In all their overwhelming variety!

In no other biological field are students required to learn biochemistry, molecular biology, genetics, electrophysiology, microscopy, histology, behavioral assays, and human imaging technologies, not to mention the basic biological and physical properties that allow these methodologies to work. It is true that each neuroscientist only practices a handful of these general techniques. However, all neuroscientists must learn to understand and appreciate one another's research, even if they don't perform the specific experiments themselves.

-Carter & Shieh, 2010
Guide to the Guide to Research Techniques in Neuroscience:

- p. 23 Imaging
- p. 81 Implants for Long-Term Access to the Brain
- p. 82-85 Measuring Neural Activity in vivo
- p. 101 I/V Curves
- p. 108 Extracellular vs. Intracellular
- p. 109 patch clamp
- p. 152 slicing
- p. 159 stains
- p. 161 antibodies
- p. 165 tracers
- p. 239 viral vectors
- p. 306 measuring proteins

Levels of Analysis

- Cognitive
- Behavior
- Systems, pathways
- Networks, circuits
- Single cell
- Subcellular, synaptic
- Molecular
- Genetic
### Categories

**Neuroanatomical**
- Whole Brain
  - Structural imaging (CT, MRI)
- Microscopy
  - Light
  - EM
- Tracer studies
  - Retrograde
  - Anterograde

**Neurochemical**
- Microdialysis
- HPLC

**Neurophysiological**
- Functional Imaging: EEG, MEG, PET, SPECT, fMRI, TCMS, Optical imaging, Optogenetics, 2-photon Ca++ imaging

**Extracellular:** single, multiunit
**Intracellular:** sharp or whole cell

**Patch clamp**

**Neuropharmacological**
- Agonists, antagonists

**Neurogenetic**
- Forward genetics
- Reverse genetics

**Molecular**
- Nucleic Acids (ISH, qRT-PCR, Northern, Southern)
- Protein (IHC, ELISA, Western)

**Behavioral**
- Lab studies
- Field studies

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![Diagram of brain and related imaging techniques](attachment:image.png)

*Cherubini and Saper (2009)*
Neuroanatomical Methods

Whole Brain
  Structural imaging (CT, MRI)

Microscopy
  Light
  EM

Tracer studies
  Retrograde
  Anterograde

Golgi Stain
Morphological Diversity

Brainbows

Jean Livit/Joshua Sanes/Jeff W. Lichtman/Harvard University
Tract tracers

Retrograde
- Horseradish peroxidase (HRP)
- Fluoro-Gold, Fast Blue
- Fluorescence-labeled microspheres

Anterograde
- PHA-L (Phaseolus vulgaris-leucoagglutinin)
- Cholera Toxin
- $^3$H-proline

Both retrograde and anterograde
- Biotinylated dextran amine (BDA)
- WGA-HRP
- Lipophilic dyes (DiI DiO DiA DiAsp)

HRP Injections in A1
LP/Pulvinar to A1 projection neurons

Transsynaptic Tracers

- (Tritiated) $^3$H-proline
- Wheat-germ agglutinin (WGA)
- Tetanus toxin, fragment C (TTC) (retrograde only)
- Pseudorabies virus (PRV) (retrograde only)
- Herpes simplex virus (HSV)
Stains

Cresyl violet (cell nuclei, used in Nissl stain; blue to purple)
Hematoxylin (cell nuclei; used in combo with eosin; blue to blue-black)
Eosin Y (cytoplasm; often used in combo with hematoxylin; pink to red)
DAPI (cell nuclei; UV light makes fluorescent blue)
Golgi (stains full neuron black at random)
Cell and fiber stains

Nissl stain

Rhesus Macaque Brain

Dentate gyrus of mouse brain section (green: retroviral labeled cells, red: doublecortin [DCX] positive immature neurons, blue: DAPI labeling nuclei of all cells).

