Lecture 4: Experimental techniques in visual neuroscience

Reading Assignments:

None!
Today we will briefly review...

- electrophysiological recording and stimulation
- visual psychophysics
- functional neuroimaging
  - positron emission tomography (PET)
  - single-photo emission tomography (SPECT)
  - functional magnetic resonance imaging (fMRI)
- optical imaging
- electroencephalography (EEG) and magnetoencephalography (MEG)
**Electrophysiology**

**Basic idea:** record electrical activity associated with neuronal activity, using electrodes inserted in the brain of an animal.

**Typical setup for visual experiment:**
- animal is either anaesthetized or awake.
- various stimuli are presented on computer screen.
- activity of one neuron or a small group is recorded for a few seconds around stimulus presentation.
- (optional): if awake, animal may be doing a visual task and give responses, e.g., by pressing a button.
- many such “trials” are acquired from many different recording sites.
- recordings are pooled and analyzed.
Typical Setup
Electrode setup

- drill hole in cranium under anesthesia
- install and seal “recording chamber”
- allow animal to wake up and heal
- because there are no pain receptors in brain, electrodes can then be inserted & moved in chamber with no discomfort to animal.
Recording setup

- Connect electrodes to amplifier & noise suppression board.
- Sample & record.
- Label & store data.

Result: sampled traces of V or I as function of time.
Multi-Electrode Arrays

Allow simultaneous recording from many locations. **Problem**: does that mean from many neurons? The answer is no. Much signal processing needed to separate sources.
Localization & source separation

Main problem: we don’t see the image below!
With individually adjustable electrodes: slowly advance them until a clear signal is obtained.
With fixed arrays: separate sources by post-processing software.
Visual Electrophysiology: Receptive Field

**Issue:** Neurons in visual processing areas do not respond to every location in visual field. Recall: “retinotopic organization.”

So, once a neuron is localized, we also need to localize its “receptive field,” that is, the region of visual space (or computer screen) in which the presentation of a stimulus will elicit a response from our neuron.

Kuffler (1953): shine a spot of light at many different locations over screen and monitor cell activity. All locations where light elicits neuronal response belong to neuron’s receptive field (RF).
Receptive field

Transient Response

ON-center OFF-surround

Stimulus: on          off

Kuffler 1953
RFs increase in size and complexity
Raster Displays and Histograms
Single-unit recording in humans!

Kreiman & Koch, 2000
Single-unit recording in humans
**Visual Psychophysics**

**Basic idea:** instead of recording from individual neurons, record from the whole organism.

**Typical setup for visual experiment:**
- animal or human subject is always awake.
- a stimulus appears on computer screen, and subject is asked to make a judgment about the stimulus.
- subject reports judgment, e.g., by pressing a button.
- experimenter monitors subject responses over many trials and modifies stimulus during experiment so that judgment becomes harder and harder to make.
- from results over many trials, experimenter can compute the subject’s “threshold,” i.e., the breaking point in the subject’s ability to make the judgment.
Example: yes/no task

Example of contrast discrimination using yes/no paradigm.

- subject fixates cross.
- subject initiates trial by pressing space bar.
- stimulus appears at random location, or may not appear at all.
- subject presses “1” for “stimulus present” or “2” for “stimulus absent.”
- if subject keeps giving correct answers, experimenter decreases contrast of stimulus (so that it becomes harder to see).
**Staircase procedure** is a method for adjusting stimulus to each observer such as to find the observer’s threshold. Stimulus is parametrized, and parameter(s) are adjusted during experiment depending on responses. Typically:

- start with a stimulus that is very easy to see.
- 4 consecutive correct answers make stimulus more difficult to see by a fixed amount.
- 2 consecutive incorrect answers make stimulus easier to see by a fixed amount.
Psychophysical threshold

The **threshold** is the value of the stimulus parameter for which a given probability of making a correct judgment is obtained.

