Behavioral Neuroscience A
6/7: Methods in Neuroscience

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https://youtu.be/OmbmdajUZsI

Lecture Video at above link.

Today’s aim: Methods

1) Introduce to methods commonly used in neuroscience

- *Measurement* methods
- *Intervention* methods

2) Understand the limitations and advantages of these methods

Measurement Methods in Neuroscience

Invasive (requires surgery):

1) Electrophysiology (“E-phys”) – *in vivo*

2) Optogenetics

3) Electrocorticogrphy (ECoG)

4) Calcium Imaging (Ca^{2+} Imaging)

5) Infrared imaging, microscopy

Non-invasive (no surgery):

1) Electroencephalography (EEG)

2) Magnetoencephalography (MEG)

3) (functional) Magnetic Resonance Imaging (fMRI)

4) Positron Emission Tomography (PET)

Measurement Methods in Neuroscience

Invasive (requires surgery):

1) Electrophysiology ("E-phys") – *in vivo*

2) Optogenetics

3) Elec

4) Calc

5) Infra

Non-in

1) Elec

2) Mag

3) (functional) Magnetic Resonance Imaging (fMRI)

4) Positron Emission Tomography (PET)

The physics behind many of these methods is *complex*.

People get Engineering Ph.D. in *only one method*.

Intervention Methods in Neuroscience

Invasive (requires surgery):

1) Lesions (micro-lesions) - non-reversible

2) Pharmacological manipulations (local)

3) Reversible cryogenic deactivation (cooling)

4) Genetic manipulation (gene knock-out etc.)

Non-invasive (no surgery):

1) Trans-cranial Magnetic Stimulation (TMS)

2) Trans-cranial Direct (Electrical) Stimulation (TCDS)

3) Pharmacological manipulation (oral etc.)

Advantages and Limitations

1) Spatial Resolution: What is the smallest "part" of the brain that you can measure/intervene?

- High Resolution: Individual neurons (E-Phys, 10 µm)
- Low Resolution: Whole brain (EEG, 100,000 µm = 10 cm)

2) Temporal Resolution: What is the shortest "event" that you can measure/intervene?

- High Resolution: Action Potentials (E-Phys, <1 msec)
- Low Resolution: Blood flow (fMRI, 1000 msec)

Other Advantages/Limitations

1) How *directly* does the method measure/affect brain activity?

fMRI (BOLD): measures blood-oxygenation, which *might* correspond to more neurons firing action potentials?

E-Phys: Directly measure action potentials

EEG: Measure signal oscillations caused by synchronized PSP changes of dendrites of a subset of pyramidal cells in cortex.

2) How much of the brain can you simultaneously measure/affect (and which parts)

EEG is limited to only parts of brain (surface)

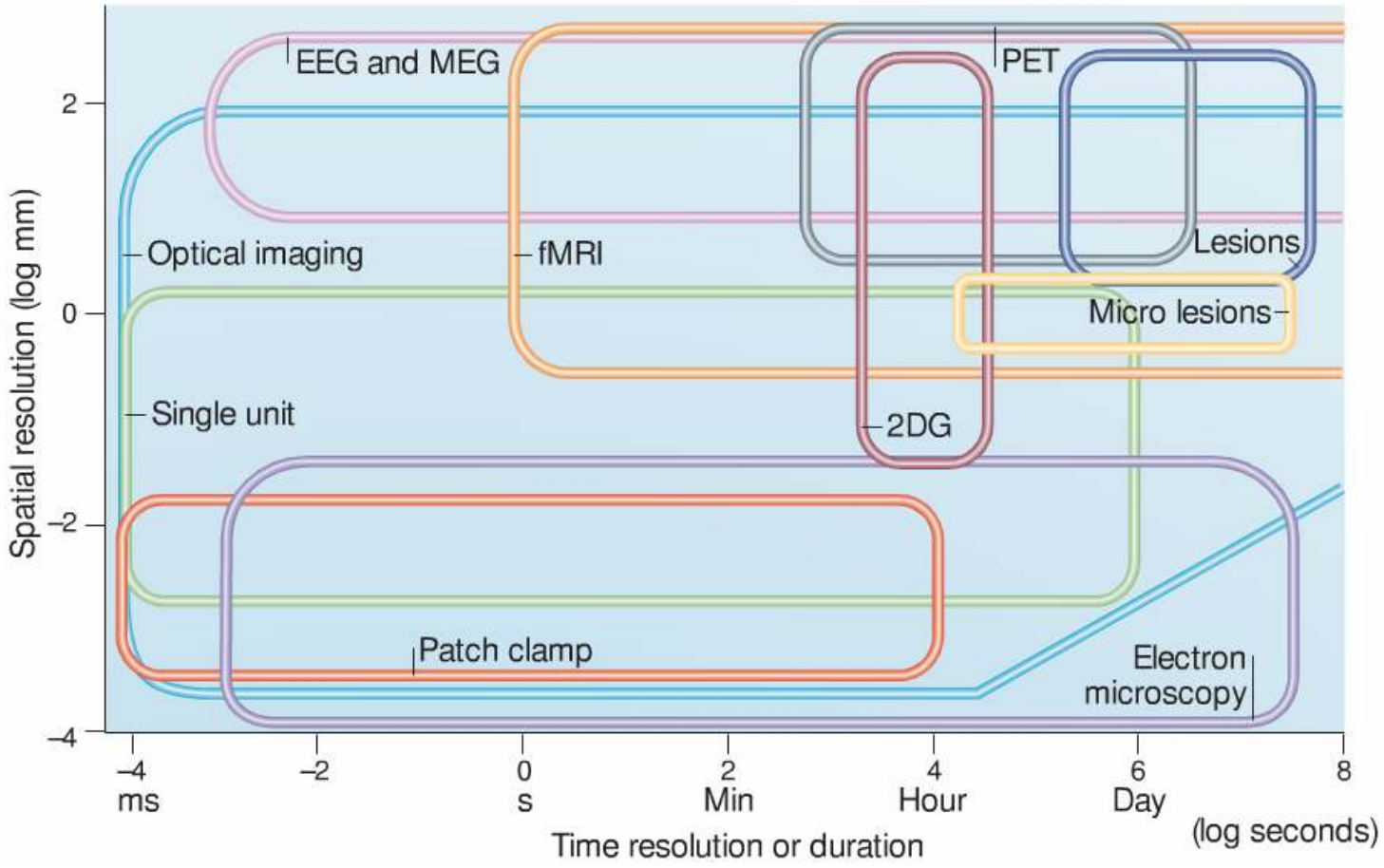
E-Phys: you can only stick so many wires in the brain so deep.

fMRI: measures all the brain at the same time!

Temporal/Spatial Resolution

In vivo – means in the alive, intact animal

In vitro – means in an experimental preparation, i.e. in a lab dish after the piece has been removed from the animal (or cultured) and kept alive artificially.

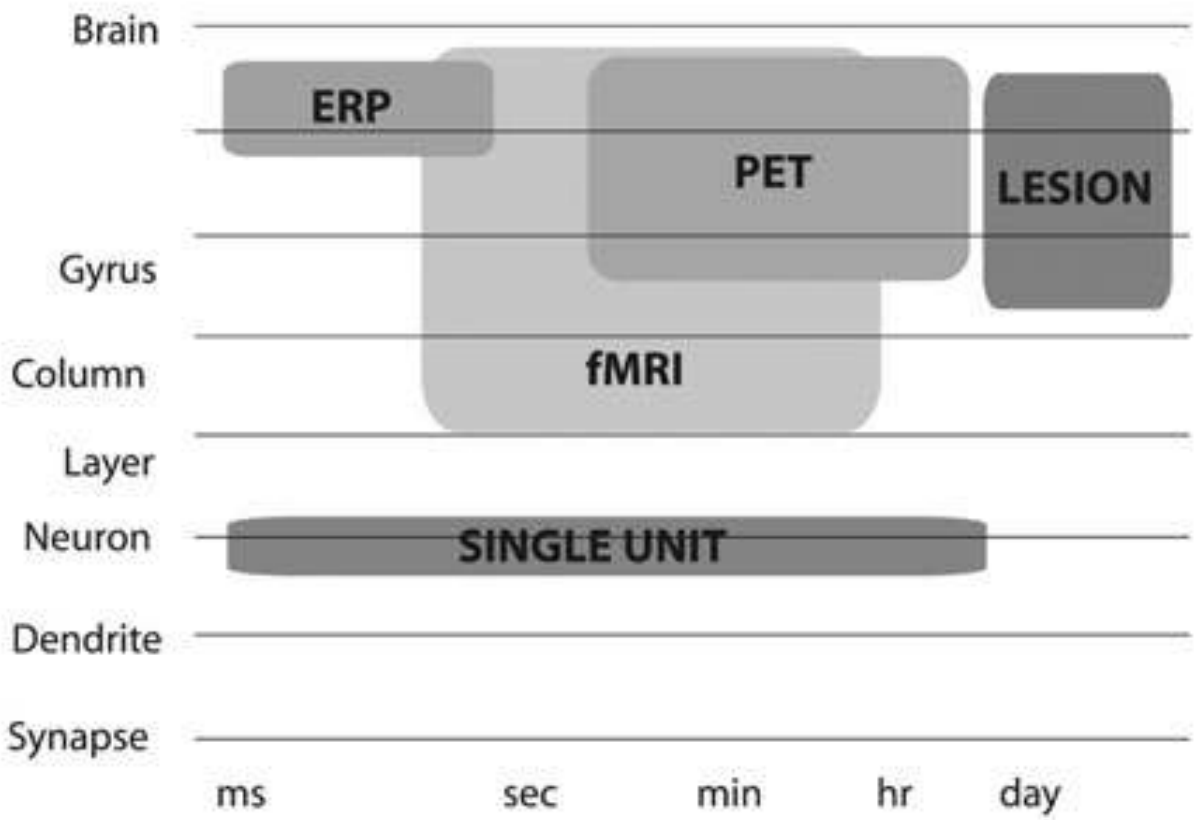


→ “Patch clamp” is recording with wire *clamped to* a single cell (very difficult *in vivo*).
→ “Single Unit” is a wire that is very close to a single cell.

https://neuroscimed.wordpress.com/2014/09/21/spatial-temporal-resolution-plots-for-neuroscience-methods/

Temporal/Spatial Resolution

A simpler representation
(fewer methods)

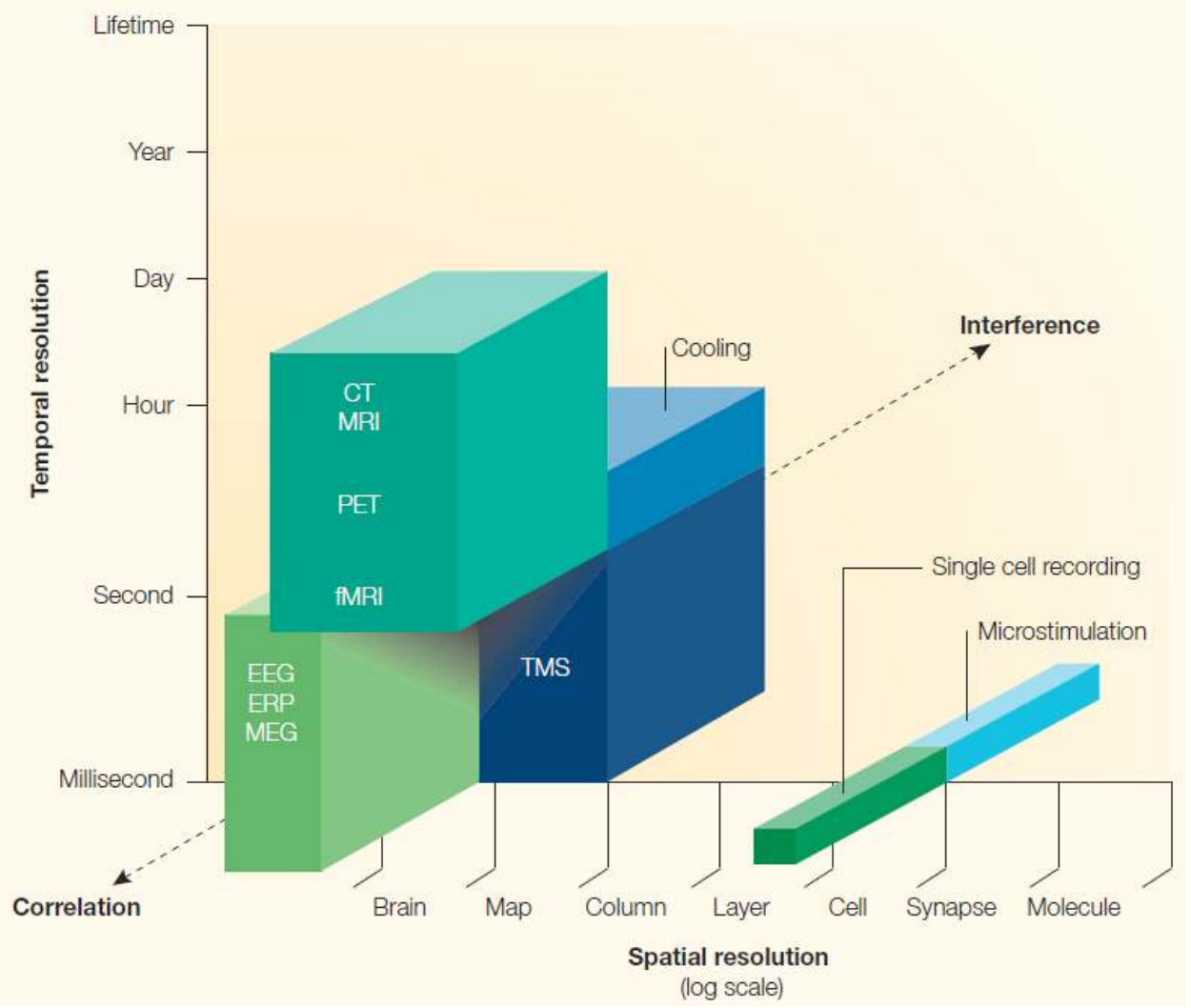


Intervention vs. Measurement

You can learn about the brain by causing lesions (damage) to a brain area and seeing how behavior changes.

You can cool the brain area (lower temperature) to reversibly deactivate it.

You can add chemicals (muscimol -- GABA agonist), that deactivate temporarily.

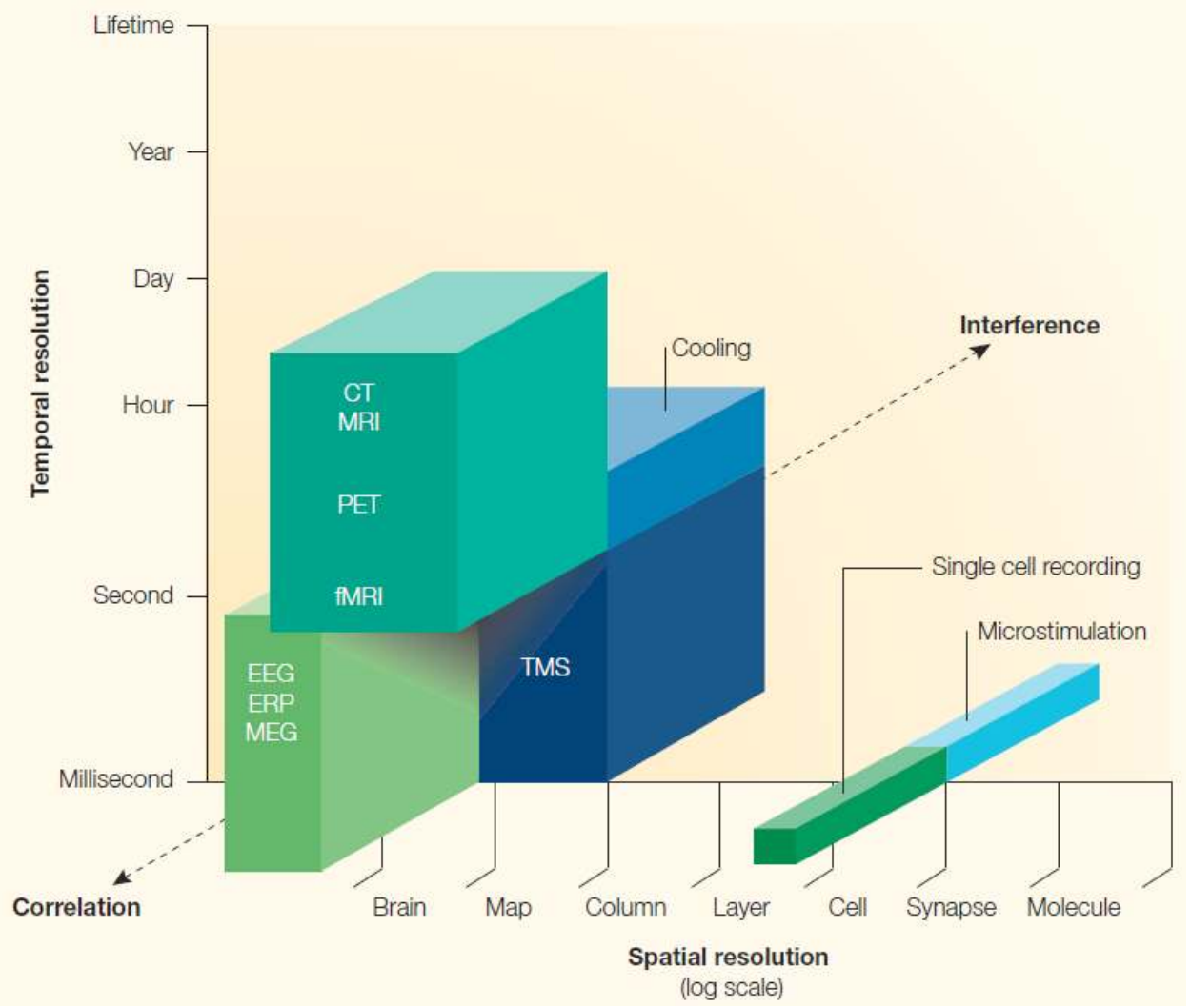


Intervention vs. Measurement

You can “stimulate” a brain area (via electrical current) and see how behavior changes.

You can “trace” specific connections between neurons using pathogens such as rabies or cholera.

