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

Lecture 3: Techniques for Studying Brain and Behavior

1

lecture 3 outline

Crown 85 Winter 2016

Visual Perception: A Window to Brain and Behavior

Lecture 3- Techniques for Studying the Brain

Reading: **Amherst College Course**
Stanford College Course

Looking: **Optogenetics**
ECoG

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

2

disclaimer !!!!!

Just a

not expecting U to become an

3

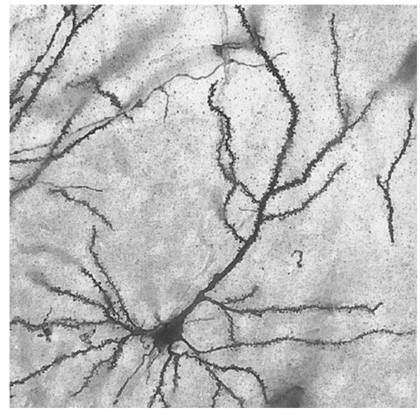
1. Anatomical
    a. Neuron staining
    b. Electron microscopy
    c. Pathway tracing

4

Anatomy: Golgi, Nissl staining of neural substrate

Camillo Golgi
1843-1926

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



http://www.nature.com/npp/journal/v32/n10/covers/index.html

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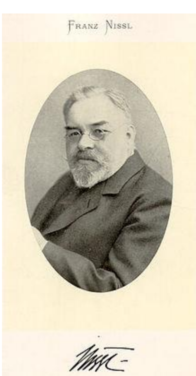
Anatomy: Golgi, Nissl staining of neural substrate



Franz Nissl
1860-1919

Nissl
staining of **cell body** with dyes (e.g. cresyl violet) that interact with RNA (and DNA) .

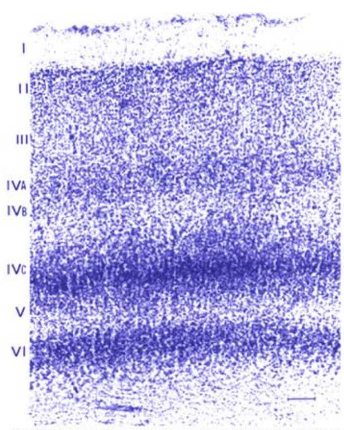


Figure 13. Nissl stain of the visual cortex reveals the different layers I through VI quite clearly.

http://webvision.med.utah.edu/imageswv/nissl2.jpg

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The Nobel Prize in Physiology or Medicine 1906
Camillo Golgi, Santiago Ramón y Cajal

Share this:

The Nobel Prize in Physiology or Medicine 1906

Camillo Golgi
Prize share: 1/2

Santiago Ramón y Cajal
Prize share: 1/2

The Nobel Prize in Physiology or Medicine 1906 was awarded jointly to Camillo Golgi and Santiago Ramón y Cajal *"in recognition of their work on the structure of the nervous system"*

OPINION

The contribution of Santiago Ramón y Cajal to functional neuroscience

Rodolfo R. Llinás

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

http://www.utdallas.edu/~tres/memory/intro/llinas.pdf

Anatomy: electron microscopy

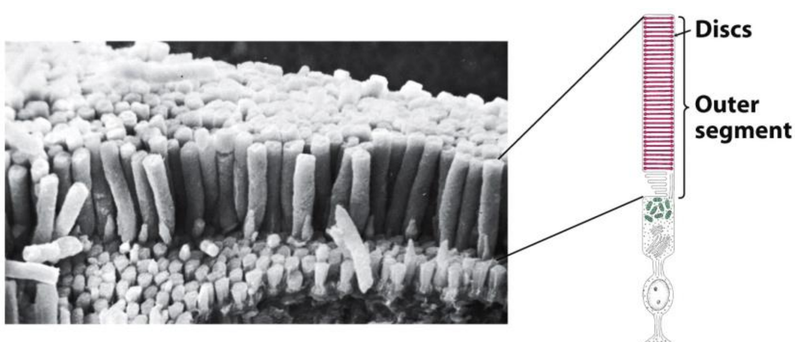


Figure 33.19
***Biochemistry*, Seventh Edition**

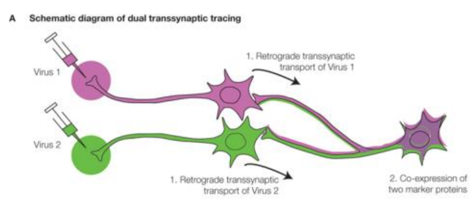
http://www.ncbi.nlm.nih.gov/books/NBK22541/

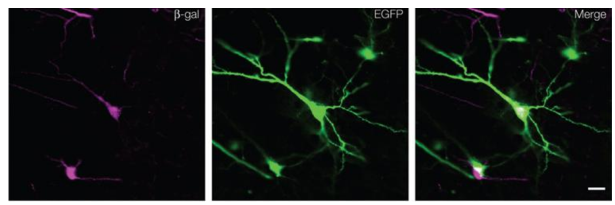
Figure 32.20The Rod Cell
(Left) Scanning electron micrograph of retinal rod cells. (Right) Schematic representation of a rod cell. [Photograph courtesy of Dr. Deric Bownds.]