Myelin stain

Rhesus Macaque Brain

Multiple Sclerosis demyelination
Imaging Methods

Structural
- Cerebral Angiography
- CAT Computerized Tomography
- MRI Magnetic Resonance Imaging
- Diffusion Tensor Imaging

Functional
- EEG Electroencephalography
- PET Positron Emission Tomography
- SPECT
- fMRI Functional MRI
- Optical Imaging

Youtube Overview

MRI Acquisition

http://www.youtube.com/watch?v=Xe3XT3ddZI0
DTI
Diffusion Tensor Imaging
Tractography

http://www.youtube.com/watch?v=9k11ida1wYE

CLARITY

http://www.youtube.com/watch?v=c-NMfp13Uug
The brain controls the flow of oxygenated blood to where it is needed (discovered over 100 years ago).

There is iron in blood which is a magnetic metal. Oxygenated and deoxygenated blood have different magnetic properties (discovered by Linus Pauling in the 1930s).

MRI was invented in the 1970s, based on the physics of magnetic resonance which was discovered in the 1940s.

The MRI scanner can be reprogrammed to pick up differences in magnetization that take place when the brain ships oxygenated blood to where it is needed.

Prof. David Heeger's Neuroimaging Lecture

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Comparing functional imaging techniques

**Comparing fMRI, PET, ERP, & MEG**

- fMRI has best spatial resolution & localization
- ERP and MEG have best temporal resolution (PET has worst temporal resolution)
- PET use of radioactive compounds is both a disadvantage (for obvious reasons) and an advantage (can radioactively label many different compounds).
- Cost and availability: ERP is the least expensive, then fMRI and MEG, and then PET.

fMRI has become the method of choice

Overview: [http://www.youtube.com/watch?v=N2apCx1rIiQ](http://www.youtube.com/watch?v=N2apCx1rIiQ)
Combining Imaging Methods for Surgery

http://www.youtube.com/watch?v=MtGMZSwl1Yk
**Optical Imaging**

![Image of optical imaging setup](image)

**Figure 27.12A** Optical imaging of functional architecture in the primate visual cortex. (A)

**2-photon Calcium imaging**

Cat vs. rat visual cortex orientation tuning

![Image of calcium imaging results](image)

*Single-cell resolution orientation maps from (a) a pinwheel in cat visual cortex [42] and (b) rat visual cortex [38**](http://www.hms.harvard.edu/bss/neuro/bornlab/nb204/papers/ohki-reid-review-curropinneurobiol2007.pdf) obtained with in vivo two-photon calcium imaging. One side is 300 mm. Cells are colored according to their preferred orientation. In (a), 1100 cells from nine different depths are overlaid. Cells are arranged up to the very center of the pinwheel in cat visual cortex. In (b), cells in one depth of rat visual cortex are displayed. Even neighboring cells are tuned to different orientations. (c) In rat visual cortex, relatively independent subnetworks are embedded in larger scale functional architecture [31]. Excitatory connections from layer 4 to layer 2/3 and within layer 2/3 define subnetworks of selectively interconnected neurons (red or blue). The excitation from layer 5 (gray triangles) and inhibition from layers 2/3 and 4 adaptive interneurons (IN, gray ovals) do not respect the subnetworks.*
Neurophysiological Methods

- Functional Imaging
  - Large scale
    - EEG, MEG
    - PET, SPECT
    - fMRI
    - TCMS
  - Finer scale
    - Optical imaging
    - Optogenetics (stimulation)
    - 2-photon Ca** imaging
- Extracellular:
  - Single unit
  - Multiunit
  - MEAs
  - Worms
  - Intracellular
    - sharp electrodes
    - whole cell patch clamp
    - traditional patch clamp

The electrophysiology “rig”
Electrophysiological recording: Mapping receptive fields

Extracellular recording
MEAs

Sharp electrodes
Patch electrodes

http://www.youtube.com/watch?v=MzwgrH43f_U
This video shows IR-DIC microscopy imaging of acute brain slices prepared from a 5 month old mouse brain comparing the conventional "neuroprotective sucrose aCSF cutting method" versus new "NMDG protective recovery method." The imaging is focused on the dorsolateral striatum, starting at the slice surface and progressing deeper in 10 micron increments examining different time points after the slicing procedure. (Courtesy of Dr. J. Ting & Dr. G. Feng, McGovern Institute for Brain Research at MIT, USA.)