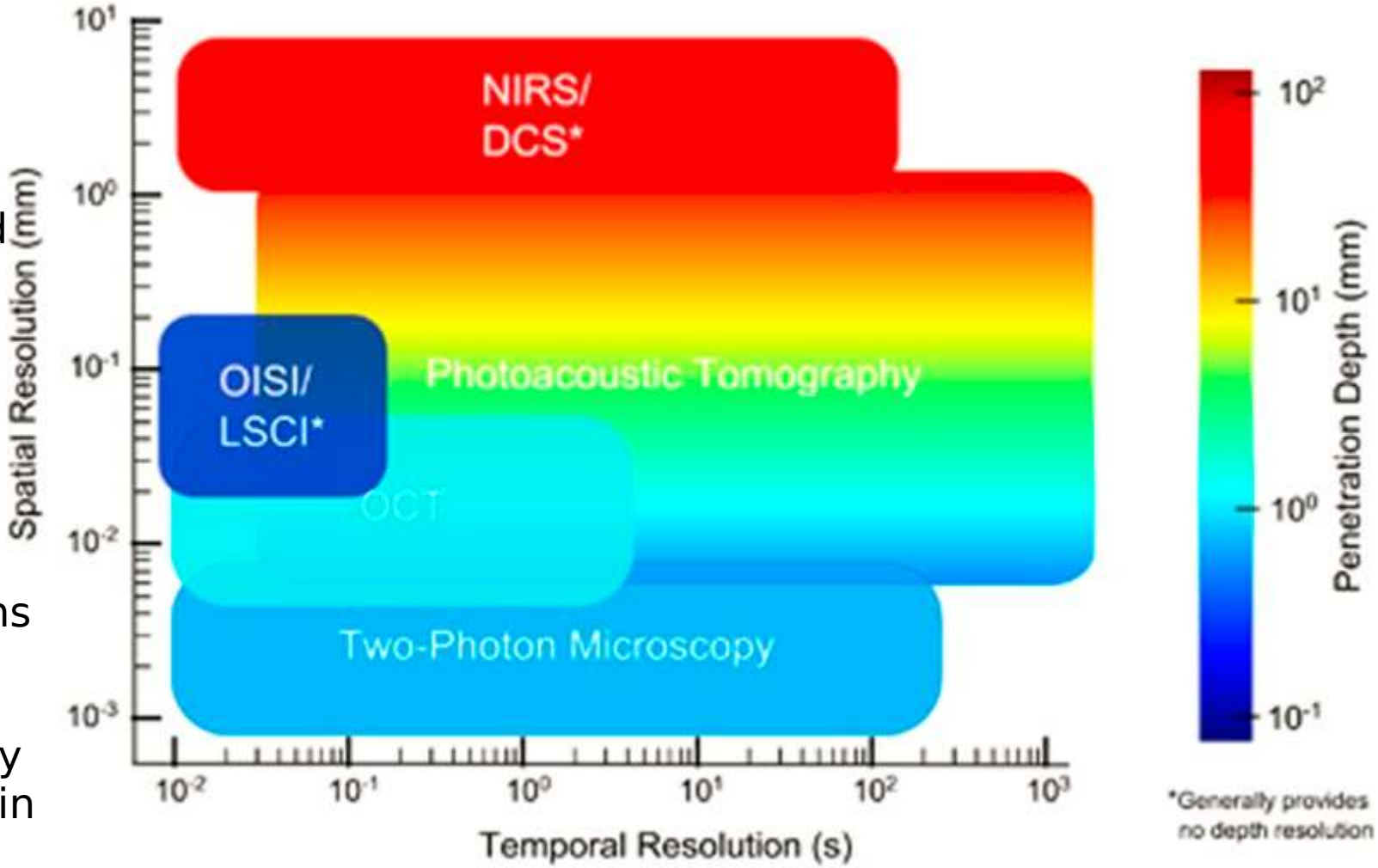
You can double infect two areas, and then reversibly inactivate just the pathway between them.



Penetration Depth

Some methods (especially light-based methods like microscopy, infrared reflection) have limitations to very superficial layer.

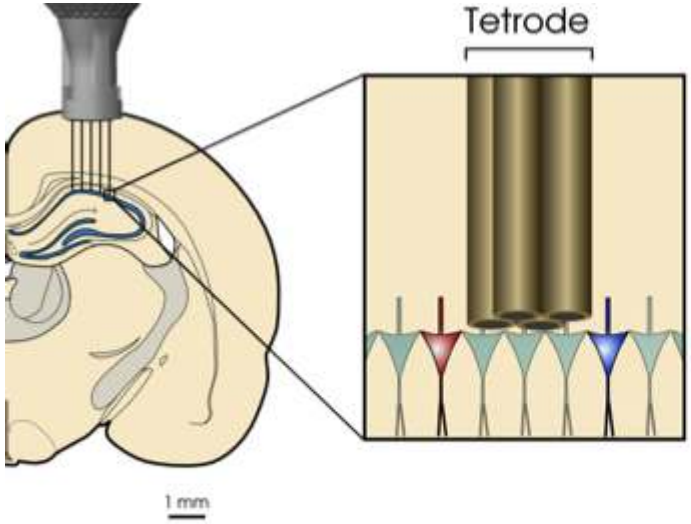
Intervention has similar limitation: Many probes/manipulations can only affect surface cortex (very difficult to accurately implant/inject deep in the brain without damage)

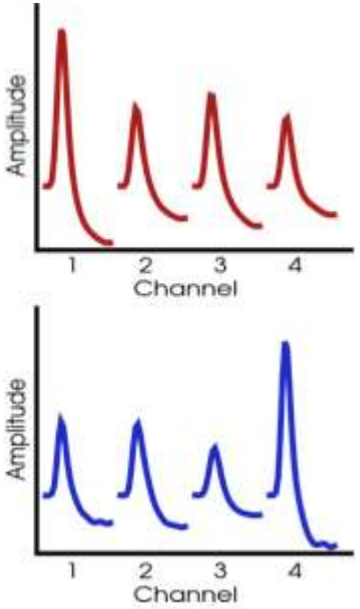


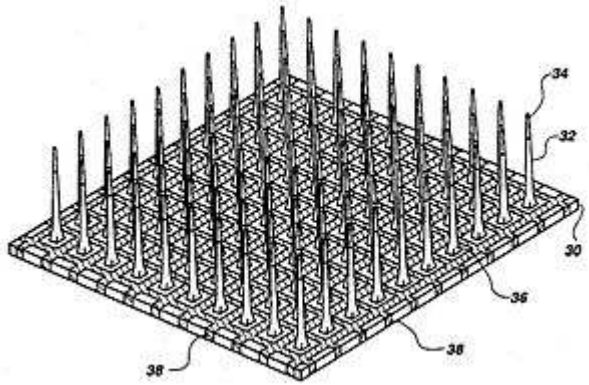
Invasive single unit/LFP recordings

Tetrode (we saw they used this to record from hippocampus to find place cells and grid cells)

Can use a guide to push deep but will damage tissue on the way in...







Microelectrode array:

These are pressed into cortex and can measure 100s of neurons at the same time, or local field potentials.

→ Can not go very deep.

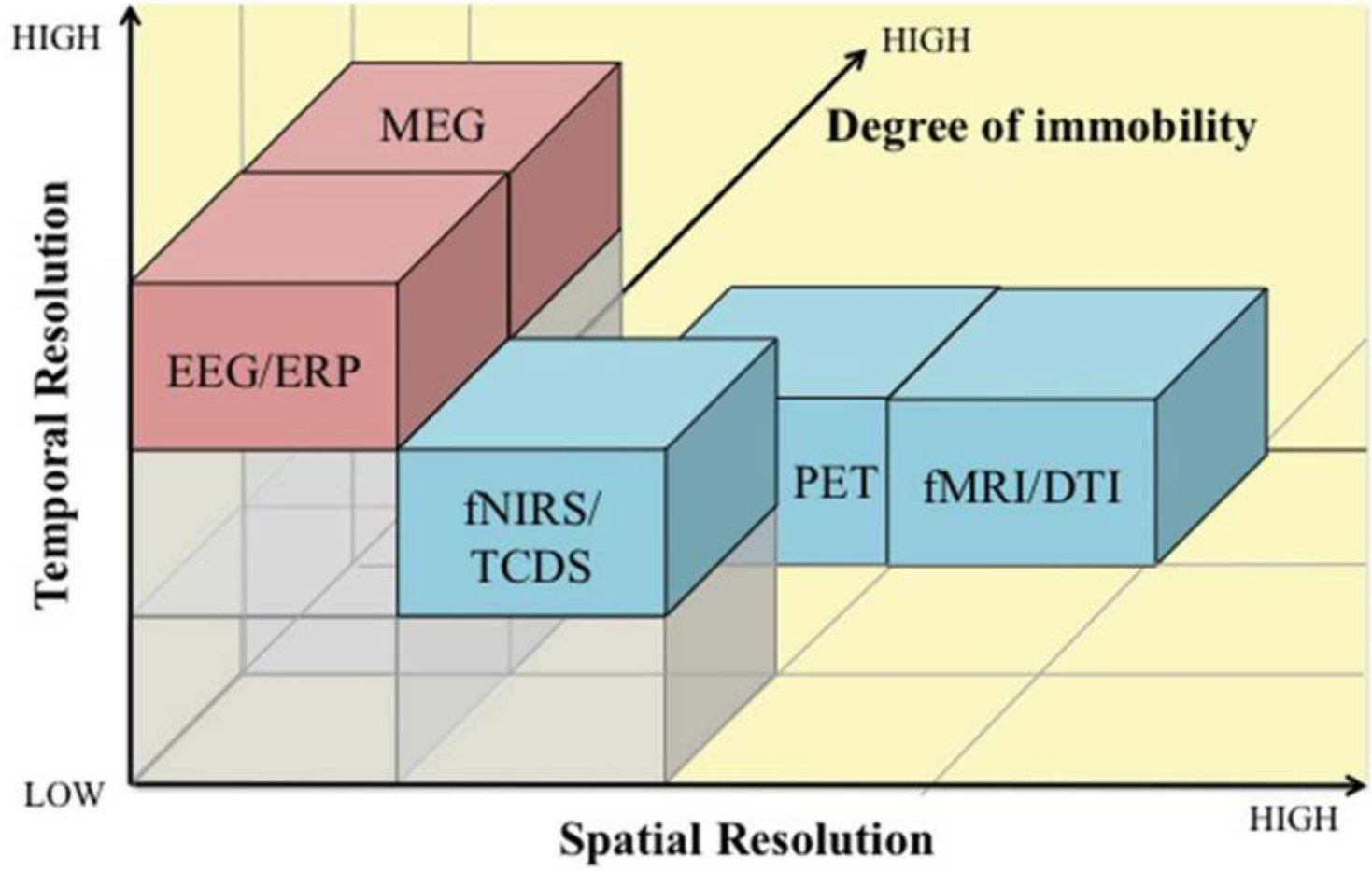
Immobility/Limitation on Behavior

Some methods limit the types of stimuli and behaviors you can measure.

For example, for FMRI, MEG, PET, the subject must stay stationary with respect to the (large, heavy) machine.

MRI Machines are loud, you can not bring metals into the room, etc.

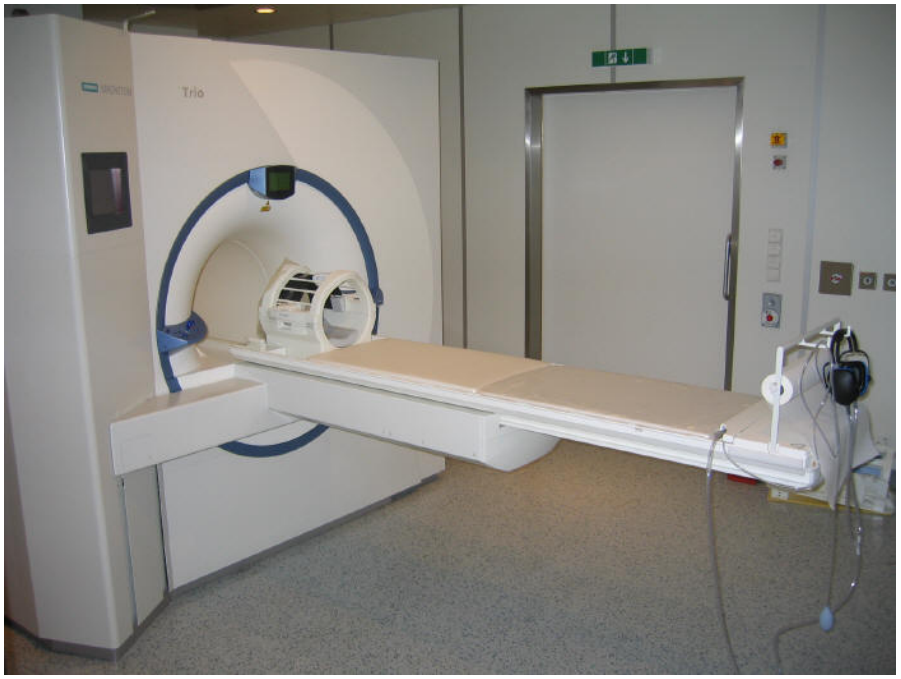
→ However, there are portable EEG machines widely available now.



https://neuroscimed.wordpress.com/2014/09/21/spatial-temporal-resolution-plots-for-neuroscience-methods/

Non-invasive methods are usually not portable

MEG – not portable!



(f)MRI – not portable!

Human brain measurement during behavior

E.g. EMOTIV machines.

For e.g. Human-Computer Interfacing

Or research…

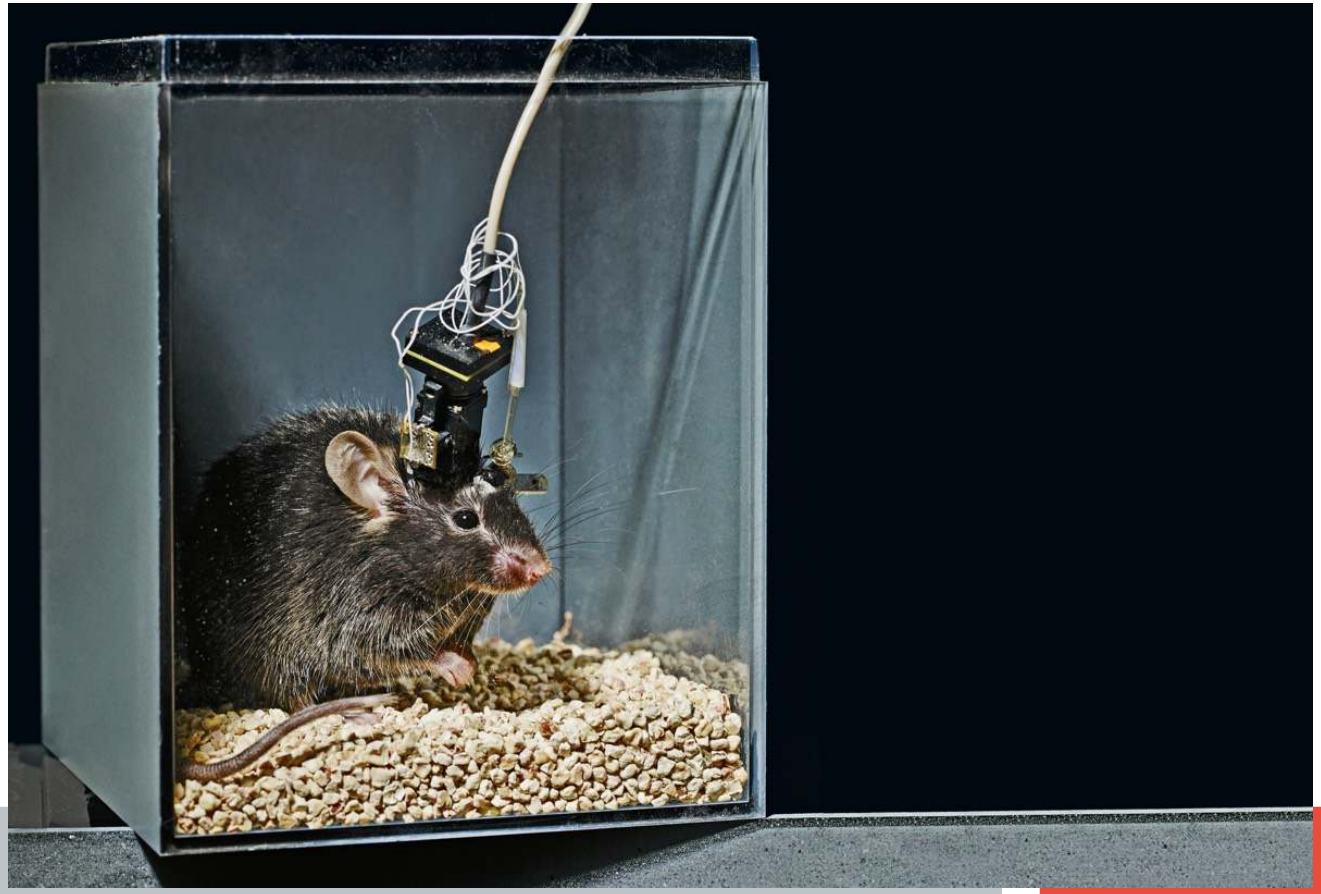
Of course, will be more noisy (in an already very noisy measurement method). So, not much accuracy.

But can measure during “natural” behavior.

E-phys during behavior

You can do optogenetics/calcium imaging/e-phys on mice/rats in limited area. Mosers and O'Keefe did this to find place/grid cells (lecture 1)!

Monkeys will use their hands to fiddle with the instrument and injure themselves. Also, much more violent and mobile (3 dimensions).



4 methods in detail

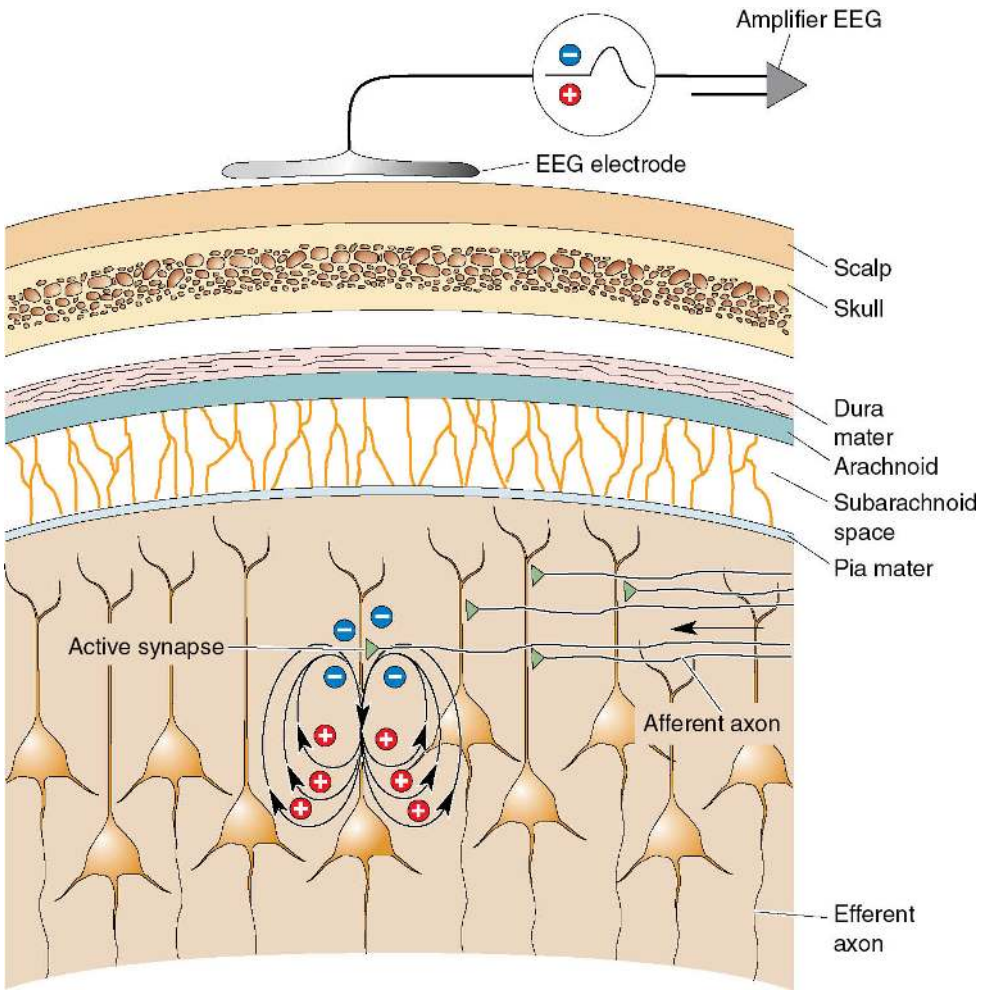
→ EEG

→ (f)MRI

→ Calcium Imaging

→ TMS

Electro-encephalography (EEG)



Excitatory Postsynaptic potentials lead to an inflow of positive (Na^+) ions into the cell. Negatively charged ions stay behind, outside the cell (-).