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

Pathway Tracing:

Injecting, via fine needle, a ‘tracer substance’ into or near a neuron which is then transported down the axon (anterograde: *soma* $\rightarrow$ *axon terminal*) or up the axon (retrograde : axon terminal $\rightarrow$ 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.





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

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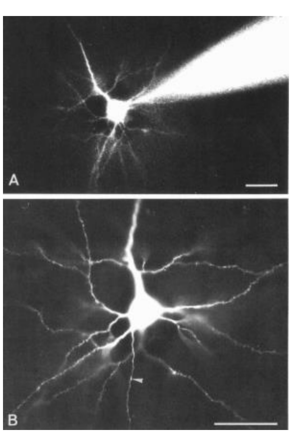
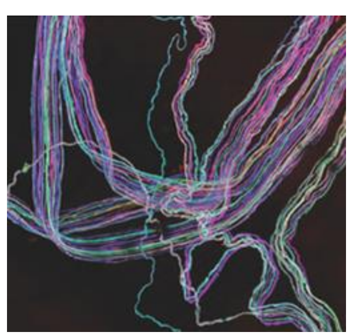
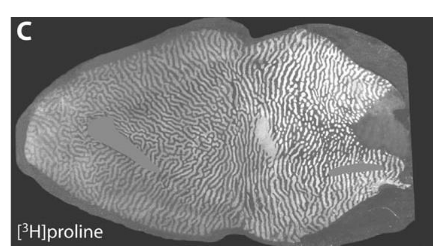
anatomy: tract tracing (e.g. HRP, ^{3}H proline, etc)







https://www.hccfl.edu/media/153423/handbook%20of%20chemical%20neuroanatomy%20vol8%20ch5.pdf

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

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

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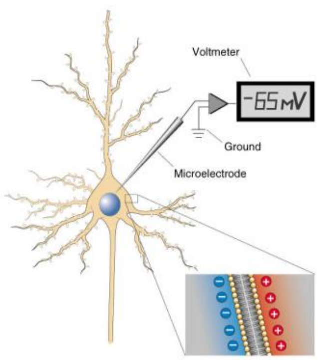
got 'em

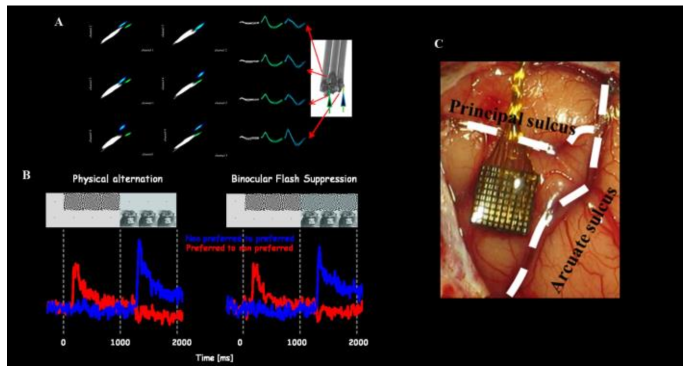
1. Anatomical
   - ✓ a. Neuron staining
   - ✓ b. Electron microscopy
   - ✓ c. Pathway tracing

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.





http://www.kyb.tuebingen.mpg.de/nc/de/mitarbeiter/details/theofanis.html

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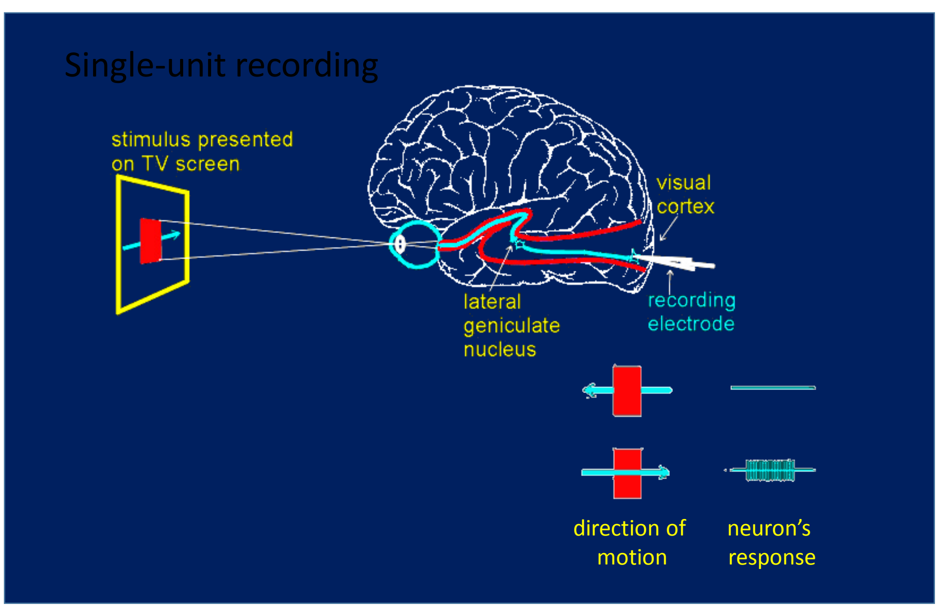
electrophysiology (neural recordings)



