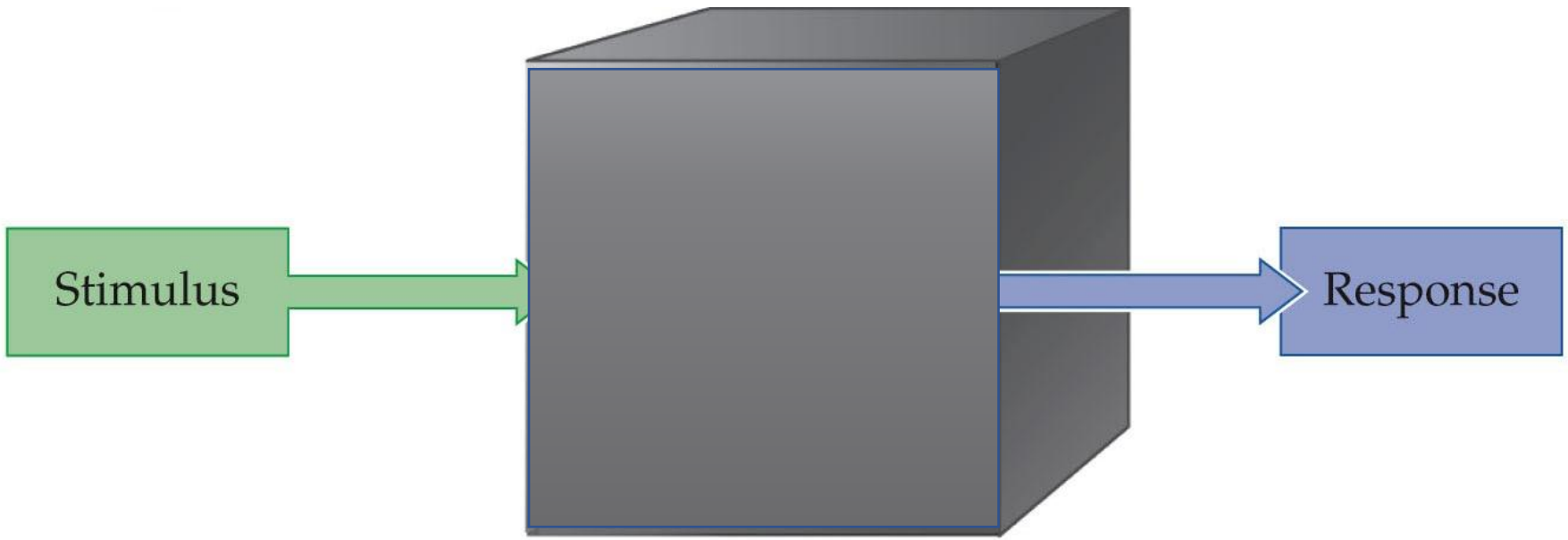
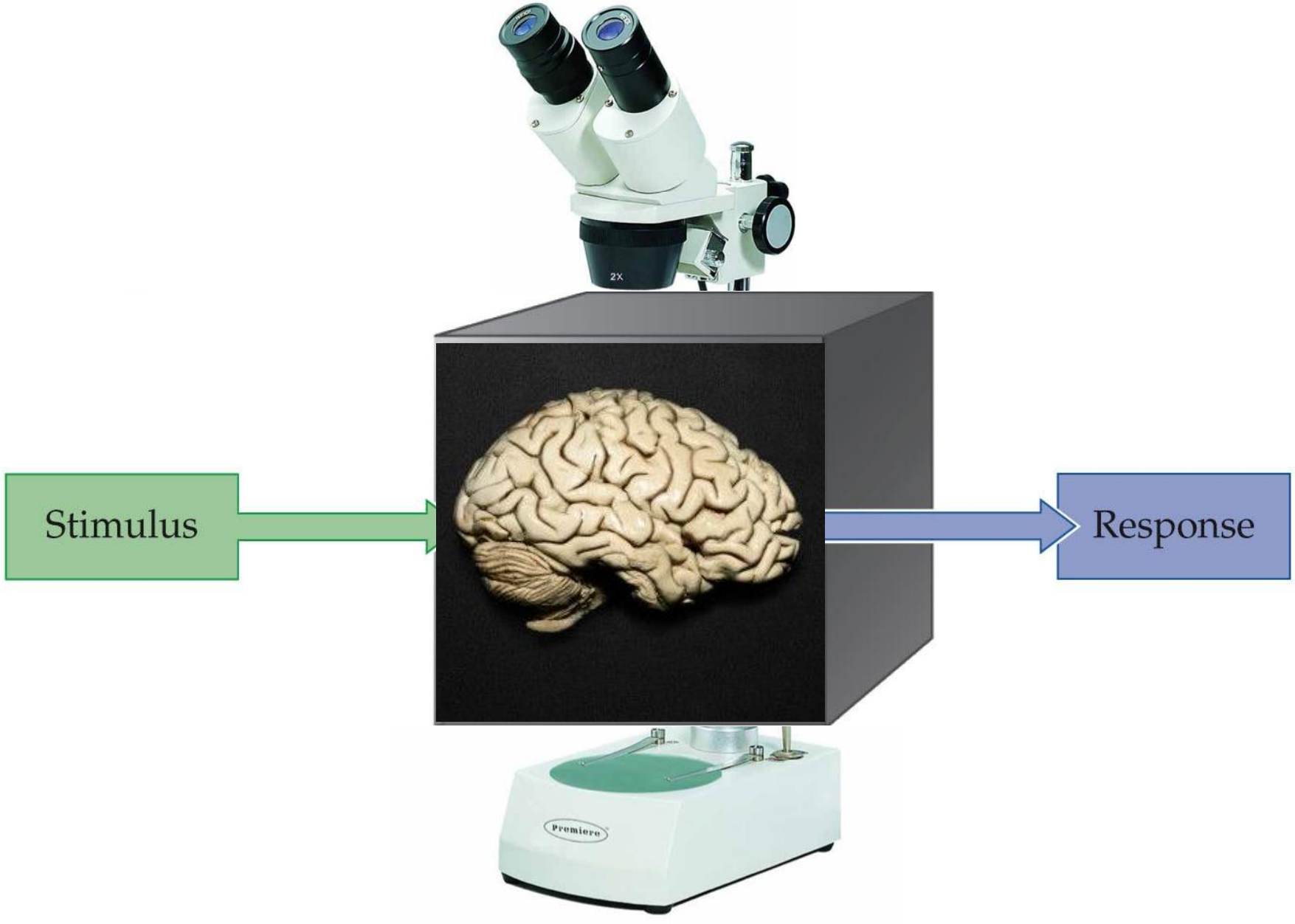
Methods and History of Cognitive Neuroscience

NEU/MOL 502A: **From Molecules to Systems to Behavior**


Stimulus
Response


Stimulus
2X
Premiere
Response

Goals for Today

- How do we observe neural signals?
  - Electrophysiology –Neural recordings and EEG/MEG
  - Imaging – Calcium and Voltage imaging
- How do we observe neural correlates (mostly in humans)?
  - PET
  - fMRI
  - fUS
  - fNIRS
- How do we causally manipulate neural activity?
  - Electrical/optical stimulation
  - TMS
  - tDCS/tACS/tRCS
  - Pharmacology
  - Cooling
  - Lesions

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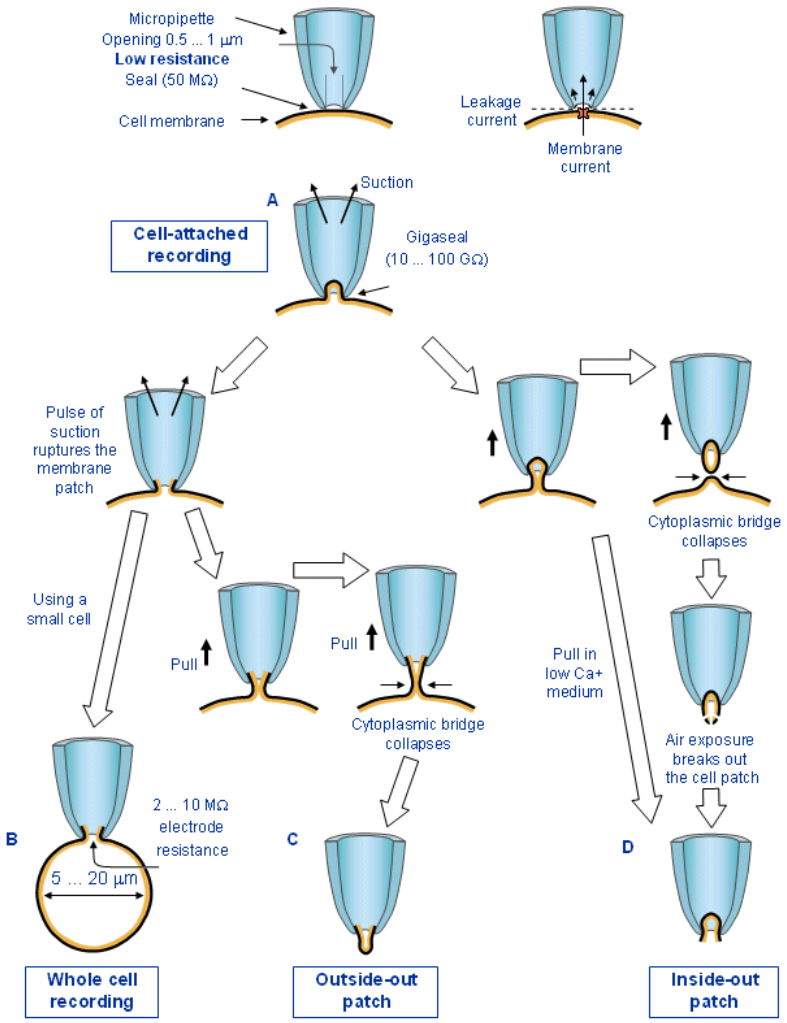
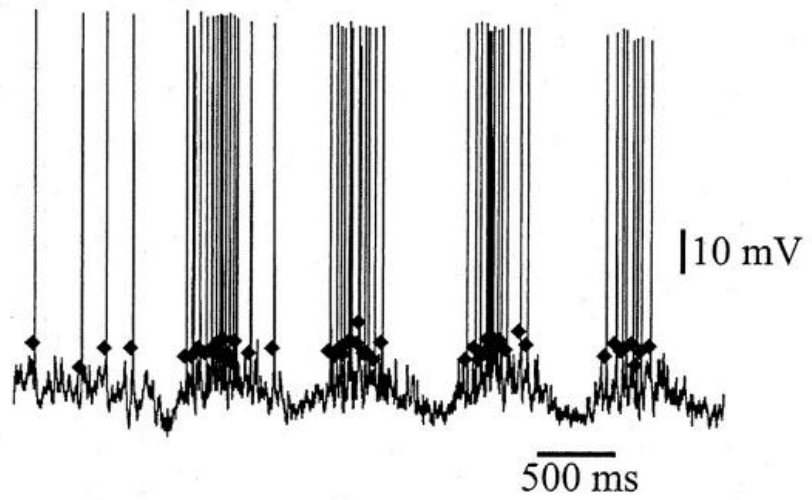
Intracellular Electrophysiology

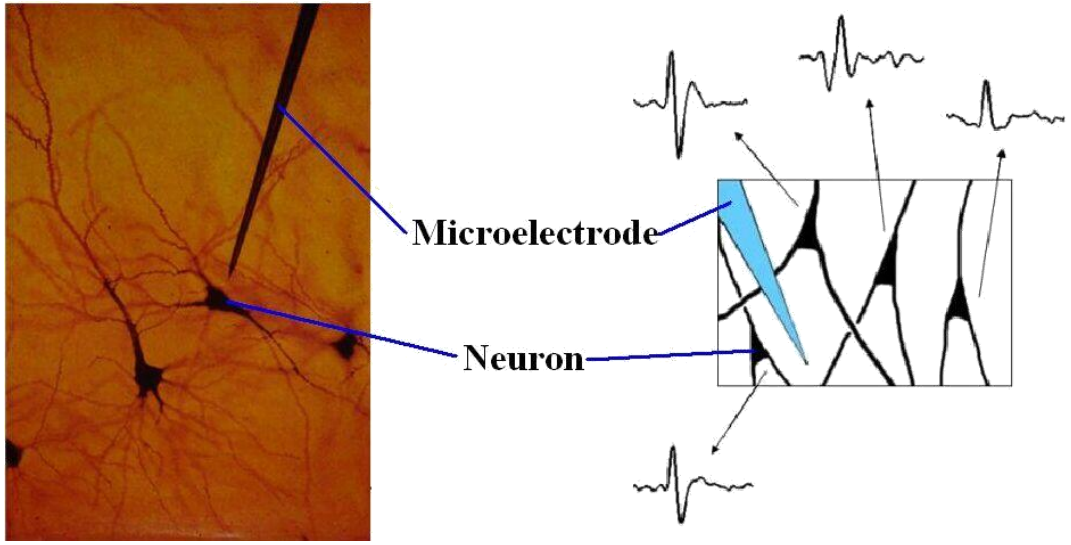


Cat striate CTX neuron

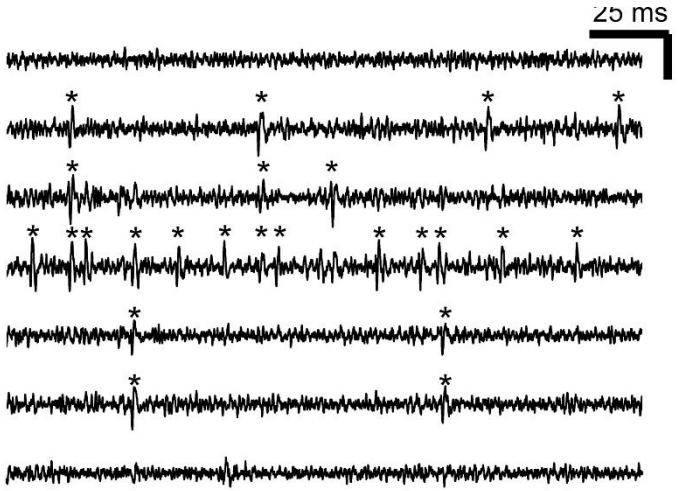


- Patch-clamping gives unparalleled access to information in the cell. Gold standard.
- Difficult to perform; takes extensive training and yield is low. Once patched, it is difficult to hold a cell, particularly *in vivo*.
- Newer patching robots lower the bar for entry and are allowing for multiple neurons to be simultaneously patched.

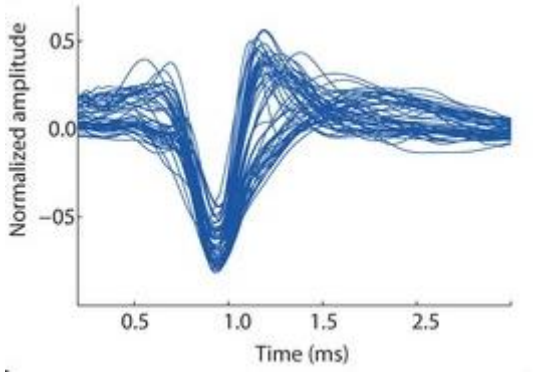
Extracellular Electrophysiology



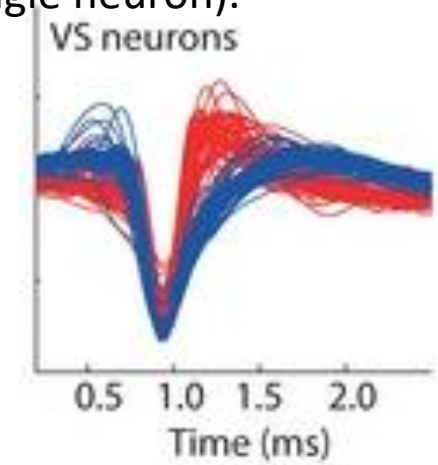
Recording this extracellular potential at high frequencies gives you a raw trace of neural signals:



You can extract action potentials from this signal:



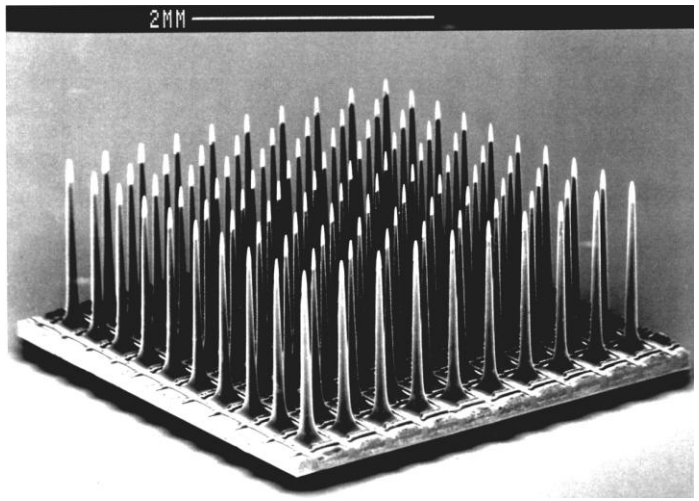
And then using various techniques to ***cluster*** these waveforms into individual ***units*** (i.e. action potentials from a single neuron).

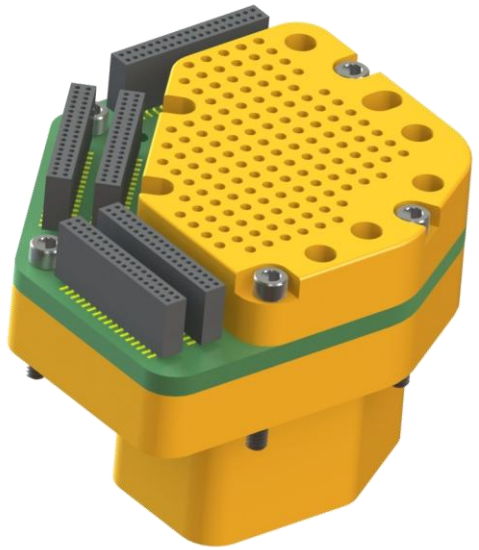


Note: People also discuss **multi-unit activity** which is the aggregate spiking activity of several neurons around the tip of an electrode.