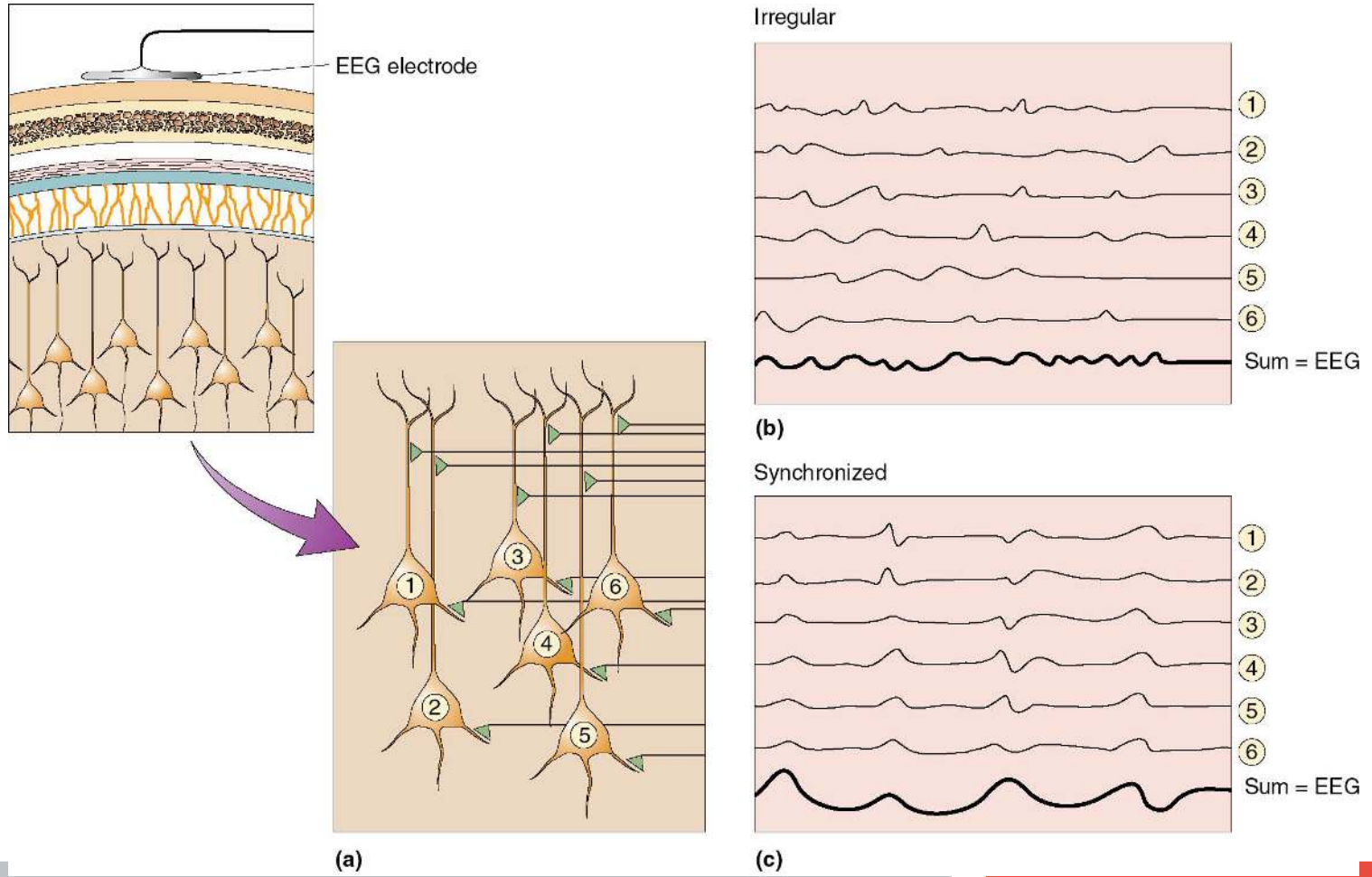
The positive current inside the cell flows down toward the soma and leaks, leading to positive charge closer to the soma.

EEG mainly measures the voltage fluctuations due to the effect of EPSPs and IPSPs (excitatory and inhibitory postsynaptic potentials) on the surrounding extra-cellular space.

Bear, Neuroscience

EEG

EEG requires synchronized activity of (tens of) thousands of neurons. The signal can be picked up by EEG when neurons are well aligned to each other (pyramidal cells) and are excited in synchrony (at the same time).



EEG/MEG

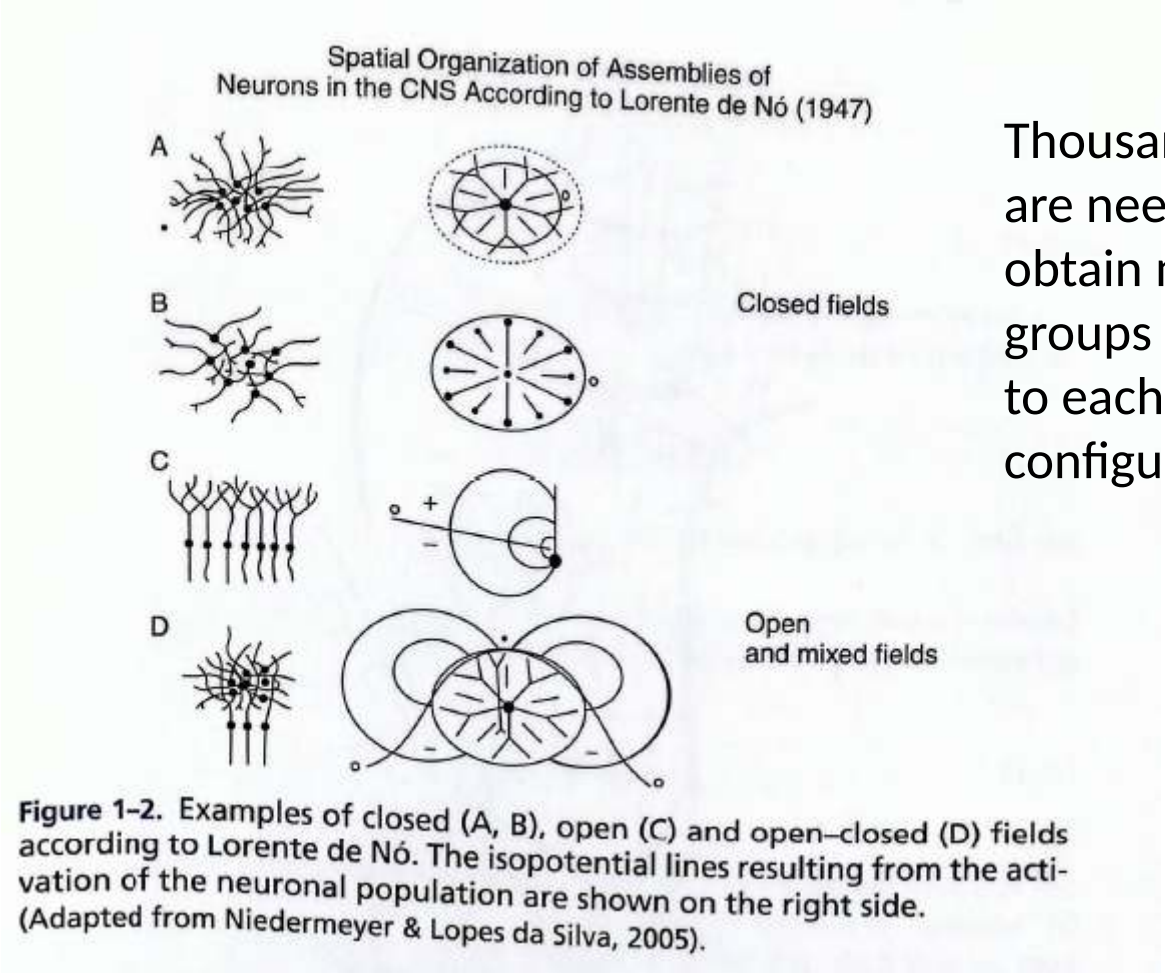
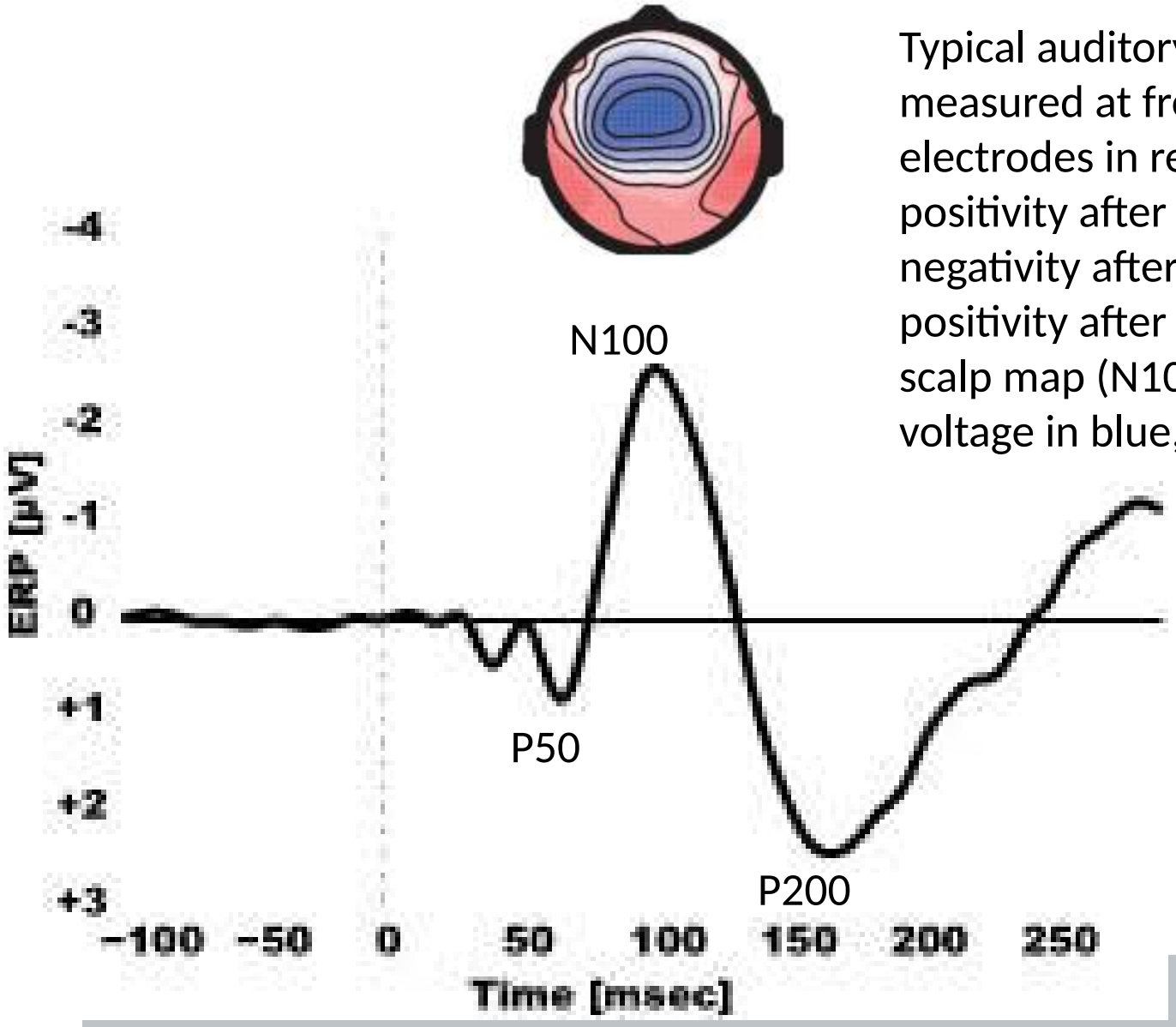


Figure 1–2. Examples of closed (A, B), open (C) and open–closed (D) fields according to Lorente de Nó. The isopotential lines resulting from the activation of the neuronal population are shown on the right side. (Adapted from Niedermeyer & Lopes da Silva, 2005).

Thousands of synchronized neurons are needed for an EEG-signal and to obtain measurable signal these groups of neurons should be aligned to each other, i.e., have an open-field configurations (figure: C).

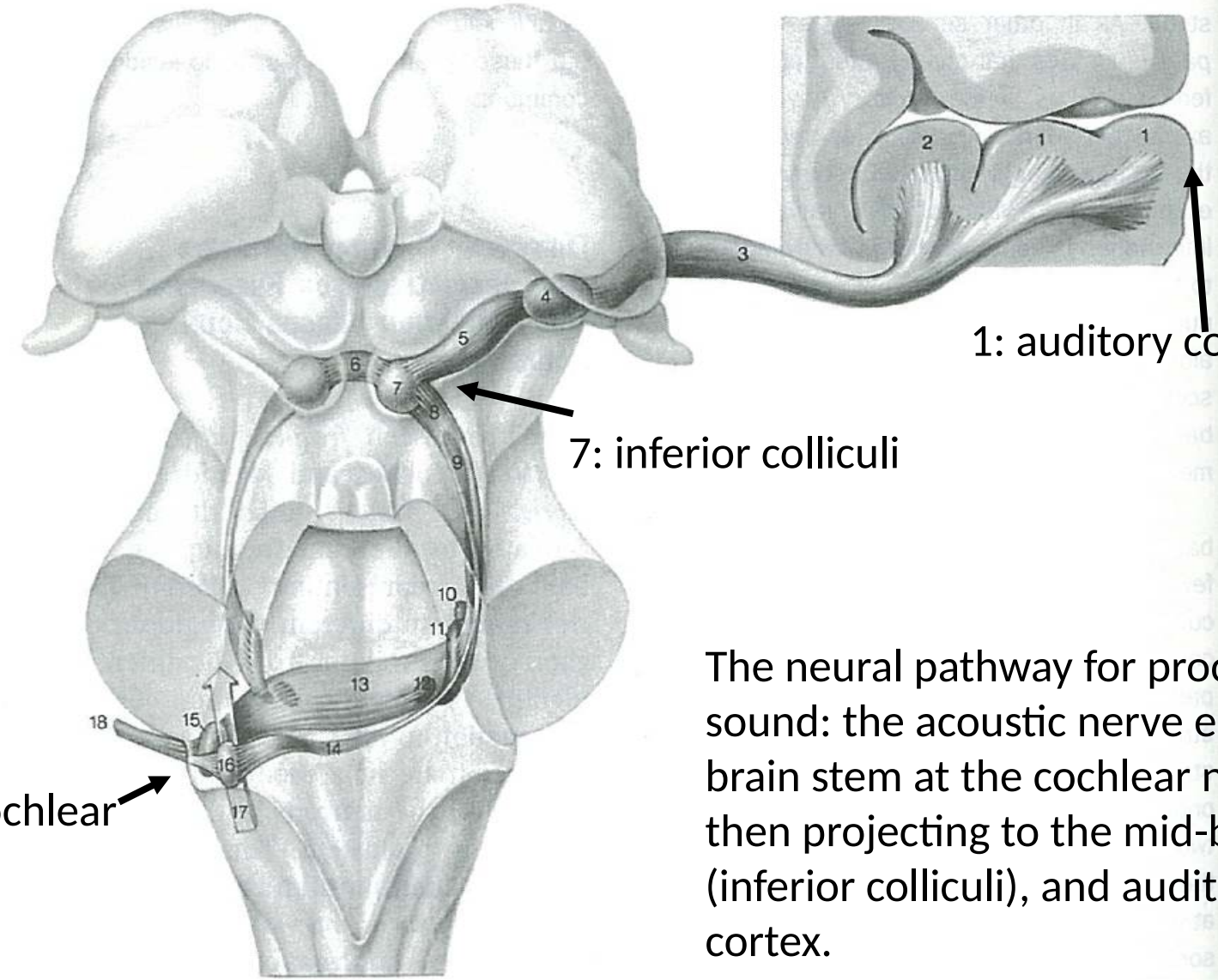
Hansen, Kringelbach, Salmelin, MEG – An introduction to methods

EEG – Evoked Potential



Typical auditory evoked potential measured at fronto-central electrodes in response to a sound: positivity after about 50 ms (P50), negativity after 100 ms (N100), and positivity after 200 ms (P200). The scalp map (N100) shows negative voltage in blue, positive in red.

EEG – Auditory Evoked Potential

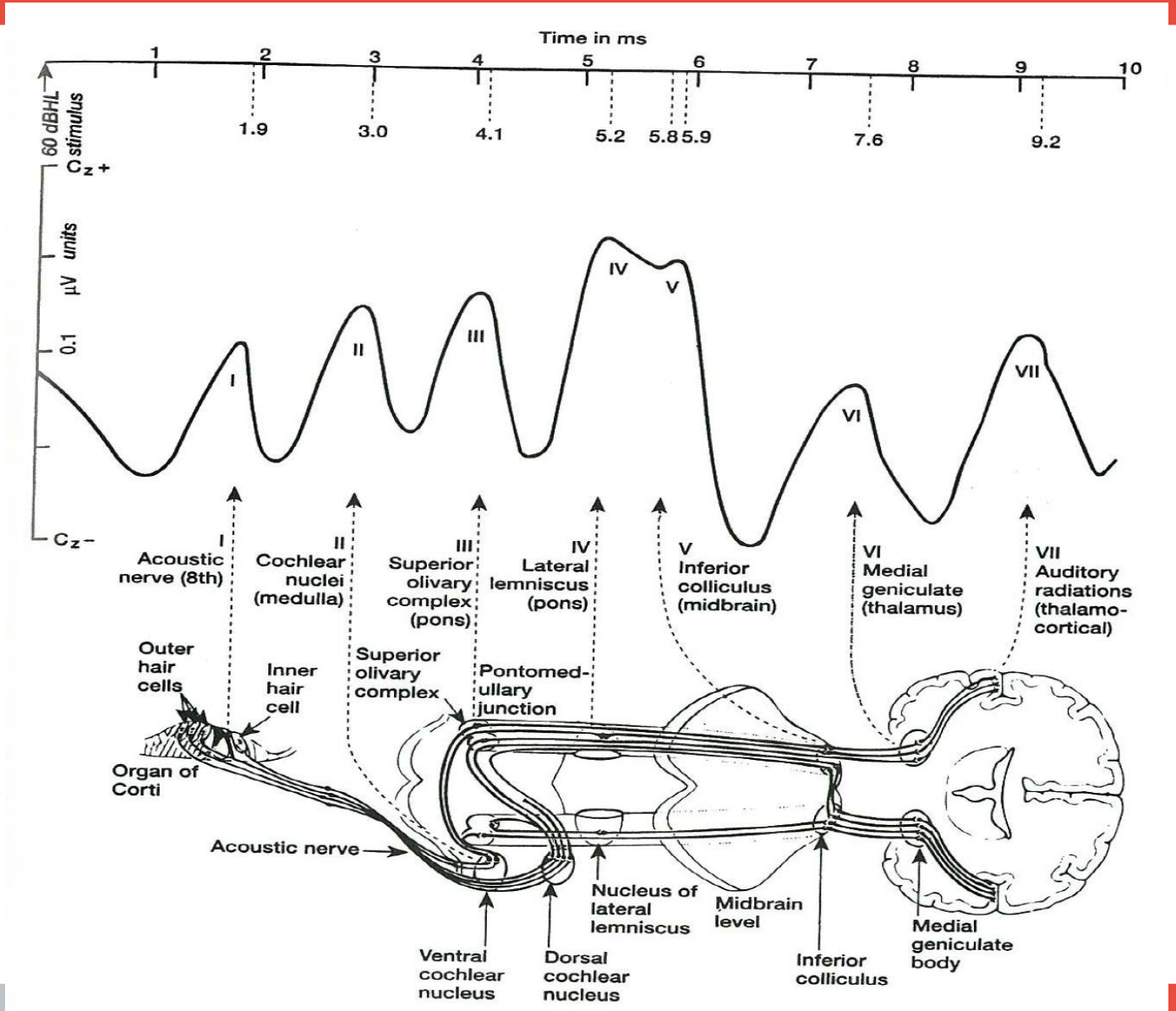


The neural pathway for processing sound: the acoustic nerve enters the brain stem at the cochlear nucleus, then projecting to the mid-brain (inferior colliculi), and auditory cortex.

EEG – Auditory Evoked Potential

Auditory brain stem responses can be measured with a large amount of stimulus (usually clicks) repetitions (1000-5000).

They are helpful to obtain an objective measure of hearing and signal processing in the brain stem.