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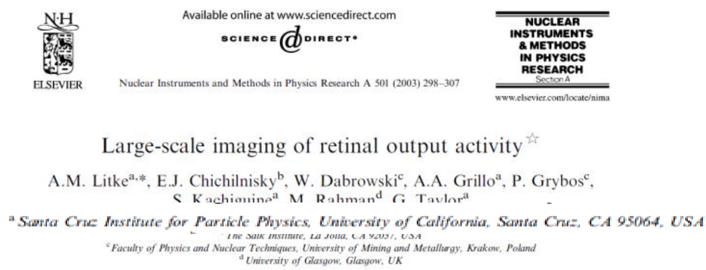
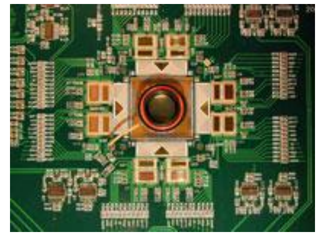
UCSC multielectrode array

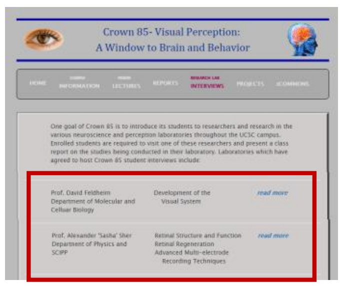

Available online at www.sciencedirect.com
SCIENCE DIRECT
Nuclear Instruments and Methods in Physics Research A 501 (2003) 298–307
NUCLEAR INSTRUMENTS & METHODS IN PHYSICS RESEARCH Section A
www.elsevier.com/locate/nima

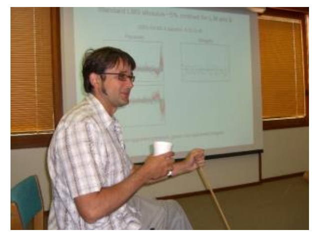
Large-scale imaging of retinal output activity☆

A.M. Litke[a,*], E.J. Chichilnisky[b], W. Dabrowski[c], A.A. Grillo[a], P. Grybos[c], S. Kachiguine[a], M. Rahman[d], G. Taylor[a]

[a] Santa Cruz Institute for Particle Physics, University of California, Santa Cruz, CA 95064, USA
[b] The Salk Institute, La Jolla, CA 92037, USA
[c] Faculty of Physics and Nuclear Techniques, University of Mining and Metallurgy, Krakow, Poland
[d] University of Glasgow, Glasgow, UK

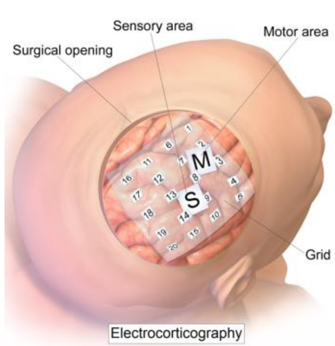




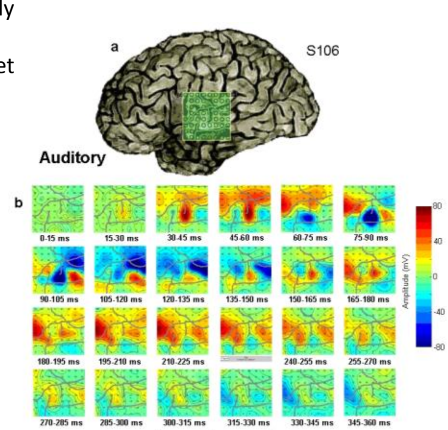


electrophysiology (neural recordings): electrocorticography (ECoG)

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.



https://en.wikiversity.org/wiki/Blausen_gallery_2014



https://www.med.nyu.edu/thesenlab/research-0/intracranial-eeg/

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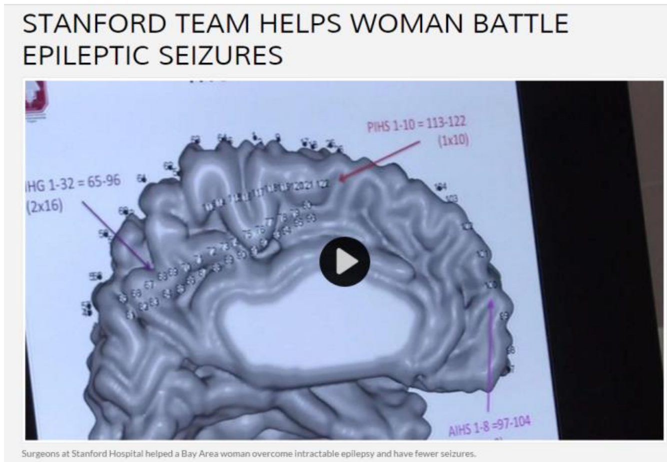
electrophysiology (neural recordings): electrocorticography (ECoG)