Calcium imaging

http://www.youtube.com/watch?v=PF5GTRX0NYB
This video shows live confocal microscopy imaging of acute brain slices prepared from a 5 month old Thy1-GCaMP3 mouse brain comparing the conventional "neuroprotective sucrose aCSF cutting method" versus new "NMDG protective recovery method." The imaging is focused on hippocampal dentate granule cells in the superficial layers and examining neuronal stimulation by bath application of high potassium aCSF.
A method is described for labeling neurons with fluorescent dyes in predetermined functional micro-domains of the neocortex. First, intrinsic signal optical imaging is used to obtain a functional map. Then, two photon microscopy is used to label and image neurons within a micro-domain of the map.

**Optical Imaging**

**Optogenetics**

Channelrhodopsins: Turning cells on (depolarizing) with blue light
Haloarhodopsins or archaerhodopsins: turning cells off (hyperpolarizing) with green or yellow light (respectively)
Uses viral vectors to deliver genes to produce rhodopsins
“Snap Shot” vs Real-Time Imaging

“Snap Shot”
- ICC (IHC)
- Western Blot
- Golgi stain (or any stain)
- In situ
- qRT-PCR

Real-Time
- fMRI
- EEG
- PET (positron emission technology)
- optical imaging

Optogenetics can be used to elicit real-time changes, but is not really imaging

Molecular Level Assays

Protein
- ICC/IHC
- Western blot
- ELISA

RNA/DNA
- ISH
- Northern, Southern Blots
- QRT-PCR
Central “Dogma” of Molecular Biology

Transcription

DNA $\rightarrow$ mRNA

RNA polymerase reads the DNA template strand in the 3’ to 5’ direction and synthesizes the new mRNA in the 5’ to 3’ direction.

Transcription begins when various proteins called transcription factors interact with regions of the genome called promoters.

After transcription terminates, a newly formed RNA molecule receives further processing called RNA splicing to remove the introns (non-coding sequences) before leaving the nucleus.
A ribosome reads an mRNA molecule in the 5’ to 3’ direction and uses this template to guide the synthesis of a chain of amino acids to form a protein.

One codon, “AUG” codes for the amino acid methionine, which is the start of any protein sequence.

Other codons (“UAA,” “UAG,” and “UGA”) serve as “stop” codons and terminate translation.
Translation

Gene Expression Assays

- Gel electrophoresis
  - Distance = size in bp of fragment (compare to “ladder”)
  - Assess purity/contamination of sample
  - Cut out and sequence bands
Gene Expression Assays

- ISH
  - spatial, relative quantity
- qRT-PCR
  - relative or absolute quantity, no spatial
- cDNA Microarray Screen:
  - assay all genes in the genome at the same time in a relatively small device

Ephrin-As are expressed in a rostrocaudal gradient that declines with postnatal age

Tadesse, Xu, Baro, Young, Pallas 2013
qRT-PCR

http://www.youtube.com/watch?v=2KoLnlwoZKU
http://www.youtube.com/watch?v=EaGH1eKfvC0&spfreload=10
http://www.youtube.com/watch?v=kvQWKcMdyS4
cDNA Microarray “chip”

http://www.youtube.com/watch?v=UgL1Pq2sk3M

Gene delivery methods

Physical
- Microinjection of DNA p 231
- Electroporation p 233
- Biolistics “gene gun” p 234

Chemical
- Calcium phosphate
- Lipofection (endocytosis) p 237

Viral vectors [Table 10.2]
- Adenovirus
- AAV
- HSV
- Lentivirus

Sagittal brain section of a transgenic brain mouse showing GAD65,67-EGFP neurons (green) and dopamine neurons stained for tyrosine hydroxylase (red). Courtesy Dr. Fu-Ming Zhou
**Transgenics**