Steward, Functional Neuroscience

MEG

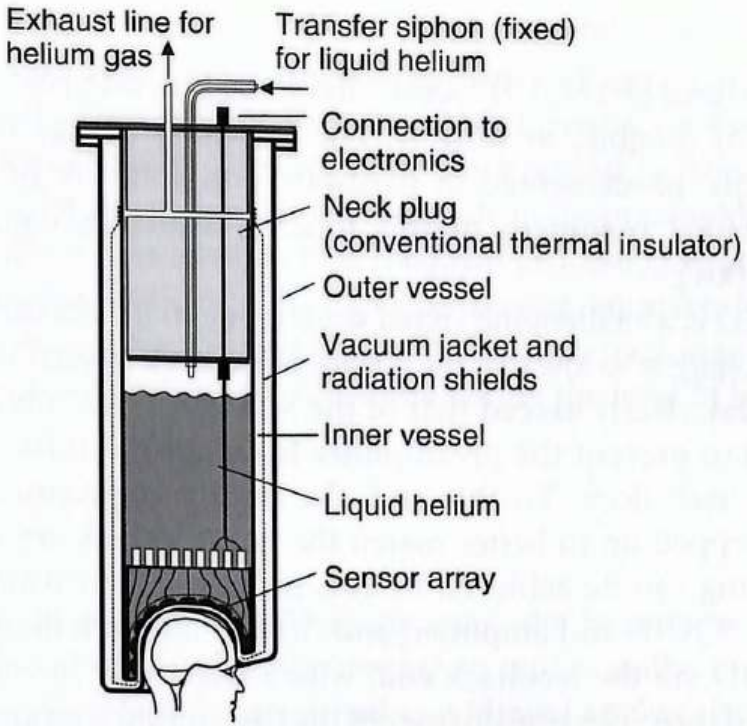


Figure 2–9. Schematic cross section of a Dewar employed in an MEG system.

MEG measures the magnetic fields surrounding the head generated by electric current flow. Since the magnetic fields are less distorted than the electrical scalp potentials (EEG), spatial resolution is better. To measure these tiny magnetic fields, super-conducting quantum interference devices (SQUID) need to be cooled down to -269˚C with liquid helium.

Hansen, Kringelbach, Salmelin, MEG – An introduction to methods

EEG/MEG Problem: Localization

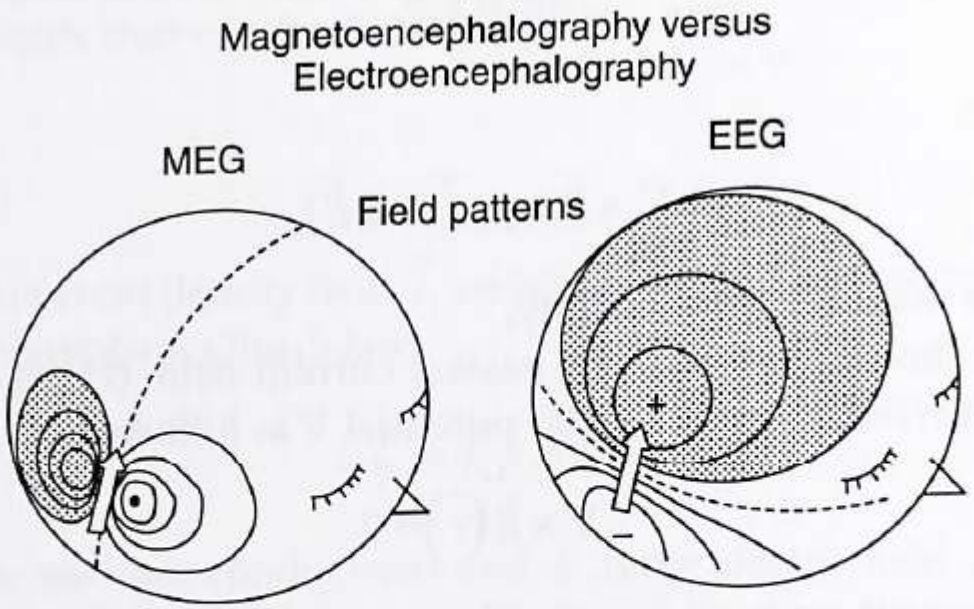


Figure 1–4. Field pattern of MEG, on the left, and EEG, on the right, caused by a current dipole model source in a concentric 4-layer spherical model of a head. The shaded areas indicate the magnetic flux out of the head (MEG) and the positive potential (EEG).
(Adapted from Hari, in Niedermeyer & Lopes da Silva, 2005).

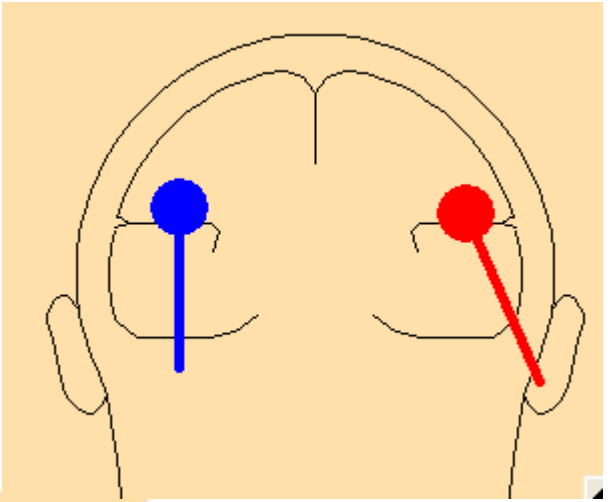
We can calculate what kind of electric potential or magnetic field topography a specific source generates (so-called forward model).

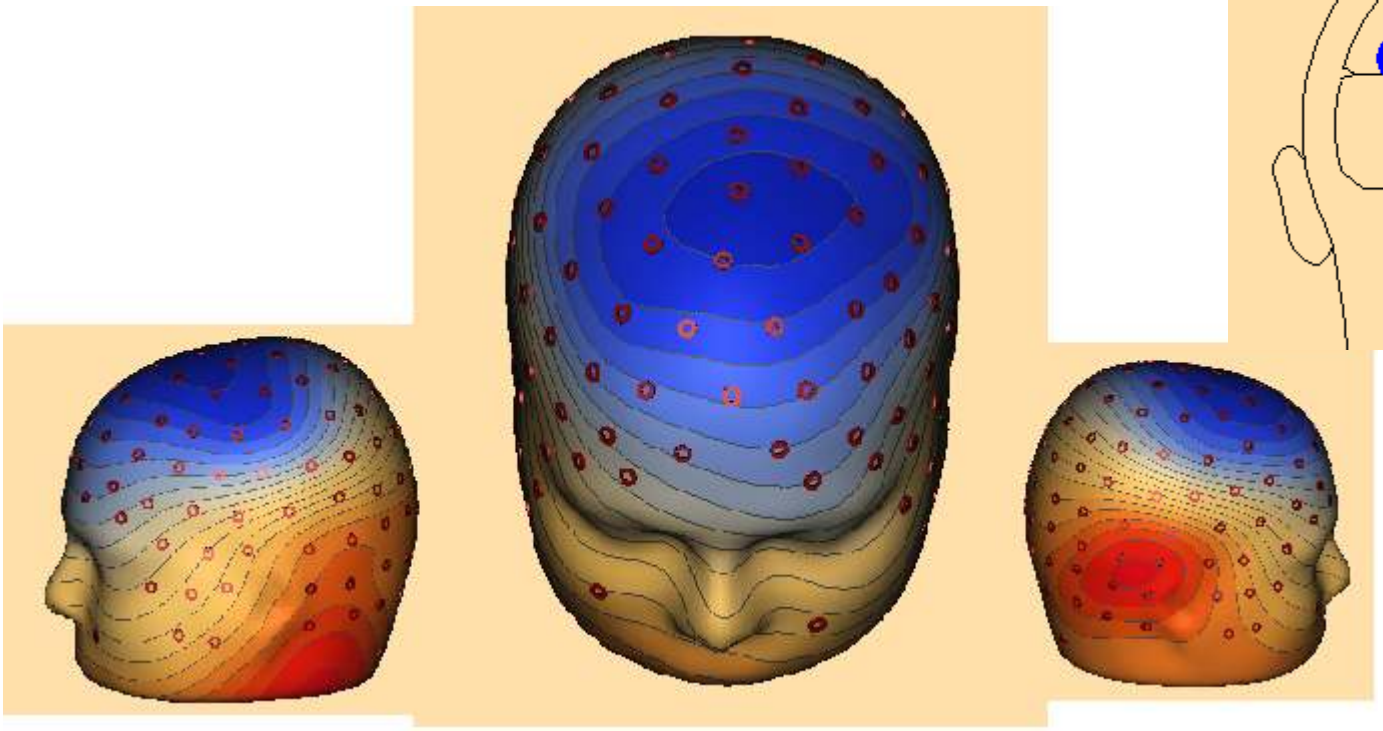
Hansen, Kringelbach, Salmelin, MEG – An introduction to methods

EEG/MEG Localization: Inverse Problem

The inverse problem, that is inferring the source location and activity, is ill-posed (has potentially an infinite number of solutions), but solvable with additional constraints/assumptions:

Here we assume two point-like symmetrical generators.

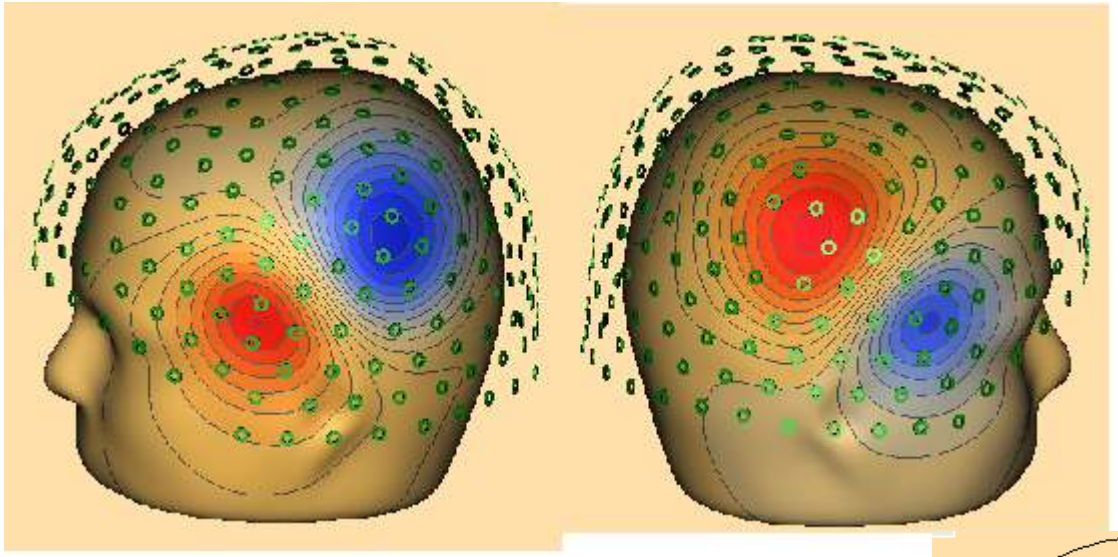


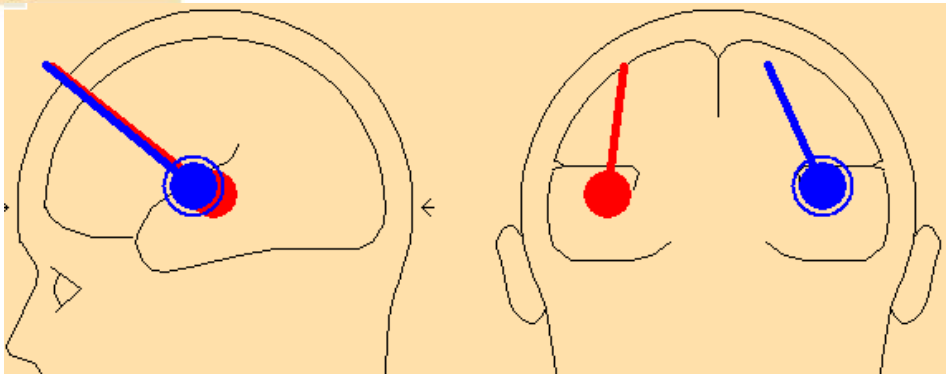


For example, two bilateral sources (Equivalent current dipoles).

Auditory evoked potential (EEG), N100

EEG/MEG Localization: Inverse Problem



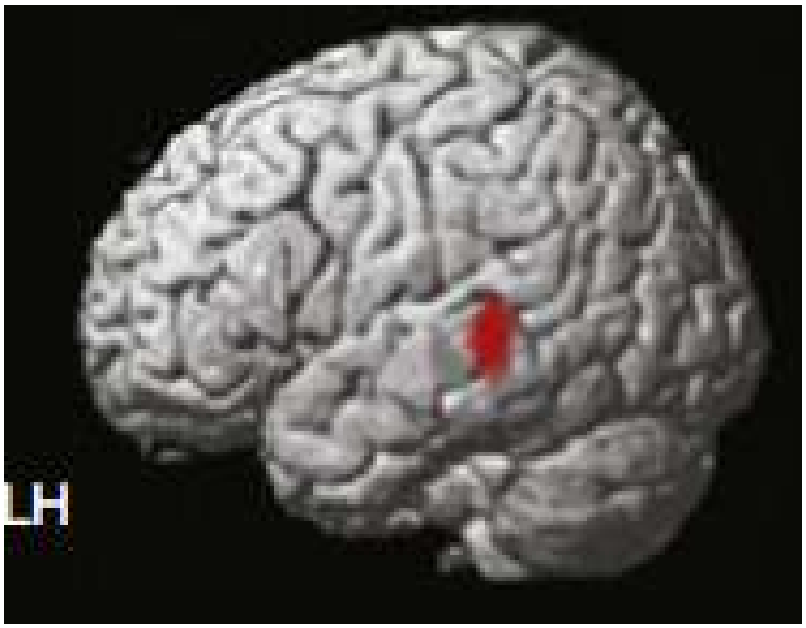


For example, two bilateral sources (Equivalent current dipoles).

Auditory evoked field (MEG), N100m

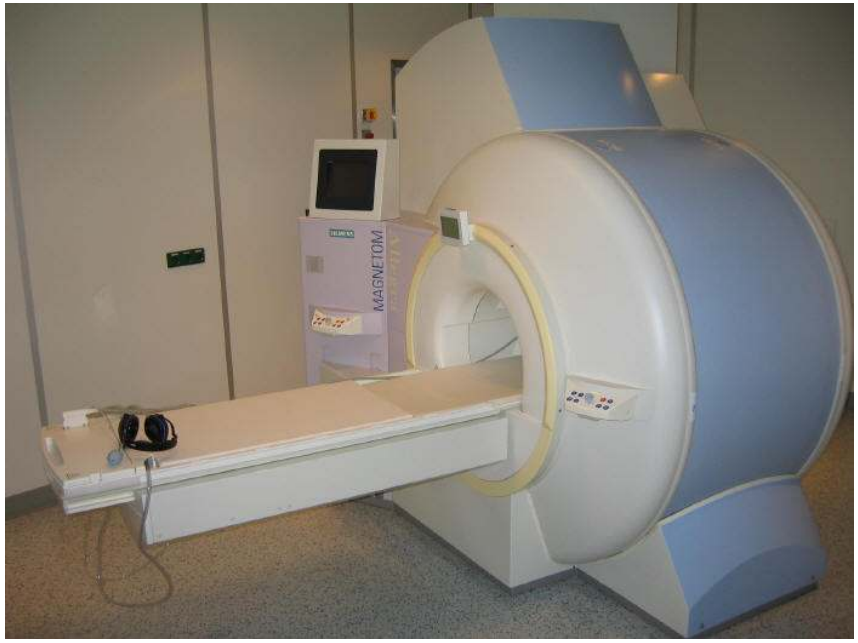
EEG/MEG Localization: Inverse Problem

Here we made different assumption: sources are distributed throughout the brain (distributed source model) and we assume maximal spatial smoothness.
Speech evoked fields (MEG), about 350 ms after speech onset.

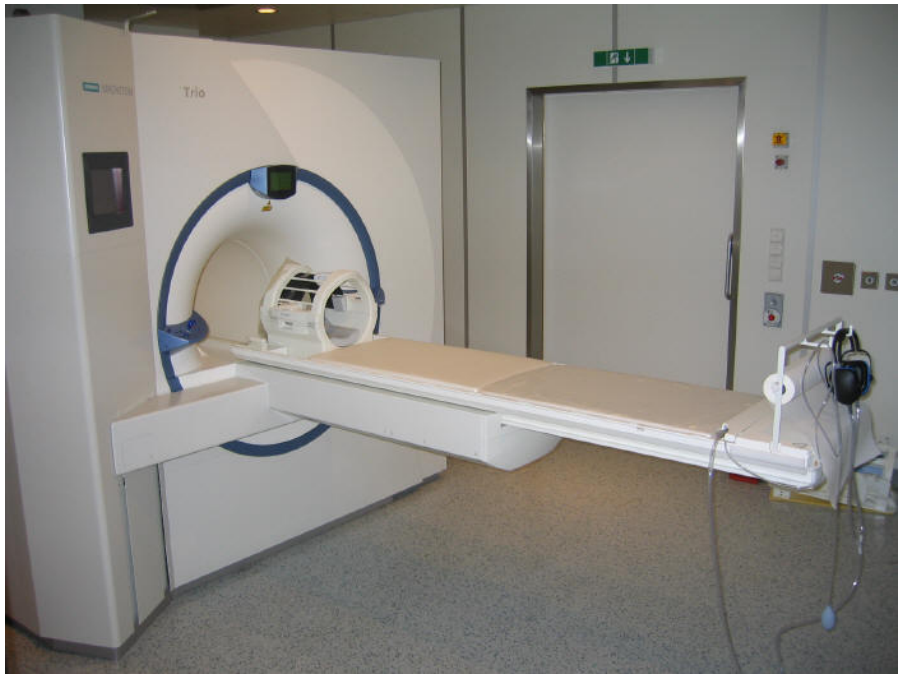


Altmann et al., Neuropsychologia, 2014

Functional Magnetic Resonance Imaging (fMRI)



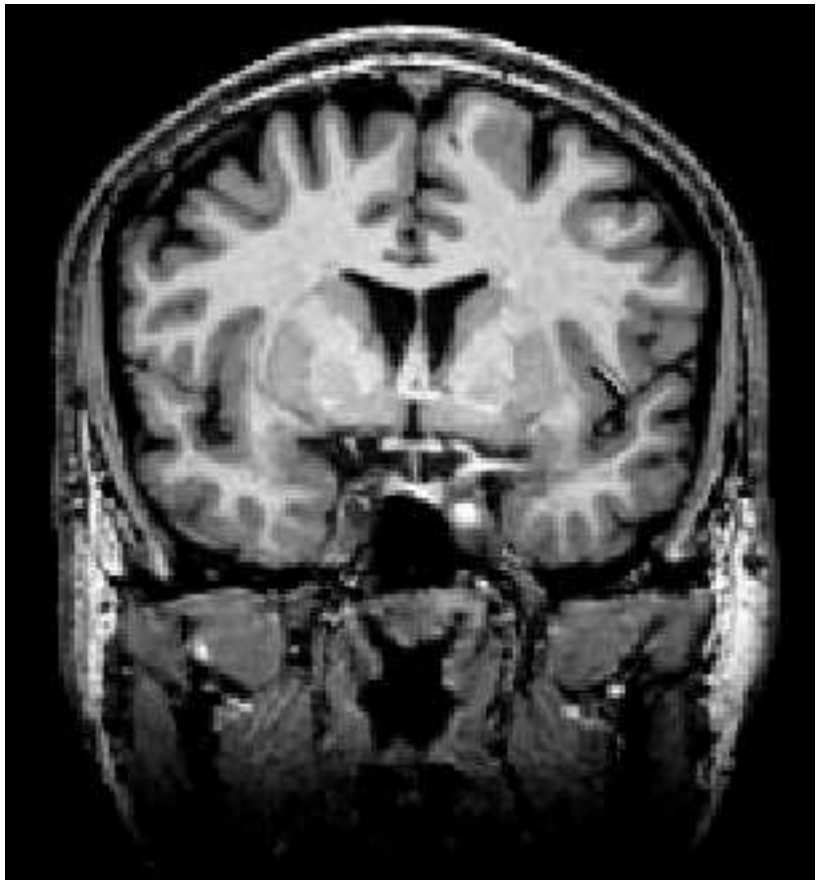
Head-Scanner (3T)



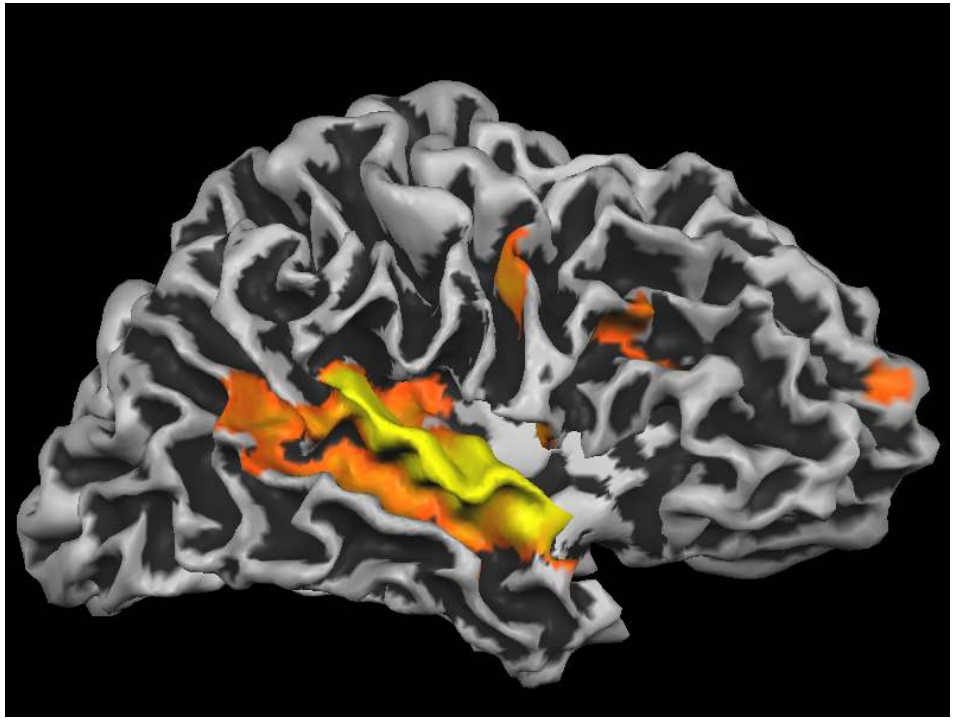
Full-Body-Scanner (3T)

fMRI has a “good” spatial resolution (mm) without the problem of ill-posed localization. However, the temporal resolution is low (100ms to seconds).