http://abc7news.com/archive/9405444/

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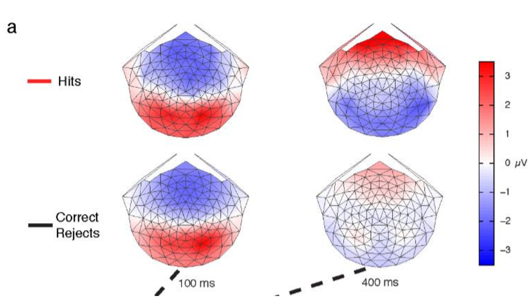
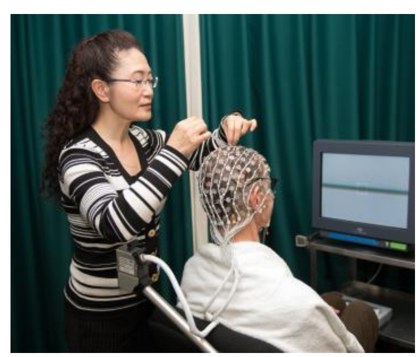
electrophysiological recording: electroencephalography (EEG)

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

http://www.st-andrews.ac.uk/~jma23/papers/alesInPressNeuroimageShape.pdf



http://www.ski.org/lab/hou-lab

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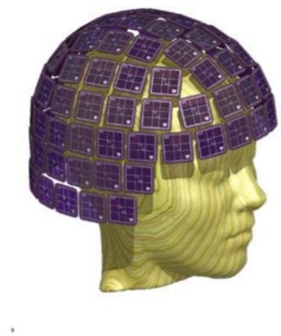
electrophysiological recording: magnetoencephalography (MEG)

MEG: measures the magnetic fields generated by neural activity



SQUID SENSOR ARRAY

http://ilabs.washington.edu/what-magnetoencephalography-meg

http://www.washington.edu/news/author/mollywmc/

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got 'em

2. Electrophysiological recording of neural activity
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3. Imaging
   a. Positron emission tomography (PET)
   b. Functional magnetic resonance imaging (fMRI)
   c. Calcium dyes
   d. Voltage sensitive dyes

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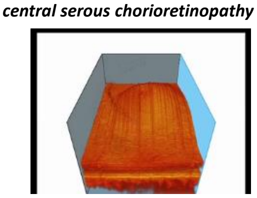
imaging: tomography (CT, PET, OCT)

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

central serous chorioretinopathy



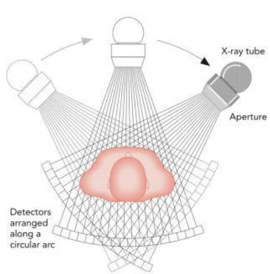
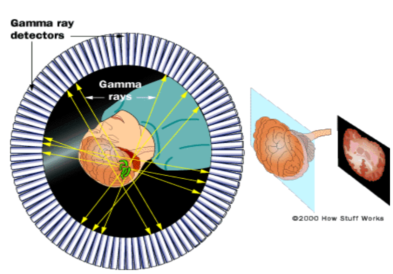


Figure 7-10 Computer tomography

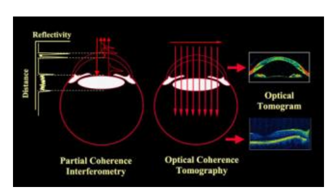
http://130.237.83.53/medicaldevices/album/Ch%207%20Medical%20images/slides/F%207-10%20Computer%20tomography.html

COMPUTER TOMOGRAPHY:
xrays absorbed



http://science.howstuffworks.com/nuclear-medicine1.htm

POSITRON EMISSION TOMOGRAPHY:
positrons emitted



http://vsri.ucdavis.edu/research/retinal/oct

OPTICAL COHERENCE TOMOGRAPHY:
coherent light

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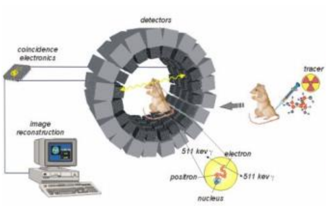
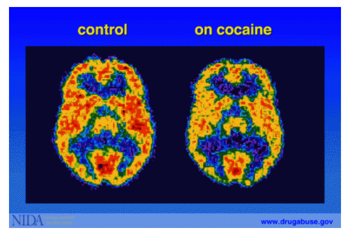
imaging (metabolic): positron emission tomography (PET)

- insert radioactive tracer into blood stream
  e.g. 2-deoxy-2-(^{18}F)fluoro-D-glucose (FDG)
- FDG *(viz 'glucose' and metabolism)* concentrates in areas of high neural activity = high metabolism
- *^{18}F isotope emits positrons ('electrons' with + charge)*
- *detectors map areas of positron emission*





http://healthyscientist.blogspot.com/2009/11/wonders-of-pet-scan.html

http://www.drugabuse.gov/publications/teaching-packets/understanding-drug-abuse-addiction/section-ii/2-positron-emission-tomography-pet-scan-person-us

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

THE BOLD SIGNAL : BLOOD OXYGEN LEVEL DEPENDENT fMRI

- Magnetic Resonance Imaging involves putting the sample *(i.e. your head!!)* in a large external magnet
- Brain imaging is most often down with a spectrometer that measures the magnetic properties of hydrogen (in water) in various parts of the brain
- The 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.
- (via the hemoglobin molecule) Deoxygenated blood is magnetic while oxygenated blood is not magnetic (TAKE CHEM 1B!!).