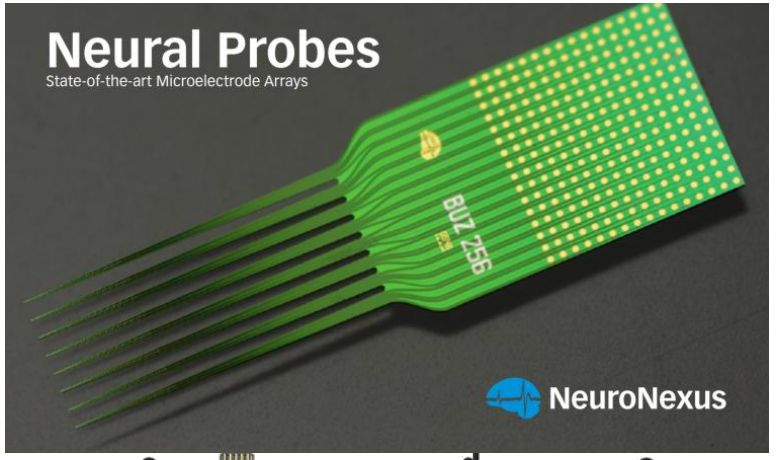
Extracellular Electrophysiology

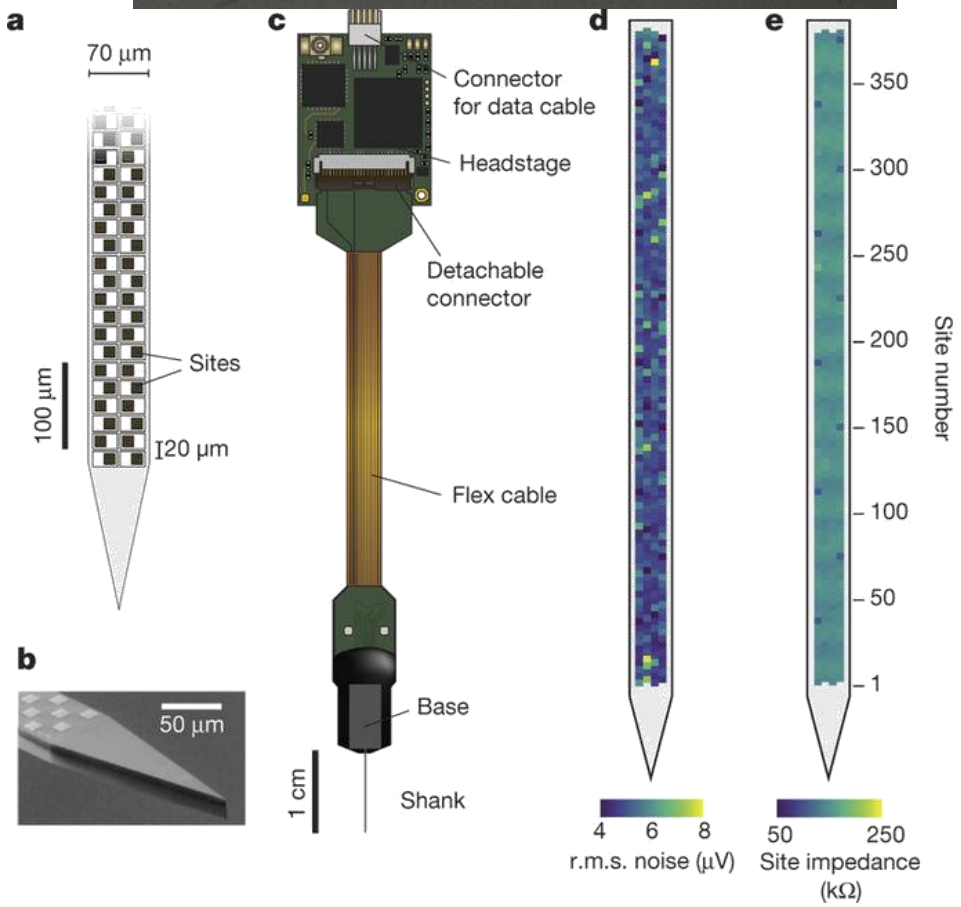
Chronic Electrode Arrays





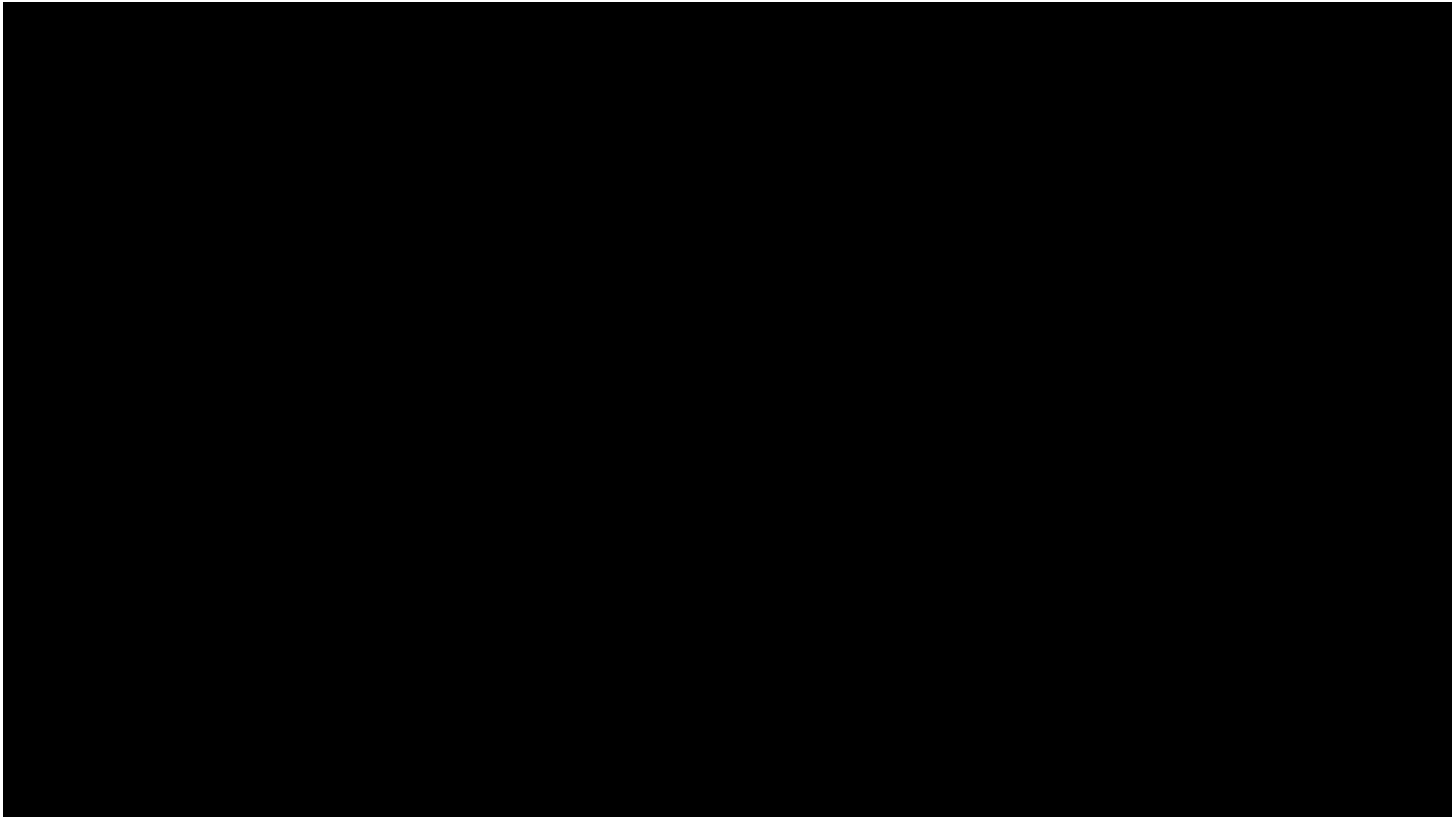
Silicon-based Electrodes



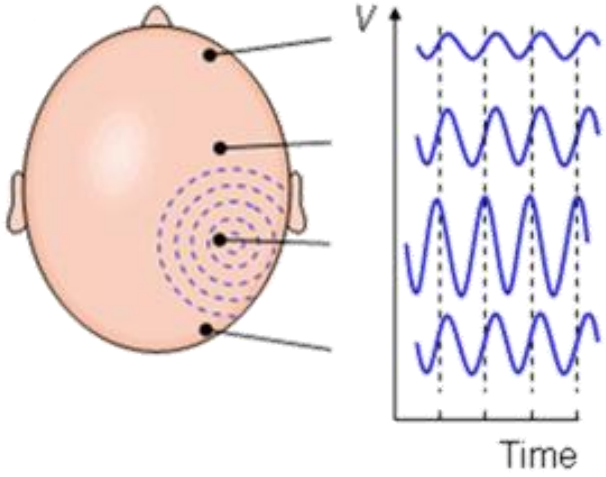


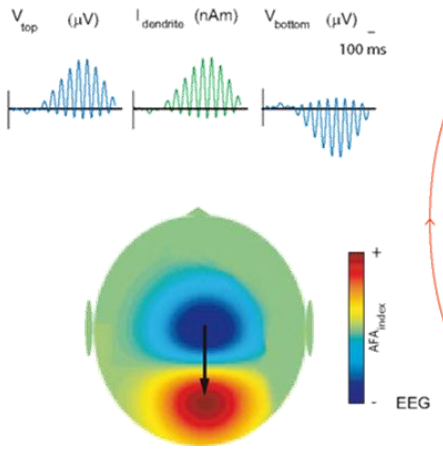
- Extracellular electrophysiology provides high spatial and temporal resolution; making it the gold-standard for systems-level neuroscience research.
- Recent advances have facilitated large-scale recordings of hundreds of neurons, across several different brain regions.
- Allows for chronic recording of activity, over weeks to months.
- Difficult to identify cell types directly (some indirect methods exist).

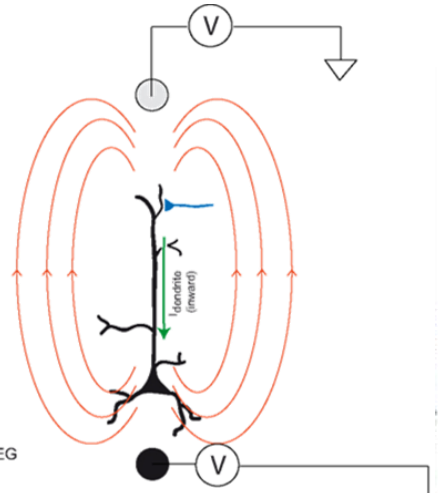
Combined Ephys, Behavior, and Imaging (1x speed) – see notes

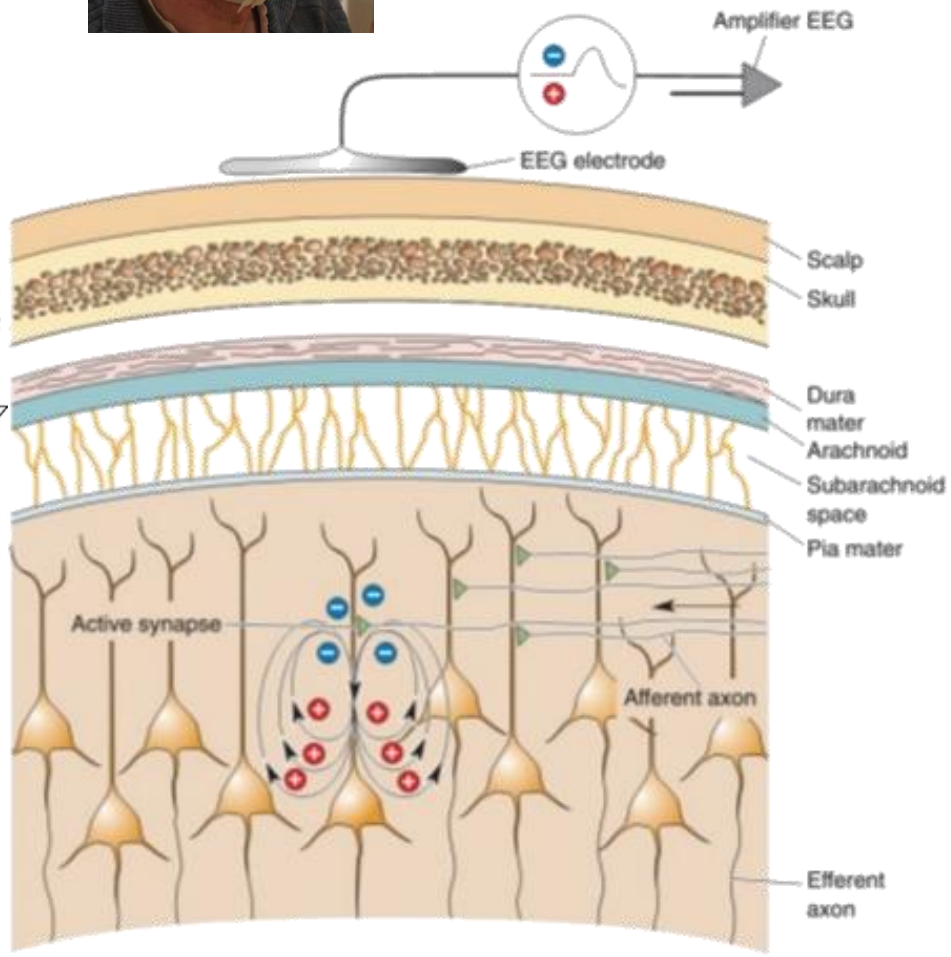


Electroencephalography (EEG)









- Hans Berger first used the technique in 1929.
- Low spatial accuracy; EEG captures the sum activity of millions of neurons over an area of ~10 cm^2.
- High temporal accuracy, but the spatial filtering of the skull can cause shifts in estimated times.
- High-density EEG recordings, combined with source modeling can improve spatial localization.

Buzsaki and Koch, Nature Reviews Neuroscience, 2012

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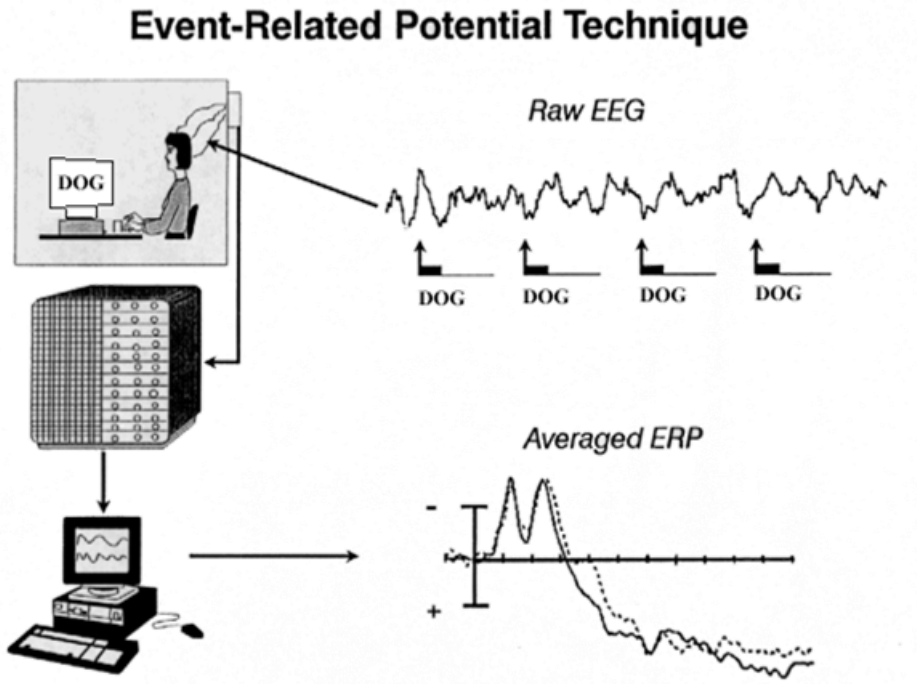

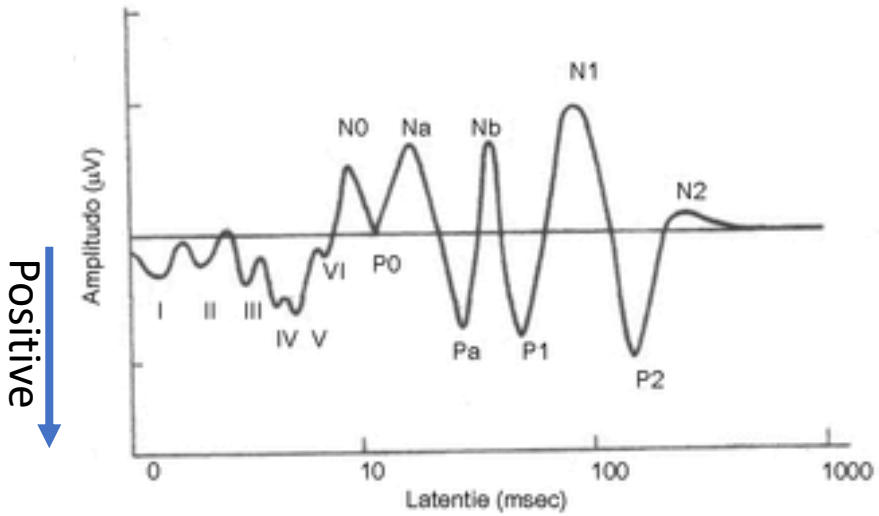

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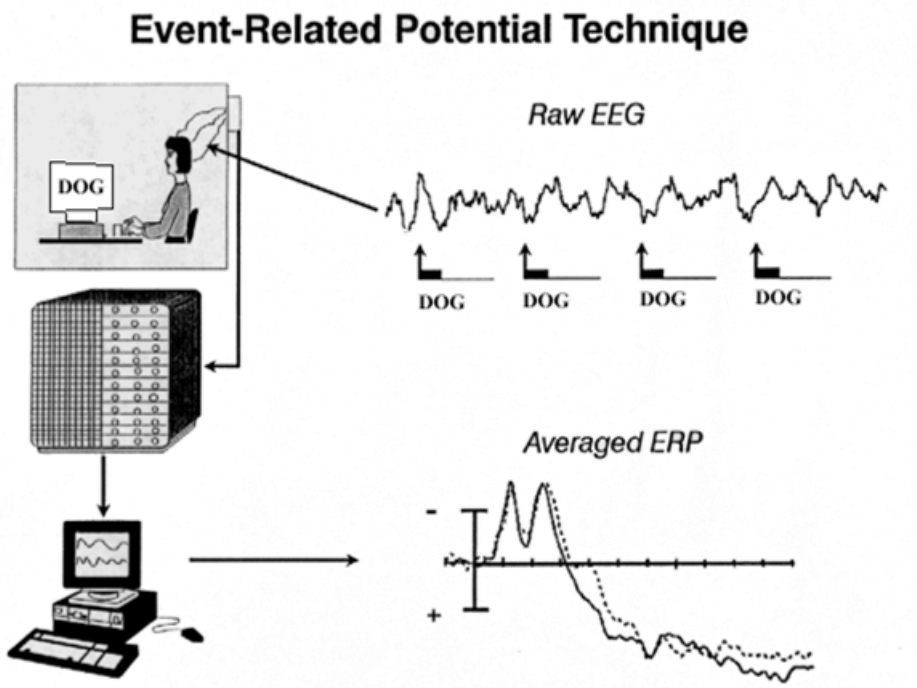

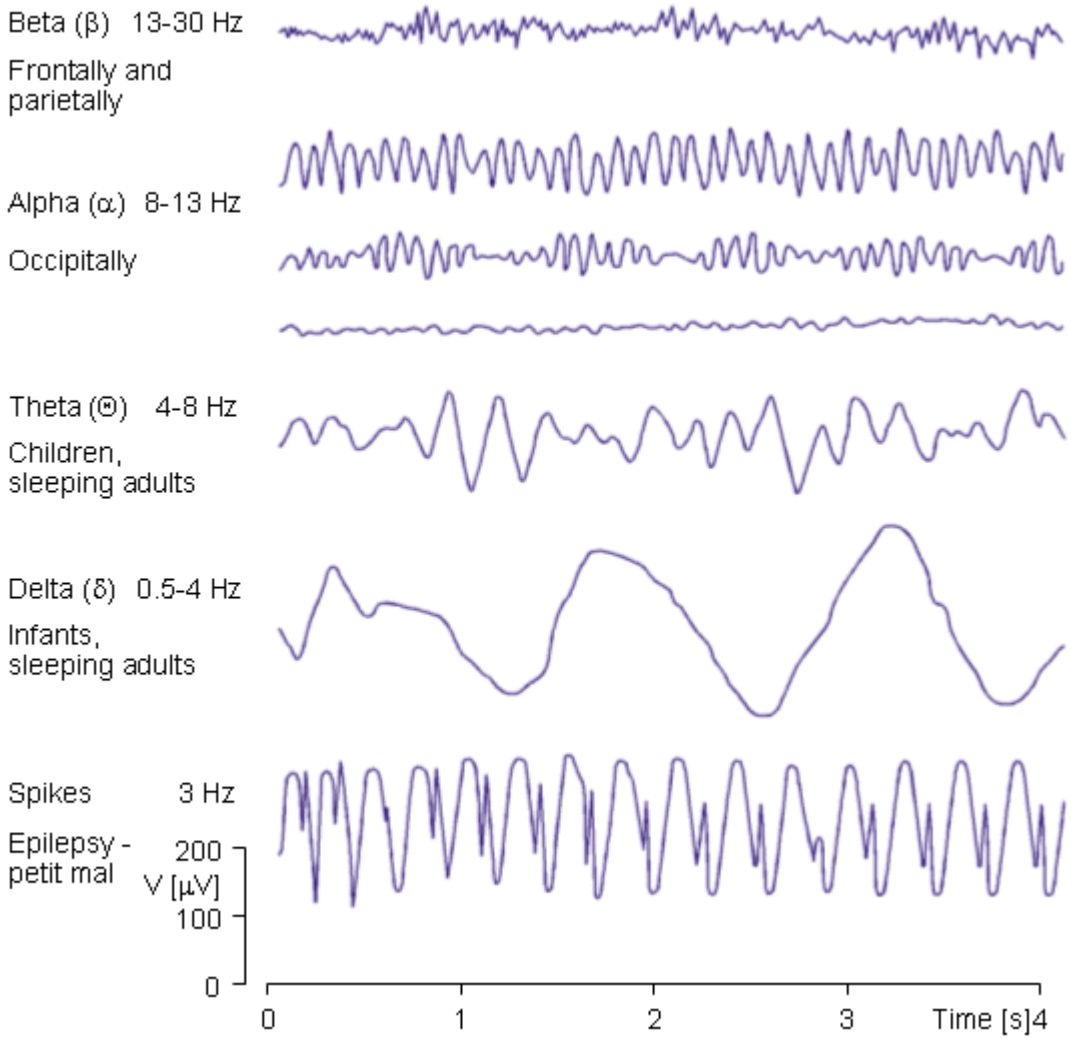

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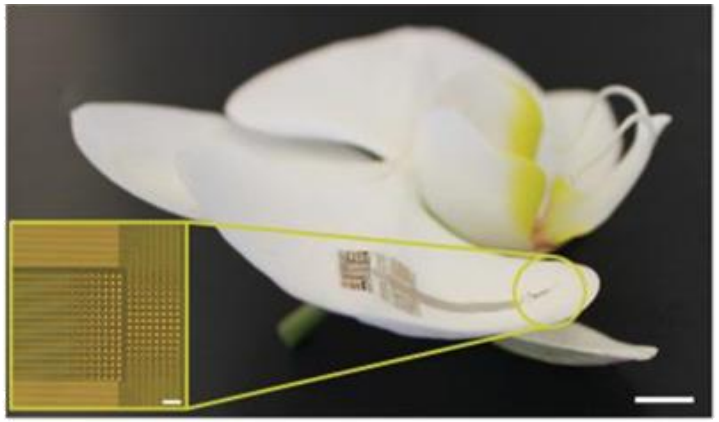
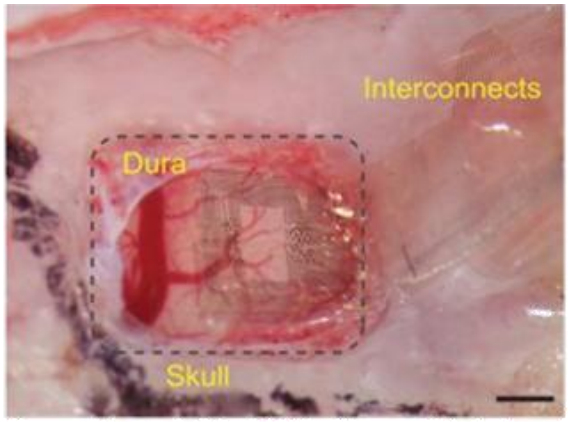
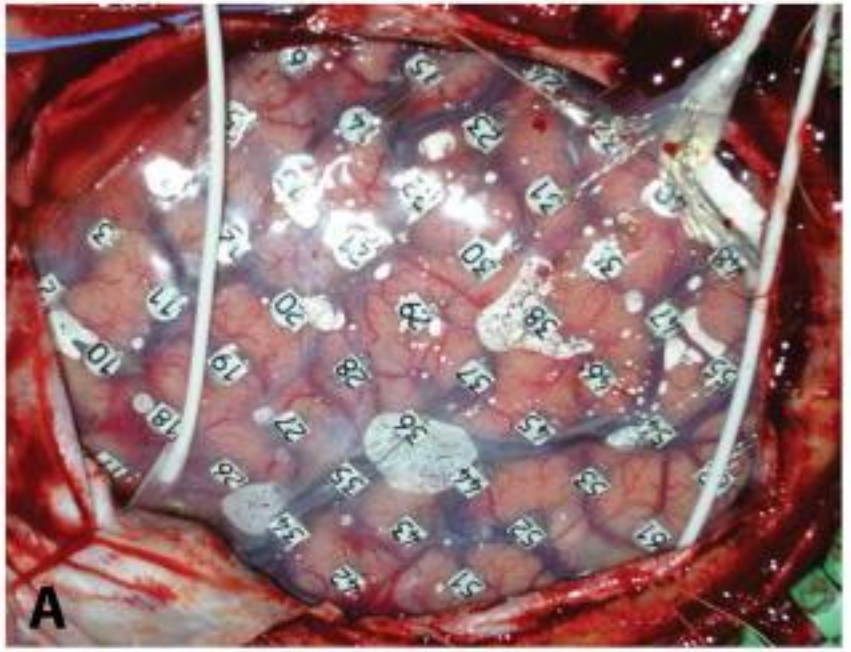
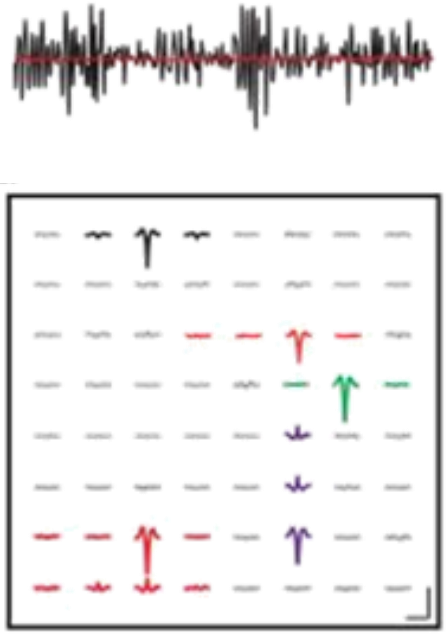
Electrocorticography (ECoG)







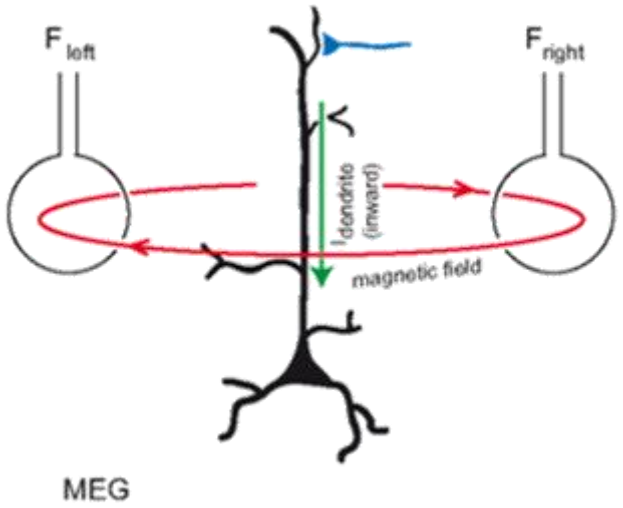


- High impedance skull is the biggest barrier to accurate EEG signals; therefore, one can improve signal by removing the skull.
- Invasive approach, only appropriate for animals and patients.
- Recent increase in density of electrodes has even allowed for detection of spiking activity.

