Introduction to neuroimaging

Course overview

What?

- This course provides an overview of cognitive neuroscience methods
- We discus most common methods applied in human neuroscience (fMRI, EEG, TMS, …)
- Plus peripheral measures, animal research, and novel developments in the field
- We will discuss the general approach, the pros and cons of each method, and the possibility to combine different methods
- And talk about research ethics and critical issues in cognitive neuroscience research

Peripheral measures

Peripheral nervous system

What? Not the brain as such …

- Somatic: Controls skeletal (striped or striated) muscles
- Autonomic: Controls smooth muscles, the heart and glands
  - Sympathetic
  - Parasympathetic

Autonomic Sympathetic (SNS): fight or flight

Can have system-wide effects (interconnected ganglia)

Autonomic Parasympathetic (PNS): rest and digest

Tends to affect one organ at a time

1. Skin conductance

What do we measure?

- Skin conductance reflects fairly pure SNS activity (fight/flight) that is nit “contaminated” by PNS influences
- Arousal stimulates sweat glands (cools down body)
- Sweat is conductive and changes the ElectroDermal Activity (EDA)
- Mostly interpreted ad an index of arousal intensity in affective or cognitive processing (not positive vs. negative valence)

Arousal in general, no dissociation in valence for example high fear or high positive emotion

How to measure?

- Two electrodes are applied to the volar surfaces of the finger or palm of the non-dominant hand (dominant hand is used for the task)
- A very small constant voltage is applied and the amount of current that passed is interpreted as skin conductivity
- Typical units are microsiemens (μS) or micromhos (μmho)

Different measures:

- Skin conductance level (SCL): a tonic measure of skin conductance during a task, large inter individual differences (absolute values not very meaningful, but interesting for block manipulations)
- Non-specific skin conductance response (NS-SCR): spontaneous phasic changes in electrical conductivity
- Event-related skin conductance response (ER-SCR): a phasic response to a certain event (stimulus-locked), latency: 1-3 sec

Example: Responses to affective events (Öhman and Soares, 1994)

- Skin conductance is often used in fear conditioning research to detect the magnitude of a person’s fear response to conditioned stimuli

Example: Decision making (Crone et al., 2004)

- Skin conductance predicts response in decision making in good performance (higher skin conductance they’re about to make a bad decision)

2. Pupillometry

What do we measure?

- Pupil size changes based on luminance, but also reflects fluctuations in the autonomic nervous system

Large pupil:

- Stimulation of SNS
- Arousal
- Surprise
- Mental effort
- “Adaptation in uncertain environment”

Underlying mechanism: noradrenergic neurons (monkey, locus coeruleus in the brainstem)

How to measure?
- An infrared light source illuminates the eye, and an infrared-sensitive camera captures the contrast between dark pupil and iris.
- When luminance is kept constant, changes in pupil size can reflect cognitive processes during an experimental task
- Avoid luminance differences between conditions in your experiment

-> More information in the technical set in the Eye-tracking lecture

Example:
- Cognitive surprise in performance monitoring (Braem et al. 2015)
- Classic conflict task: judge central target, ignore distractors
- Pupil size measured after congruent and incongruent trials

Although eye-tracking uses the same setup as pupillometry, is different from the other peripheral measures and more related to classic behavioral measures (reaction time and accuracy) in that it is somewhat more ‘controlled’.
Skin conducting, pupil size both rise and fall, we measure when the peak is
Eye tracking is more controlled, pupil dilation is implicit

3. Cardiac activity

What do we measure?
- The heart transports oxygen from lungs, as well as nutrients, waste products, and regulatory substances (endocrines)
- What does the heart respond to most? Exercise! (has to be controlled during “psychological” experiments)
- Cardiac cycle: Initiation of cycle via sino-atrial (SA) node (pacemaker), intrinsic rythm of 105 bpm, slowed down by the PNS, sped up by the SNS
- further signal conduction via atrial-ventricular (AV) node

Main methods and measures of interest
- Electrocardiography (ECG):
  - Heart rate (HR)
  - Heart rate variability (HRV): longer period of time
- Impedance cardiography / Impedance variation (ICG)
  - Pre-injection period (PEP)

HRV is genetic, like blood pressure, not possibly to induce a high HRV
PEP is selective

Electrocardiography (EEG)
- Recording of electrical activity via surface electrodes
- No direct measure of action potentials, but reflects production and conduction of action potentials in the heart during cardiac cycle

Measure of interest: Heart rate (HR)
- Based on RR interval, beats per minute (bpm)
- HR = 60 / (RR interval in seconds)
- Sensitive to emotional processes, influenced by SNS and PNS

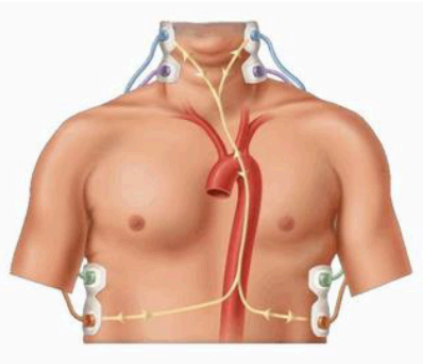
Example: Affective processing (Bradley et al. 2012)
- Heart rate drops for emotional events (especially negative ones)
- In contrast to skin conductance (elavated for emotional events)