Also See: What is Functional Magnetic Resonance Imaging (fMRI)?, Hannah Devlin
fMRI an Introduction: Michael Firbank

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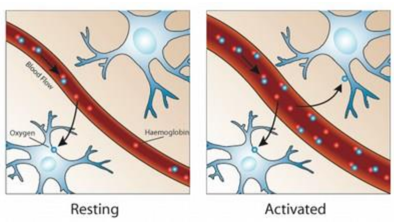
imaging (metabolic): functional Magnetic Resonance Imaging (fMRI)

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

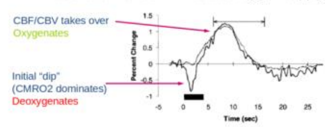


http://psychcentral.com/lib/what-is-functional-magnetic-resonance-imaging-fmri/

fMRI

BOLD and Haemodynamic effects

- Increased energy consumption extracts oxygen
- Vasodilation and CBF increase oxygen supply



- Hemodynamic response time of ~3s

http://www.slideshare.net/christina101/fmri-introduction-newcastle-university-newcastle-upon-tyne

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

The neural correlates of maternal and romantic love

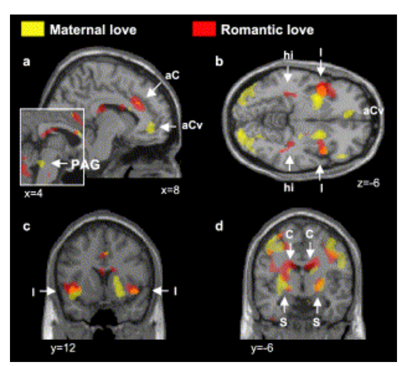

Fig. 3. Overlap between activity of maternal love and romantic love. Activity obtained in this study (contrast: cO vs. cA) was colored in yellow and overlaid on sections through a template brain, along with activity obtained in our previous study on romantic l...

Andreas Bartels, Semir Zeki

NeuroImage, Volume 21, Issue 3, 2004, 1155–1166

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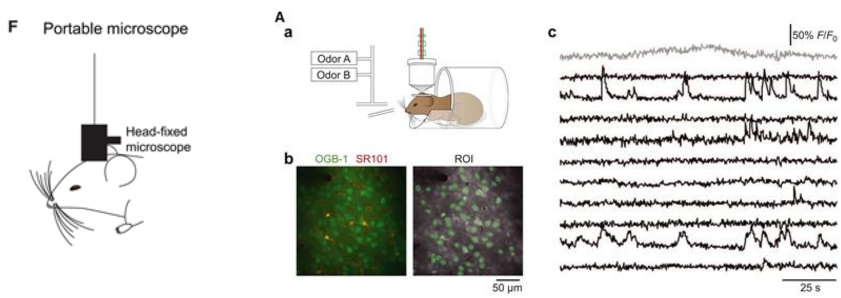
imaging (binding agents): Ca^{2+} imaging

- 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’



Imaging Calcium in Neurons
Christine Grienberger
Arthur Konnerth
Neuron 73, pp 862-885 (2012)

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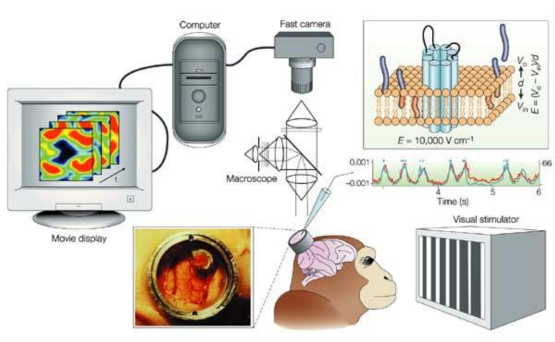
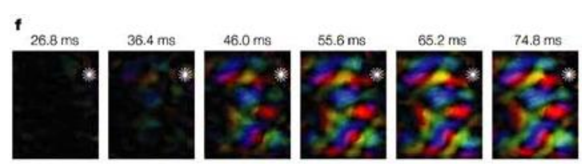
functional imaging: voltage sensitive dyes

- 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





http://www.nature.com/nrn/journal/v5/n11/full/nrn1536.html

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done with 3

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

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

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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**