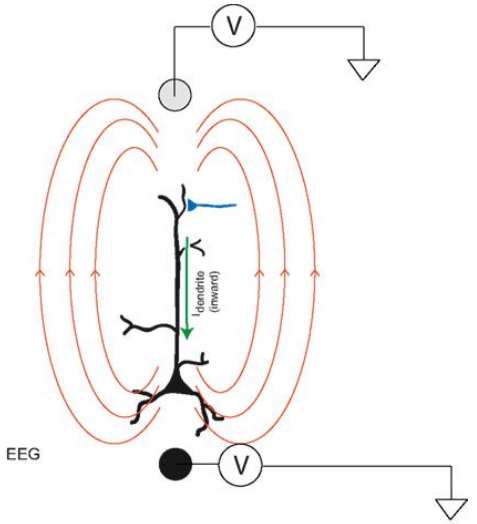
Dalal et al, J Neurosci Methods, 2008
Khodagholy et al, Nat Neuro 2015

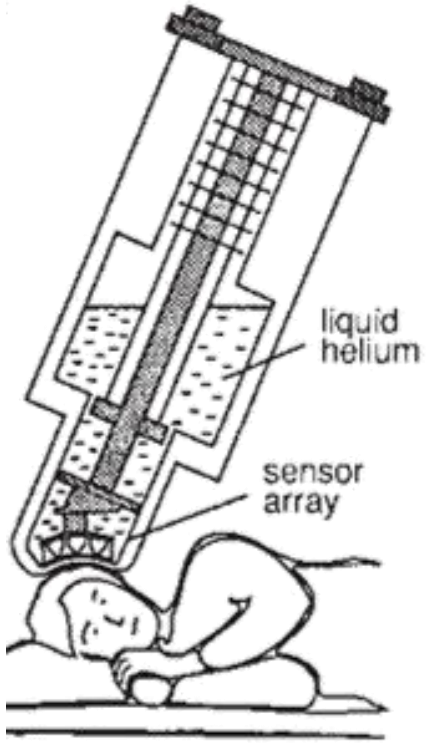
Magneto-encephalography (MEG)

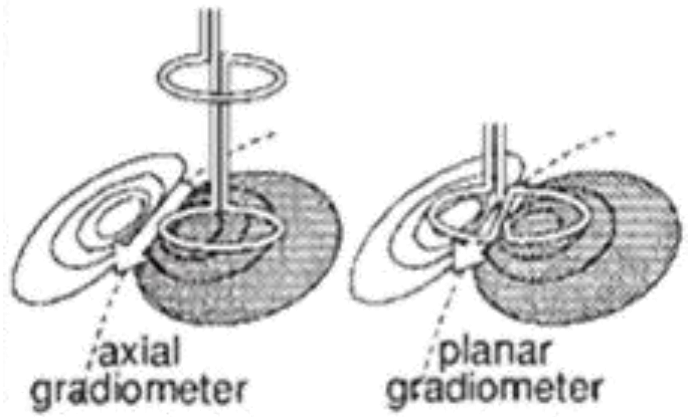
Magnetic Field

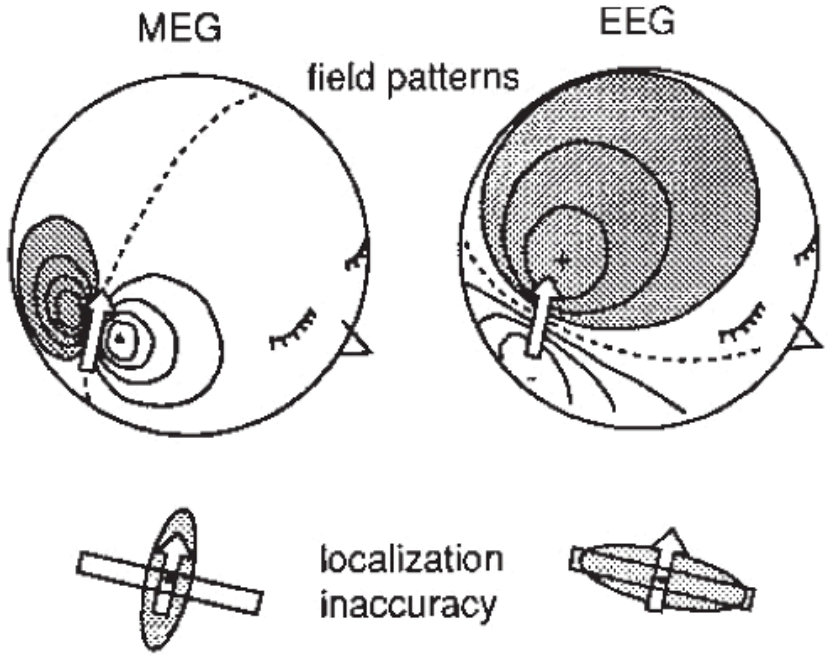


Electric Field









- Measures magnetic field from current flow.
- Very expensive (to purchase and run).
- Better spatial resolution than EEG as B-fields are not smoothed by skull/tissue.

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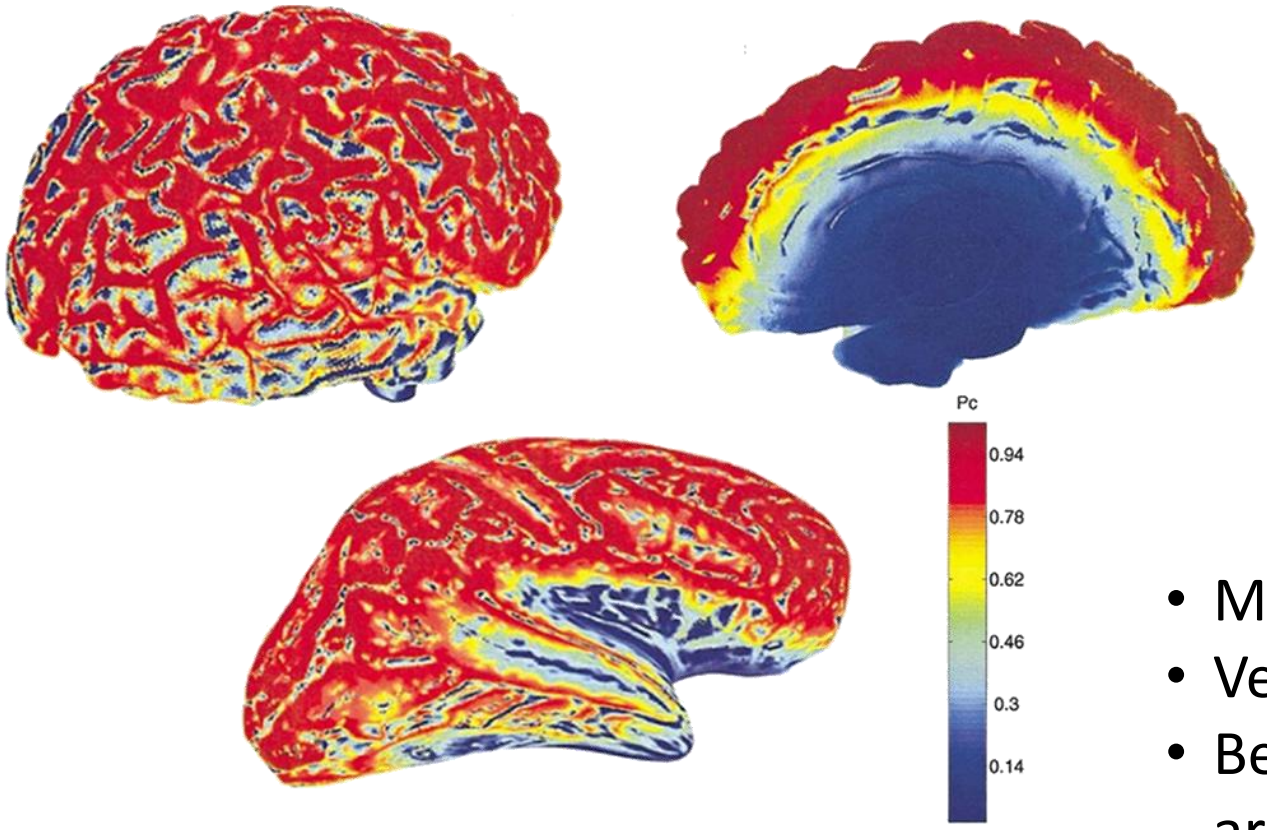
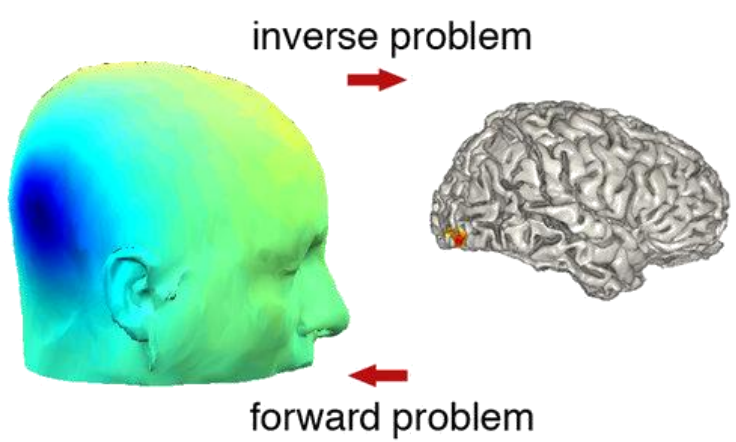
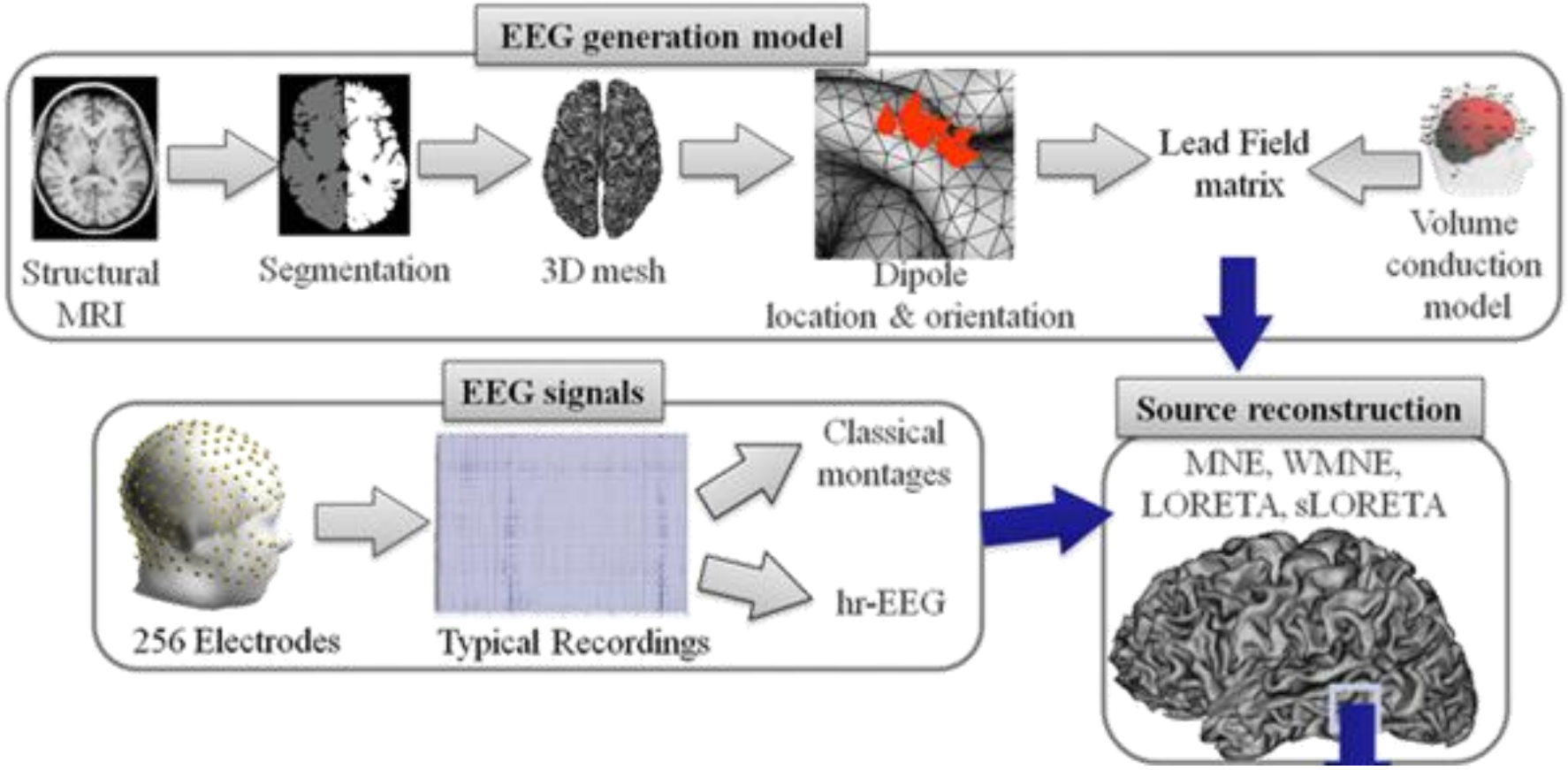


- Measures magnetic field from current flow.
- Very expensive (to purchase and run).
- Better spatial resolution than EEG as B-fields are not smoothed by skull/tissue.
- Like EEG, has difficulty measuring activity in deep structures.

Source Localization with EEG & MEG

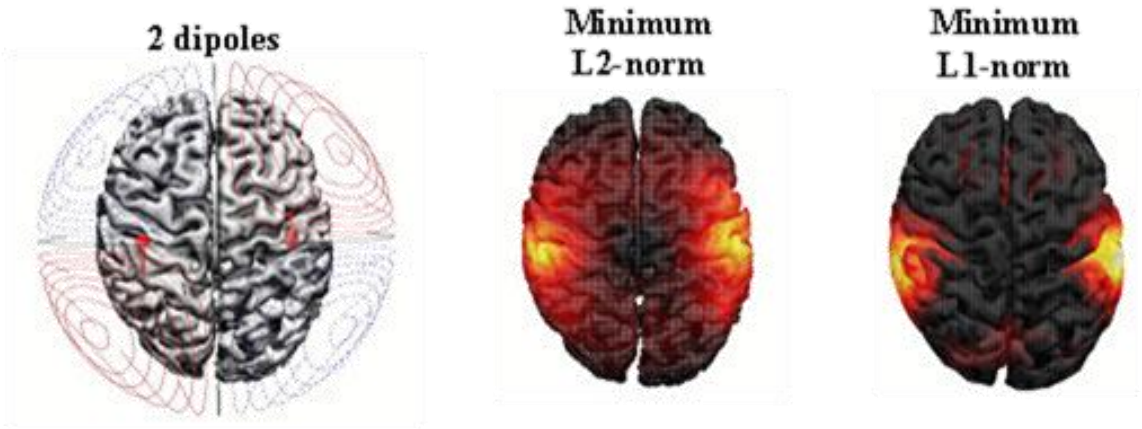
Ideally, one would like to determine the neural source of a signal observed outside the brain.





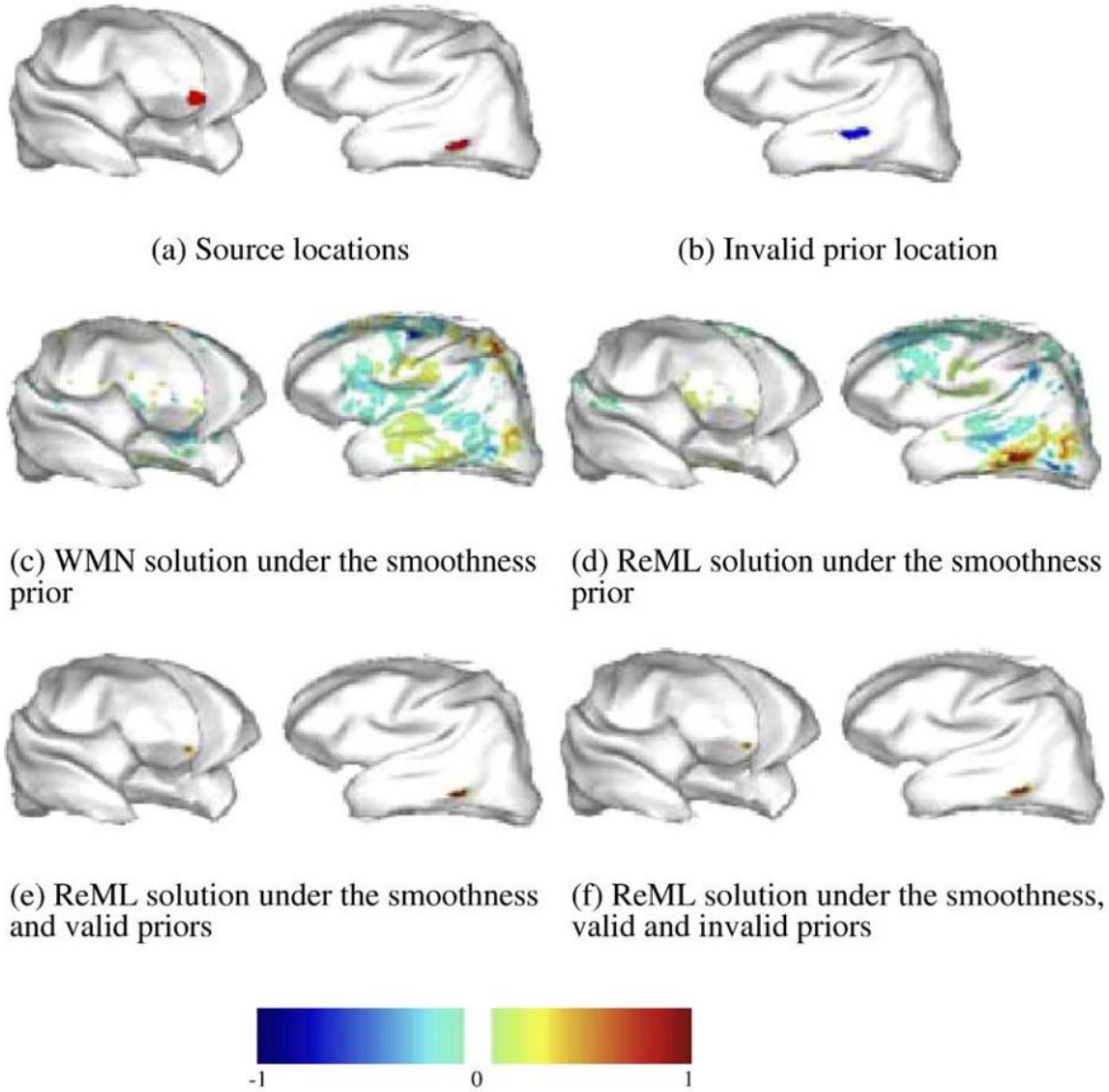
Source Localization with EEG & MEG

Regularization Provides Constraints



- Source localization is either over-constrained (placing of a few dipoles) or severely under-constrained when using a surface of dipoles.
- Under-constrained fitting requires constraints of some type, often structural and mathematical.

Bayesian Approaches to Localization



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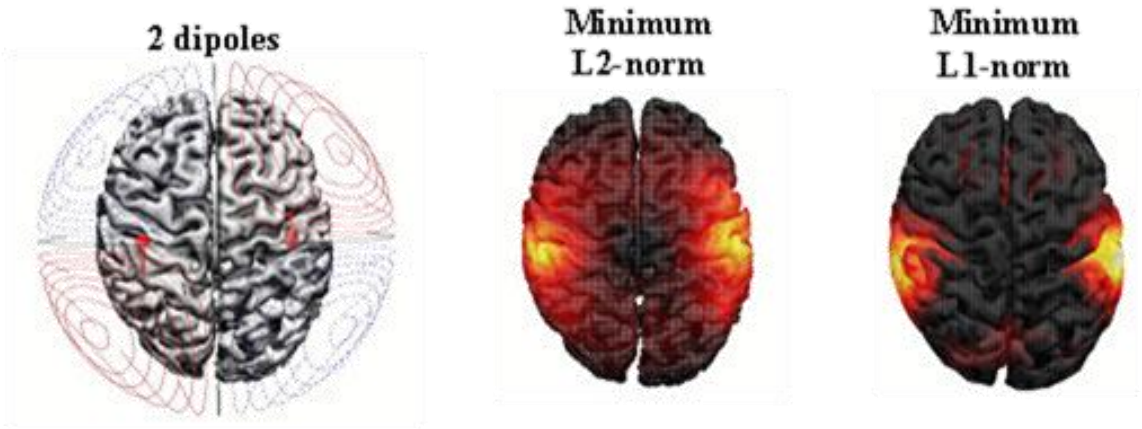

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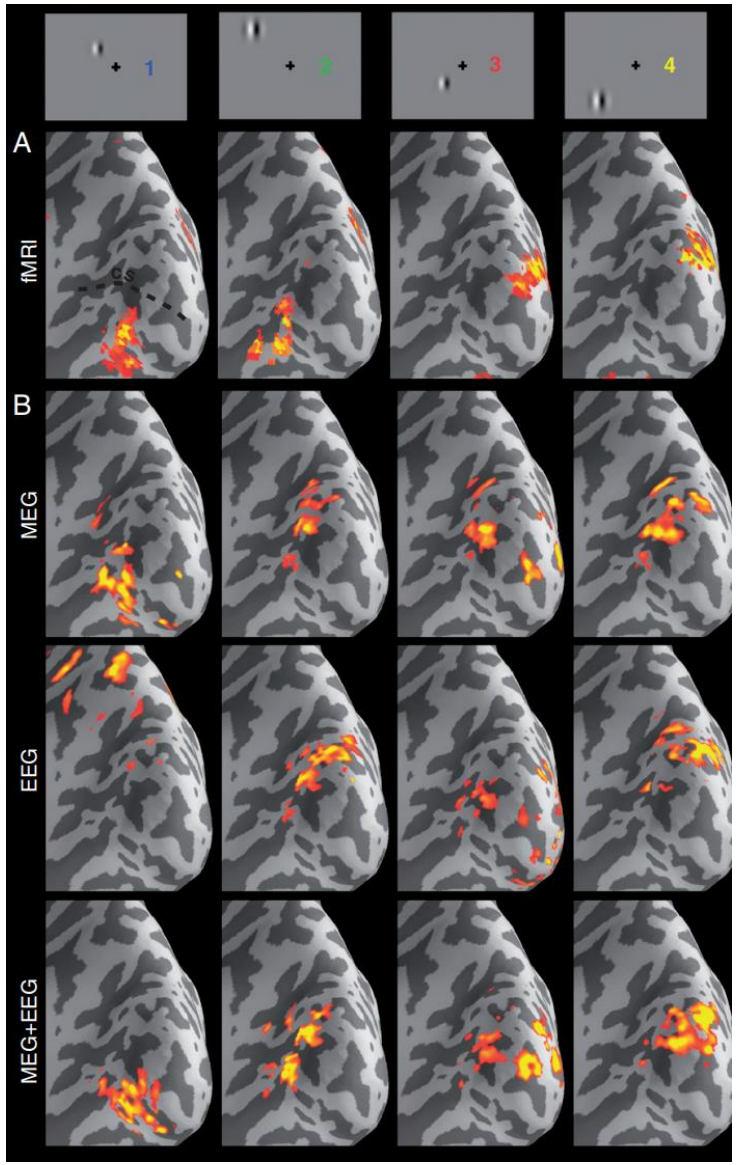
- Under-constrained fitting requires constraints of some type, often structural and mathematical.
- Combining information from multiple sources significantly improves localization.