Structural imaging vs Functional Imaging

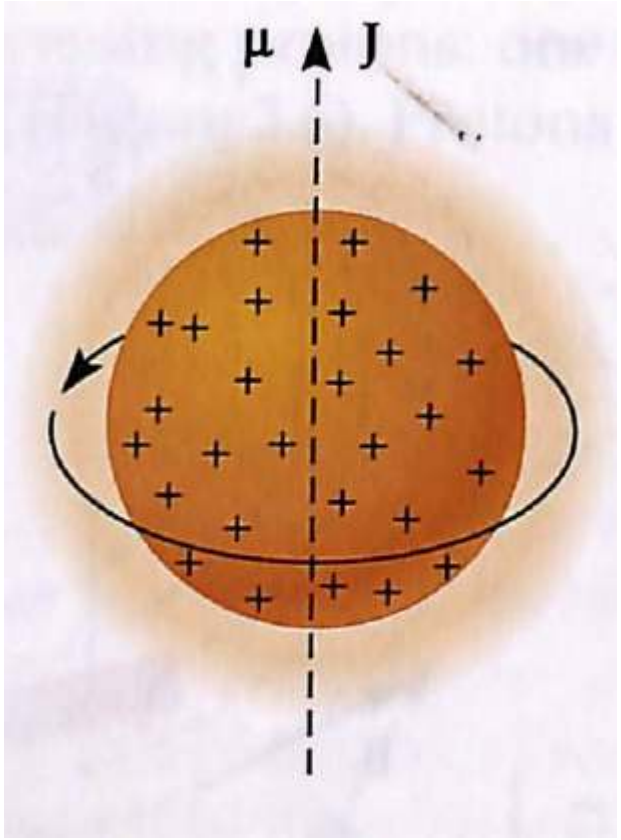


Structural Image
(T1-weighted)



Functional statistical map
overlaid onto 3D-reconstruction of the cerebral cortex

Principle of MRI



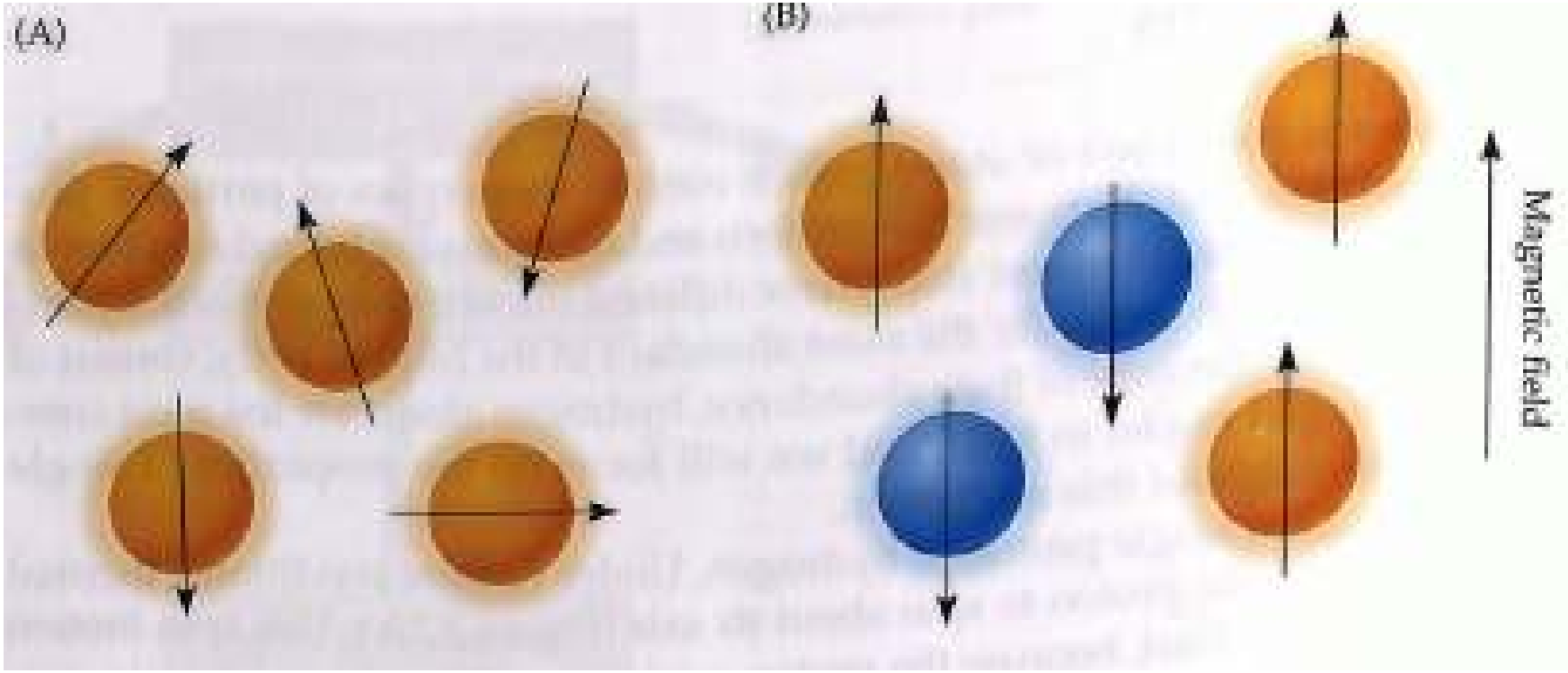
Consider a hydrogen proton:
(hydrogen is abundant in the human body and so the most important proton when it comes to MRI)

Protons have “spin”:
They rotate (well, don’t actually rotate, but they have angular momentum) around a spin axis.

Since protons are positively charged, their motion induces an electrical current.
→ This in turn leads to a magnetic moment (μ).

Huettel, Functional Magnetic resonance imaging

Principle of MRI

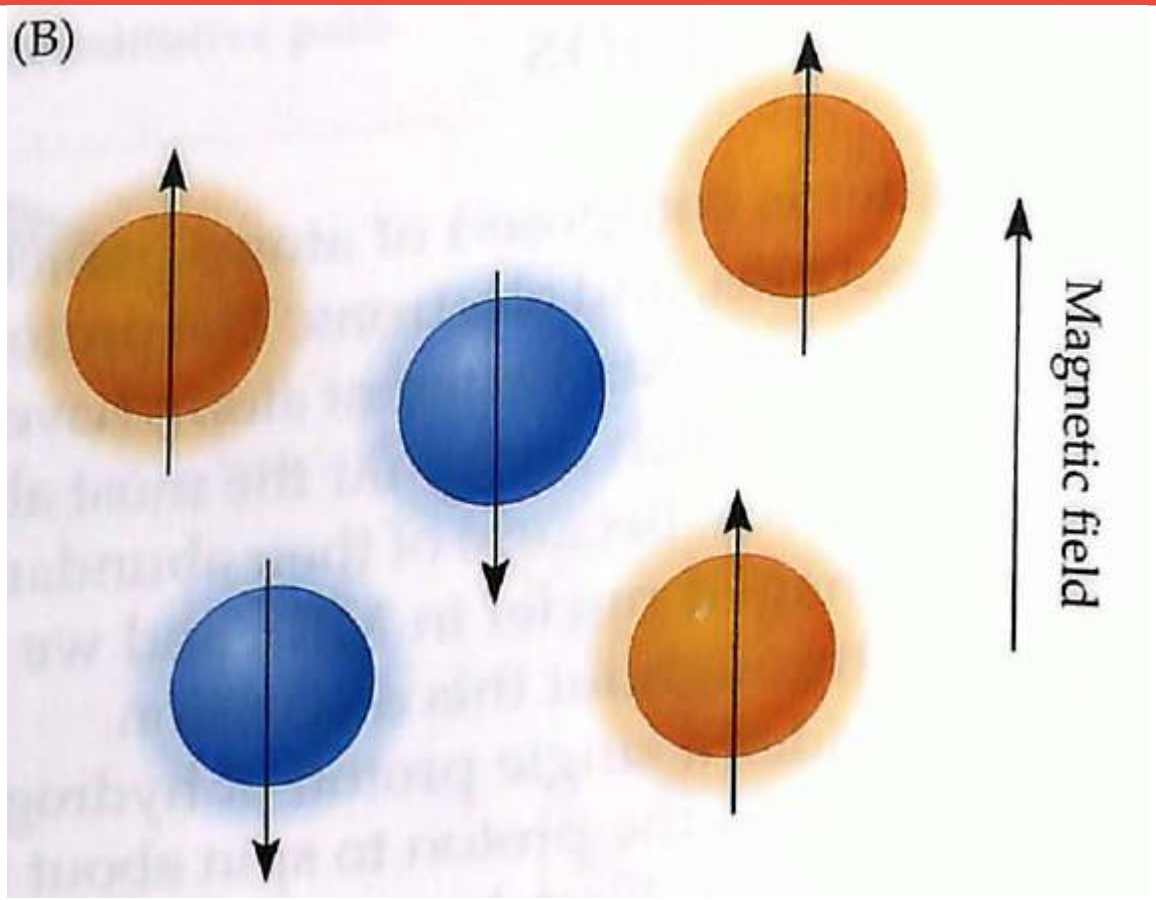


Usually, the protons are oriented randomly and their magnetic moments cancel each other out.
Net magnetization = 0

Placed into an external magnetic field, they will align either parallel or antiparallel to the magnetic field.

Huettel, Functional Magnetic resonance imaging

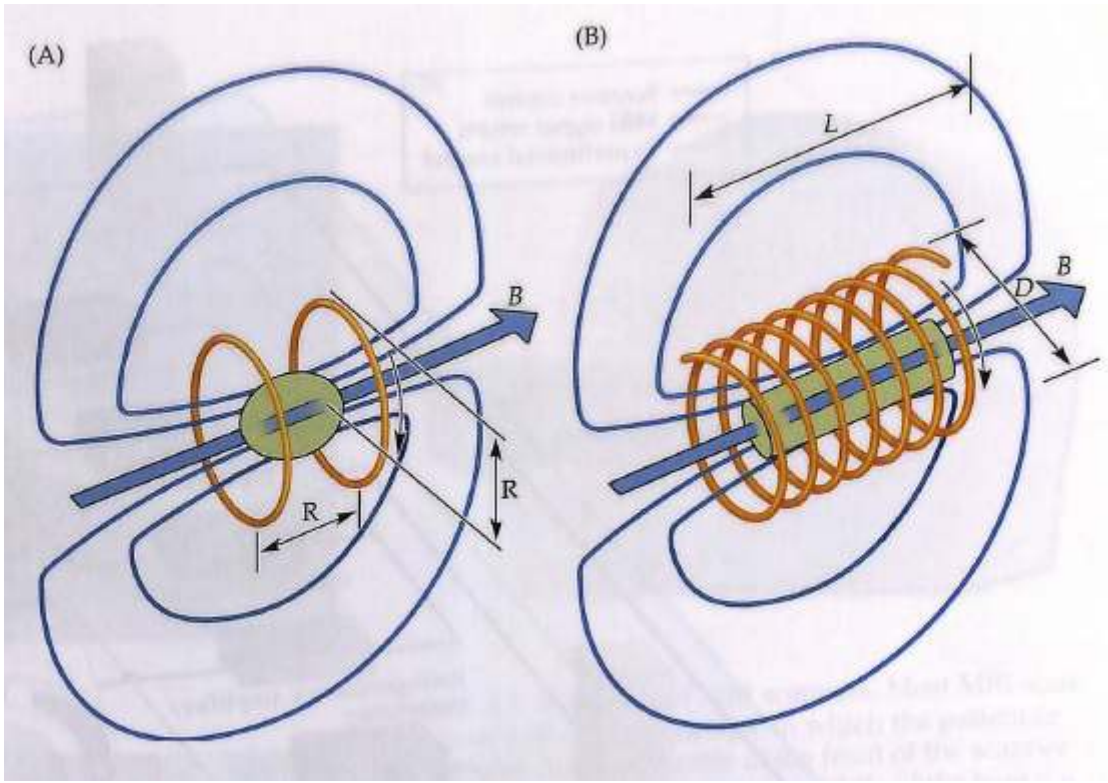
Principle of MRI

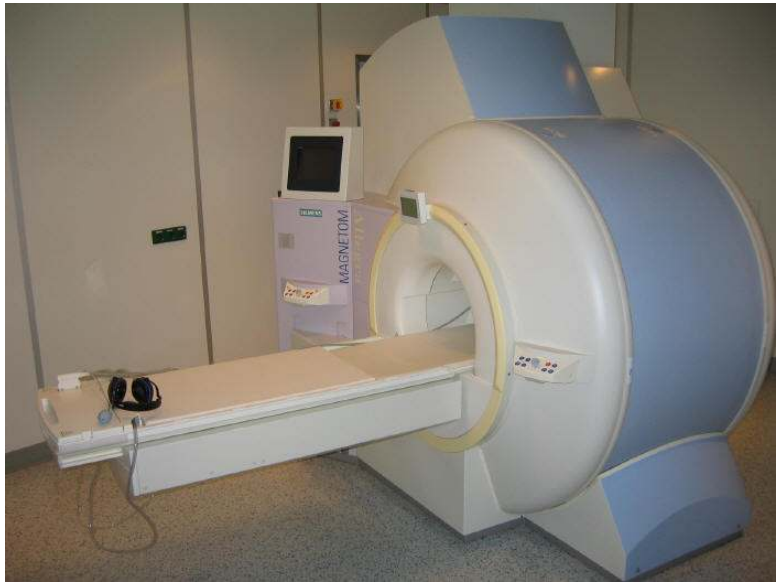


The parallel state has a lower energy level than the antiparallel state.
-> More protons will be aligned in parallel.
-> Net magnetization > 0

Huettel, Functional Magnetic resonance imaging

Principle of MRI



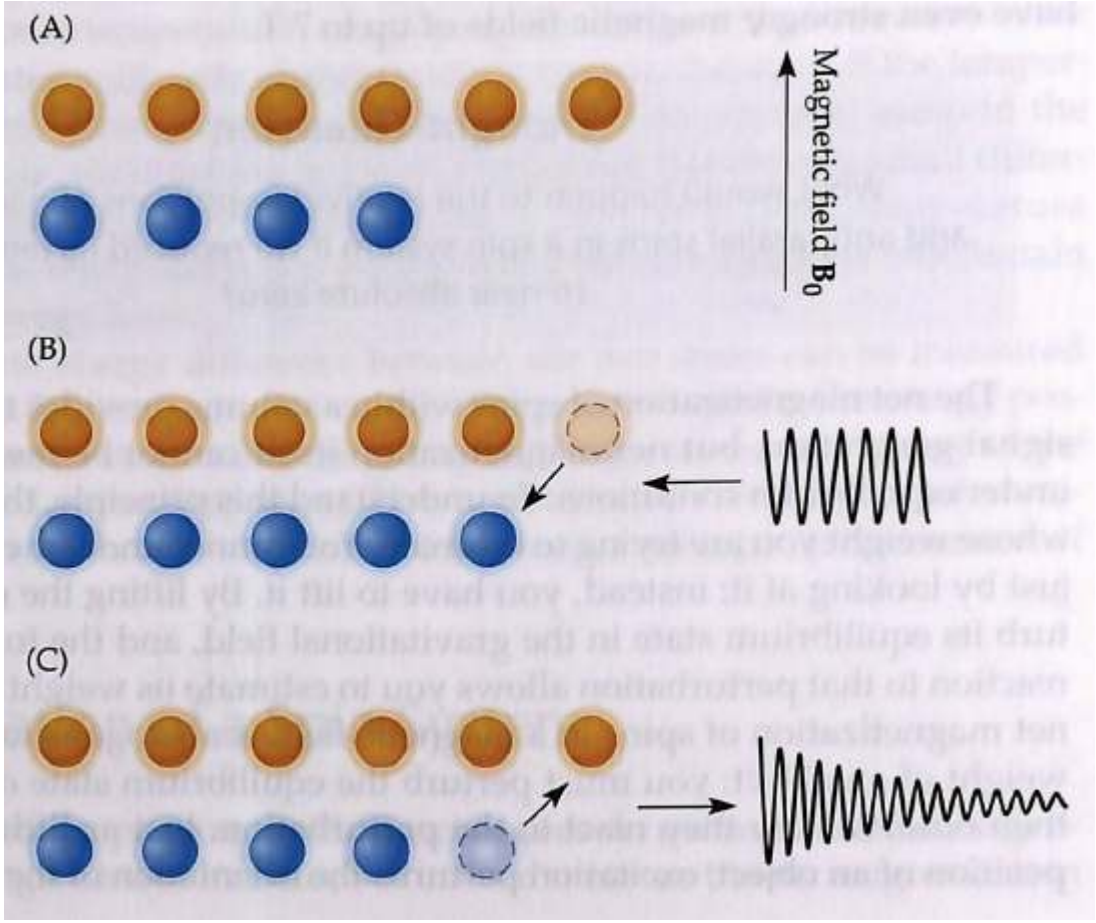


The magnetic external field (strong and static) is generated by an electrical current in (superconducting) coils.

Field strengths are 1.5 or 3 Tesla (7T recently installed at KU).
Earth: 30-60µT; small magnet: 5mT

Huettel, Functional Magnetic resonance imaging

Principle of MRI



So, in a static magnetic field, more protons align themselves parallel than antiparallel to the axis of the external field.

A radio-frequency (RF) pulse can "push" some protons to a higher energy level (antiparallel).