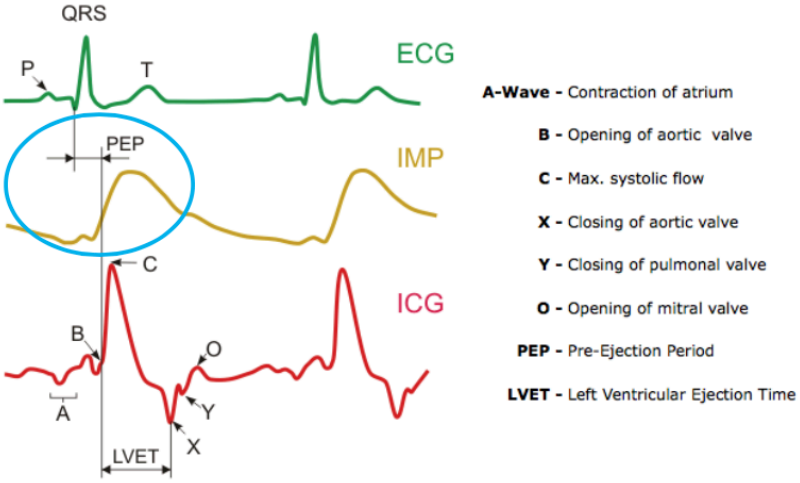
Measure of interest: Heart rate variability (HRV)

- Changes in the time intervals between consecutive heartbeats (RR interval); termed Inter-beat Intervals (IBIs)
- Reflects general measure of the influence of the PNS on the heart

Impedance cardiography (ICG)
- Total electrical conductivity of the thorax and its changes over time
- High-frequency current is flowing between an electrode pair (via aorta)
- Impedance changes are picked up by a second pair
- Returns Impedance pulse wave (IMP) and the ICG curve (1st derivative)

Measure of interest: pre-ejection period (PEP)
- Time interval from the electrical stimulation of the ventricles to the opening of the aortic valve (~Pumping performance)
- Thought to reflect the effect of the SNS on the heart





Example: HRV and PEP in auditory attention (Giuliano et al., 2018)
- High HRV and short PEP reflect selective attention (EEG)
- Both PNS and SNS influence attentional processing

4. Respiration

Respiratory activity
- Measured via belt around chest
- Relationship between respiration and heart rate

Different measures of interest:
- Respiratory rate (most relevant for cognitive neuroscience)
- Inspiration time
- Expiration time
- Inspiration to Expiration ratio

Example: Respiration in cognitive tasks (Backs & Seljos, 1996)
- Effects of working memory and inter trial interval on respiration
- Respiration rate increases with cognitive load and paradigm speed

5. Muscle activity

Electromyography (EMG)
- Measures electrical field changes due to muscle action potentials (~allows us to be in close contact with the motor cortex)
- Always on striated muscle (e.g. different from the cardiac muscle discussed earlier), needs stimulation to contract (motor nerves)

Different measures of interest
- Startle blink ~ probe for surprise and negative valence
- Facial EMG ~channel for emotion expression
- Pre-response motor EMG ~ partial errors
- [EMG during motor TMS]

Example: Startle blink (Lang et al. 1990)
- Surface electrodes below eye
- Startle reflex (e.g., unexpected noise) is increased for unpleasant events

Example: Facial EMG Cannon, Hayes, & Tipper, 2010)
- Participants rated objects (garage or kitchen) with left or right response
- Grip of the object could be compatible or incompatible with response
- Zygomaticus muscle to smile, surrogates muscle to frown
- More smiling when same side, more frowning when dissociation

Example: motor EMG and partial errors (Burle et al. 2002)
- Simon task: respond to stimulus color and ignore the position (conflict, blue button on left and green on right)
- Surface electrodes to record activity of finger muscles
- EMG reflects intended but not executed responses (partial errors)

Why record peripheral measures?
- Immediate reactions of the peripheral nervous system (e.g., arousal) can complement behavioral and neuroimaging data
- They are cheap, mostly univariate, and straightforward to analyze
- Although most of the methods are relatively old (e.g. used in emotion research), there is a new interest in basic and applied neuroscience today (e.g. affective processing of effortful tasks)
- Some measures can/should be considered as “nuisance” variables that influence other measures we are interested in (e.g. heart rate and respiration modulate the hemodynamic response in fMRI)
  - PPG gebruikt tijdens fMRI om het effect van de hartslag (ruis) weg te werken
- From all the ones mentioned, pupil dilation and eye-tracking are most often used in the research conducted in our department

Animal research
Animals as basis for humans (we aren’t always allowed to stick needles in humans)

1. Why discuss animal research?
This course is on human neuroimaging, but we need the animal-model perspective
- Human cognitive neuroscience is heavily influenced by animal research
- Human neuroimaging methods are less precise (lower spatio-temporal resolution)
- We often need to relate our results to animal models for a comprehensive view)

2. The basis
Neuronal activity
- Sodium-potassium pump in the membrane pumps sodium (Na+) out and potassium (K+) in. Negative resting potential (more Na+ exported)
- Neurotransmitters (released from pre-synaptic cell) open NA+ or K+ channels: excitatory or inhibitory stimulation
- Based on the summed inputs, the electrical membrane of the neuron rapidly rises and falls: Action potential
- The Action potential travels along the axon and contributes to activating the next neuron...