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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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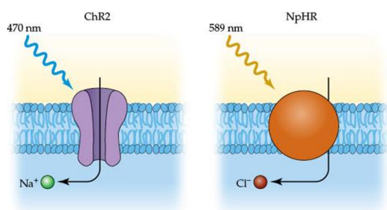
neural excitation by external stimulus: optogenetics

- **genetically modify neurons to express LIGHT SENSITIVE ion channels e.g. Na^+ or Cl^- channels**



Channelrhodopsin-2 (ChR2) and halorhodopsin (NpHR) and light-sensitive proteins used in optogenetic studies. ChR2 is a cation channel that opens in response to blue light, whereas NpHR is a chloride pump that is activated by yellow light.

http://sites.sinauer.com/psychopharm2e/webbox09.03.html

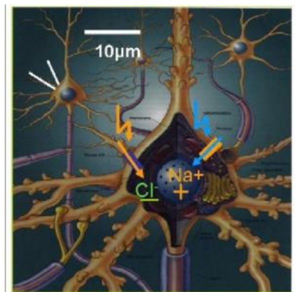


Fig 1a: Schematic representation of the action of Channelrhodopsin2 and Halorhodopsin on neural cells.

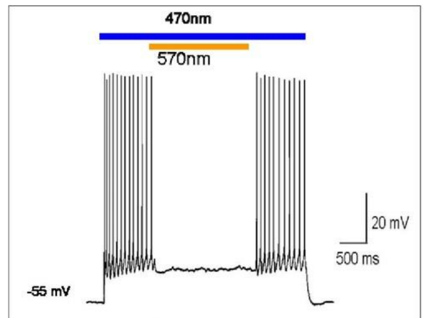


Fig 1b: Activation of action potentials by ChR2 (blue light) and inactivation by NphR (yellow light) of a cultured hippocampal cell

http://optogenetics.weebly.com/why--how.html

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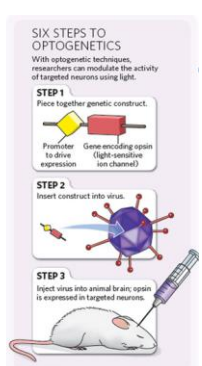
neural excitation by external stimulus: optogenetics

- **genetically modify neurons to express LIGHT SENSITIVE ion channels e.g. Na^+ or Cl^- channels**



http://optogenetics.weebly.com/why--how.html

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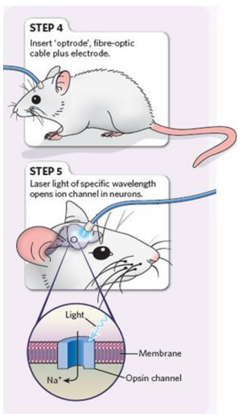
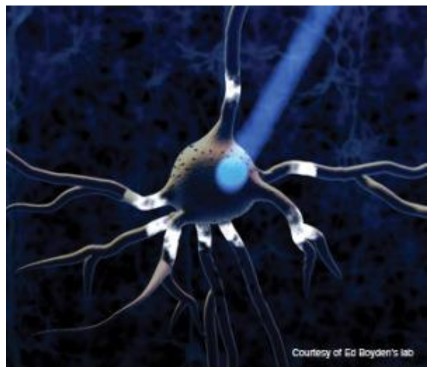
neural excitation by external stimulus: optogenetics

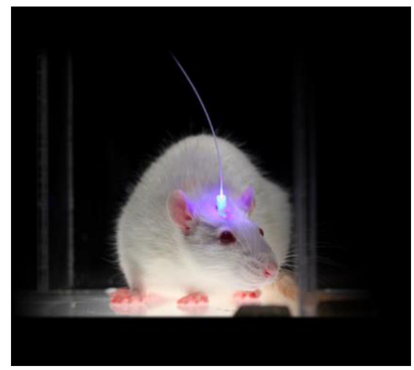
- **activate neuron (depolarize or hyperpolarize) by light source (e.g. laser)**



http://optogenetics.weebly.com/why--how.html



http://sciencecalling.com/2011/08/12/light-science-optogenetics/



http://web.stanford.edu/group/dlab/optogenetics/

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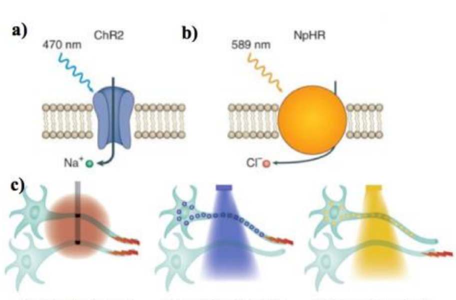
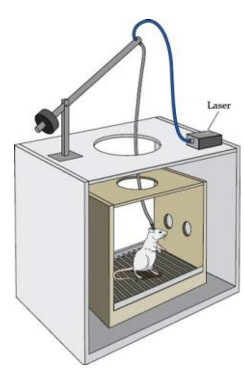
neural excitation by external stimulus: optogenetics