Report genes

Death-inducing genes

Activity reporters

Optogenetics

Function-blocking

Over-/mis-expression

Conditional expression
- Gal4/UAS
- Cre-Lox
- Flp/Frt
- tTA/tetO

See Table 11.1 p 245

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**Viral Vectors**

See Table 11.1 p 245
Gene reduction

Knockout/in
RNAi
  shRNA
  siRNA
Antisense oligonucleotides (morpholinos)
Dominant negatives

Protein Assays
Immunological detection of proteins

- Immunohistochemistry (IHC),
- Immunofluorescence (IF),
- Enzyme-Linked Immunosorbent Assay (ELISA),
- Western Blotting (WB),
- Immunoprecipitation (IP),
- Fluorescence Activated Cell Sorting (FACS)

Principle of recognition
primary antibody binds to specific epitope (one or several) in the protein

Principle of detection
primary antibody or secondary antibody that recognise primary antibody is labelled
(examples: HRP for IHC and Western blotting, fluorescent dye for IF and FACS)

www.methods.info/TMR24/Protein%20analysis%20IV_kzhyshkowska.ppt

Immunocytochemistry/Immunohistochemistry (ICC/IHC)

1) Add species #1 serum with detergent (e.g. Triton-X)
2) Add primary antibody (derived from species #2)
3) Add secondary antibody (same species as #1 serum)
4) Marker for secondary antibody

(wash steps would be in between each of these major steps)
**IHC and IF: overlapping terms**

<table>
<thead>
<tr>
<th></th>
<th>Direct</th>
<th>Indirect</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Advantages</strong></td>
<td>Cheap</td>
<td>Only limited number of labeled primary antibodies are available commercially</td>
</tr>
<tr>
<td></td>
<td>Fast</td>
<td></td>
</tr>
<tr>
<td><strong>Disadvantages</strong></td>
<td>Wide range of labeled secondary antibodies are available commercially</td>
<td>It is always possible to design combination for double and triple staining</td>
</tr>
<tr>
<td></td>
<td>Takes more time, sometimes is more expensive</td>
<td>Additional control for the background staining is absolutely necessary</td>
</tr>
</tbody>
</table>

**Controls**

1. Antibody-independent of non-specific signals

   - **IHC**
   - Background signal coming from substrate

   - **IF**
   - Auto fluorescence

2. Antibody-dependent non-specific signals/cross-reactions for IHC and IF:

   2.1 Non-specific signal coming from antibody alone

      Solution: optimization of concentration of secondary antibody (not signal has to be observed when primary antibody is not applied)

   2.2 Non-specific signal coming from primary antibody.

      Following controls for primary antibody have to be used and concentrations have to be optimized:
      - Isotype control for monoclonal antibody
      - Preimmune serum for polyclonal antibody-containing serum
      - Matching Ig for purified polyclonal antibody

      **Important note:**
      by optimization working concentrations has to be calculated NOT dilution
Commonly used antibodies

Neurons: NeuN
Dendrites: MAP2
Axons: Tau-1
Synapses: PSD95; synapsin
Glia:
  Astocytes: GFAP
  Oligodendrocytes (myelin): MBP

Morphology of GABA/Calbindin-ir Neurons Changes in Cross-Modal A1
**CLARITY, Transgenics, and IHC**

Three-dimensional view of intact hippocampal tissue block showing enhanced yellow fluorescent protein-expressing neurons (green), parvalbumin-positive neurons (red), and glial fibrillary acidic protein GFAP (blue). *Image courtesy of Kwanghun Chung and Karl Deisseroth*

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**Behavioral Assays**

Validity of animal models for human conditions

- Face validity-similarity
- Construct validity-mechanistic similarity
- Predictive validity-treatment similarity

Types of behavior

- Natural behavior (Neuroethology)
- Sensory
- Motor
- Learning and Memory
- Social
  - Reproductive
  - Aggressive
- Affective
  - Anxiety
  - Depression