When the RF pulse is turned off, some protons will fall back to the lower energy state and emit energy.

The frequency of the RF pulse depends on the atom of interest and field strength. For hydrogen and 3T: ~128 MHz.

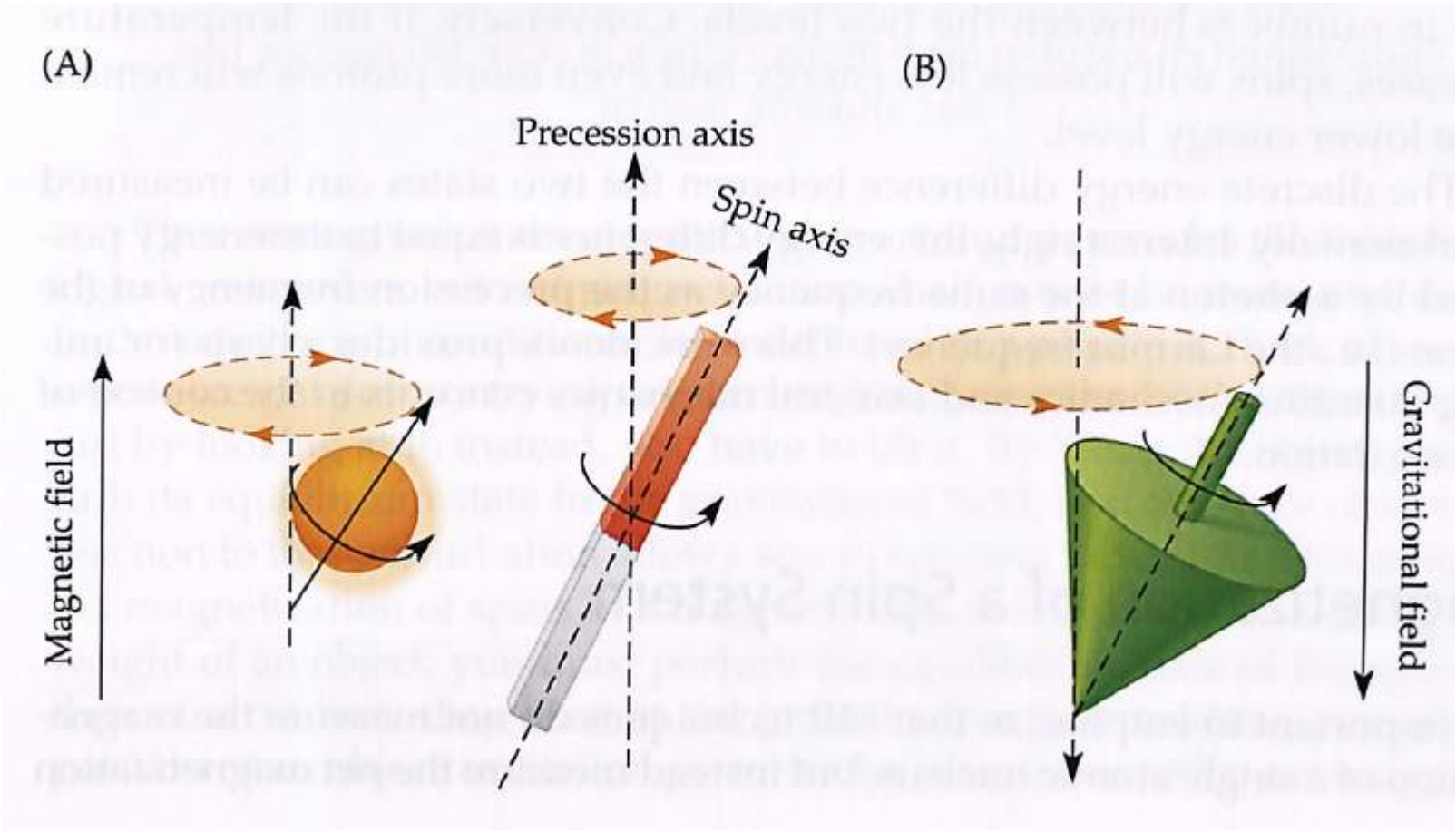
Huettel, Functional Magnetic resonance imaging

MRI Head Coils

The images show some coils used to emit RF pulses and receive MR signal (electromagnetic oscillation).
Left: surface coil, is attached directly to the head or body-part.
Middle and right: head-coils.

Huettel, Functional Magnetic resonance imaging

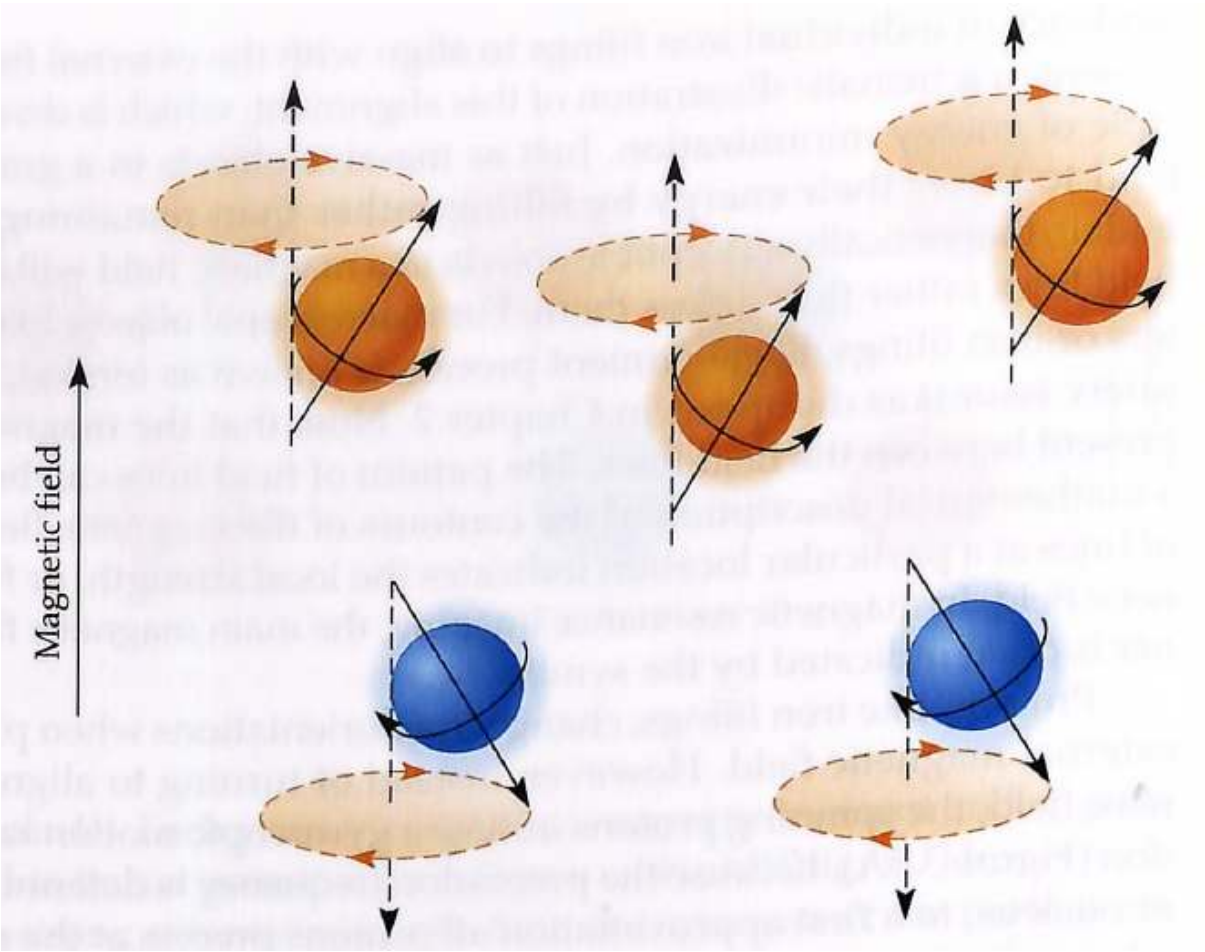
MRI Principle



The protons not only align themselves to the external magnetic field, but their rotation axis will wobble around it: this is called precession.
Think of a top spinning in the gravitational field.

Huettel, Functional Magnetic resonance imaging

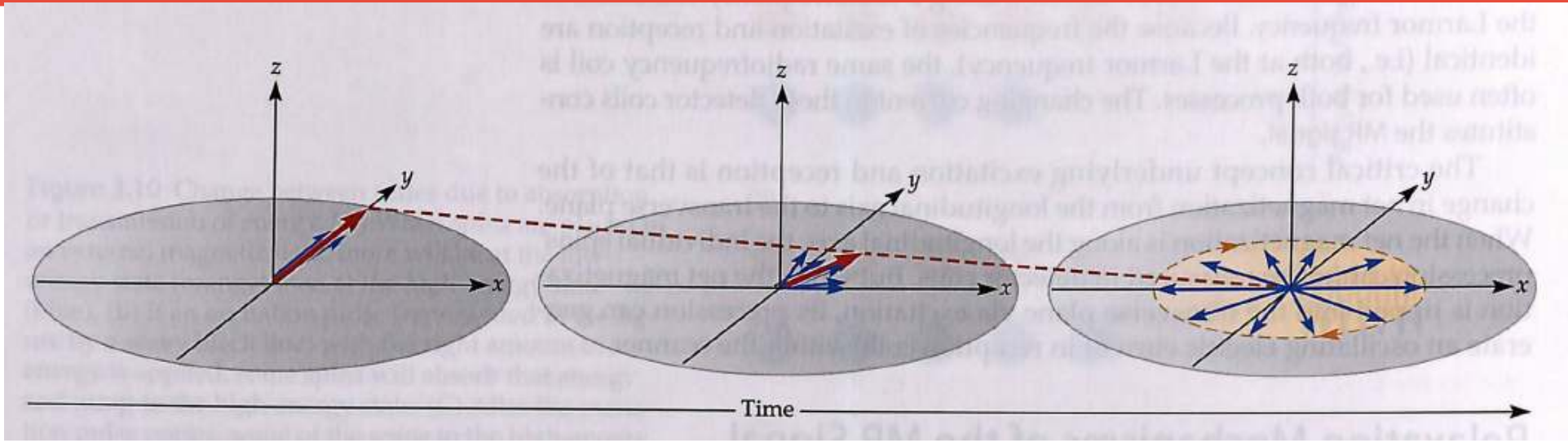
MRI Principle



Precession of protons aligned antiparallel (blue, high energy state) and parallel (red, low energy state) to the external magnetic field.

Huettel, Functional Magnetic resonance imaging

T2 (transverse relaxation)

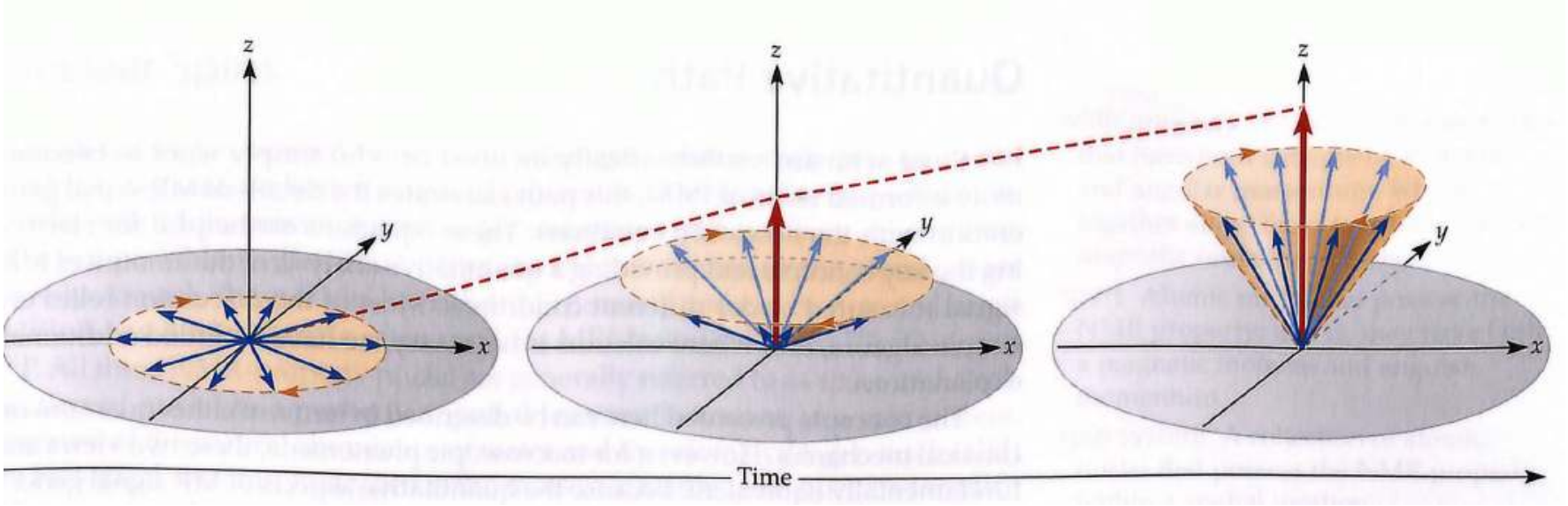


The RF pulse tips magnetization toward the transverse plane (x-y-plane, 90˚-pulse).
Spins are first coherent and produce strong net magnetization (red vector) in the transverse plane.
This transverse magnetization can be measured by the receiver coil. → "Magnetic Resonance" (MR) signal

After a few 100 ms, coherence is lost and net magnetization decreases.

Huettel, Functional Magnetic resonance imaging

T1 (longitudal recovery)

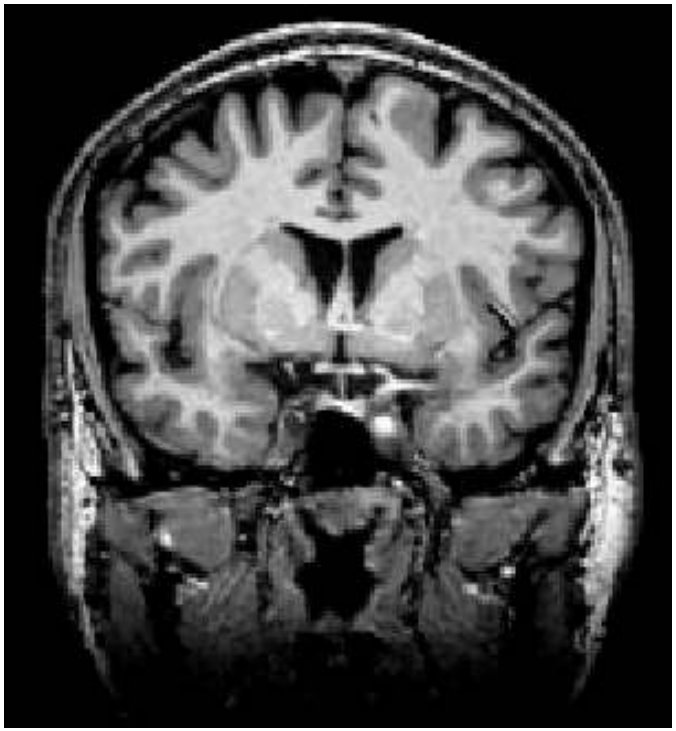


The RF pulse tips magnetization toward the transverse plane (90˚-pulse).

After a few seconds, the spins return to being aligned to the external magnetic field (z-coordinate).
Net magnetization recovers along the longitudinal axis.

Huettel, Functional Magnetic resonance imaging

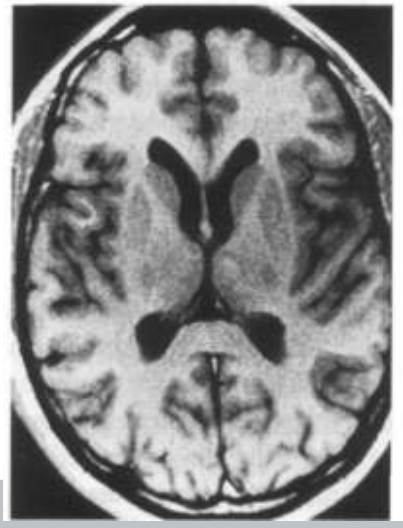
T1 vs T2



T1-weighted image

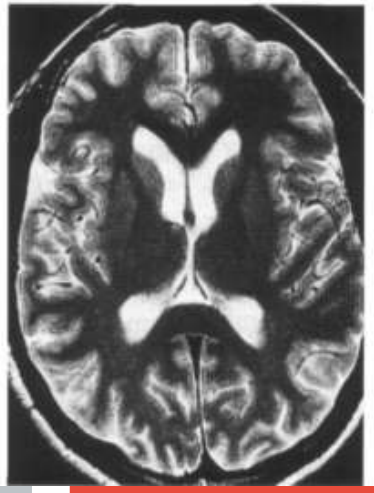
T1 and T2-parameters depend on the molecular environment of the hydrogen atom.

Gray matter, white matter, and cerebrospinal fluid have different time constants. These can be used as a contrast between these tissues.
→ By adjusting image acquisition parameters (repetition time TR, echo time TE), we can change the weights of T1/T2-parameters in image formation and thus change contrast between tissues.



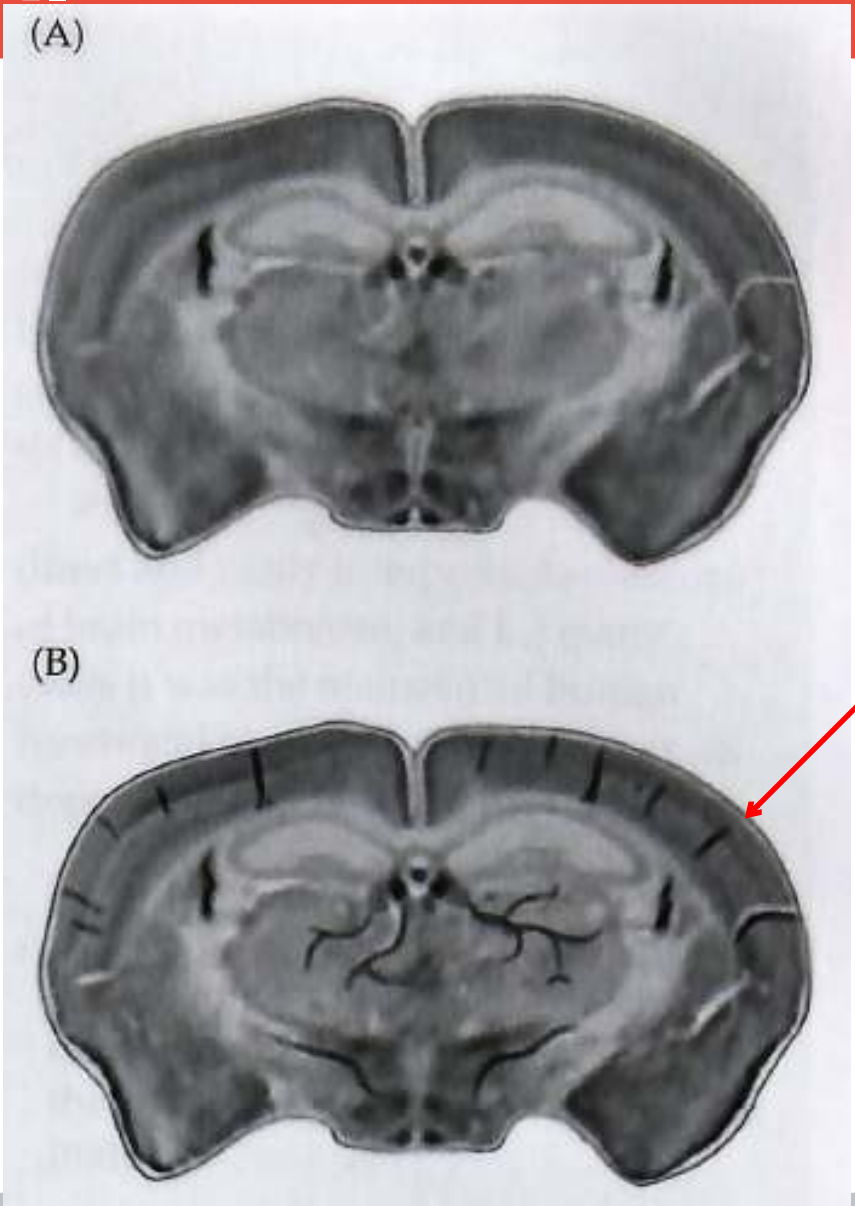
T1-weighted image

www.fmri-easy.de



T2-weighted image

fMRI



Deoxygenated hemoglobin (deoxyHb) leads to an MR signal loss compared to oxygenated hemoglobin (oxyHb).