Typically, in a yes/no task:
- chance level = 50% correct.
- perfect judgment every time = 100% correct.
- threshold set at 75% correct.
Confusing terminology

The **threshold** is the value of the stimulus parameter for which the **threshold performance** (e.g., 75% correct) is reached. This notion thus is highly task-dependent.

For example:

A “**contrast threshold**” may be the value of contrast for which 75% correct discrimination in the task “was there a stimulus?” is obtained.

An “**orientation threshold**” may be the angle for which 75% correct discrimination in the task “was the stimulus vertical or tilted?” is obtained.

and so on…
Better presentation techniques: 2AFC

One problem with the yes/no paradigm is that observers may develop a bias in their judgment (e.g., always answer yes when not sure).

The (temporal) two-alternative forced-choice (2AFC) paradigm eliminates this problem by always showing two stimulus alternatives, one after the other, in random order, and by forcing the observer to report on the order in which those two alternatives appeared.

e.g., a vertical and tilted gratings appear in random order; the observers answers “was the stimulus vertical then tilted?”

In the spatial 2AFC, both stimulus alternatives appear simultaneously, next to each other.
Example of psychophysical data
**Functional Neuroimaging**

**Basic idea:** monitor brain activity using an external, non-invasive machine, and do it simultaneously for the entire brain (at the cost of a fairly low spatial and temporal resolution).

**Typical setup for a visual experiment:**
- subject lies in scanner and views stimuli on a screen.
- an image of the brain is taken at rest.
- subject does a visual task.
- an image of the brain is taken during or just after task.
- the difference between rest and task images tells us what changed in subject brain because of task.

Thus, the subject (at rest) is his/her own reference for detecting task-related activation.
Different imaging techniques

- **Nuclear medicine (PET and SPECT):** inject a radioactive tracer into the subject’s blood stream. Tracer will get trapped into those neurons which are active. Thus, imaging the radioactive regions in the subject’s brain reveals those areas where strong neuronal activity was present during experiment.

- **functional MRI:** most commonly, exploit the property that oxyhemoglobin and deoxyhemoglobin have distinct magnetic properties; thus regions where magnetic changes are seen indicate higher consumption of oxygen, and, by inference, higher neuronal activity.

- **perfusion MRI:** inject a paramagnetic tracer into blood stream; thus, regions where magnetic properties change are where a lot of blood arrives, presumably because of neural activity.

- **MR spectroscopy:** different chemicals have different resonance frequencies; thus, by sweeping over those frequencies and recording resonance responses, we measure concentration of chemicals.
Single-Photon Emission Tomography

Basic physics:
- uses gamma-radioactive elements: those emit a gamma photon as they transition to a lower energy state.
- the imaging tracer is a complex chemical, in which one element is gamma-radioactive.
- gamma-ray detectors placed around the subject’s head detect the gamma photons; this is done from different viewpoints (e.g., by rotating an array of detectors around the subject).
- from the multiple projections of the subject’s head, a 3D volume can be reconstructed by tomographic reconstruction.

Typical tracers: HMPAO (\(^{99m}\text{Tc}\) hexamethyl propylene amine oxime enters neurons and is metabolized and trapped); \(^{201}\text{Tl}\)-based agents enter tumors; ECD (\(^{99m}\text{Tc}\) ethyl cysteinate dimer) is similar to HMPAO; but also tracers to specifically image lungs, bone, specific glands and organs, etc.
Tomographic reconstruction
Example SPECT images

HMPAO
Calibrated Xe / HMPAO
99m Tc MIBI
201 Thallium
**Positron Emission Tomography**

Basic physics:
- some radioactive elements emit a positron (e+) as they transition to a lower energy level.
- the positron (an anti-particle) soon collides with a nearby electron (e-), yielding an annihilation.
- energy is liberated during the annihilation by the emission of two gamma photons traveling in exactly opposite direction.
- a ring of gamma light detectors around the subject’s head captures the photons.
- because we know that annihilation yields two opposed photons, the reconstruction algorithm can take this into account to eliminate scatter. Results in better resolution than SPECT.