Synaps is where electrical signal is transformed into chemical signal -> transformed into conduction again
Baseline negative resting potential

Measuring and manipulating neuronal activity at different levels (Single cell -> general)
- Recording and inducing neuronal activity: recording and inducing electrophysiological activity just outside the cell(s)
  - > related to action potentials
- Optogenetic imaging: Manipulating cell function with light via genetically modified neurons
- Pharmalogical manipulations: inducing receptor agonists and antagonists; changing re-uptake, synthesis, break-down (also somewhat done in humans)

- Local field potentials (LFPs): measuring the summed dendritic synaptic current in the tissue (not action potentials as such, more general)
- (Intracranial) EEG and fMRI ("neuroimaging)
  - Intracranial EEG is mostly done in monkeys
- Behavioral observation

2. Selected techniques

2.1 Recording and inducing neuronal activity (inc. ontogenetic imaging)

-> activity patterns of neurons and neuron clusters provide insights into a region's function

Recording neuronal activity via electrodes

- Microelectrodes (tip 1-10 µm) can isolate activity of a single neuron: voltages generated in the extra cellular matrix when an action potential is generated in the cell ("spikes")
  - You hear spike pattern with single-cell recordings
- Carrier device mounted on skull; electrodes are moved down through the target area until well-isolated neuronal activity is observed (supported by stereotactic reference frame)

Example: single-cell recordings from dopaminergic neurons in the substance nigra (black part in the brain) during reinforcement learning (Schultz et al. 1997, 2001)

- Increase in firing rate when reward (R) is delivered
- Increase in firing rate when a conditioned stimulus (CS: light that accompanies food) reliably predicts this reward (replaces the response to R itself)
- Decrease in firing rate when R is predicted but does not occur: omission response/ disappointment
- These changes in neuronal activity lead to varying dopamine release in the target regions (e.g. nucleus accumbens)
- This model of complex neuronal coding of reward information became the basis of human motivation studies using fMRI and PET

To humans you typically don't give food but money

1 needle records one to 10 neurons/one neuron bundle (noise from other neurons)

Inducing neuronal activity via electrodes

- The same set-up can be used to stimulate the neurons in the vicinity of the electrode which van provide insights into the function of an area and its projected regions

Example: Eletrical stimulation of the septal area (Olds & Milder 1954)

- Electrodes were placed in the septal area of the rat forebrain, part of the dopaminergic system
- The electrodes induced electrical pulses every time the rats pushed a certain lever in their box
- The rats "enjoyed" the stimulation instead of avoiding it; some rats even neglected eating and drinking in favor of the simulation
  - > electrical stimulation could be used as an operant reinforcer
- Demonstration that electrical stimulation of neurons can be used as an operant reinforcer (and evidence for the existence of some kind of "pleasure center")
- Functionally, the stimulation of the septal area lead to dopamine release in the nucleus accumbens, similar to the effects of primary rewards

Inducing neuronal activity via optogentic imaging

- Method to control and monitor the activities of individual neurons in living tissue in freely-moving animals
- Genes for light-activated ion channels (opsins) are introduced to a population of cells by an engineered virus

Change neutron structure -> light outside the brain leads to opening/closing of some membranes of channels in the brain

Blue light activates ON opsin (channelrhodopsine)

Yellow light activates OFF opsin (halorhodopsin)

Risks are very high -> not done in humans (very complicated surgery)

2.2 Pharmalogical manipulations (and lesions)

Pharmalogical manipulations

Example: Manipulating dopamine transmission during effort-based choice (Salamone et al. 1994)

- T-maze with a high (HD) and low (LD) reward density arm; one group had to cross a barrier to reach the food in the HD arm (effortful choice)

- The rats mostly chose the HD arm, even those in the barrier group
- Dopamine depletion (6-hydroxydopamine) and dopamine receptor blocking (haloperidol) in the nucleus accumbens abolished these high effort choices
- Disrupting dopaminergic signalling did not alter reward, evaluation, but biased the decision process towards investing less effort
- Dopamine is responsible for deciding wether climbing the barrier is worth the effort to get the food

Lesions
Example: the role of the anterior coingulate during effort-based choice (Waton et al. 2003)
- In this study the same T-maze task was used as on the previous slide.
- Anatomical lesions via toxin injection (anterior cinglulate: ACC, limbic cortex: PL-IL, sham lesion: surgery without lesion).
- Only the ACC lesion rats stopped climbing the barrier to get to the high reward. Same result as dopamine depletion.