Combining EEG and MEG Improves Localization



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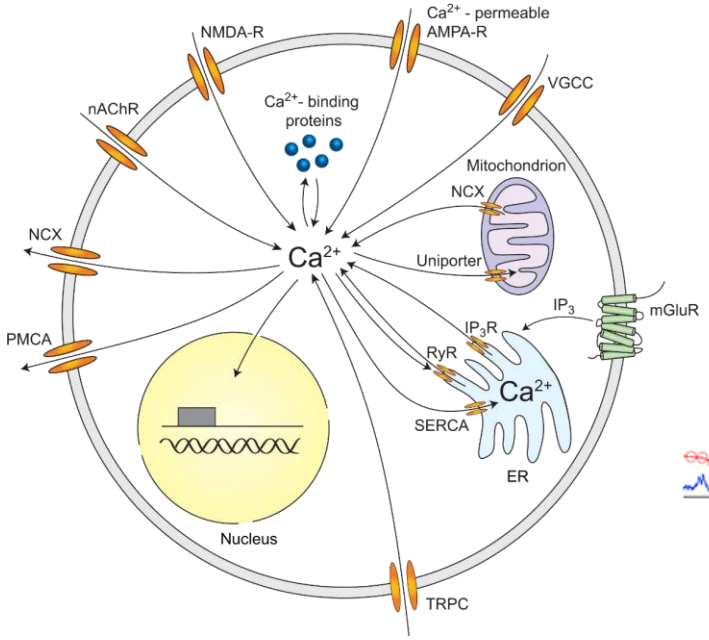
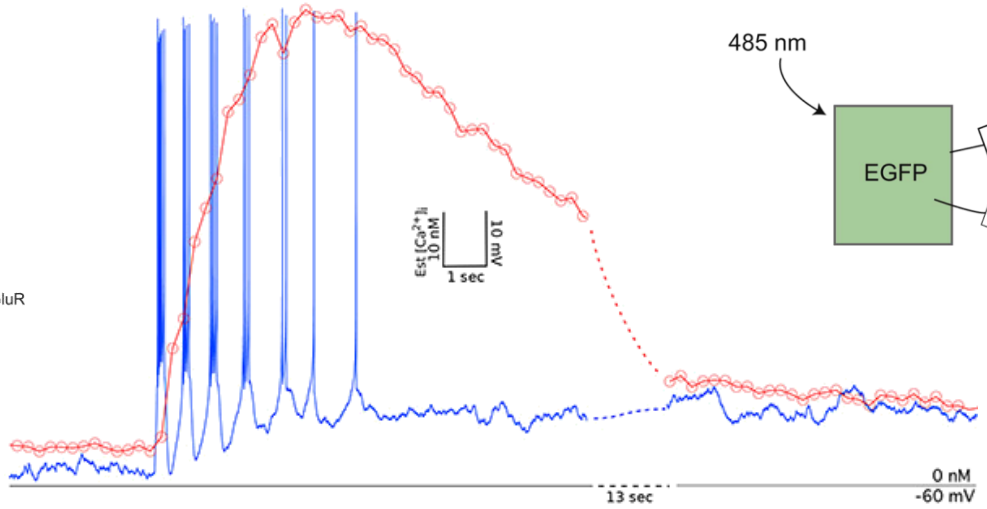
Calcium Imaging

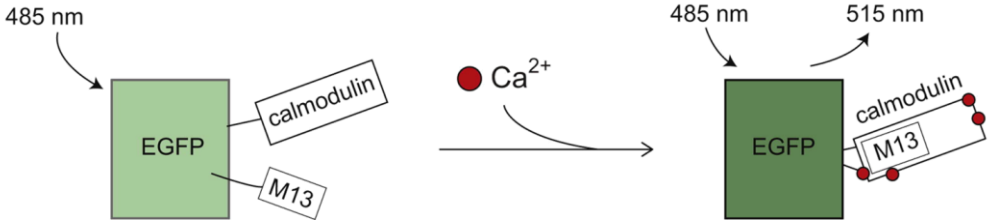
There is a calcium influx when neurons are stimulated:



This is integrated over time:

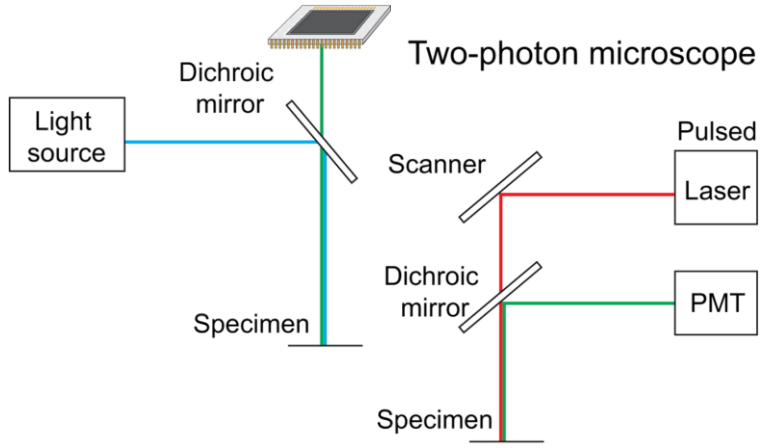


Multiple different calcium indicators can be used:



And calcium signals can be imaged in multiple ways:

CCD-based camera

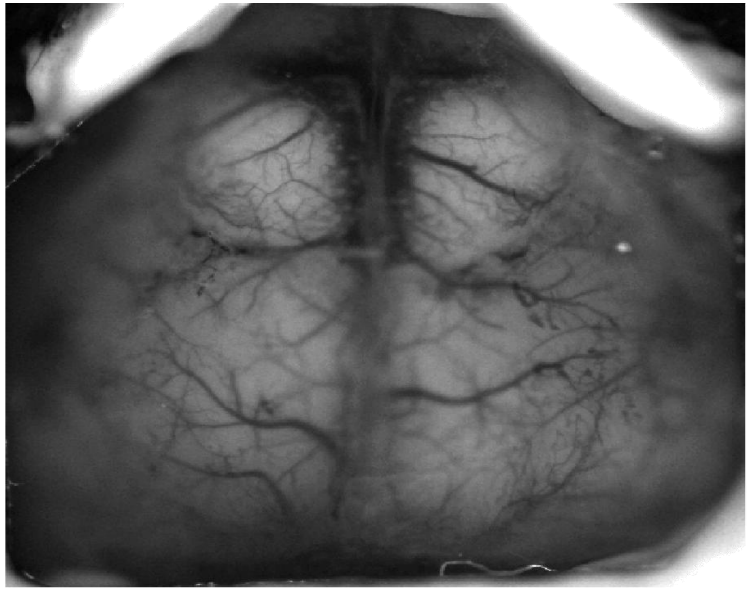


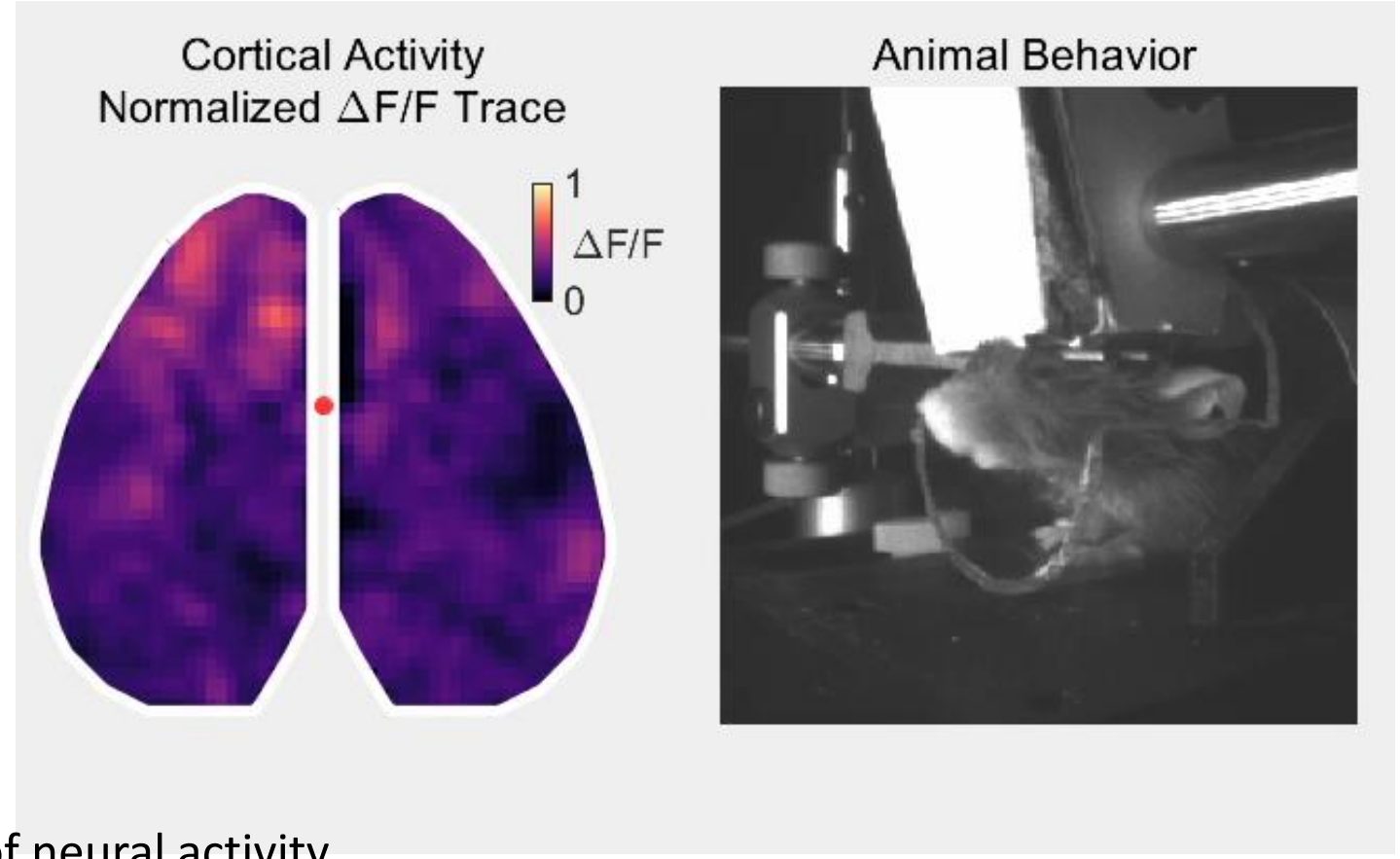
- Allows for non-invasive, low-noise imaging of neural activity.
- Can image very large populations of neurons at many different scales (from dendrites to populations).
- Slow temporal dynamics; signal is not directly tied to action potentials.
- Calcium indicators can buffer calcium, disrupting signaling (epilepsy).
- Can combine with other techniques to allow cell-type and/or projection-specific recording.

Grienberger and Konnerth, *Neuron* 2012.
Irwin and Allen, *J Vis Exp* 2013;

Calcium Imaging

Newer approaches are allow for unprecedented access to neural populations:





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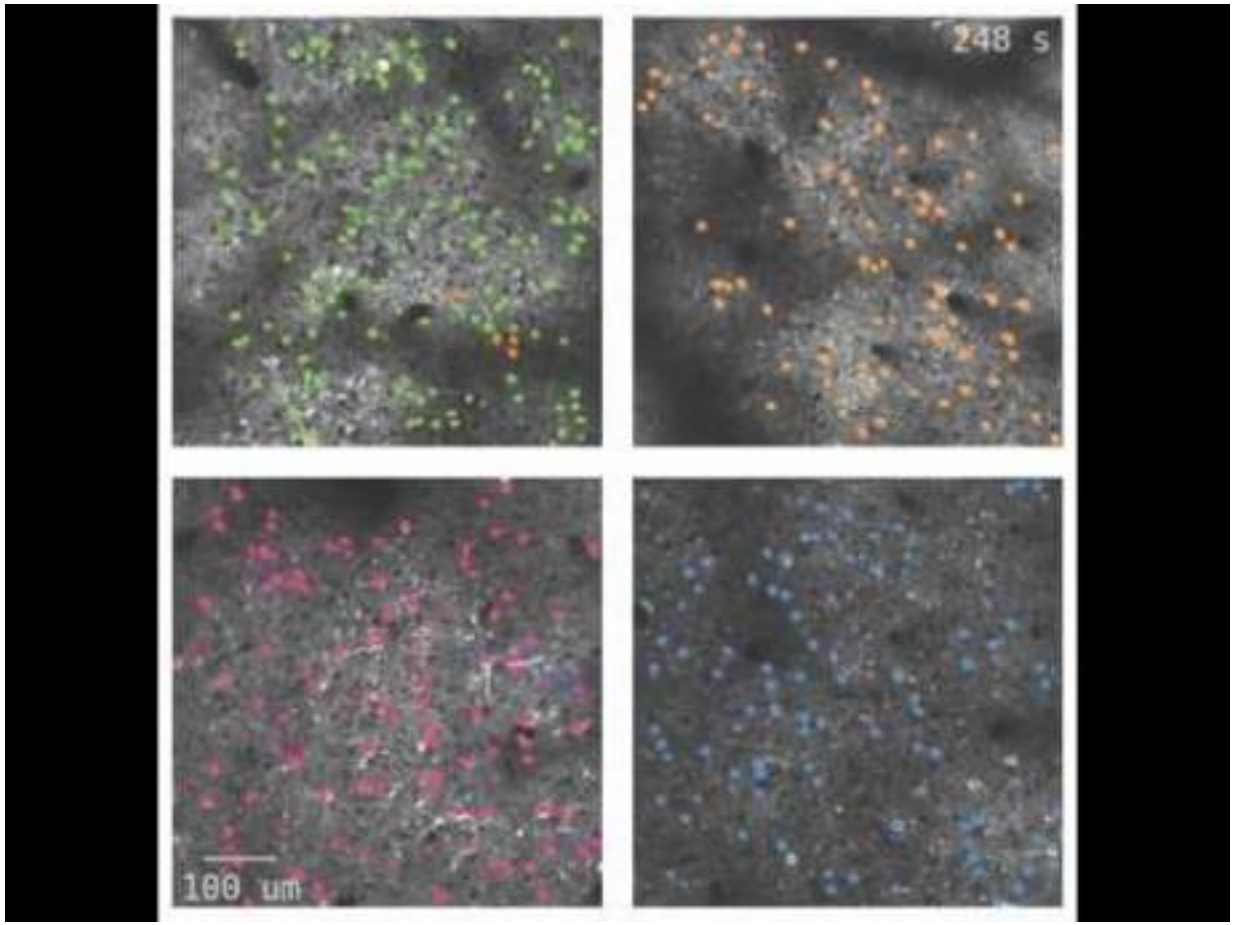
Calcium Imaging

Two-photon imaging allows for recording of 1000s of neurons simultaneously.

Improvements in optics is consistently increasing our yield, giving unprecedented ability to see the dynamics of information in the neural population.



Sofroniew et al, *eLife* 2016

Voltage Imaging

Voltage indicators have been around for a while. However, early versions were toxic to the brain and so only suitable for short surface recordings.

More recently, genetically encoded voltage indicators have become faster and more stable, allowing for imaging over extended periods of time.

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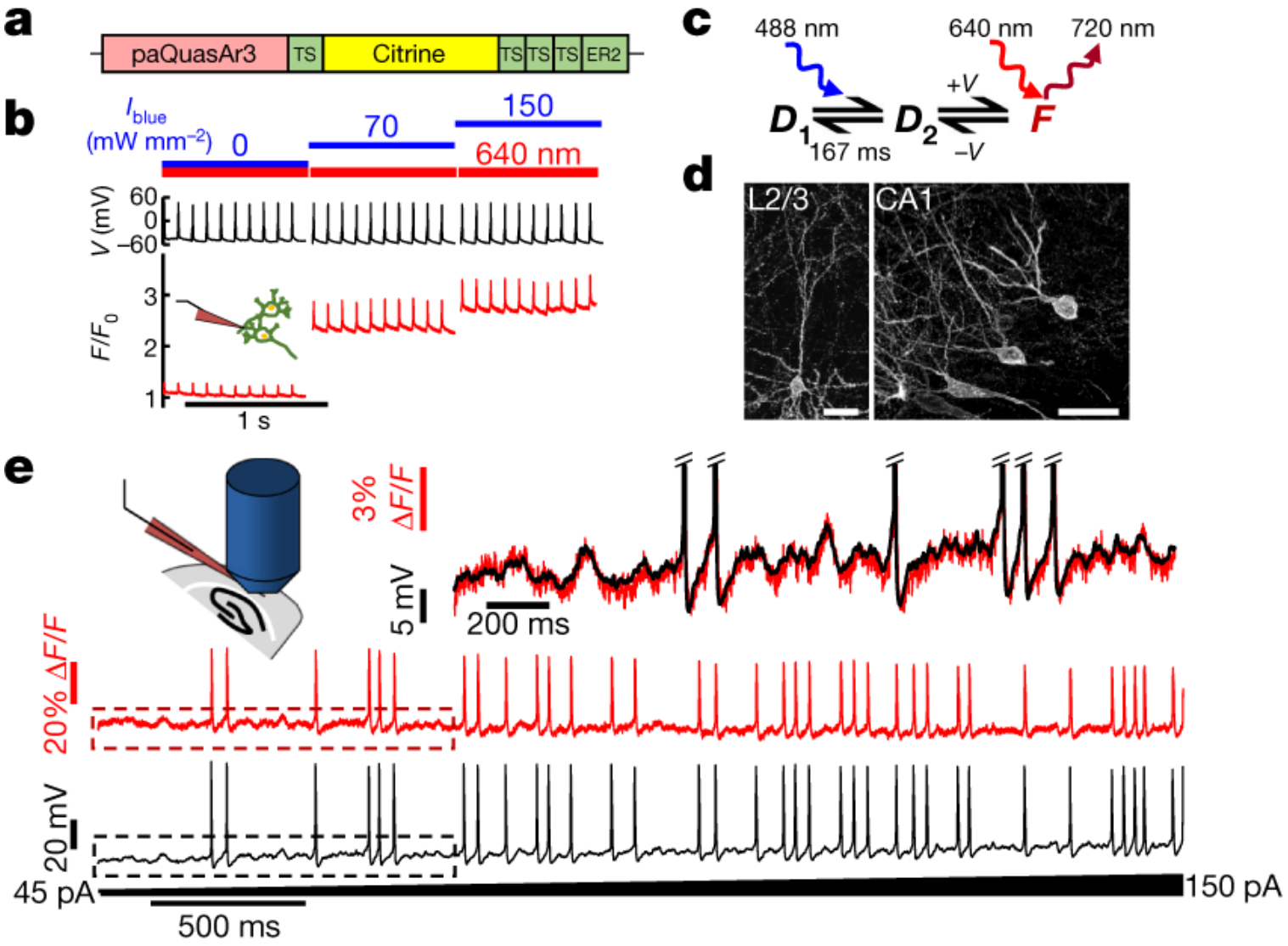
- Provides insight into subthreshold signals and/or dendritic signals.
- Could be combined with other techniques to allow cell-type and/or projection-specific recording.
- Protein expression may cause issues?



Adam et al, Nature 2019

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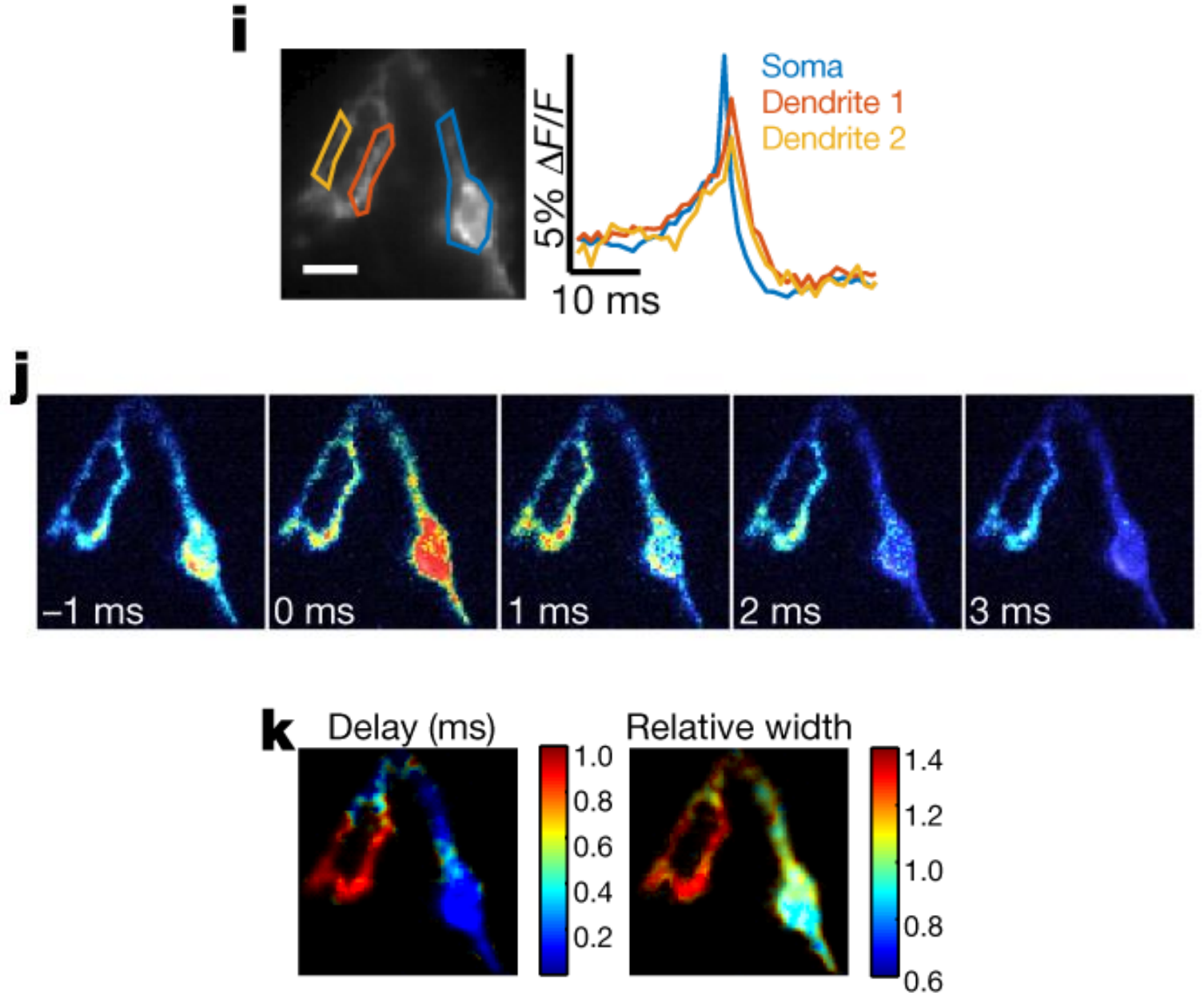