Typical tracers: radioactive labeled oxygen (\(^{15}\text{O}\)) or glucose (\(^{18}\text{F FDG; fluoro-deoxy glucose}\)).
Reconstruction using coincidence
Example PET image
Use in activation studies

Show difference image between, e.g., rest and task, superimposed onto a structural scan (here MRI), possibly normalized to a standard coordinate system (here Talairach).

Maguire et al., 1997
Use in activation studies


A

B

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Exp. techniques in visual neuroscience
**PET vs. SPECT**

Positron-emitting elements are to create and have very short half-life (a few minutes). PET scanners require cyclotron on premises. SPECT tracers easily created by mixing two stable reactants.

Coincidence in PET yields better resolution and less scatter.

SPECT tracers applicable to wider range of studies (lungs, bone, grands, etc).

SPECT tracers decay more slowly so scans cannot be made as often as PET.
Magnetic Resonance Imaging

**Basic idea:** protons in brain have a magnetic moment; when placed in a magnetic field the moments “align” with the field and precess around it; an RF pulse can kick them out of alignment; their precession can then be picked up by sensitive coils (dynamo effect).

Use in visual neuroscience:
- regular MRI provides very detailed anatomical information
- functional MRI

for more info: [http://www.cis.rit.edu/htbooks/mri/](http://www.cis.rit.edu/htbooks/mri/)
PROTON SPIN
and MAGNETIC MOMENT

Cohen
The Resonance Phenomenon

When an RF pulse is applied at the Larmor frequency, the proton will precess about the axis of the RF pulse.

Cohen
Free Protons
Protons in Applied Field

Applied Magnetic Field
An RF Pulse Converts Longitudinal Magnetization to Signal

Longitudinal Magnetization

90° RF Pulse

MR Signal

Precession

Cohen
In-Phase Precession

Cohen

NMR Signal
Out of Phase Precession

Receiver

NMR Signal

Cohen

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The Larmor Relation

Frequency (Megahertz)

Magnetic Field (Tesla)

Cohen
Imaging System Components

- Magnet
- RF Receiver
- Viewing Console
- Gradient Power Systems
- RF Transmitter
- Scan Controller

Cohen
Frequency Selective Excitation
3D T1 Images

**TE = 3.2**
**TR = 14.4**
124 slices
1.25 mm thick
1 NEX
Flip Angle 20°
**TI = 500**
**BOLD effect**

Blood-oxygen level-dependent effect: the presence of fresh (oxygenated) blood affects the MRI signal.

The magnetic moment of deoxyhemoglobin leads to an increase of the magnetic field in the surrounding of the erythrocytes.
The magnetic properties of blood change with the amount of oxygenation resulting in small signal changes.
Vascular System

arteries
arterioles (<0.1mm)
capillaries
venules (<0.1mm)
veins
The exclusive source of metabolic energy of the brain is glycolysis:

$$C_6H_{12}O_6 + 6 \text{ O}_2 \rightarrow 6 \text{ H}_2\text{O} + 6 \text{ CO}_2$$
BOLD Contrast

stimulation

neuronal activation

metabolic changes

hemodynamic changes

local susceptibility changes

MR-signal changes

signal detection

data processing

functional image
Note about BOLD effect

The observed change in the MR signal indicates an increase of oxygenation in the activated areas!

So, what we measure is an overshoot in the brain’s vascular response to oxygen consumption.

neural activity =>
  oxygen consumption =>
  oxygen depletion =>
  vascular response: increase blood supply =>
    more oxygenated blood arrives at site of activity =>
      increased concentration of oxyhemoglobin picked by MRI

BOLD measures a hemodynamic (change in blood supply) response.
**Fast response & early dip**

**BOLD Signal Response**

- "impulse" response to a brief stimulus
- more sustained positive BOLD response (larger scale flow changes, excess of HbO2 created, reduction in conc. of Hbr)
- brief initial "dip" (HbO2 → Hbr, local flow changes)
- post-stimulus undershoot, return to normal flow but slow CBV recovery (giving effective increase in [Hbr])

![Graph showing the BOLD signal response over time.](image)

fMRI "dip": Menon et al., MRM 33:453; Ernst & Hennig, MRM 32:146; Hu et al., MRM 37:877
Experimental paradigms

Two basic classes:

- **Blocked**: rest for a while, do task for a while; repeat. Subtract average activity during rest from that during task.