Dopamine has multiple roads
In animals bleeding is most often caused by toxins

2.3 Local Field Potentials (LFPs), (intracranial) EEG, and fMRI

-> more indirect than single-unit recordings, but comparable with human data
Why use indirect techniques such ad EEG and fMRI in animals, when we can record single cell activity directly?
Local Field Potential (LFP's): Transient electrical signals reflecting the summed electrical activity of individual neurons. Recorded close by the generating cells. LFP's are no action potentials!
Combining EEG and fMRI in animals with LFP recordings reveals what these indirect measures reflect in the human brain

LFPs and EEG
Relate EEG to neuronal activity in the same monkey -> compare EEG data of monkeys and humans

FPS and fMRI
Relate fMRI data to neuronal activity in the same monkey -> compare fMRI data of monkeys and humans
- Presenting visual stimuli while recording fMRI, multiple-unit activity, and local field potentials
- fMRI signal (red) corresponds most likely to local field potentials (blue) (summed dendritic activity)

LFPs fire as long as pictures are there
fMRI has a lag, blood flow is much slower than neural activity

3. Notes on comparability

Comparability in terms of function
What can we actually learn?
-> different research questions require different animal models
Rodents in the lab (mostly mice and rats)
- Rodent models are highly valuable for cognitive neuroscience especially for processes related to "old" brain structures (brainstem, basal ganglia, hippocampus)
- Not comparable to humans on the neocortical level and more limited regarding complex cognitive tasks, valuable when you look at very basic functions
- Cheap, easy in terms of breeding, handling, training

Primates in the lab (mostly macaques and rhesus)
- Primate models are more comparable on the neocortical level and thus more valuable for investigating higher cognitive functions (e.g. monkeys can perform comparable computer tasks)
- Still, there are neuroanatomical differences on the cortical level, which is why comparative studies often use the expression: "monkey homologue" of a region
- Much more time consuming and challenging to breed and train primates

Comparability in terms of methods
Animal research procedures (mostly invasive):

- Closer to the actual neuronal substrate (firing rate, LFPs, receptor binding, etc)
- Provide insights into causal relationships (pharmacological intervention, lesions, postmortem histology, …)

Human research procedure (mostly non-invasive)
- Recording / including neuronal activity: only as part of therapeutic approach (e.g. Parkinson's patients)
- Pharmalogical manipulations: mild pharmacological manipulations (healthy) and treatment (patients)
- Lesions: "virtual lesions" TMS (healthy); real lesions (e.g. stroke); therapeutic lesion (e.g. epilepsy)
- EEG and fMRI: in healthy population and patients

See lecture on Clinical groups
In human neuroscience, we rely on 'indirect' measures of neuronal activity and need to integrate results from animal research

Research ethics

1. Some history on human research ethics:

Landmark for today's human research ethics:
- 1947 Nuremberg Code: subject consent and the right to quit, no unnecessary harm, scientific foundation, performed by experts. This was in response to "medical" experiments by the Nazi regime without any ethical standards.
- 1950-70 US research scandals: without consent, sick/dependent participants, and/or clearly deceptive. e.g. Willowbrook hepatitis study, Ohio prison & Brooklyn Hospital cancer studies, Obedience study (Milgram), Tearoom trade study (Humphreys)
- 1964 Declaration of Helsinki: ethical principles for human experimentation (World Medical Association)
- 1966 Protection of Human Subjects policy: independent review boards (National Institute of Health)
- 1972 Tuskegee Syphilis study revealed: selection of poor powerless african-amaerican, painful procedures, omission of treatment, unethical financial benefits (1930's-1970's)
- 1974 National Research Act: formation of the National Commission "Protection of Human Subjects in Biomedical and Behavioral Research"
- 1979 Belmont Report: principles for human subject research: Respect for person, Beneficence, Justice
- 1991 Common Rule: establishing the Institutional Review Boards to ensure ethical procedures

US research scandals: Most common problem is absent of consent

Basic rules for human (neuroscience) research today:
- Ethical Board approval: each study has to be approved by the local ethical review board, which checks for accordance with overarching guidelines.
- Informed consent: human subjects have to give their voluntary consent after being informed properly (procedure, risks, benefits), and they have to be able to give consent
- Unbiased selection of subjects: same procedure, benefits, rights (no "minority" studies)
- Special protection for special groups: children, patients, prisoners
- Minimal risk: no health risks for the subjects, both physically and mentally. Seemingly not very relevant for the majority of experimental psychology studies, but there are cases where ut is (e.g. physical pain manipulations; fear conditioning; food/water deprivation; addiction; depression; obedience, etc.)
- Subjects have the right to quit: at any time without negative consequences

While this seems even more relevant in clinical studies, these basic rules apply to all studies conducted in our faculty
Questions on ethics get more complicated as there is more data collected
If your study is very standard you can say it falls under the GEP (general ethical procedure) and you don't have to get special approval
TMS sometimes causes unpleasant feeling on the skin -> important to disclose on beforehand
Pain simulation: you have to prove it is very mild

3. Guidelines animal research ethics

Animal guidelines are less strict

Transcranial Magnetic Stimulation (TMS)

1. TMS basics:

History
Older than you might think
First use was in 1902 for clinical use
1910 was experimental use, but wasn't developed well
Better development in 1985

Principles
1. A strong brief electrical current is passed through the stimulating coil, held on or near the person's head.
2. A transient magnetic field is generated perpendicular to the paddle, and penetrates the brain.
3. Variations in the magnetic field induce an electric current in the brain, stimulating/depolarising the neurons in the stimulated region.