Adam et al, Nature 2019

Goals for Today

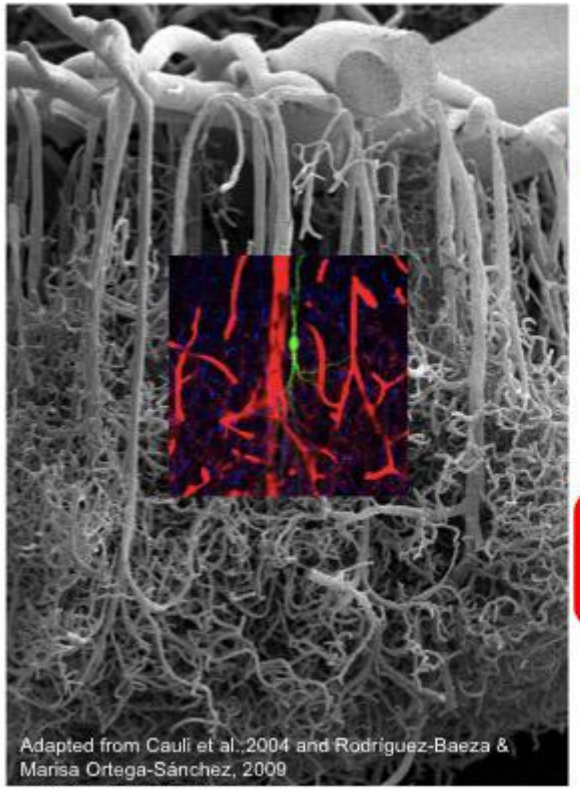
- How do we observe neural signals?
  - Electrophysiology –Neural recordings and EEG/MEG
  - Imaging – Calcium and Voltage imaging
- **How do we observe neural correlates (mostly in humans)?**
  - PET
  - fMRI
  - fUS
  - fNIRS
- How do we causally manipulate neural activity?
  - Electrical/optical stimulation
  - TMS
  - tDCS/tACS/tRCS
  - Pharmacology
  - Cooling
  - Lesions

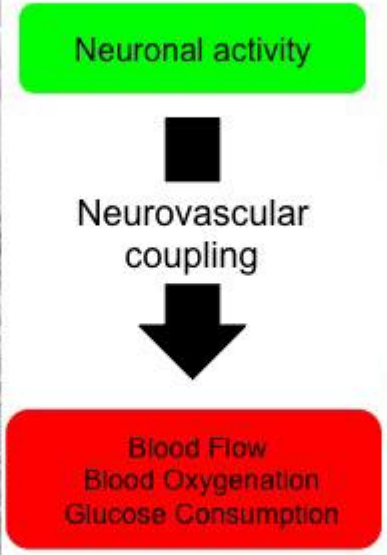
Non-invasive Functional Imaging

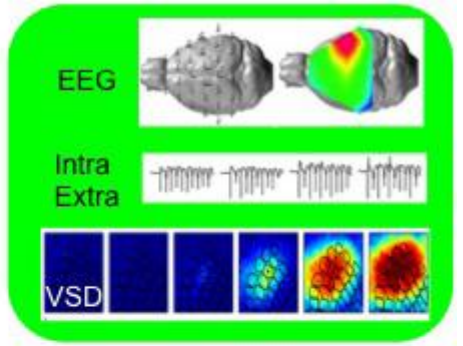
Brain uses large fraction of body's energy (20% of glucose and 0_2)

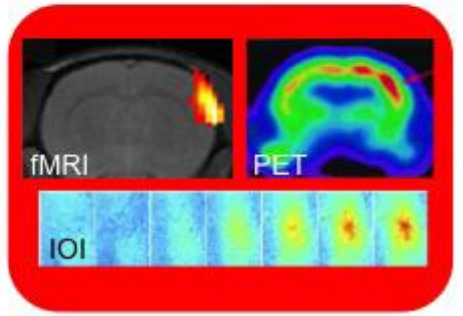
Brain imaging detects local fluctuations in energy consumption. Relies on the coupling between neural activity and non-electrical signals that are correlated with neural activity.

- PET
- fMRI
- fNIRS
- fUS

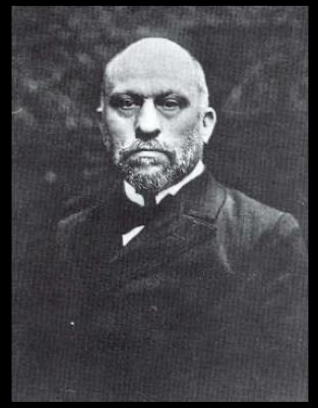




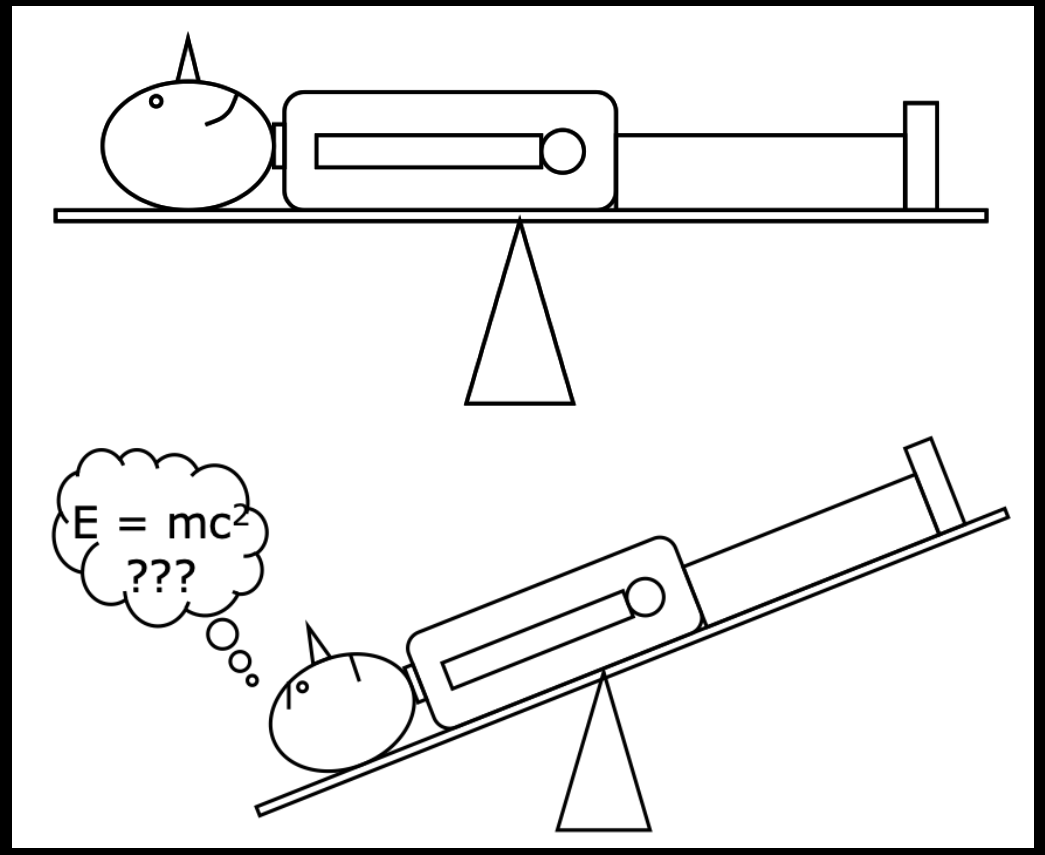




The first “brain imaging” experiment



Angelo Mosso
Italian physiologist
1846-1910



“[In Mosso’s experiments] the subject to be observed lay on a delicately balanced table which could tip downward either at the head or at the foot if the weight of either end were increased. The moment emotional or intellectual activity began in the subject, down went the balance at the head-end, in consequence of the redistribution of blood in his system.”

-- William James, Principles of Psychology (1890)

The first “brain imaging” experiment

Brain Advance Access published January 9, 2014

doi:10.1093/brain/awt352 Brain 2014: Page 1 of 6 | e1

BRAIN
A JOURNAL OF NEUROLOGY

LETTER TO THE EDITOR

Weighing brain activity with the balance: a contemporary replication of Angelo Mosso’s historical experiment

David T. Field[1] and Laura A. Inman[2]

The first “brain imaging” experiment

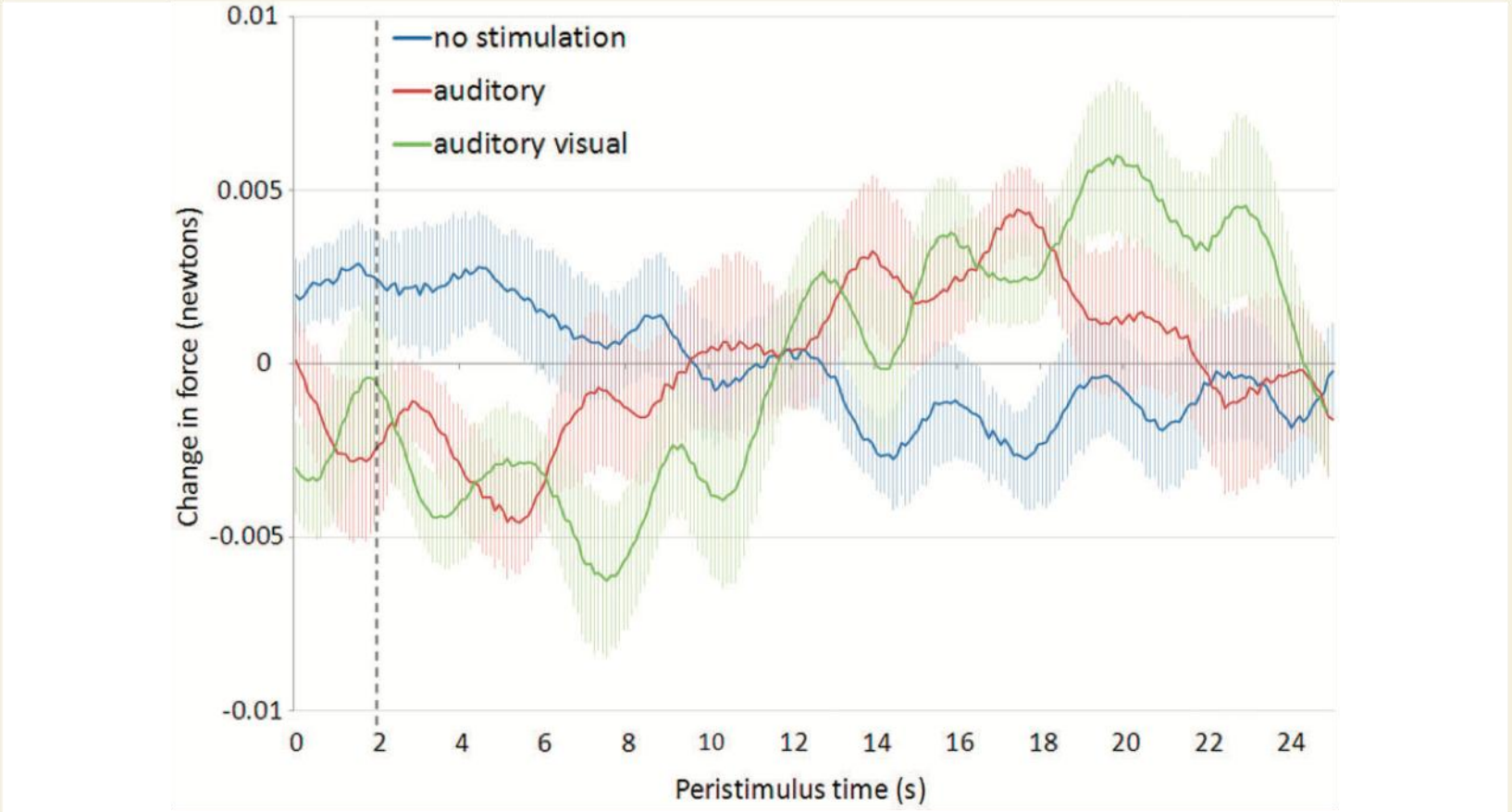
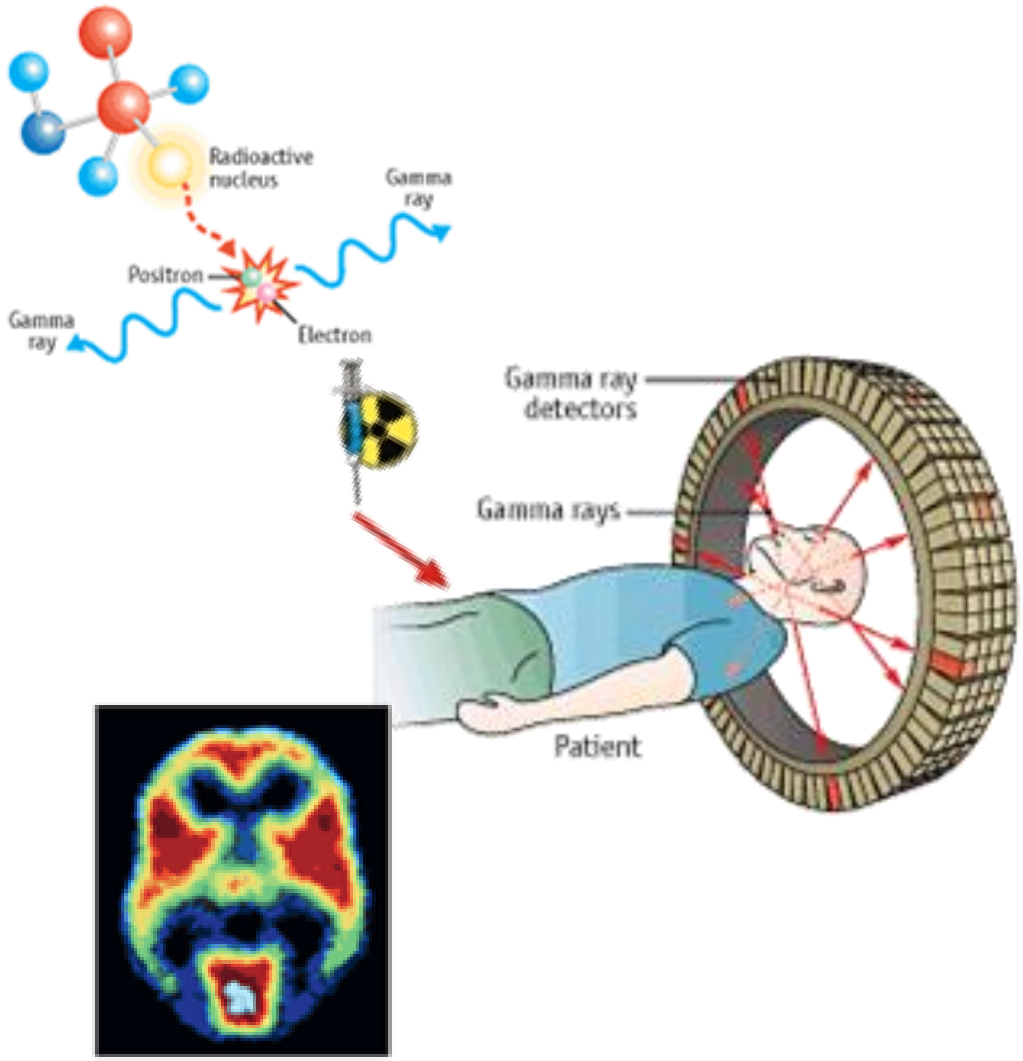


Figure 4 Peri-stimulus time plots averaged across 14 participants from the functional experiment. Stimulation occurred between 0 and 2 s for the auditory (red) and combined visual and auditory (green) conditions. In the baseline condition (blue) there was no stimulation. The end of the stimulation period is indicated by the dashed line. Raw data for each participant was preprocessed by removing the linear drift and demeaning, followed by smoothing out the respiration and cardiac signals using a 12 s Hanning window. Event-related averages were created for each condition for each individual to feed forward to the group average. For each data series change in force exerted is relative to the mean of that data series. Error bars represent ± 1 standard error based on between-participant variation.

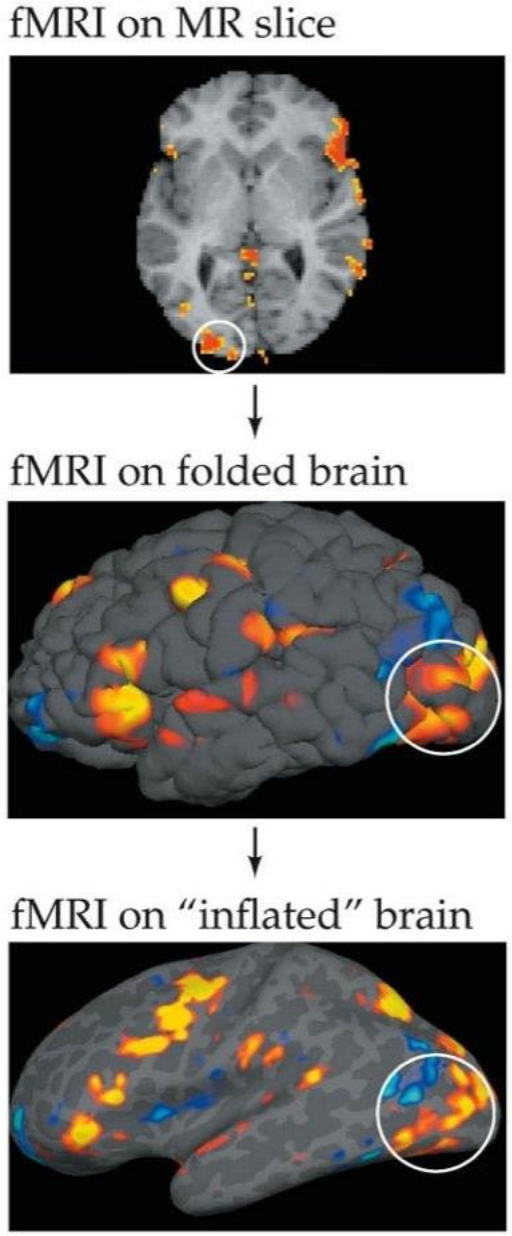
Positron Emission Tomography (PET)



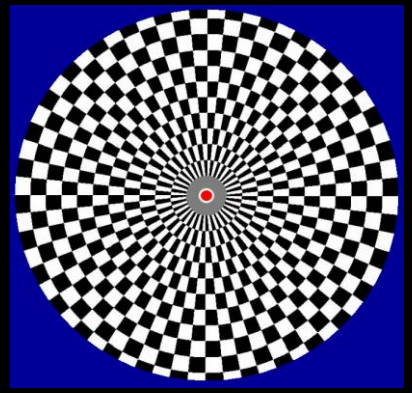
- Uses radioactive-labeled glucose to measure energetic consumption in the brain (blood flow).
  - Injected; must be synthesized in cyclotron nearby.
  - Oxygen-15 (half-life two minutes), also F-18, C-11
- Accumulates in metabolically active areas
- Isotope decays, proton breaks into neutron and positron
- Positron goes a few mm, collides with electron, emits gamma rays
- Gamma ray detectors arrayed around subject's head → reconstruction
- Other radiolabeled drugs can be used to measure receptor concentration. This can be used for diagnostic purposes or for experimental ones (like tracking neurotransmitters)
- Poor spatial and temporal resolution.
- Moderate radioactive dose (~2x CT scan)

fMRI

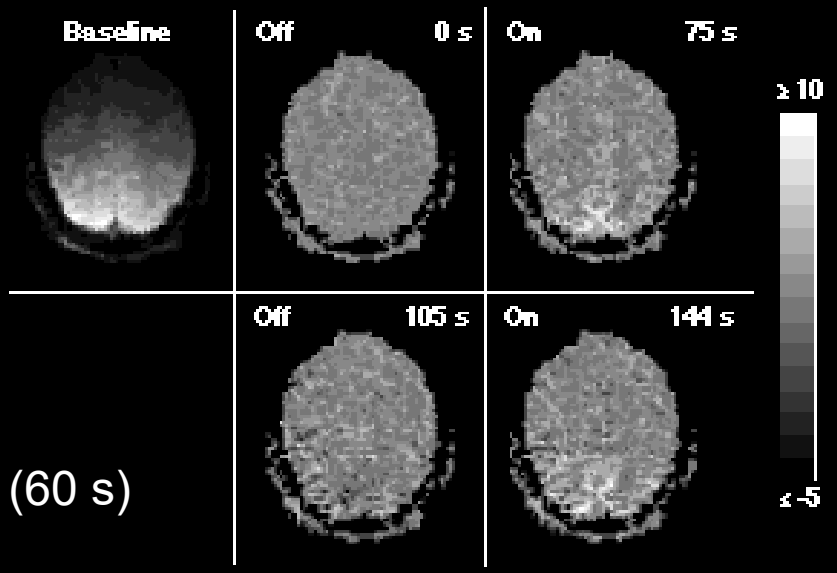
- Versus PET:
  - No need to inject anything
  - Better spatial localization (several mm vs. ~1 cm)
  - Much better temporal resolution (a few seconds versus many)



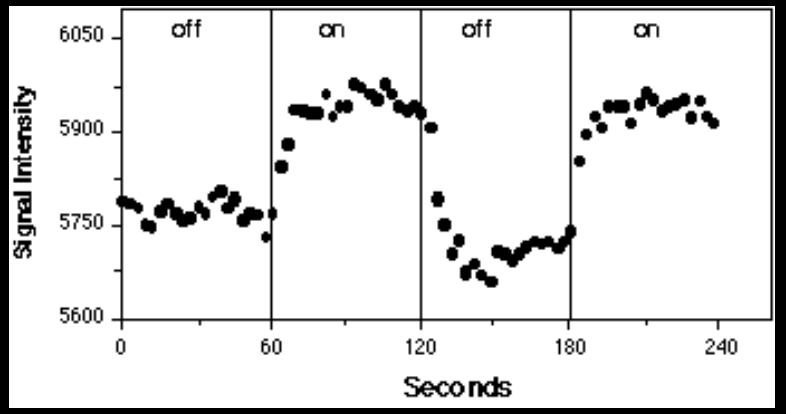
fMRI Activation



Flickering Checkerboard
OFF (60 s) - ON (60 s) -OFF (60 s) - ON (60 s) - OFF (60 s)



