- sensory input (light, sound, rat whiskers!!)
- pharmacology (drugs, etc.)
- stimulating electrode (as in neuron lectures)
  - optogenetics
  - intracranial electrical stimulation
  - transcranial magnetic stimulation

neural excitation by external stimulus: optogenetics

- genetically modify neurons to express LIGHT SENSITIVE ion channels e.g. Na⁺ or Cl⁻ channels
- activate neuron (depolarize or hyperpolarize) by light source (e.g. laser)
- image subsequent neural activity (e.g. Ca²⁺ imaging) or behavior in awake animal

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**CROWN 85: Visual Perception: A Window to Brain and Behavior**

**Lecture 3**

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**Optogenetics: videos**

- **Method of the Year 2010**
  - [Link](http://www.nature.com/nmeth/video/moy2010/index.html)

**Prof. Kliger interview**

- [Link](http://news.ucsc.edu/2015/07/optogenetics.html)

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**Neural excitation by external stimulus: intracranial electrical stimulation**

- **Intracranial electrical stimulation**: direct electrical stimulation of brain in awake subjects either with temporary or implanted electrodes (in consenting patients often those with epilepsy) in order to:
  - map brain areas to guide surgical procedures
  - monitor brain function in patients
  - explore cognitive responses

- [Link](http://golbylab.bwh.harvard.edu/intracranialEEG/EEG.html)

**Neural excitation by external stimulus: transcranial magnetic stimulation (TMS)**

- **Transcranial magnetic stimulation (TMS)**: external magnetic coil put on head, pulses direct magnetic field to stimulate brain areas

- First developed in 1985, rTMS has been studied as a possible treatment for depression, psychosis and other disorders since the mid-1990’s. Clinical trials studying the effectiveness of rTMS reveal mixed results. When compared to a placebo or inactive (sham) treatment, some studies have found that rTMS is more effective in treating patients with major depression but other studies have found no difference in response compared to inactive treatment.

  - [Link](http://www.nature.com/nrn/journal/v8/n7/full/nrn2169.html)

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**Brain images from a study that used positron emission tomography (PET) to measure metabolic activity. The colour coding shows the areas in which activity after a 25 min session of real 1 Hz is less than that seen after a sham rTMS session. There are significant decreases in activity after real rTMS at the site of stimulation (outlined in red) as well as at many distant sites. L, left side of the brain.**

- [Link](http://www.nature.com/nrn/journal/v8/n7/full/nrn2169.html)
4. Neural activation by external stimulus
   - a. Optogenetics
   - b. Intracranial electrical stimulation
   - c. Transcranial magnetic stimulation (TMS)

Psychophysics is the scientific study of the relationship between stimuli (specified in physical terms) and the sensations and perceptions evoked by these stimuli. The term psychophysics is used to denote both the substantive study of stimulus-response relationships and the methodologies used for this study.

http://www.cis.rit.edu/people/faculty/montag/vandplite/pages/chap_1/ch1p2.html

**Comparison of attributes of some brain recording techniques**

| Method                    | Fast or slow | Resolution | Local or Global | Invasive
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<tbody>
<tr>
<td>Single cell recording</td>
<td>Fast</td>
<td>High</td>
<td>Local</td>
<td>Invasive</td>
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<tr>
<td>Electrophysiology (EEG)</td>
<td>Fast</td>
<td>Low</td>
<td>Global</td>
<td>Noninvasive</td>
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<tr>
<td>Magnetoencephalography (MEG)</td>
<td>Fast</td>
<td>Moderate</td>
<td>Global</td>
<td>Noninvasive</td>
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<tr>
<td>Positron emission tomography (PET)</td>
<td>Slow</td>
<td>Low</td>
<td>Global</td>
<td>Noninvasive (but involves radioactive material)</td>
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<tr>
<td>fMRI</td>
<td>Slow</td>
<td>Low</td>
<td>Global</td>
<td>Noninvasive</td>
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<tr>
<td>Ca²⁺ dyes</td>
<td>Fast</td>
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What types of neural processes would each of these be suited to measure?