Electromagnetic induction (Faraday's law, 1831)
An electrical current in a conductor is associated with a perpendicular magnetic field.
This magnetic field causes an electrical current in a second conductor within the field.
Brain is a conductor, magnetic field induces a movement of electrical charges in the neural tissue.
-> Induces membrane depolarisation (action potentials).

Induced neural activity ("neural noise") interferes with coordinated neural processing
TMS forces neuron patch to fire -> added neural noise
-> hard to coördinate different parts of the brain because of degraded percept

Why use TMS?
Establishing causal relationships (brain area -> cognitive function)
How? By interfering with cortical activity and then observing changes in behavior.
What determines this interference?
A. Location of brain region
B. Coil (shape, position, orientation)
C. Experimental parameters
D. State of neurons at time of stimulation
TMS is non-invasive but allows to research causal relations
This is not a physics class, don't worry about the technical aspects

2. TMS methods: Location

Sensorimotor stimulations
- Primary effects:
  - Motor cortex = measure motor-evoked potentials (MEPs).
  - Visual cortex = induce phosphenes, transient scotomas, block motion processing.
- Secondary effects:
  - Interference in neighboring regions (often stimulating more than the visual cortex)
  - Interference in directly connected regions
    - The visual cortex is also highly connected with other brain regions, there is downstream activation from the visual cortex

-> be careful interpreting results, it is never the effect from one brain region

Associative cortical stimulation
- Neuronavigation
- fMRI-guided TMS

"Virtual lesion": knock out task-related cortical region, see how it affects task performance
Broca's area stimulated: interferes with high level speech, he would be able to sing
For individuals Broca's area is positioned differently (it might not even be in the left hemisphere), variability is quite high
Neuronavigation: with fMRI during speech you first find where in the brain speech is located

Shape of the coil
Determines locality (and depth) of the stimulation.
Stimulation depth isn't high, can only stimulate close to the skull

Focality: how big the area is
Circular and figure of eight are most commonly used
Figure of eight is more focal (with big peak where the circles meet)
If you go deeper (with circular coil), you will also stimulate a much bigger area
Either deep or focal stimulation, you cannot get both

Position of the coil
Determines the stimulated region
Where you put the coil, even within the same region matters, especially in the motor cortex

Orientation of the coil
Affects locality and position of the region
If you change the orientation of the coil, the magnetic field also changes
Keep the coil perpendicular
Direction of the coil also has an effect
Tiny differences have an effect
Skill: finding the correct way to hold the coil to get the best stimulation

Intensity
Experimenter control strength of current in the coil.
This changes how much electrical current is induced in the brain.
TMS is a pretty safe method, but if you have (family) history of epilepsy, you are not allowed to participate
If you were to dump enough electricity in the brain, you will trigger an epilectic seizure

Motor treshold
Lowest TMS intensity (M1) needed to evoke MEPs in 50% of trials
Differs for e.g. healthy vs clinical populations (MS, stroke)
Configure the intensity for every individual (for example if their is hair in the way, …)
Tweak intensity so half of the pulses generate visible motor function (= motor threshold)
For phosphenes the logic is similar

Phosphene threshold
Lowest TMS intensity (V1, V5) needed to evoke phosphenes in 50% of trials
Differs for e.g. healthy vs clinical populations (migraine)

Frequency
Single pulse
M1 (MEPs), V1 (phosphenes)

Double pulse
Intracortical inhibition or facilitation; TMS interacts with neural learning
ISI-dependent effects: short (1-4ms) -> inhibition
1-4 ms is still within the refractory period (neurons need time to return to original state)
Long (7-20ms) -> facilitation

Paired pulse
Two pulses to two different brain regions using two coils
Examine functional connectivity between cortical areas.

Repetitive TMS
A train of TMS purses (same intensity) applied to a single brain region (1-20+ pulses per second).
Higher frequency / intensity -> more disruption of cortical function.
Long-term modulation of cortical excitability (inhibition or facilitation)

Timing
On-line TMS
Single pulse or repetitive
Applied during task performance
Effects last only while task is performed

Off-line TMS
Only repetitive TMS (< 1Hz = inhibition; > 1Hz = facilitation)
Applied before the task
Effect lasts beyond the period of stimulation.

Neuronal state
We know the stimulation interferes with neuronal activity.
But how are the underlying neural circuits affected by applying TMS?

Within-individual factors (Pasley et al.,2009)
Structure of the neural circuit
Neural activity during application
- Higher pre-TMS activity -> stronger TMS effect (Pasley et al. 2009)
- Less active neurons most strongly affected, more active neurons show saturation effects. (Perini et al. 2012)
- Noise-induction depends on TMS intensity, interacts with stimulus intensity
- Strong signal -> TMS has less effect
- Weak signal -> TMS has strong effect (neurons were already struggling)

Cell types (e.g., projecting vs. local neurons)
Induced changes in efficacy of synaptic connections

Between-individual factors
Healthy vs. patient populations

Difficult to isolate specific neural mechanisms underlying TMS effects

3. Experimental designs

MEPs
TMS over M1 -> muscle contractions
Amplitude of evoked potentials (measured with EMG) quantifies portico-spinal excitability.
Mirror neuron system
Single TMS pulse -> moving index or little finger in contralateral hand