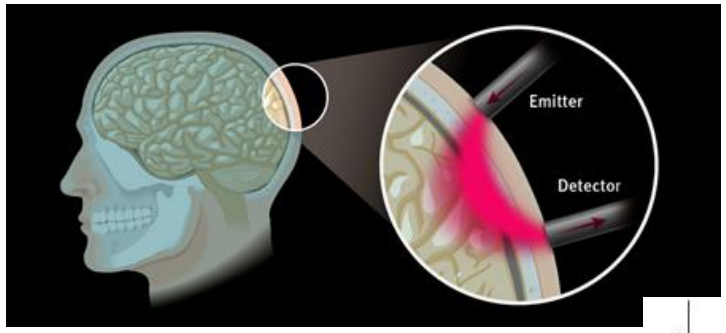
Brain Activity

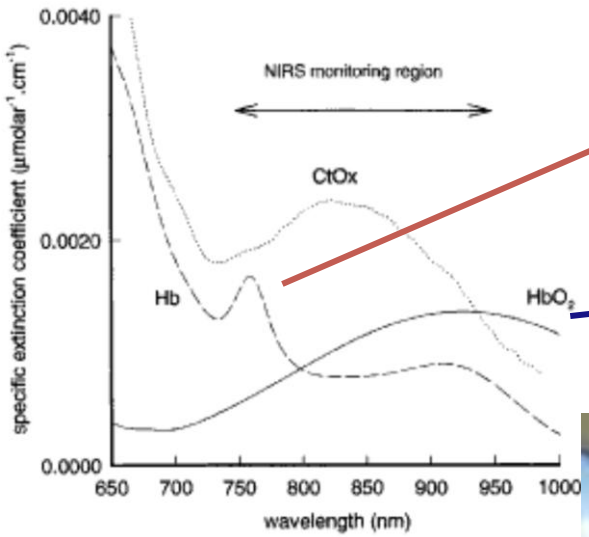


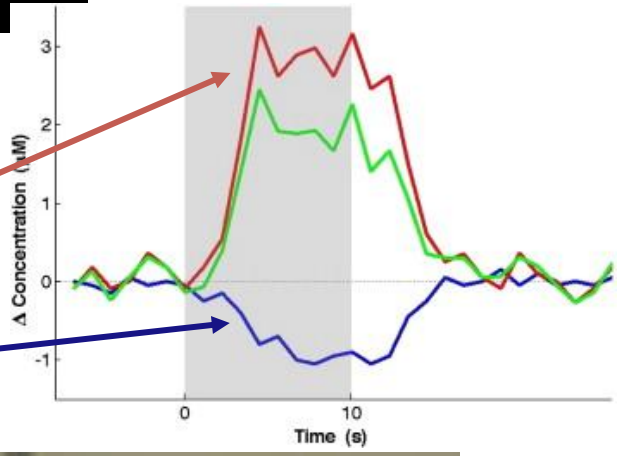
Time ⇨

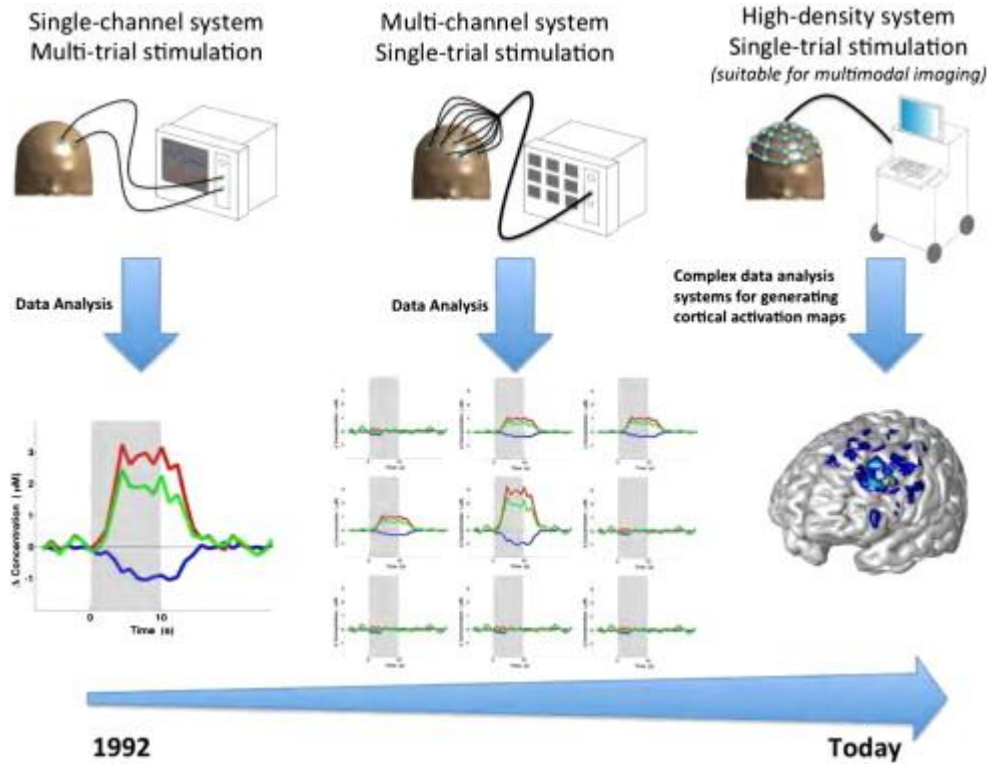
Source: Kwong et al., 1992

Functional Near-Infrared Spectroscopy (fNIRS)





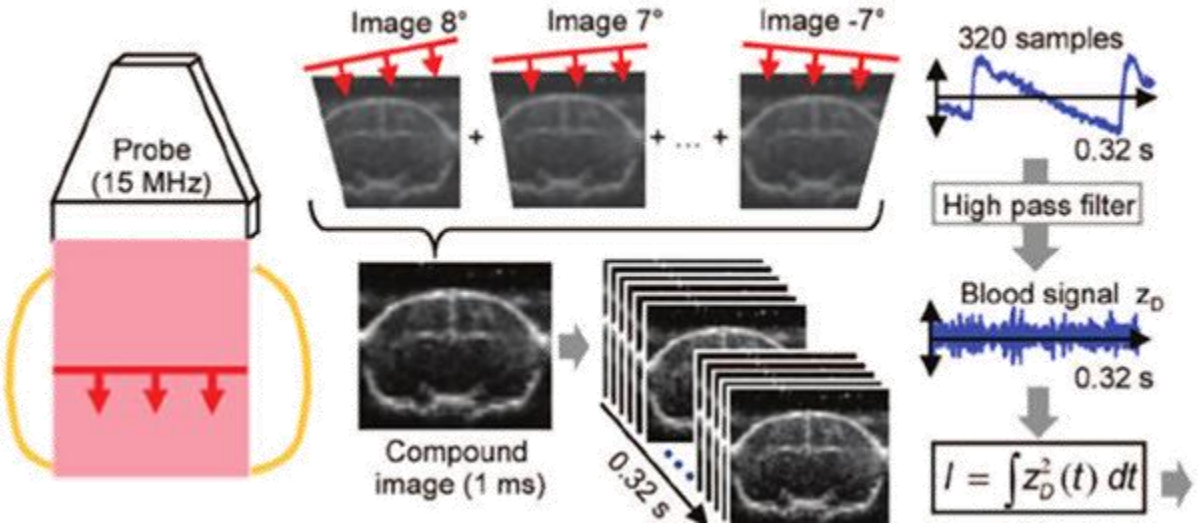


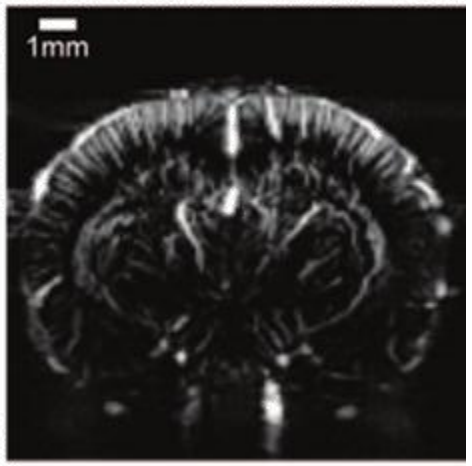


- Relies on same BOLD signal as fMRI, but can sample at much higher frequency.
- Cannot penetrate very deep; much worse spatial resolution than fMRI.
- Fiber optics can move source/detector away, making for a very light and flexible cap. Good for infants/toddlers/patients. Even some recent wireless models.

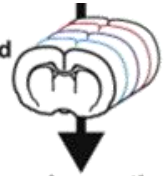
Delpy and Cope, Phil Trans B 1997;
Ferrari and Quaresima, NeuroImage 2012

Functional Ultrasound (fUS)



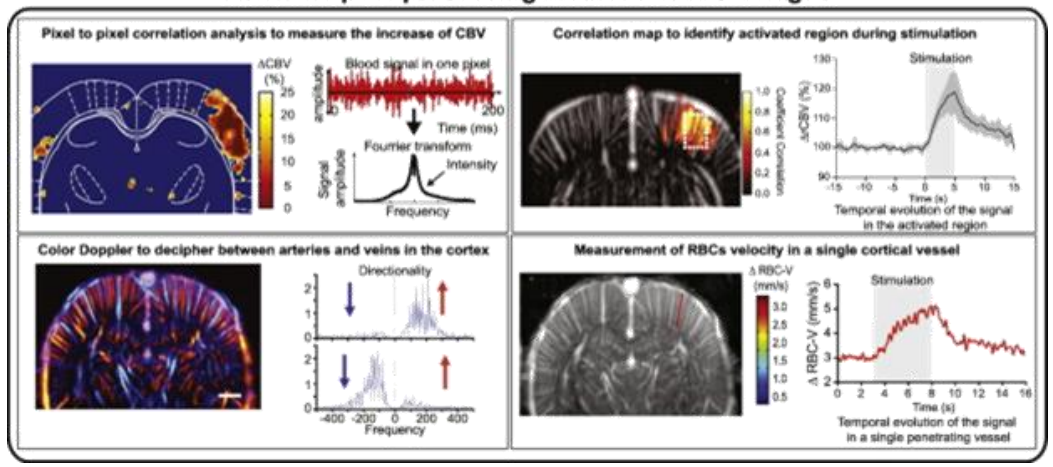


SPATIAL
Acquisition can be performed in a single plan or in multiple plan (scan)



TEMPORAL
Acquisition can be performed from 0.5s seconds to several hours (continuous imaging)

Available post-processing methods of fUSi images



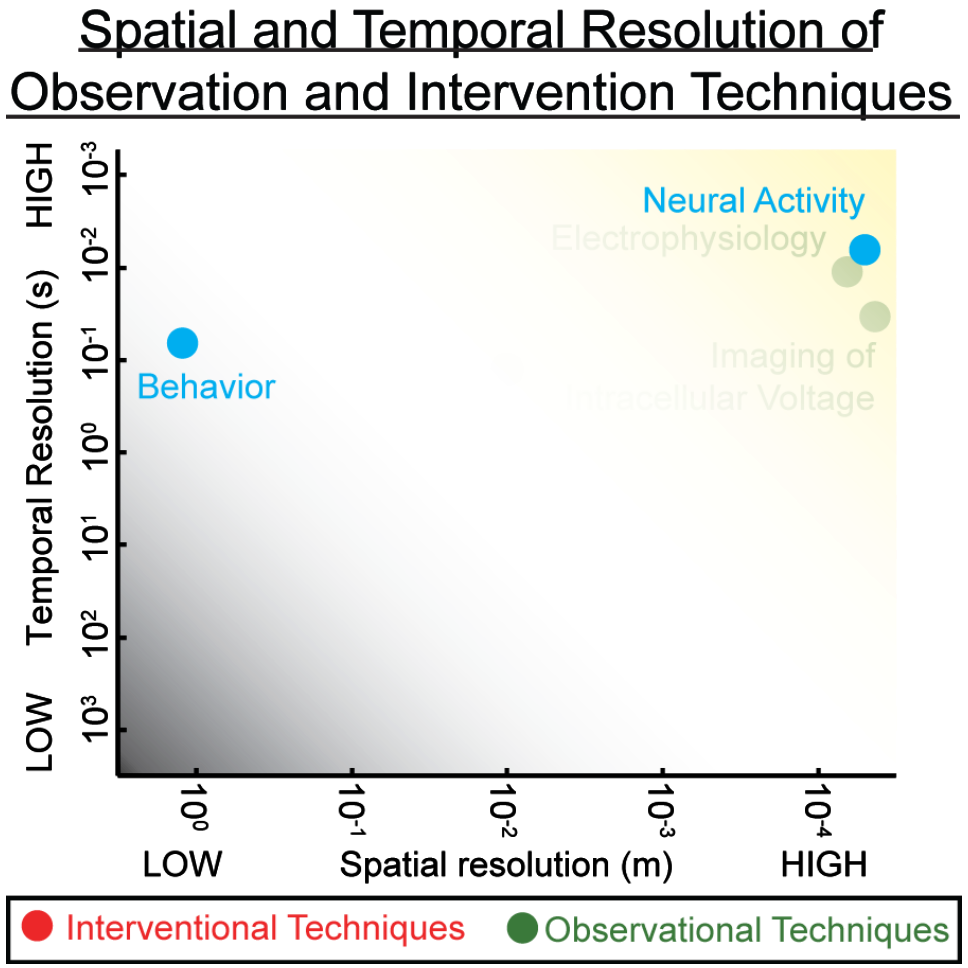
- Relies on the same neuro-vascular coupling as fMRI and fNIRS, but samples at much higher spatial and temporal frequency.

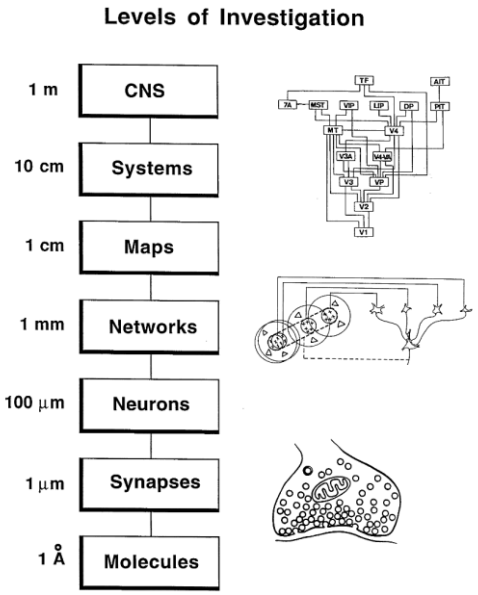
Mace et al, 2013

There is no ideal technique; there are trade-offs between different approaches:

- Spatial accuracy
- Temporal accuracy
- Invasiveness
- Cell-type/projection specificity
- Scale of recording

Combining techniques can yield complementary insights.





Perturbation of Neural Circuits

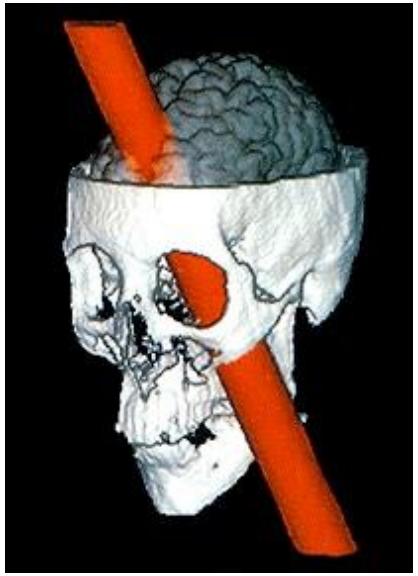
Causal manipulations are critical to testing hypotheses; without them we have nothing but correlations.

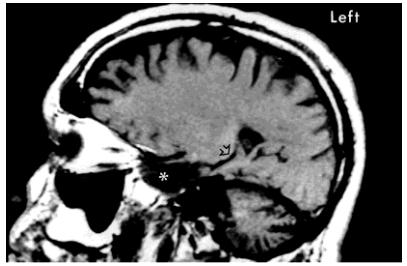
Just as importantly, these methods form the basis for many interventional treatments.

- Lesions
- Cooling
- Pharmacology
- Electrical microstimulation
- Optogenetic stimulation
- TMS
- tDCS/tACS/tRCS

Lesions

- Oldest approach: Look at patients with brain damage
  - Correlate signs/symptoms with lesion location
  - Examples: Language lateralization, Frontal lobe and planning, Perceptual disorders, Memory, Emotion...
  - Limitation:
    - Not under experimental control in humans
    - Compensatory mechanisms in the brain

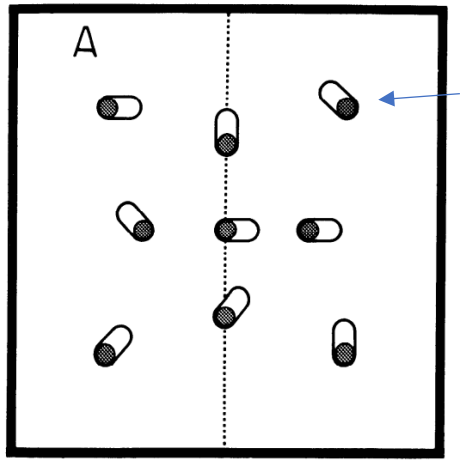




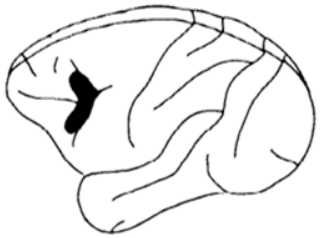
Lesions

Lesions to Frontal Eye Fields (FEF) temporarily disrupts eye movements.

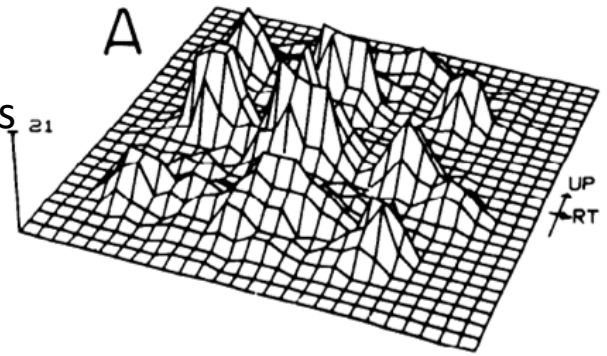
Foraging Task



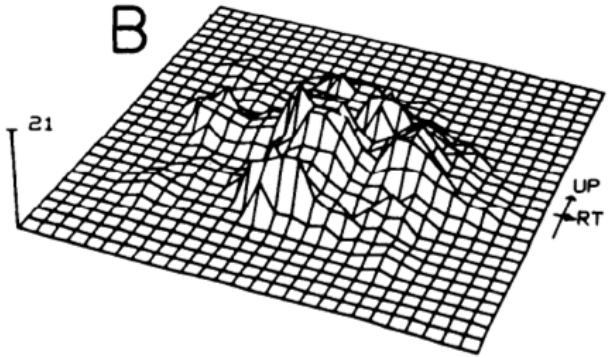
Sagittal view



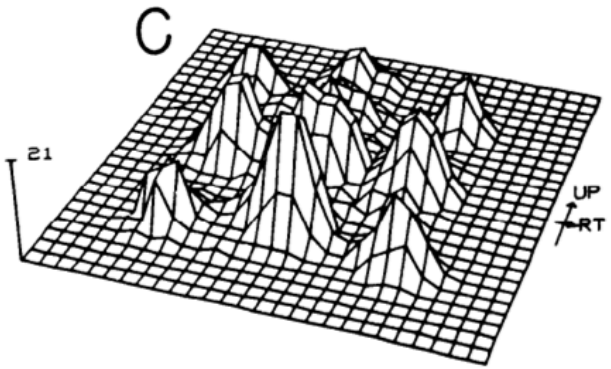
Eye movements before FEF lesion

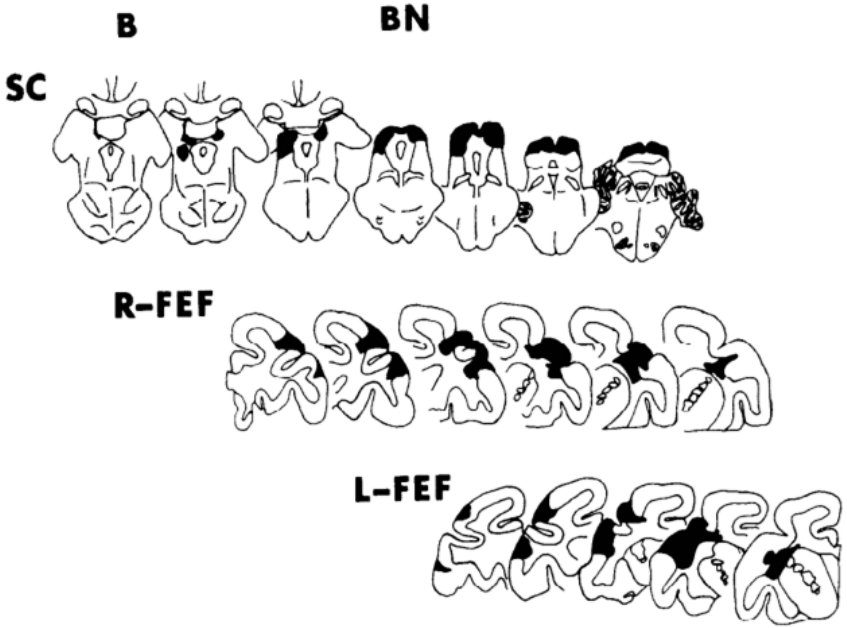


4 days after lesion



24 days after lesion



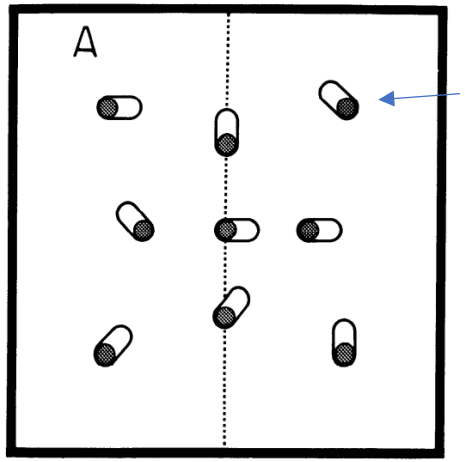


Schiller et al, *J Neurophys* 1980

Lesions

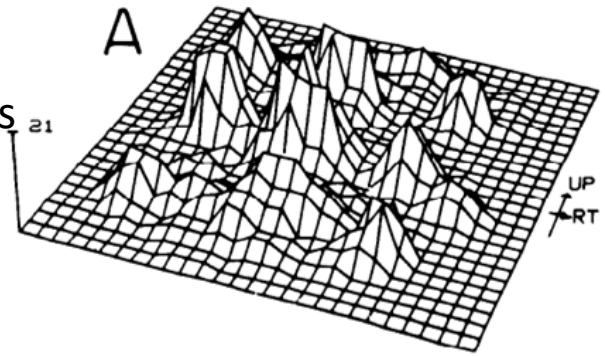
Lesions to Frontal Eye Fields (FEF) temporarily disrupts eye movements.

Foraging Task

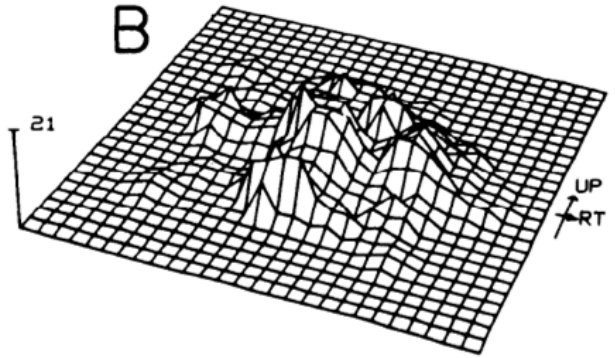


apple pieces

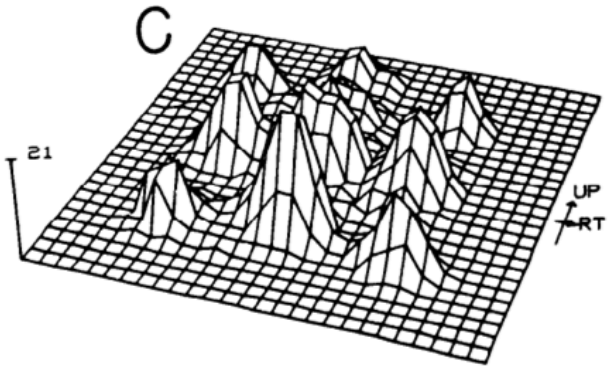
Eye movements before FEF lesion



4 days after lesion

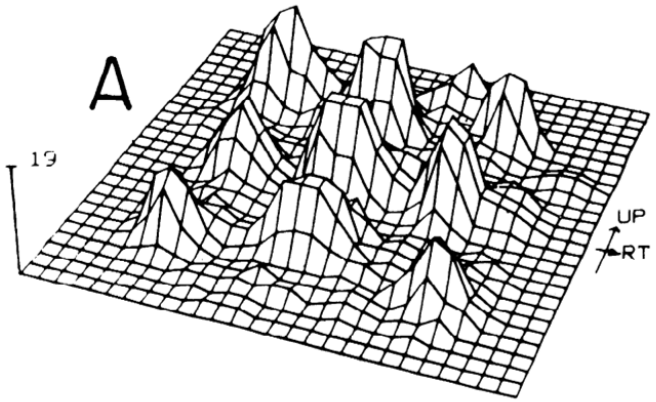


24 days after lesion

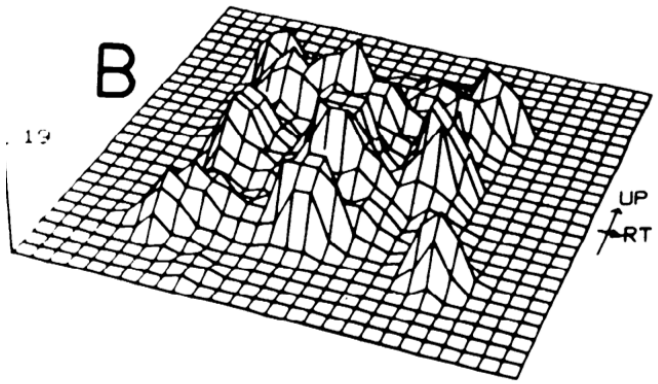


Lesions to Superior Colliculus (SC) causes little disruption.