A: rats breathing pure oxygen
B: rats breathing normal air

effect of deoxygenation: blood vessel appears darker (lower intensity), because deoxyHb leads to magnetic field distortions (and signal decrease) due to its paramagnetic properties.

Huettel, Functional Magnetic resonance imaging (cites Ogawa, 1990)

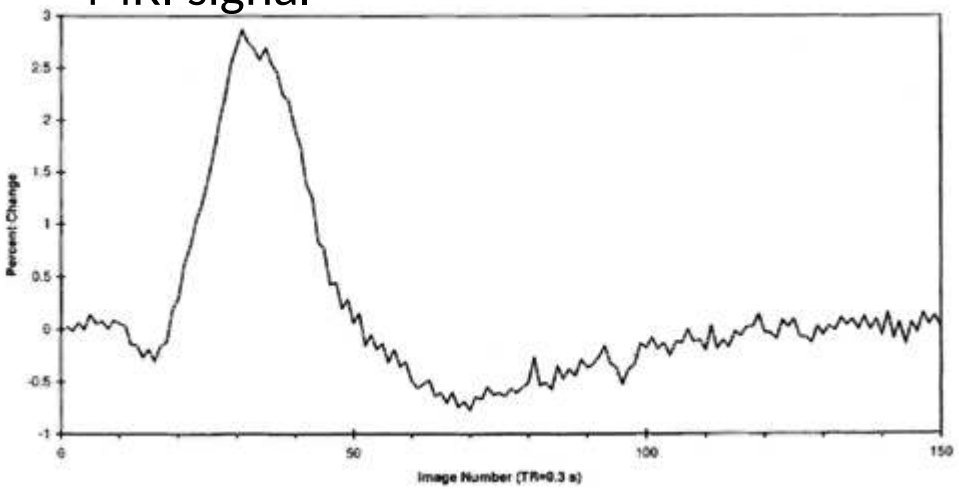
fMRI

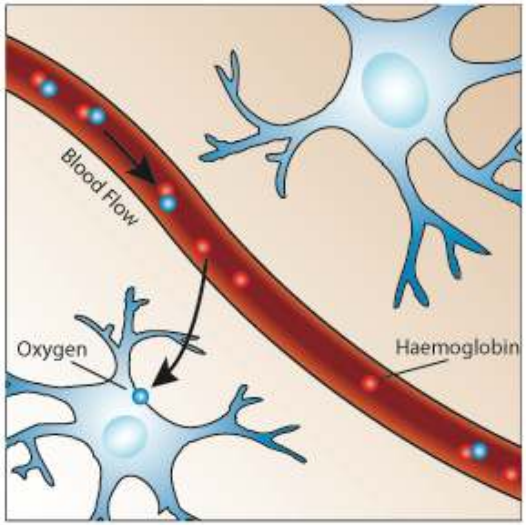
Local neural activity (for example, due to sensory stimulation or some cognitive task).

Result: increase in regional cerebral blood flow (which overcompensates for the increased oxygen demand) and an increase in the oxyHb/deoxyHb ratio.

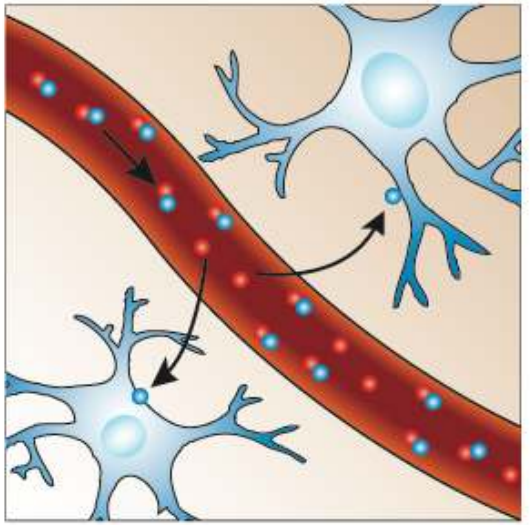
Oxygenated hemoglobin (oxyHb) leads to less MR signal loss-> signal increase: Blood-oxygenation-dependent effect (BOLD)

MRI signal



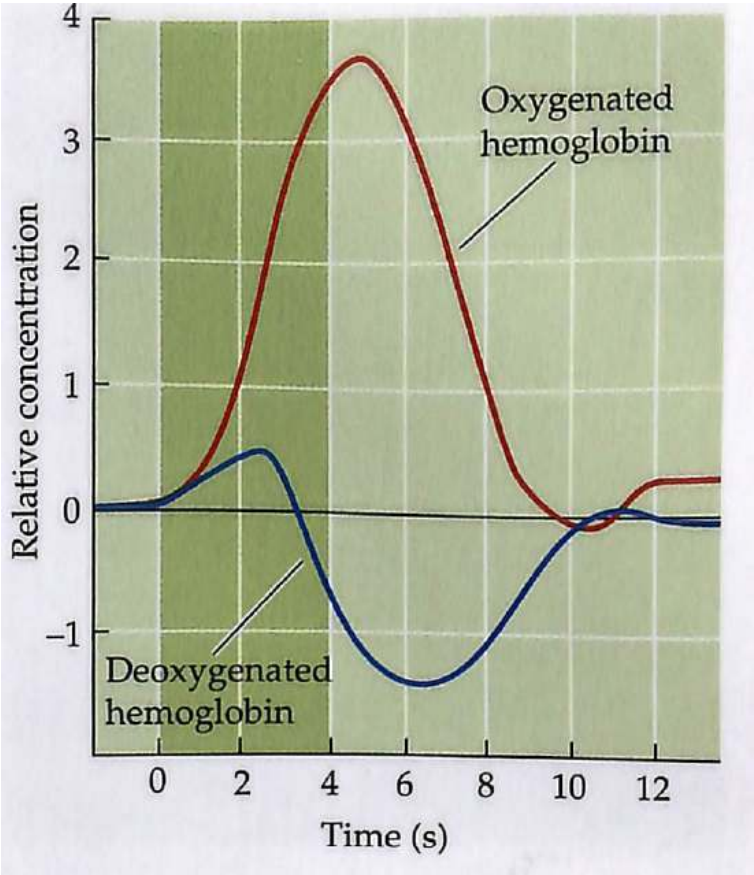


Resting

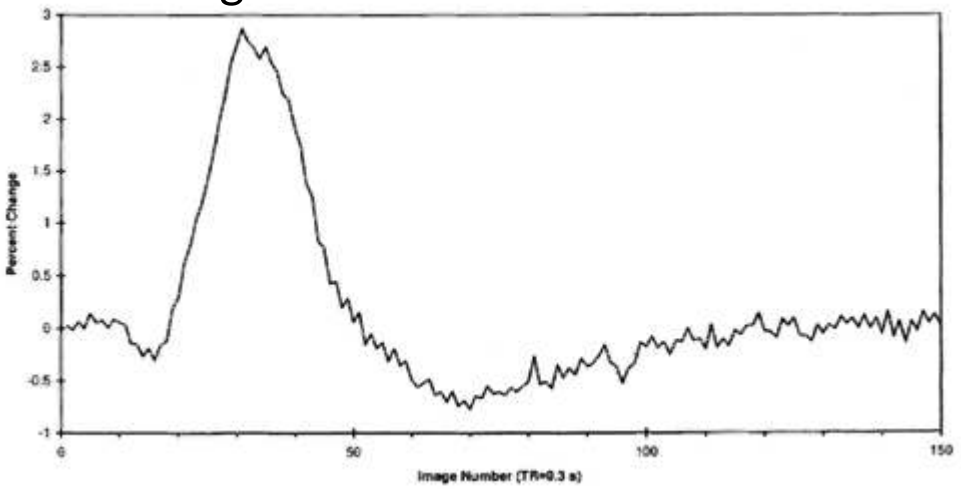


Activated

fMRI



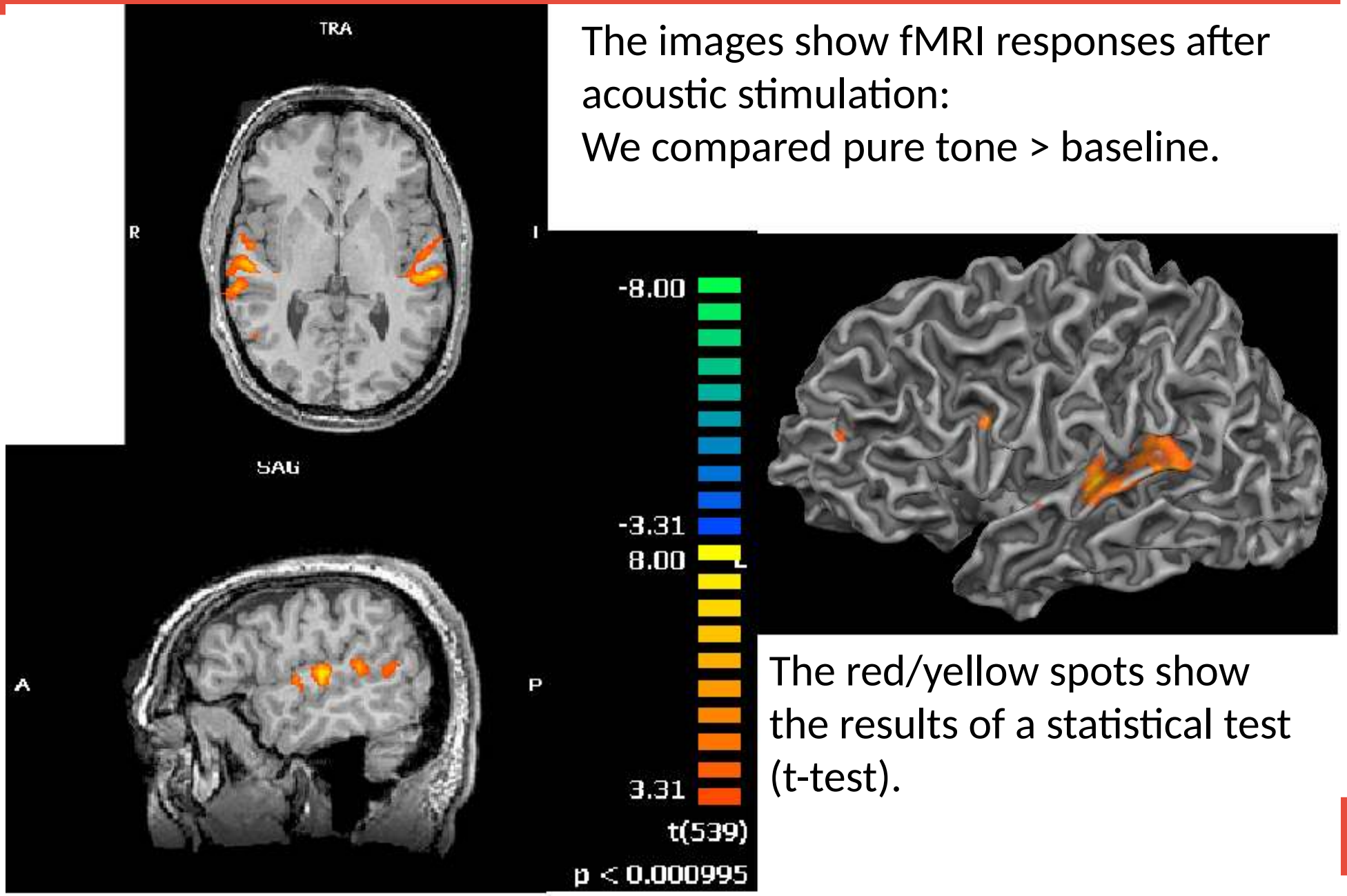
MRI signal



So, the increase in MR signal in an activated area is due to an inflow of more oxygenated hemoglobin.

Huettel, Functional Magnetic resonance imaging

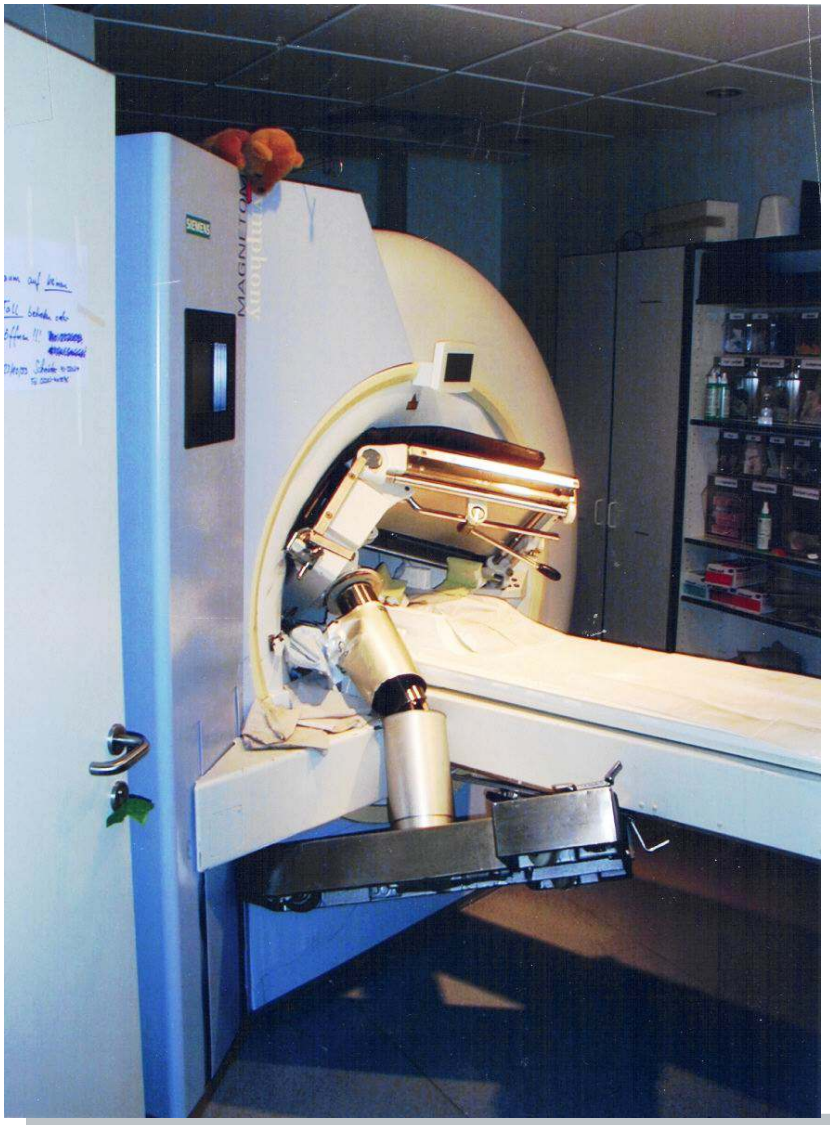
fMRI



The images show fMRI responses after acoustic stimulation:
We compared pure tone > baseline.

The red/yellow spots show the results of a statistical test (t-test).

fMRI – Big, Dangerous, Fragile Machine



Strong static magnetic field
→ metal moves, projectile effect.

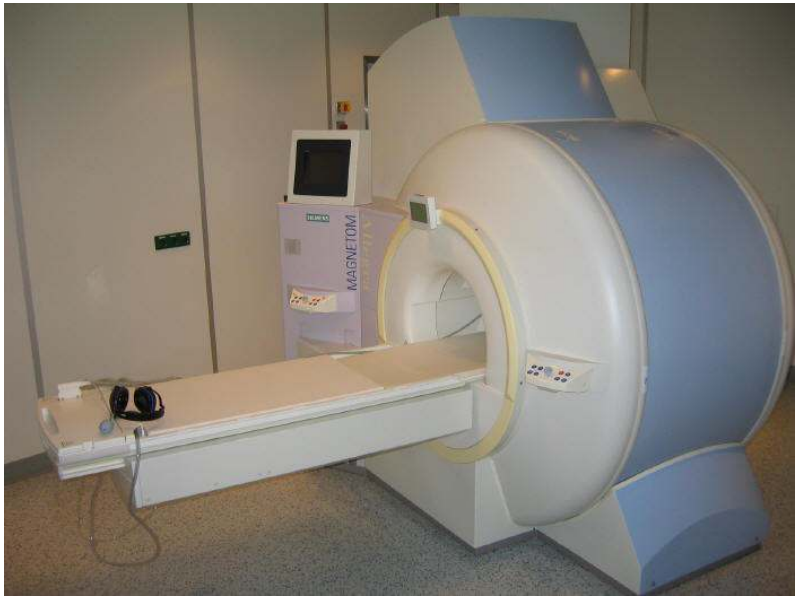
Magnetic gradients used for spatial encoding can induce electrical currents
→ pacemakers might malfunction.

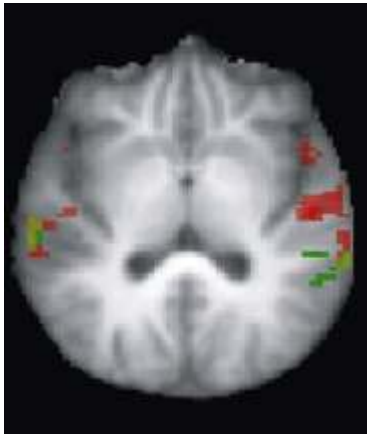
Radio Frequency (RF) pulse can heat tissue.

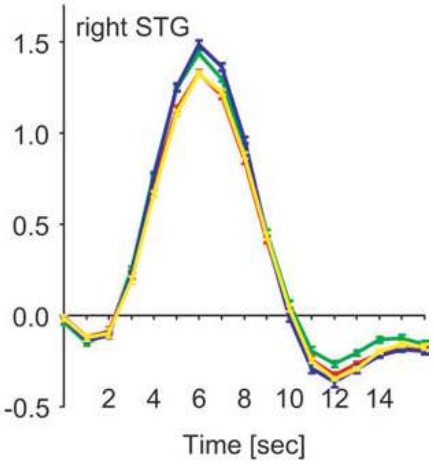
Acoustic noise can damage ears.