Phosphenes
TMS over V1 -> light flashes
Multi-sensory integration
Measure phosphene threshold
Task: report if you see a flash of light, and where (left/right)
TMS (left/right) just below threshold x auditory stimulation (left/right)
Sound either at the left or right side
TMS stimulation in the auditory cortex
0: light and sound at same time

Visual awareness
Motion processing
How do V1 and V5 interact to create visual awareness for motion?
Feedforward: onset -> V1 -> v5
Feedback: V5 -> V1?
Onset -> V1 (40-60ms) -> V5 (60-80ms) -> V1 (80-100ms)

Neuronavigation: feed info in program

fMRI-guided TMS
Numerical cognition
Side effects of TMS: facial contractions

4. Clinical applications

Therapeutic uses
- Stroke

    - Aphasia
    - Motor disability
        - Rapid rTMS (> 1Hz) over affected hemisphere
        - Enhanced excitability
        - Increases in MEPs and improved finger motor task
- Tinnitus
- Anxiety disorders
    - Panic disorder
    - OCD
- Amyotrophic lateral sclerosis
- Multiple sclerosis
- Epilepsy
- Movement disorders
    - Parkinson's, Huntington's
    - Dystonia: muscle tone is too high, muscles stay contracted -> TMS inhibits contractions
        - Increase inhibition in M1
        - Slow rTMS (1Hz)
        - Improvement in motor co-ordination
- Schizophrenia
    - Shizoaffective disorder
    - Auditory hallucinations
        - "Voices" may be due to over activation of temporal regions (involved with speech)
        - Increase inhibition in temporal regions
        - Slow rTMS (1Hz)
        - Sustained reductions activity
- Addiction
- Major depressive disorder
    - In treatment-resistant patients:
        - High frequency rTMS of the left DLPFC (hypoactivity)
        - Low frequency rTMS of the right DLPFC (hyperactivity)
        - > try to balance out the difference between the two hemispheres
- Migraines
- Epilepsy
- Hemispatial neglect

In clinical uses mostly repetitive

Control group
Always need a control group (sham stimulation)!

Mechanisms
rTMS shows long-term effects (synaptic plasticity).
Multiple possible mechanisms:
- Direct targeting
    - > alter cortical excitability of dysfunctional area
- Distant effect
    - > alter upstream or downstream region's excitability

Usefullness
Effects are not permanent, but even temporary relief is relief.
Repair vs interaction models
- Repair: rTMS corrects an imbalance, an effect that wears off
- Interaction: rTMS support neuronal plasticity, helps the brain to restore function

5. Summary

Pros
- Allows causal interference
- Great temporal (ms) and spatial (mm) resolutions
- No long-term side effects
- Clinical applications

Cons
- Shallow stimulation (max 2 cm deep)
- Exclusion criteria for safety reasons
- Can be a little painful/annoying (e.g. twitching of face/neck muscles)

Remember!
- High number od degrees of freedom (stimulation timing and frequency, protocol) -> multiple comparison problem: try a bunch of different things and see what works -> bad practice
- Need a good theoretical a-priori hypothesis!

Transcranial current stimulation (tDCS/tACS)

1. tDCS basics

History
Roman Empire
Scribonius Largues described how placing a live torpedo fish over the scalp relieve headache in patients

18th century
Galvani invented DC battery (Galvanic battery)
Aldini (his nephew) used DC for clinical applications (major depression)

Today
Seminal studies of Priori et al. (1998) and Nitsche et al. (2003) paved the way to modern tDCS.

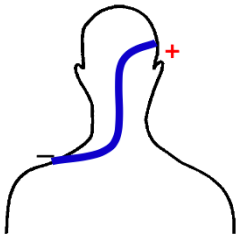
Principle
Apply constant, low direct current (1 to 2 mA) to scalp via electrodes
(De-)polarizes targeted brain regions)
2 electrodes + stimulation device

Polarity dependent modulation
Anodal (+) tDCS
- Increases cortical excitability
- Depolarizes resting membrane potential -> more spontaneous neuronal firing

Cathodal (-) tDCS
- Decreases cortical excitability
- Hyperpolarizes resting membrane potential -> less spontaneous firing

Nitsche et al. 2008

Extracephalic reference



Montage
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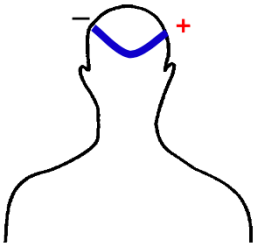
Bipolar montage (two active electrodes)



Procedure
On-line: During task
Off-line: Before task
Both is also possible

Related methods: tDCS, tACS, otDCS

2. tDCS clinical use

Effective in treating depressions (Nitsche et al., 2009)
- Chronic and acute pain
- Stroke rehabilitation
- Drug addiction

3. tDCS Experimental example

Does anodal tDCS enhance performance in a sequential-letter working-memory task?
tDCS over dlPFC (associated with WM)
N-back task (is letter the same as three letters back?)

Active -> fewer errors
Difficult to replicate
Each subject was tested during sham and active stimulation. The two tests runs were randomized within subject and the order (active versus sham stimulation) was counterbalanced across subjects. Fregni et al. (2009)
There was a significant difference in the mean number of errors between sham and active stimulation.