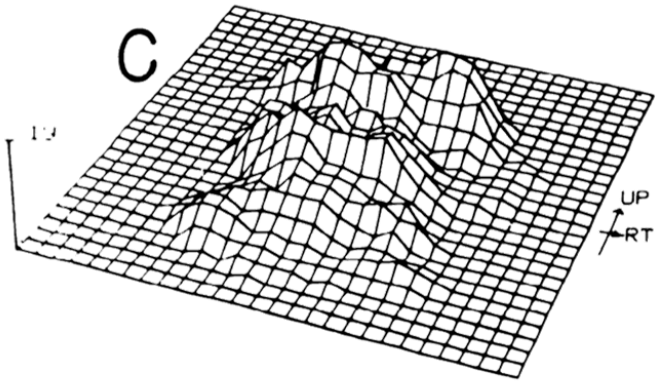
Eye movements before SC lesion



4 days after lesion



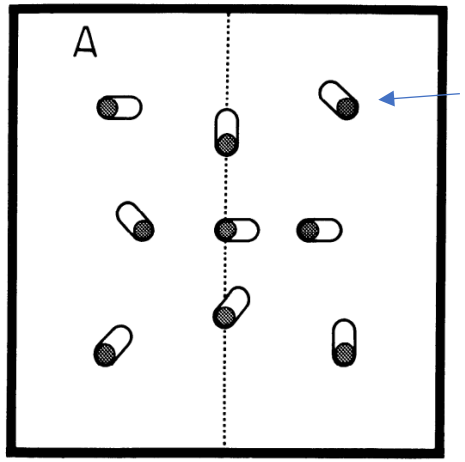
24 days after lesion



Schiller et al, *J Neurophys* 1980

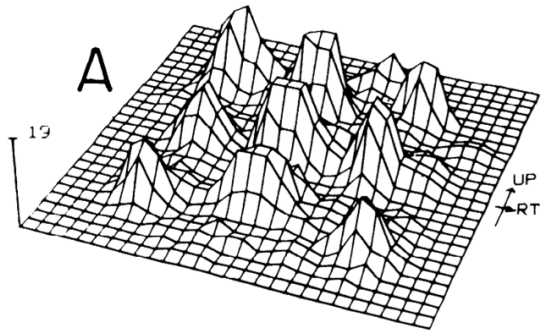
Lesions

Foraging Task

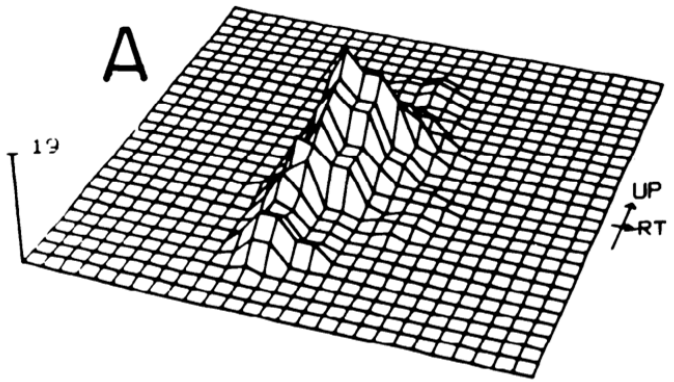


apple pieces

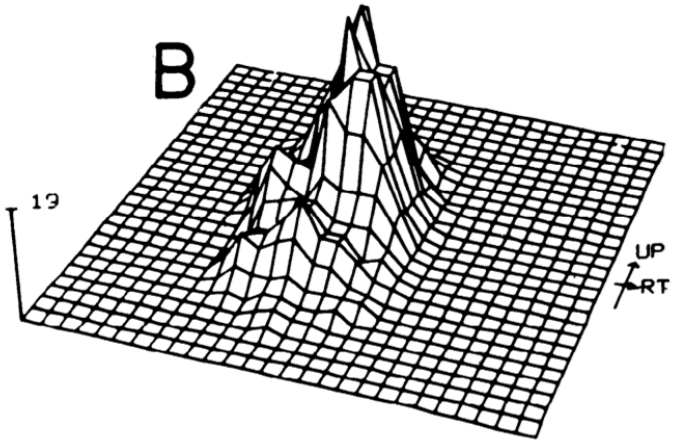
Eye Movements



57 days after SC & FEF lesion



134 days after SC & FEF lesion

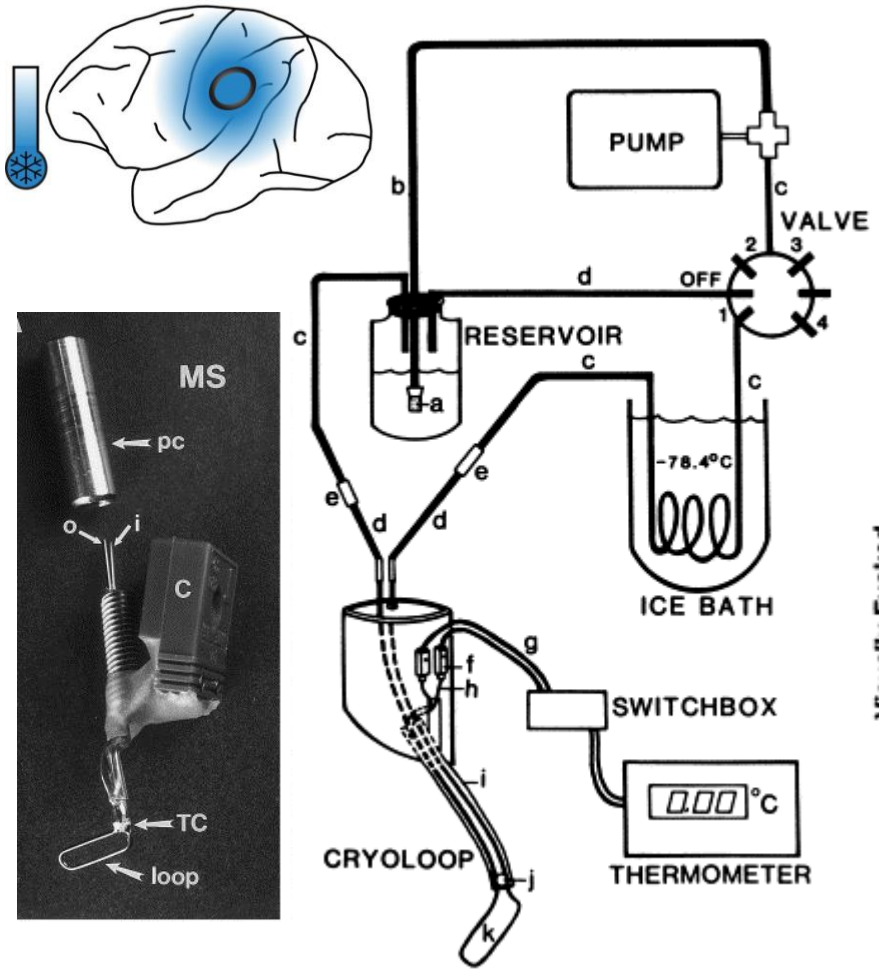


- Perhaps the oldest technique in neuroscience.
- Provides some insight into the anatomical localization of function, although care must be taken to develop proper controls that isolate the desired function.
- Spatially and temporal imprecise.
- Deficits often recover over time, complicating insights.

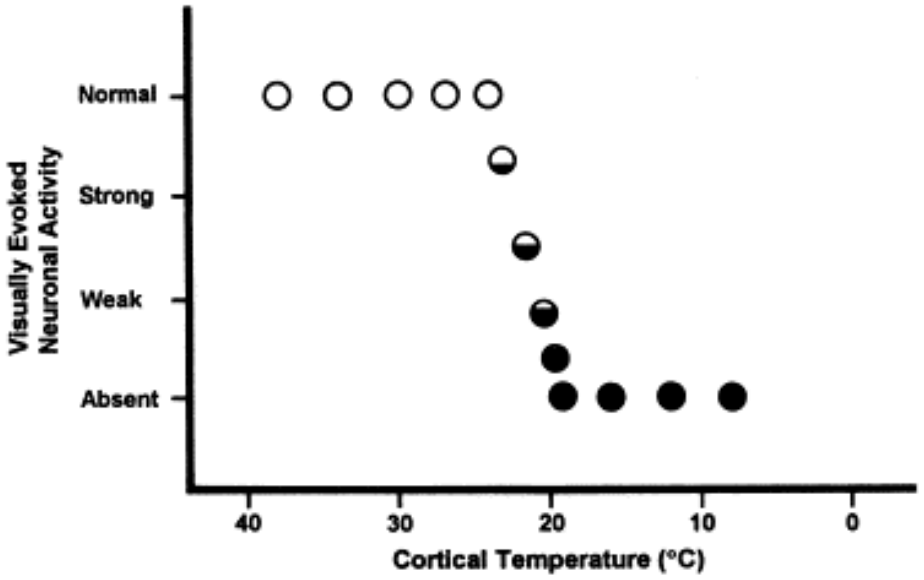
Schiller et al, *J Neurophys* 1980

Cortical Cooling

Example Cryoloop



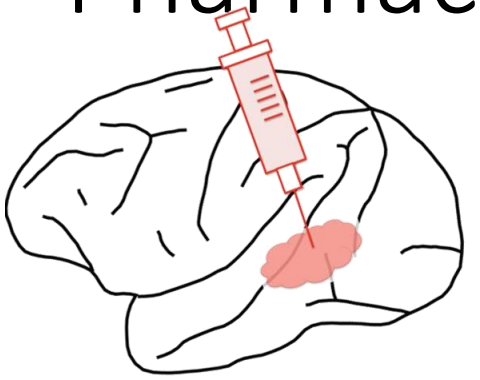
Cooling the cortex by a few degrees reduces/eliminates spiking activity:



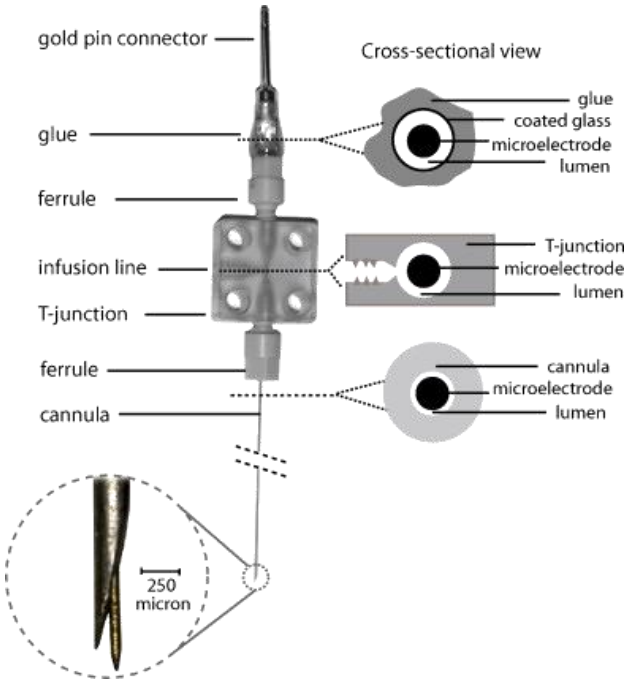
- Allows for temporary inactivation of a fairly large volume of cortex in under a minute.
- Washout is just as fast.
- No long-term effects have been observed.
- **Warming** of cortex can also *speed up* activity!

Schiller et al, *J Neurophys* 1980

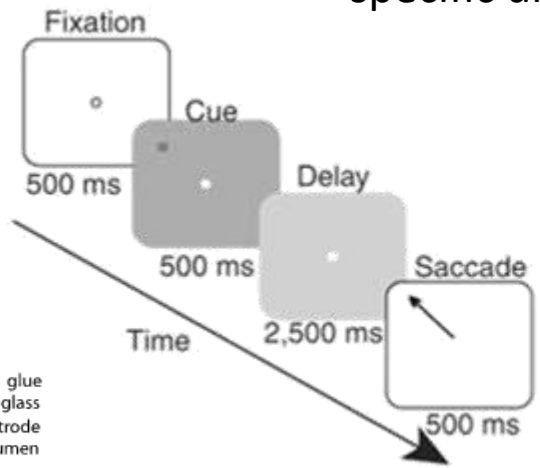
Pharmacology

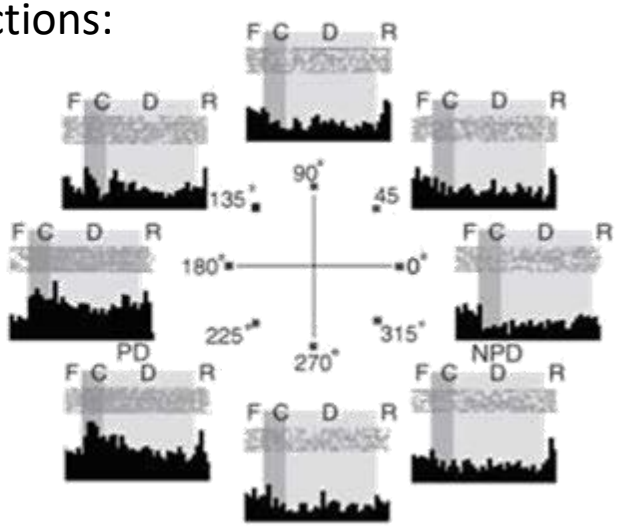


‘Injectrode’

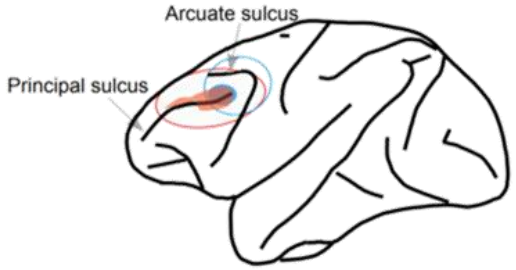


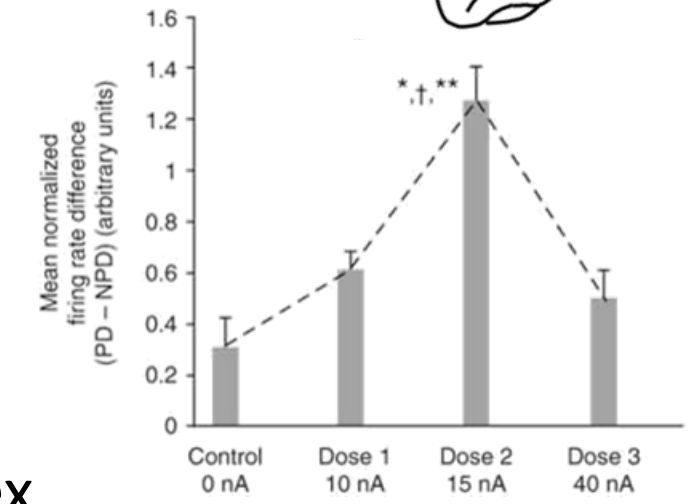
PFC neurons are tuned for remembering specific directions:





D1 agonists in PFC have an ‘Inverted-U’ effect on working memory encoding:



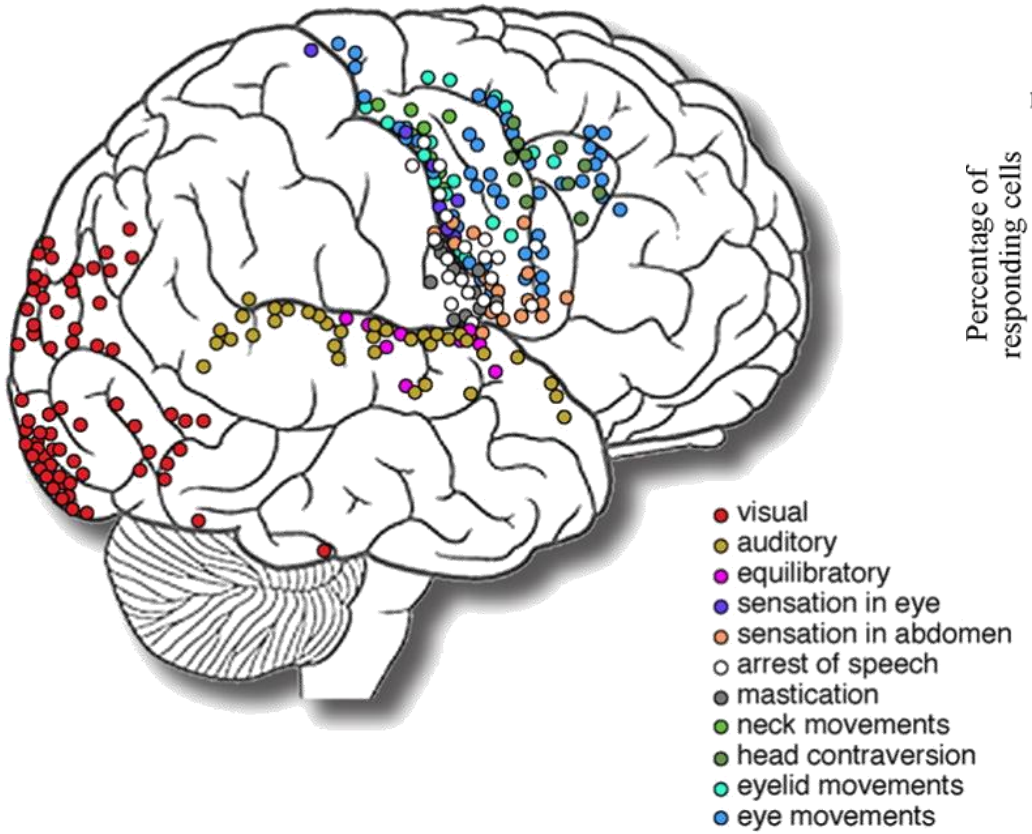


- Modulation of a large volume of cortex.
- Uniquely allows for certain types of causal manipulations (neuromodulators, receptors, transcription, etc).
- Induction is slow and non-linear; washout is slow; long-term effects can occur.

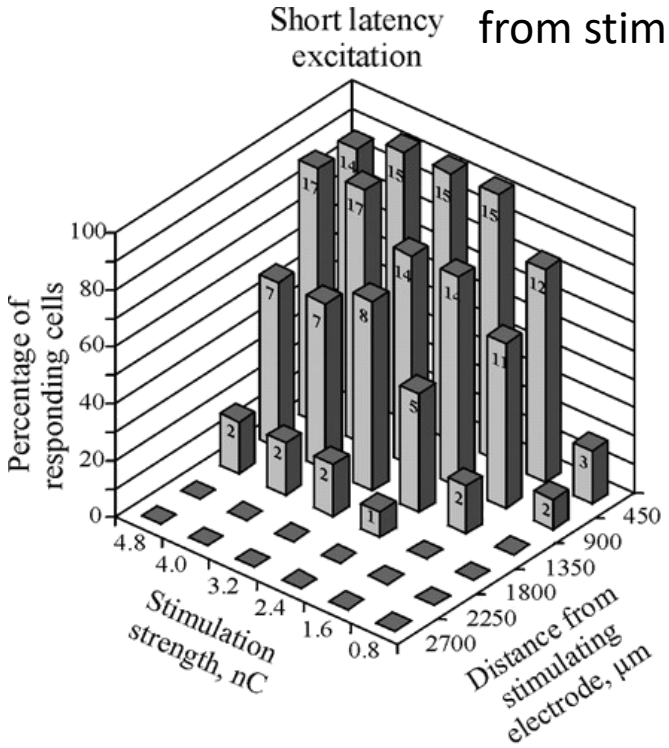
Malpeli et al, *J Neuro Meth* 1999
Noudoost and Moore, *J Neuro Meth* 2011
Vijayraghavan et al, *Nat Neuro* 2007

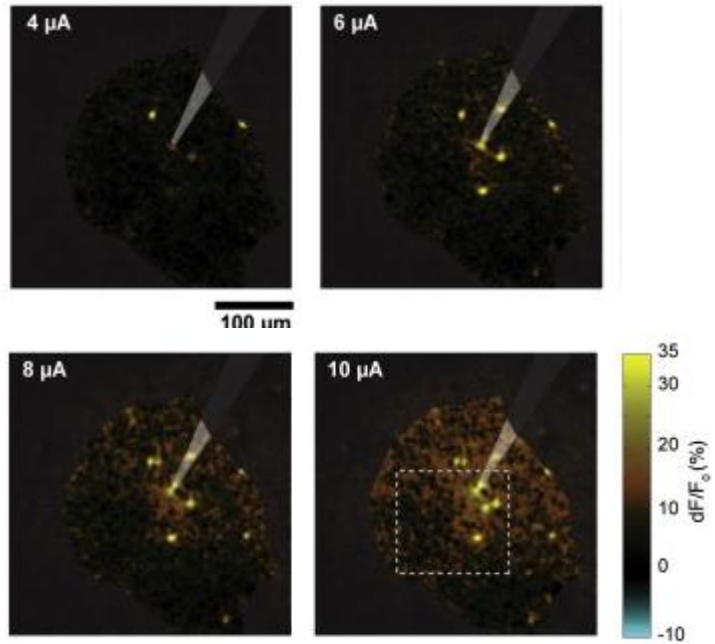
Electrical Microstimulation

Penfield's microstimulation experiments helped map the brain:



Stimulation affects neurons up to ~1 mm from stimulation electrode.