Human Brain Imaging

fMRI

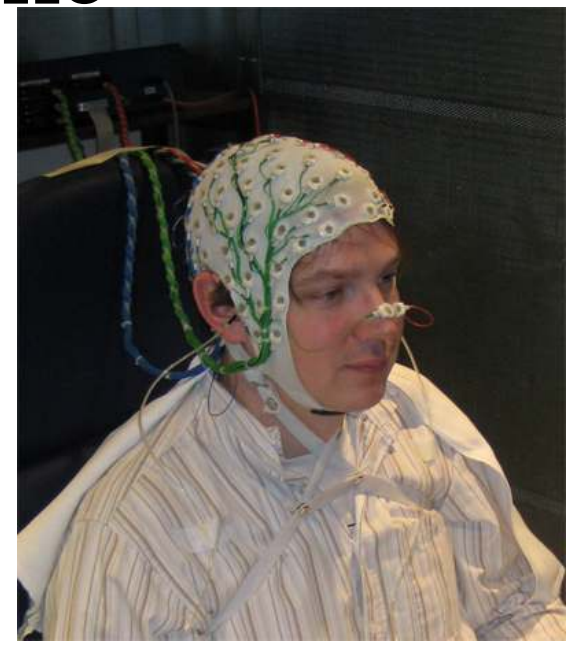


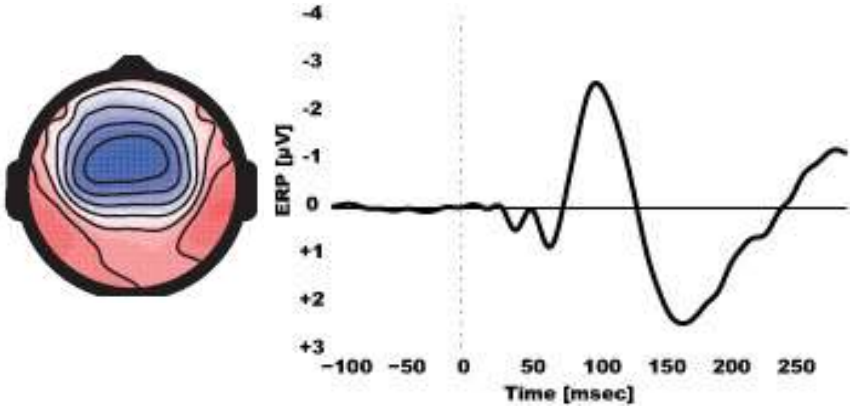




Spatial resolution: ~1mm
Temporal resolution: ~1s

EEG

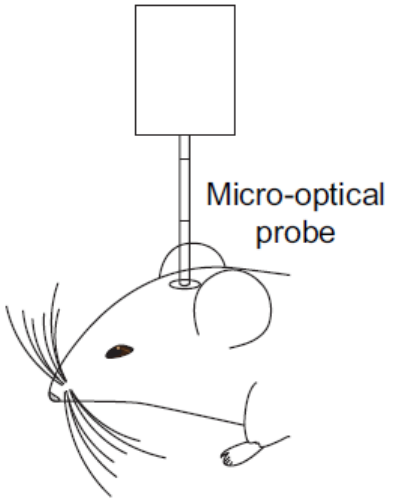




Spatial resolution: ~2-3cm?
Temporal resolution: ~1ms

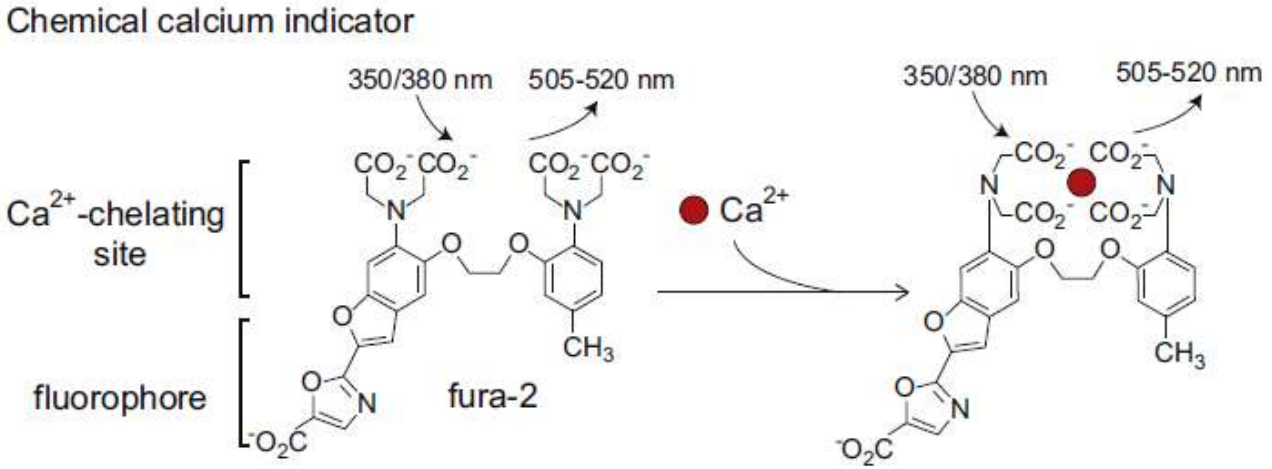
More invasive methods: Calcium Imaging

Neuronal activity (in a single or many cells) can be measured by imaging calcium (which streams into the presynaptic cell when an action potential arrives at the synapse).



For this, we need a calcium indicator: e.g., fura-2 is a fluorescent chemical that absorbs ultraviolet light (350/380 nm) and emits at 505-520 nm (green). The emission rate depends on binding to Ca^{2+}.

Excitation can be done with two longer wavelength (lower energy) photons -> two-photon microscopy.



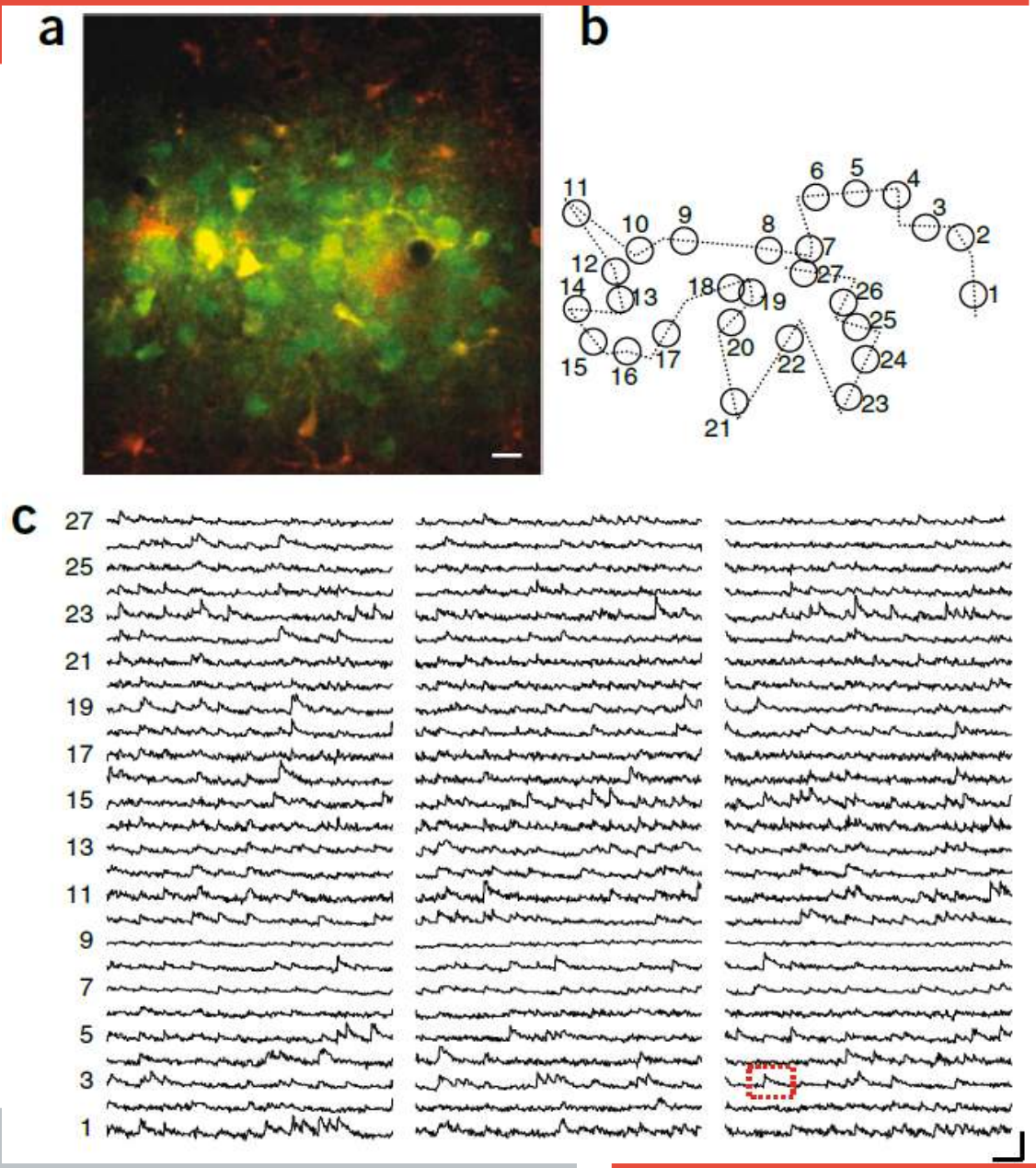
Grienberger and Konnerth, Neuron, 2012

Calcium Imaging

a) Mouse auditory cortex has been “loaded” with fluorescent dyes (neurons: green, alive specimen).

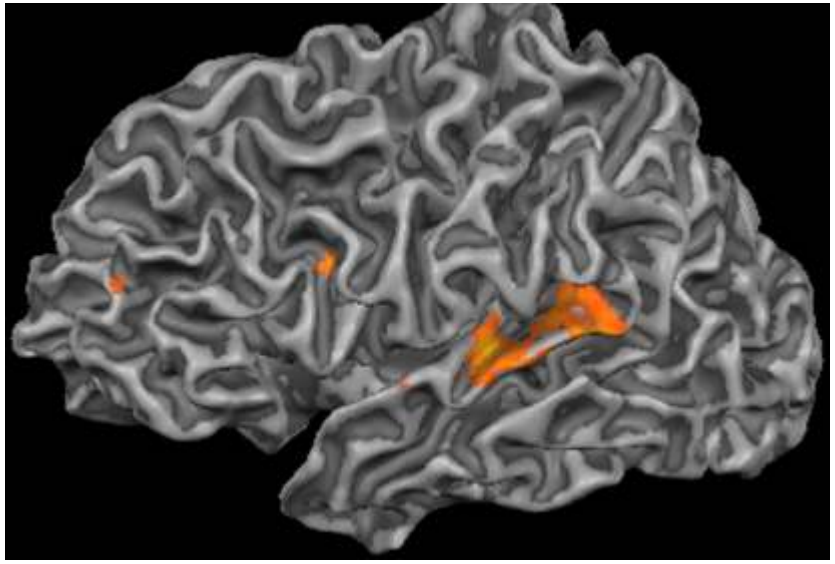
b) After the neurons have been identified, for the experiment only some (27) are scanned continuously.

c) Changes in calcium concentration within the neurons are shown during stimulation of the mouse with tones (red dashed square).



Rothschild et al., Nature Neuroscience, 2010

Correlation is not Causation…



In this fMRI example, we see areas in the left cortical hemisphere that show a significant increase in BOLD signal after acoustic stimulation.

Question: are all these areas critical for the task (hearing)?

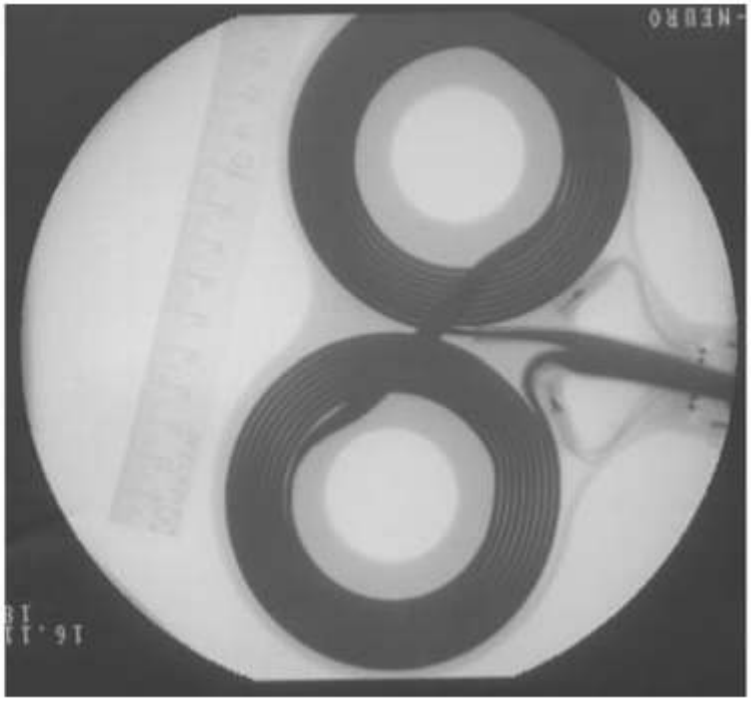
We do not know, maybe some areas are only co-activated.

To investigate the necessity of a brain area for a cognitive task, we should have some way to turn it off…

Intervention in Human Brain – TMS

TMS provides us with a tool to affect the brain by inducing an electric current with a strong magnetic field. A short single pulse can lead to excitation (e.g., finger twitch). Longer-lasting repetitive TMS (rTMS: stimulation with pulse trains) can be used to reduce excitability in an area ("virtual lesion").

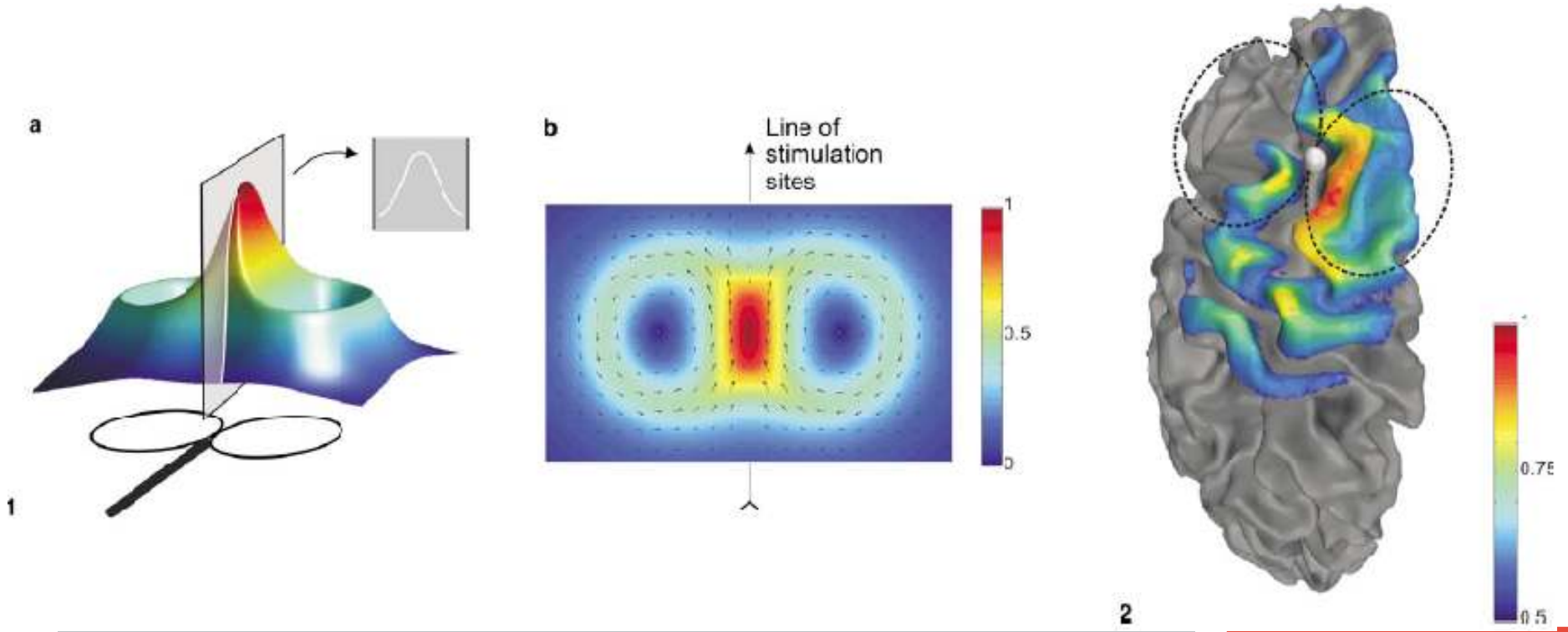
TMS coil – Figure of eight



Thielscher and Kammer, NeuroImage, 2002

TMS Bad Spatial Resolution

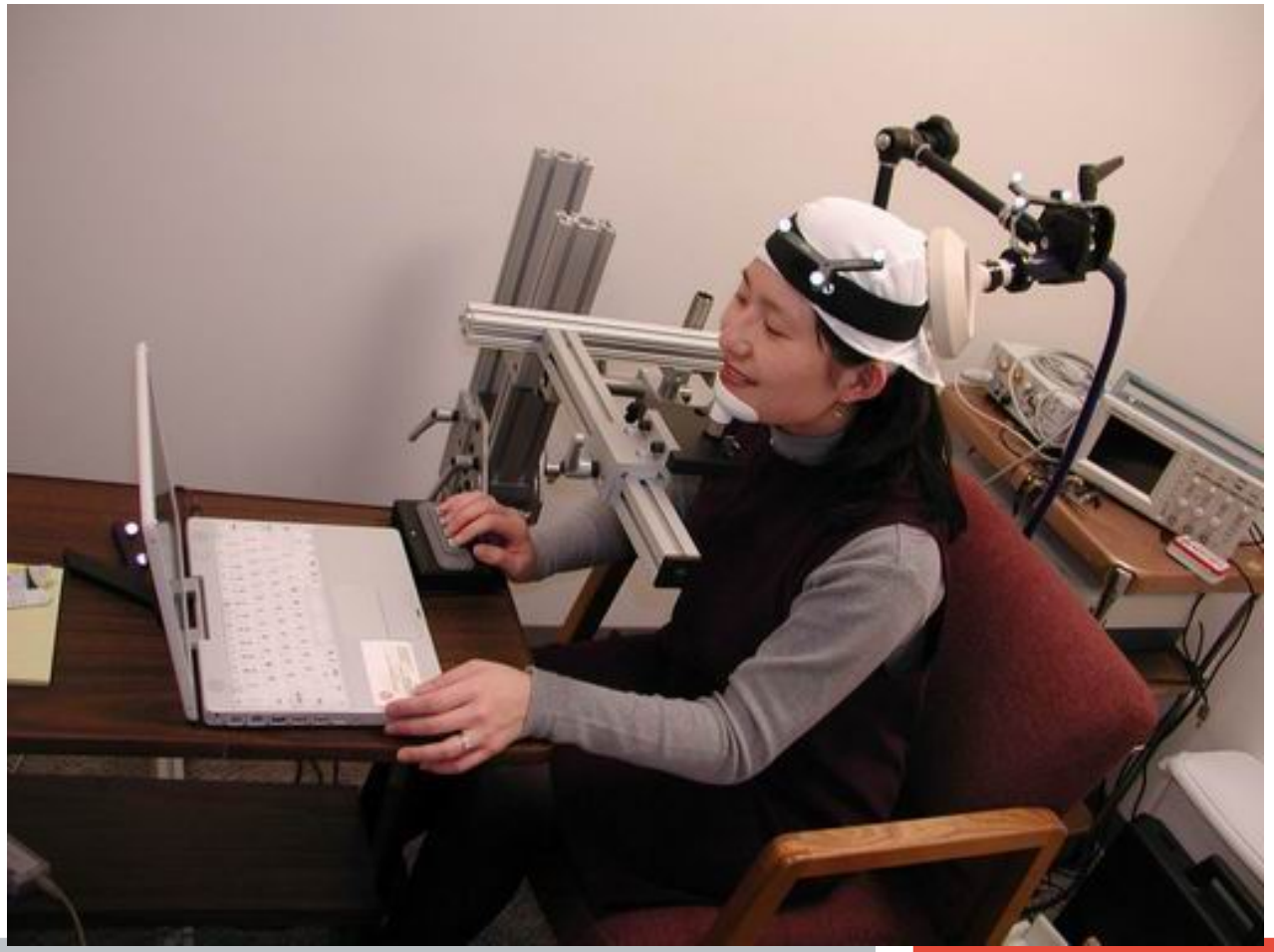
The effect of TMS is not very focused spatially . Even though a figure-of-eight TMS coil is designed to have a focal point, its effects still extend over several cm (right image shows electric field strength when TMS is applied to frontal cortex).



Thielscher and Kammer, NeuroImage, 2002

TMS

With TMS specific brain areas can be excited/inhibited before or during a cognitive task.



Summary

There are many methods in neuroscience for both measuring brain activity and intervening in brain activity.

These have different advantages and limitations in terms of spatial and temporal resolution and their portability.

EEG, fMRI, and TMS are used in humans because they are non-invasive. There are more invasive methods (lesions, calcium imaging, E-phys) which are used in animals studies for more accurate (high res) data.