4. tDCS Controversies

No evidence of reliable effect of tDCS on cognition in healthy populations (59 studies review)
Only reliable effect: MEP amplitude modulation
MEP: motor evoked potential

5. tACS: Experimental example

Can we use tACS to manipulate rhythmic brain activity?
Rhythmic brain activity is important for stimulus processing.
Participants watch a flickering light.
Visual cortex activity synchronizes with the flicker frequency.
Difference between direct and alternating current
Alternating: switches the polarity (+/-) with a specific frequency
Rithmic brain activity is important for stimulus processing
Alternating stimulation current while participants watch flickering lights
Flickering (5Hz) induces rhythm and tACS (5Hz) induces rhythm -> stronger effect
Manipulate synchrony between flicker and tACS stimulation
Simultaneous: strong neural response
The more in synch: the stronger the response

Summary: tDCS/tACS pros and cons
Pros
- Easy to apply
- Cheap
- Easy sham condition
- Less “adverse” effects

Cons
- tDCS: low spatial and temporal resolution
- tACS: low spatial resolution
- Questionnable efficacy in healthy population
- Exclusion criteria

Introduction to neuroimaging: eye tracking

A brief history of eye tracking

Louis Émile Javal (late 19th century)
- ‘naked eye’ observations
- Noticed that eye movements are not fluent while reading
  - When you read it’s not like a spotlight following someone, but chaotic jumps
  - Alternation between ‘jumps’ (saccades) and ‘pauses’ (fixations)
- Edmund Huey (1908)
  - “The Psychology and Pedagogy of Reading”
  - First mechanical eye movement tracking: cup was placed on eyeball, linked to pencil

Guy Thomas Buswel (1937)
- First researcher to use reflection of light beams in the eye + recordings
- Still what we use today as eye-tracker

Alfred Yarbus (1950s-1960s)
- System with camera’s and mirrors
  - (In addition to eye suction cups)
- Eye tracking breakthrough for psychology

Modern age eye tracking: How does it work?

Eyetracking shines infra-red light, bounces back differently depending on how the eye is positioned
We can't see infrared light
Infrared bounces of cornea
Corneal reflection is on opposite of what side particpants are looking at
Calibration procedure: look at sides and corners and middle of screen -> computer tracks distance and angle between reflection and pupil -> computer can calculate what person is looking at

High spatial resolution
- Degrees of visual angle
- 0.25° - 0.5°
- In practice eye tracking is much more noise

High temporal resolution
- 1000 Hz - 2000 Hz
- 1000 Hz is sufficient for eye tracking

Monocular or binocular
Mostly one eye that is tracked (only not in for example dyslexia because there is a hypothesis that the fixation is not simultaneous for dyslexic people)

Types of eye trackers

3 types of eye trackers
Table mounted - head fixed in head and chin rest
Temporal resulotion is high -> check what brain activity was at that point
More accurate (1000-2000 Hz), but less flexible

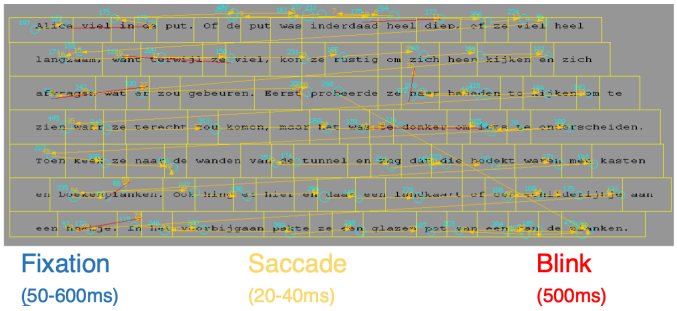
Table mounted - head free

Head mounted
Less accurate (50-500 Hz), but more flexible

Combination with other imaging techniques possible: fMRI, EEG
E.g., identification of eye-movement artifacts; accurate timing of visual inspection of stimuli; ...

Eye tracking measures

Output
Blue circles are fixation
4 saccades per second (virtually blind, too fast for the brain to process)



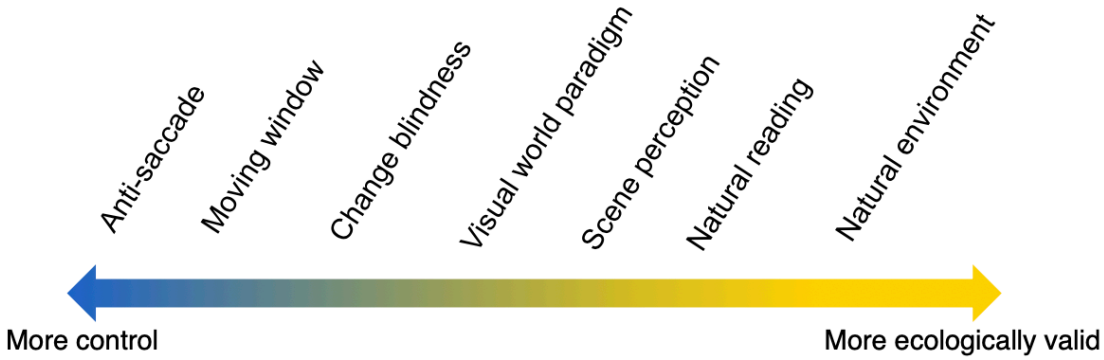
Quantitative measures
Times measures
- First fixation duration
- Total reading time