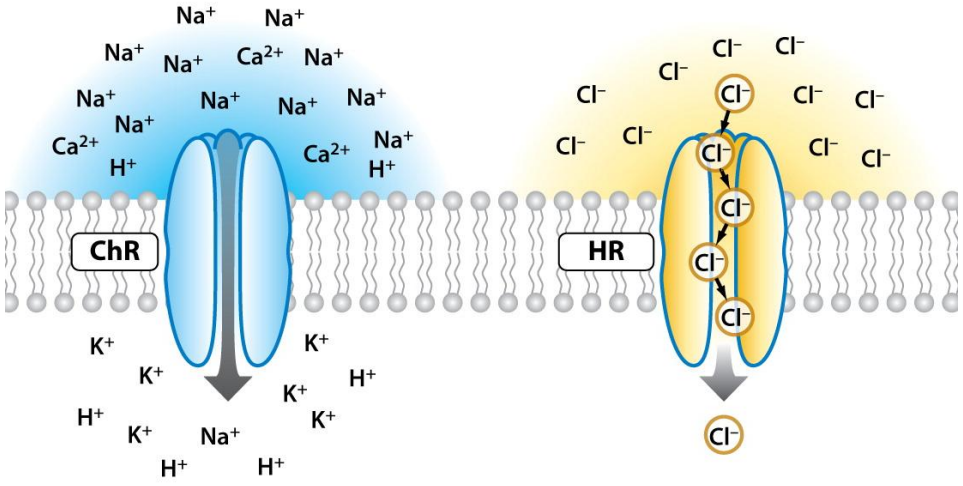


- Moderate spatial resolution; high temporal resolution.
- Can be done through recording electrodes (making it easier to complement electrophysiology).
- Unknown specificity of targets likely biases results in an unknown manner.

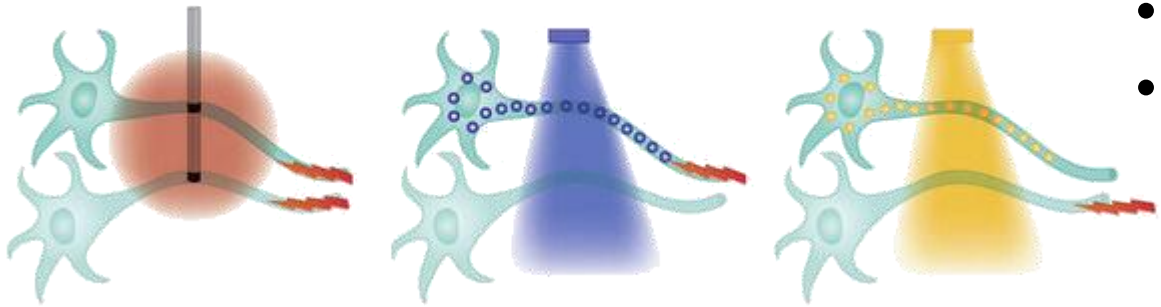
Histed, Ni, and Maunsell, *Prog Neurobio* 2013

Optogenetics

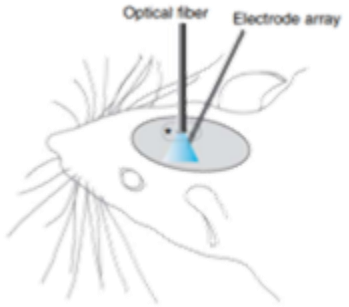
Genetically encoding light-sensitive ion channels/pumps provides control over neurons:

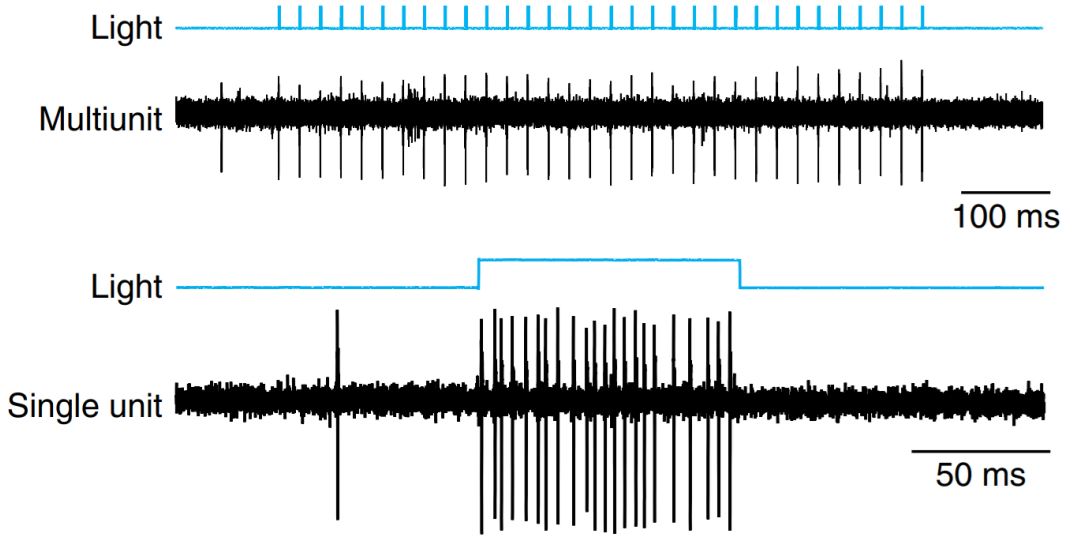


Electrical stimulation | Optogenetic excitation | Optogenetic inhibition



Genetically encoding light-sensitive ion channels/pumps provides control over neurons:



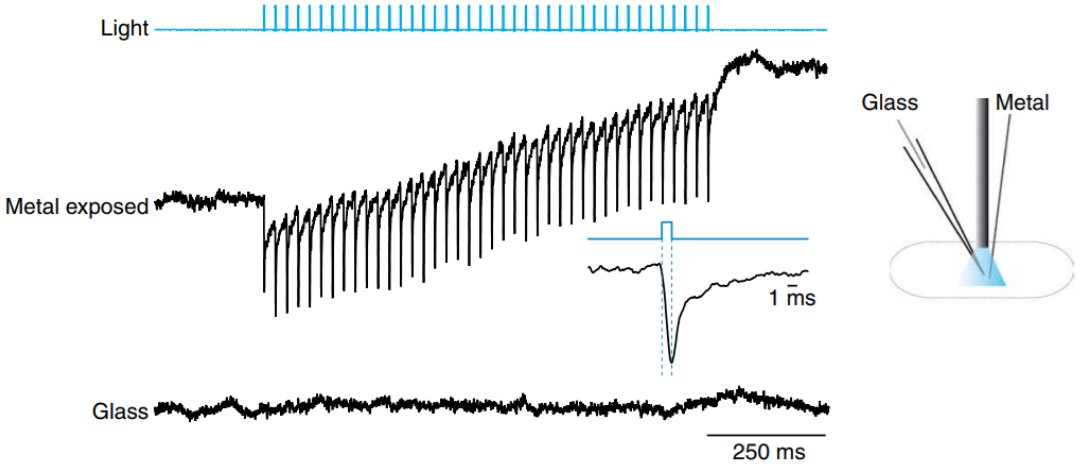


- High spatial and temporal resolution.
- Combination of genetic techniques with light control allow for highly specific control of cell-types, projections, and neural selectivity.

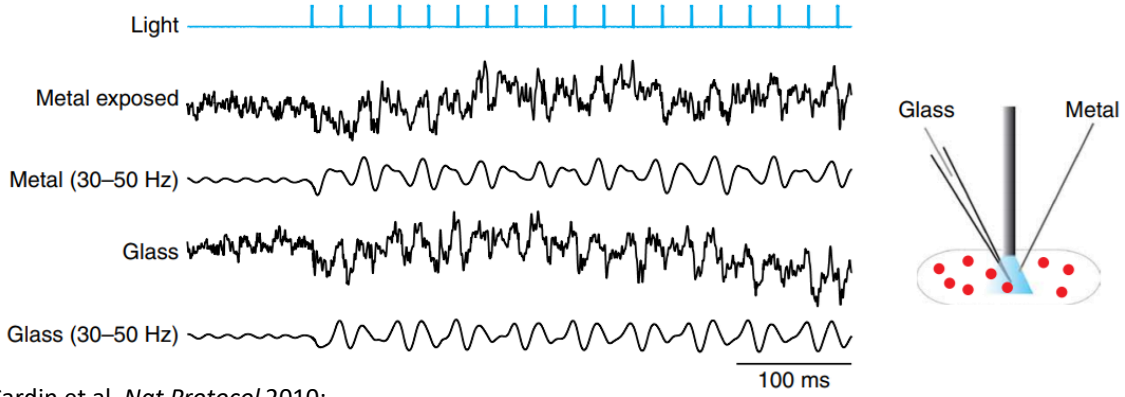
Fenno, Yizhar, and Deisseroth, *Ann Rev Neuro* 2011

Optogenetics

Powerful laser light can cause a photo-electric effect in electrodes:

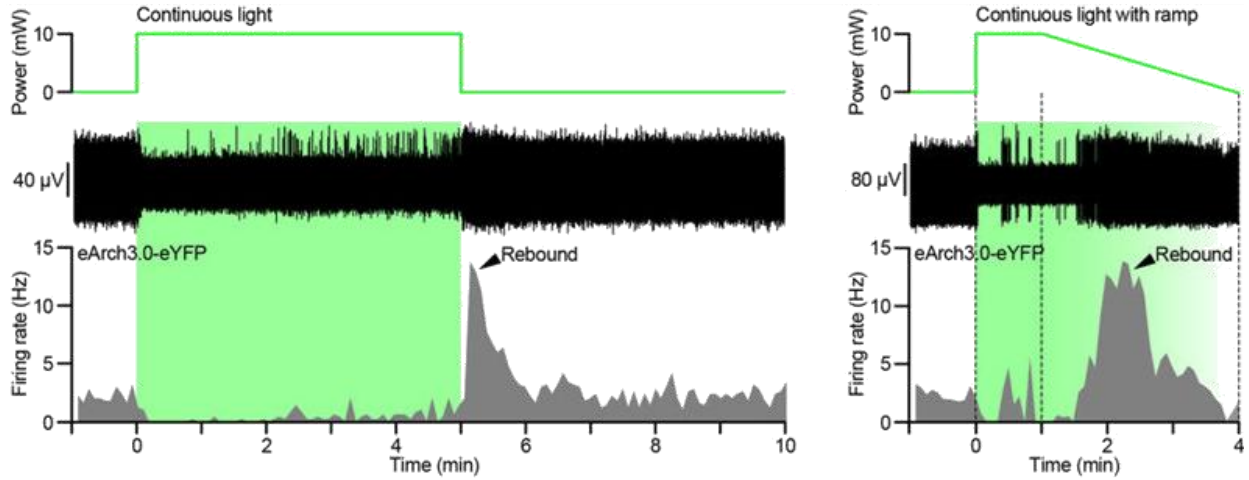


This can be mitigated by careful placement of electrode outside light path:



Cardin et al, *Nat Protocol* 2010;
Takeuchi et al, *Nature* 2016

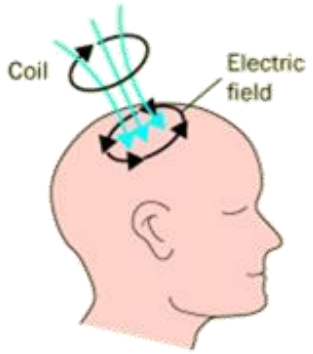
Inhibitory opsins will often cause an excitatory rebound after light is extinguished. This can cloud interpretation of effects of inhibition:



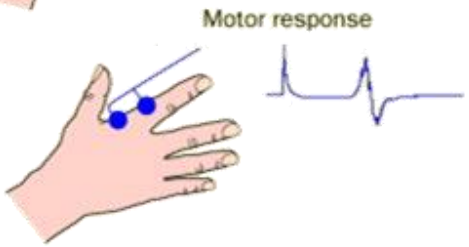
- High spatial and temporal resolution.
- Combination of genetic techniques with light control allow for highly specific control of cell-types, projections, and neural selectivity.
- Laser light can cause artifacts in certain electrodes.
- Strong inhibition can cause a rebound in activity.

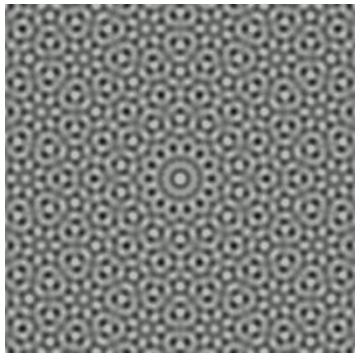
Transcranial Magnetic Stimulation (TMS)

TMS is essentially the inverse of MEG: you use a fluctuating magnetic field to induce a current in the brain:



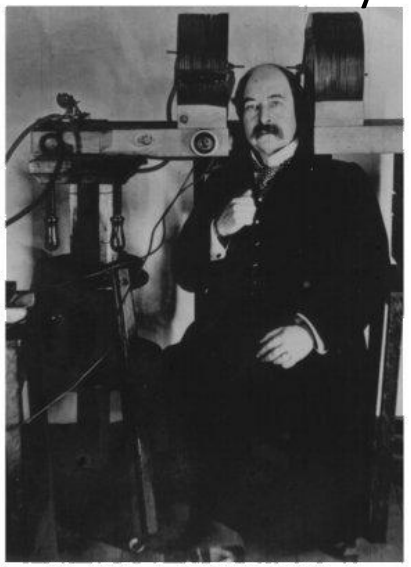
Evoked response depends on the region stimulating:





Thut and Pascual-Leone, *Brain Topography* 2010

This is a surprisingly old approach, dating back over a hundred years:

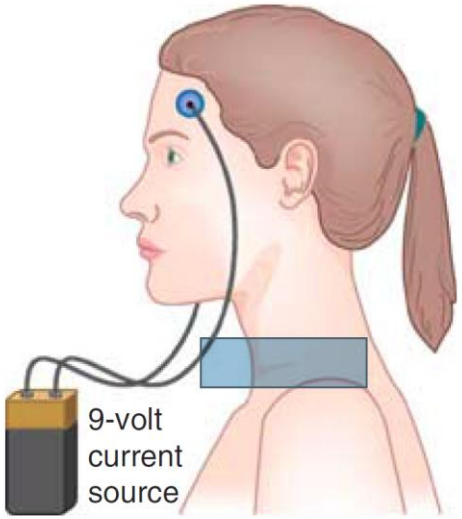


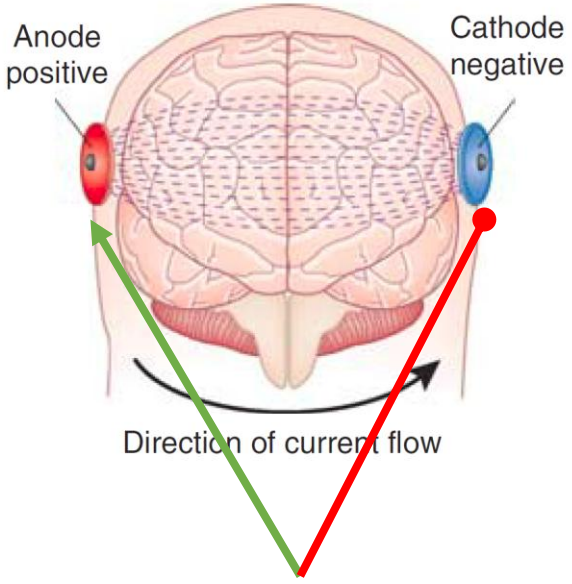
Silvanus Thomson in 1910

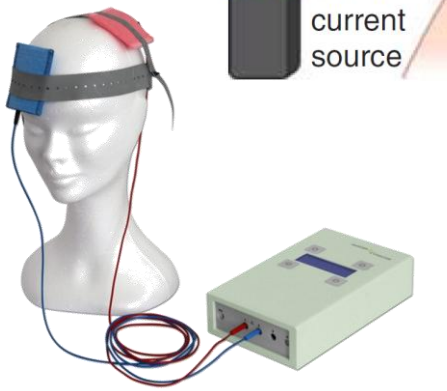
- Non-invasive, can be used experimentally and clinically.
- Poor spatial localization; single-pulse can have good temporal resolution.
- The effect of TMS is not entirely known; different pulse sequences can have different effects.
- Really hard to blind subjects to the pulse as it induces contraction of muscles in head (this can even be painful).
- Emerging clinical uses; for example for depression (but low efficacy, ~25% compared to 10% in sham, and low retention rate <10% after a few weeks)

Transcranial Electrical Stimulation (tDCS/tACS/tRNS)

Transcranial electrical stimulation passes low levels of current between two locations on the skull:







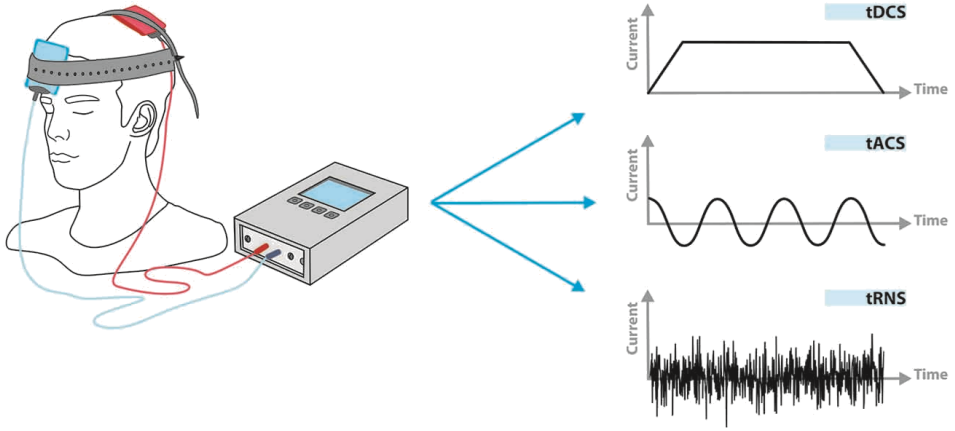
Generally, anode region is ~excited; cathode is ~inhibited. However, this appears to be dependent on region and protocol

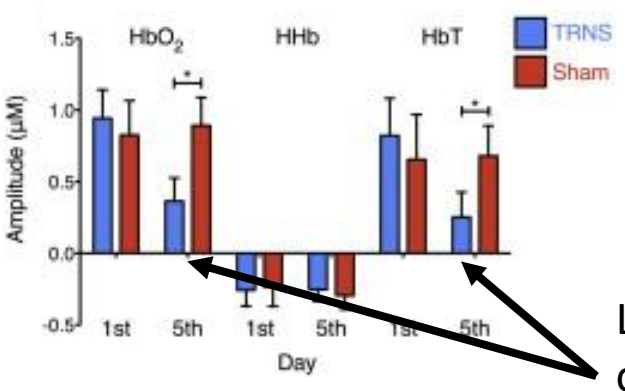
- Non-invasive, can be used experimentally and clinically.
- Very poor spatial localization; poor temporal accuracy.
- The effect is unknown and highly debated. Meta-analyses suggest a small but consistent effect (even after attempting to compensate for publication bias).

George and Aston-Jones, *Neuropsychopharmacology* 2010

Transcranial Electrical Stimulation (tDCS/tACS/tRNS)

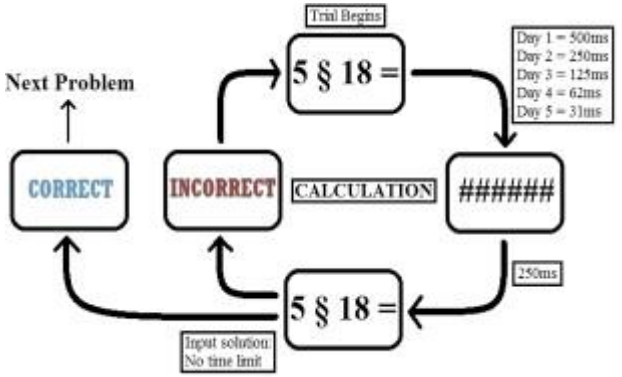
Stimulation can either be direct current (tDCS), alternating current (tACS), or random (tRNS):

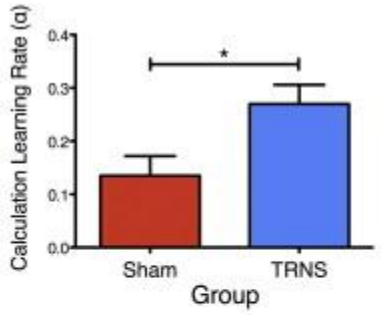


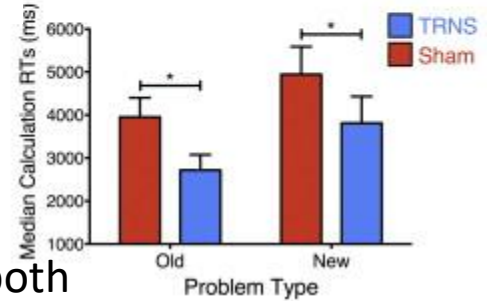


Less activity in PFC (measured with fNIRS, both oxy-Hb and total-Hb) during calculation after tRNS stimulation.

Many different types of effects have been attributed to stimulation (of many different protocols). For example, both tDCS and tRNS of parietal cortex has been found to improve learning of 'math':



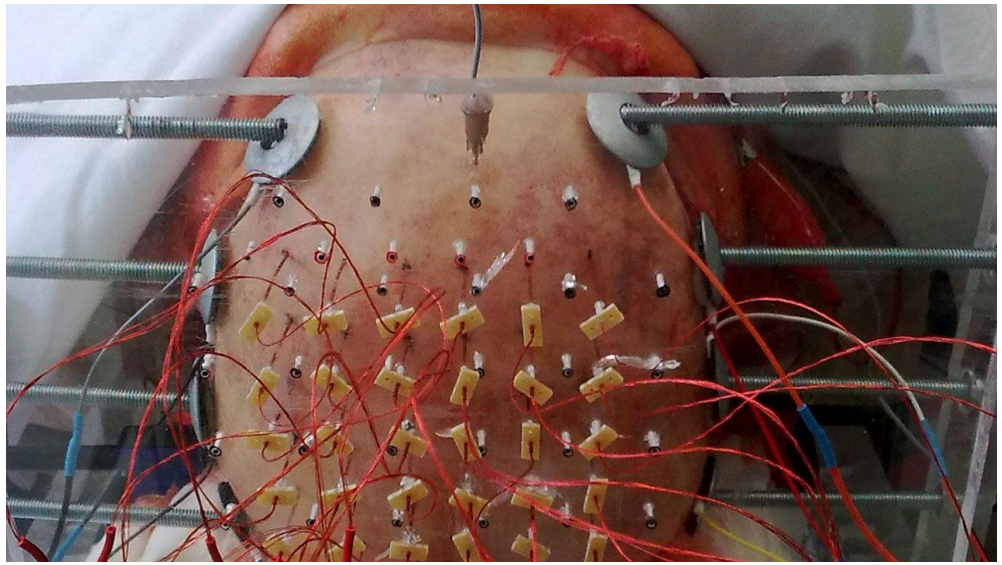




Yavari et al, *Front Hum Neuro* 2017

Transcranial Electrical Stimulation (tDCS/tACS/tRNS)

Buzsaki and Berenyi found that 90% of current just passed through the skin. The skull, having high impedance, really mitigates the effect of stimulation.



They find that to generate an action potential, you would need **5 mA** of current. FDA approves 2 mA, 4+ mA is ***painful***.

- So what might be going on?
  - Maybe just potentiating cells?
  - Maybe affecting astrocytes?
  - Vagal nerve stimulation?
- Why are there 100s of studies showing an effect?
  - Publication bias?
  - Bad science?
- Why do people want it to work so badly?
  - **$$$$**
  - Sub-culture of DIYers. Youtube, Reddit, …

There is no ideal technique; there are trade-offs between different approaches:

- Spatial accuracy
- Temporal accuracy
- Invasiveness
- Cell-type/projection specificity
- Scale of recording

Combining techniques can yield complementary insights.

Spatial and Temporal Resolution of Observation and Intervention Techniques