- **image subsequent neural activity (e.g. Ca^{2+} imaging) or behavior in awake animal**



http://nmbl.stanford.edu/research/experiments/optogenetics.htm



http://sites.sinauer.com/psychopharm2e/webbox09.03.html

Neurons expressing ChR2 or NpHR can be exposed to light of the appropriate wavelength while the animal is behaving freely in an apparatus such as an operant chamber. Performing an operant response such as a nose-poke turns a laser light generator on for a short period of time to activate light-sensitive proteins in the vicinity of a previously implanted optic fiber

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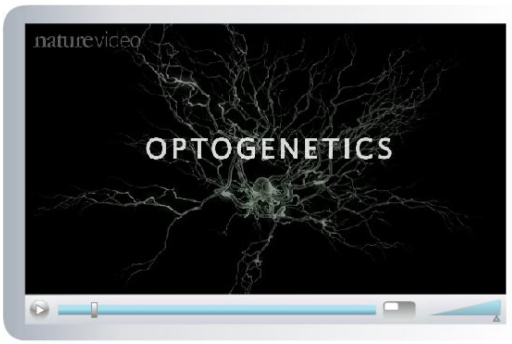
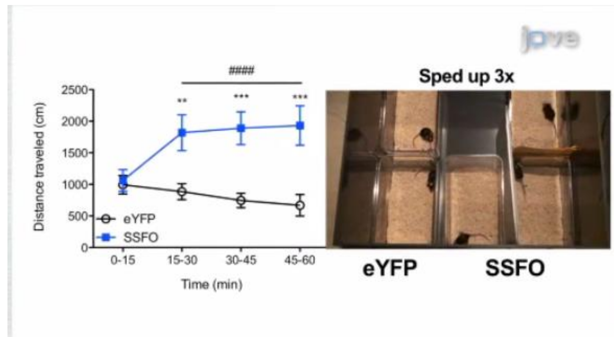
optogenetics: videos

Method of the Year 2010





http://www.nature.com/nmeth/video/moy2010/index.html

http://www.jove.com/video/51483/in-vivo-optogenetic-stimulation-of-the-rodent-central-nervous-system

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optogenetics: Prof. Kliger interview

UNIVERSITY OF CALIFORNIA SANTA CRUZ NEWSCENTER

Ion channel mechanics yield insights into optogenetics experiments

July 06, 2015
By Tim Stephens

SHARE THIS STORY:

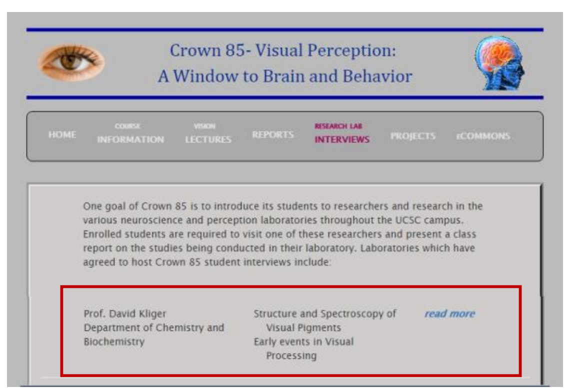
Optogenetics techniques, which allow scientists to map and control nerve cells using light stimulation, are being used to study neural circuits in the brain with unprecedented precision. This revolutionary technology relies on light-sensitive proteins such as channelrhodopsins, and researchers at UC Santa Cruz have now determined the molecular mechanism involved in the light-induced activation of one of these proteins.

The new findings, published July 3 in two papers in the *Journal of Biological Chemistry*, can help scientists create tailor-made proteins optimized for use in optogenetics, said David Kliger, senior author of both papers and a professor of chemistry and biochemistry at UC Santa Cruz.

"Little was known about the functional mechanism of these proteins even though they are widely used in optogenetics," Kliger said.

The researchers used fast laser spectroscopy to study the function of Channelrhodopsin-2, which is found in a type of marine algae and is widely used in optogenetics experiments. Channelrhodopsins are ion channels that control the flow of ions across cell membranes. There are many kinds of ion channels that serve different purposes in different types of cells. Nerve signals involve ion flow across the

David Kliger's lab uses fast laser spectroscopy to study biologically important molecules such as channelrhodopsin, an ion channel widely used in optogenetics experiments.



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

HOME | COURSE INFORMATION | VISION LECTURES | REPORTS | RESEARCH LAB INTERVIEWS | PROJECTS | eCOMMONS

One goal of Crown 85 is to introduce its students to researchers and research in the various neuroscience and perception laboratories throughout the UCSC campus. Enrolled students are required to visit one of these researchers and present a class report on the studies being conducted in their laboratory. Laboratories which have agreed to host Crown 85 student interviews include:

Prof. David Kliger Department of Chemistry and Biochemistry	Structure and Spectroscopy of Visual Pigments Early events in Visual Processing	*read more*

http://news.ucsc.edu/2015/07/optogenetics.html

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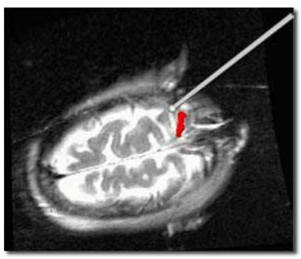
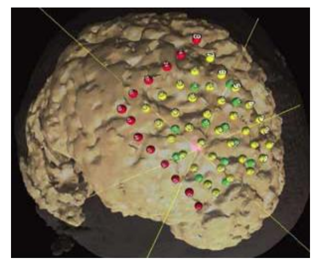
• *neural excitation by external stimulus: intracranial electrical stimulation*