- **Single-event**: do a single trial of task once in a while (possibly at randomly distributed times); record activity associated with each event; re-align all recordings and compute statistics on the average.
Example of Blocked paradigm

Gandhi et al., 1999
First BOLD-effect experiment

Kwong and colleagues at Mass. General Hospital (Boston).
Stimulus: flashing light.

Fig. 1. Adapted from Kwong et al. (20). BOLD contrast signal change is shown for a region of visual cortex during stimulation (on) and during rest (off). These data originally were used to demonstrate the application of BOLD contrast fMRI in normal human subjects. As can be seen, the rise time of the signal (indicated with arrows) is very rapid and has occurred after just a few seconds of stimulation, indicating that shorter stimulus events should be detectable.
Example: ball-tracking

Attentional Tracking

3 Targets
7 Distractors

Passive Viewing

0 Targets

Ernst et al., 2000
Ball-tracking: activation

8 Women
7 Men
No gender differences, or lateralization

z > 7.5
Single-event responses

![Graph showing single-event responses](image-url)
Optical imaging

Basic idea: reflectance properties of neurons change with activity.
Optical imaging of V1
Optical imaging of V1
V1 orientation & ocular dominance columns

Optical Imaging

Orientation preference and ocular dominance maps from the same patch of cortex.

Black for contralateral and white for ipsilateral eye preference.

Scale bar, 1 mm.
Electroencephalography (EEG)

**Basic idea:** detect electrical fields generated by neural activity, using electrodes placed at surface of skin.
**EEG terminology**

- VEP = visually-evoked potentials
- ERP = event-related potentials (are all EEGs)
- OSP = omitted-stimulus potentials
Magnetoencephalography (MEG)

**Basic idea:** detect magnetic fields generated by brain activity, using an array of very sensitive coils.

**Difficulty:** magnetic field generated by the conjoint activity of 100,000 neurons is on the order of a few femtotesla \((10^{-15})\); in comparison, earth magnetic field around \(10^{-5}\) Tesla.

**Hence:** use very sensitive magnetic detectors (SQUID: Superconducting Quantum Interference Devices that work in liquid helium at \(-269\) degrees C), and place machine in magnetically shielded room.

**Inherent limitation:** coils can only pickup the component of the magnetic fields that is perpendicular to them.
MEG machine
Raw MEG data
One trace is shown per detector. Activity is found in auditory cortex about 80ms after onset of auditory stimulus.
Mapping MEG results onto anatomy

Localization of auditory activation source with respect to array of detectors.
MEG source localization

A mix of activity from several sources is detected by several detectors; hence we have a source separation problem.
Combination of techniques

from Rosen et al., 1998
Summary

Physiological Correlates of Brain Electrical Activity

**electrical activity**
- excitatory
- inhibitory
- soma action potential

**hemodynamic response**
- ↑ blood flow
- ↑ blood volume
- ↑ blood oxygenation

**metabolic response**
- ↑ glucose consumption
- ↑ oxygen consumption

- FDG PET
- autoradiography
- $H_2^{15}$O PET
- NIRS
- optical imaging
- fMRI

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Summary 2

Functional Mapping Methods

- MEG + ERP
- fMRI
- Optical dyes
- PET
- Lesions
- 2-Deoxyglucose
- Microlesions
- Single unit
- Patch clamp
- Light microscopy

Non-Invasive

Invasive

Log size (m)

Log time (s)