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
- to monitor brain function in patients
- to explore cognitive responses





http://golbylab.bwh.harvard.edu/intracranialEEG/EEG.html

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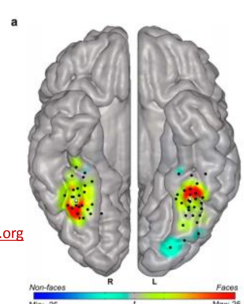
• *neural excitation by external stimulus: intracranial electrical stimulation*

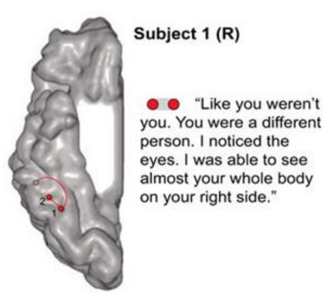
Electrical Stimulation of the Left and Right Human Fusiform Gyrus Causes Different Effects in Conscious Face Perception

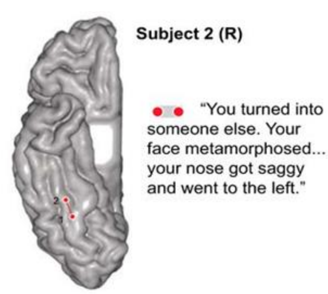
Vitha Rangarajan[1,2], Dora Hermes[2], Brett L. Foster[1,2], Kevin S. Weiner[2,3], Corentin Jacques[2,3,4], Kalanit Grill-Spector[2,3,5], and Josef Parvizi[1,2,5]



http://www.jneurosci.org/content/34/38/12828.long

Bilateral face-selective ECoG responses in the fusiform gyrus. ***a***, Face-selective HFB responses in the FG were measured bilaterally





Stanford MEDICINE | Parvizi Lab
Neurology & Neurological Sciences

http://med.stanford.edu/parvizi-lab/projects.html

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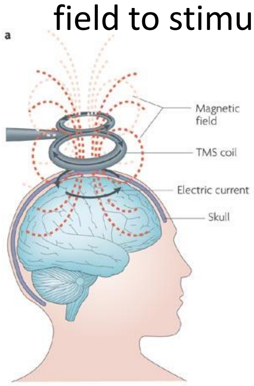
neural excitation by external stimulus: transcranial magnetic stimulation (TMS)

• transcranial magnetic stimulation (TMS)

- external magnetic coil put on head, pulses direct magnetic field to stimulate brain areas

a



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.

http://www.nature.com/nrn/journal/v8/n7/full/nrn2169.html

http://www.nimh.nih.gov/health/topics/brain-stimulation-therapies/brain-stimulation-therapies.shtml

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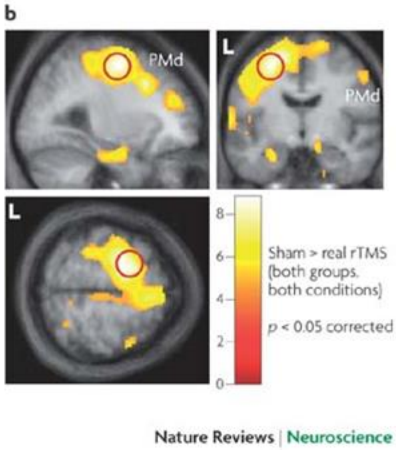
repetitive transcranial magnetic stimulation (rTMS) reduces activity

b



b | 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.

http://www.nature.com/nrn/journal/v8/n7/full/nrn2169.html

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almost done

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

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psychophysics

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

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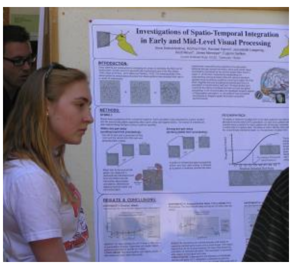
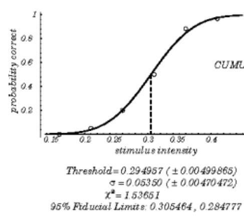
psychophysics in the switkes group





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comparison of attributes of some brain recording techniques

	fast or slow	resolution	local or global	Invasive nonInvasive
Single cell recording	fast	high	local	invasive
Electroencephalography (EEG)	fast	low	global	noninvasive
Magnetoencephalography (MEG)	fast	moderate	global	noninvasive
Positron emission tomography (PET)	slow	low	global	noninvasive (but involves radioactive material)
fMRI	slow	low	global	noninvasive
Ca^{2+} dyes	fast	high	intermediate	invasive
optogenetics	fast	high	intermediate	invasive

what types of neural processes would each of these be suited to measure ?

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Finis

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