Probabilistic measures
- Skipping probability
- Regression probability

Other measures
- Saccadic amplitude
- Number of regressions
- Number of fixations
- Pupil size
- Proportions (fixations, fixation time, …)
- Path (1st - 2nd - 3d - … fixation)
- …

Qualitative representations: spots were participants was looking

Eye tracking paradigms



Anti-saccade task
Used in research on inhibition (executive functions)
How much erroneous prosaccades are made in anti saccade trials?
Prosaccade: make a saccade in the same direction of the arrow
Antisaccade: other direction
We are very prone to following arrows
About 20% Erroneous prosaccades (Mokler & Fischer, 1999)
- Of which on average 50% goes undetected! (very automatic, involuntary)

Competition between voluntary antisaccade and involuntary pro saccade
- Nowadays for example used in research on psychiatric / neurological disorders

Moving window paradigm
Used in research in visual attention
- How much information can be processed simultaneously: the perceptual span
  - Theres a maximum amount of letters we can process simultaneously
- Text is masked except for gaze-contingent “visible box”
  - Size of the box can be varied
- How does the reading pattern change by varying the size of the visible box?
  - E.g. saccadic amplitude, fixation duration, number of fixations, etc.

Perceptual span in western languages: about 13-15 characters to the right; 3-4 to the left (McConkie & Rayner, 1975; Rayner 1998)
Related paradigms
- Moving mask
- Invisible boundary

Change detection
Paradigm in domain of visual attention (e.g., McConkie & Currie,1996; Rensink, 2002)
Participants have to look at a scene and detect what is changing by looking at its position for a certain amount of time (e.g. 1)
-> How long does it take participants to detect the change?
Original paradigm: induce change during saccades
- “Blindness” during ± 40 ms, 3-4 times per second
- Participants indeed wouldn’t notice these changes

Determine when someone made a saccade -> match change to saccade
New paradigm: flash when change is induced
- ‘Simulate’ the temporal blindness of a saccade

Also in the new change detection paradigm, participants were often blind to the changes
- Inspection of the images up to 1 min without detecting the changes!

Important illustration of a constraint due to selective attention
- We do not perceive everything that is going on in our direct environment

Visual word paradigm
Paradigm used in auditory perception (e.g., Altmann & Kamide, 1999)
“The boy will move the cake” vs “The boy will eat the cake”
What is the proportion of fixations on each of the objects?
Proportion of fixation to the cake in comparison to the other object

When verb is enunciated there is already a large difference
Listeners can predict upcoming information from previous information / context

Scene perception
Paradigm used in field of visual attention and action control: where do we look at in a scene? (e.g., Yarbus, 1967, Henderson & Ferreira, 2013)
- Are eye movements random? Or mainly driven by bottom-up processes?
- Variable of interest: fixation locations
- Dependent on nature of action (e.g., Castelhano & Henderson, 2009
  - Memorization task vs visual search task

More ecologically valid
How old? -> people look exclusively at the faces
Asked to remember the clothes -> looking at the clothes

Natural reading
Paradigm for example used in written language comprehension and more specifically visual word recognition
- Participants simply read what is presented to them (e.g., Cop, Drieghe, & Duyck, 2015), while eyes are tracked

What does the reading pattern look like?
- Multitude of possible independent variables
  - E.g. language, word length, word frequency, predictability, etc.
  - Read slower in second language than first language
- Dependent: number of fixations, fixation durations, regressions, saccades, …

Informative of word recognition processes:
- Shorter first fixation / less skipping
  - > Faster lexical acces
- Shorter total reading time / less regressions
  - > easier integration of the word in sentence

Complex sentences have more regressions
Example: word frequency effect
High frequency words have shorter fixation times
Difference becomes smaller when you are more proficient in a language

Natural environment
Participants perform actions as they occur in daily life
Widely applicable paradigm for many research purposes
- Shopper research (e.g., Wästlund et al., 2015)
  - Does the arrangement of groceries / lighting / price / location / … influence shopping behavior?
  - Fixation location and duration of interest
  - Are people looking longer at expensive/cheap products?
  - People look mostly at products on eye-level

Widely applicable paradigm for many research purposes
- Alertness while driving and calling handsfree (Desmet & Diependaele, 2017)
- During hands-free calling you are looking a lot less at your own speed, road signs, other cars, the road, …

How to?

Programming
- Most developers of eye trackers have their own software package (e.g., experiment builder by SR Research)
- Python-based coding (e.g., Pylink for eye-link)

You can use eye movements as triggers

Data collection
Difficulty: this type of program already determines what a fixation and what a saccade is (recording at 1000 Hz)
You can do this yourself, but you have to determine your own rules (speed, …)

Data processing
- Most developers of eye trackers have their own software package (e.g., dataviewer by SR Research)
- Packages in R(studio) / Python / …

Data analysis
Up to you! Depends on …
- Research question
- Eye-tracking variables of interest
  - Quantitative vs qualitative
  - Timed / probabilistic / …
- Personal preference for software package
- Current state-of-the-art analytical technique
- …

The downside

Eye tracking: downside
- Expensive: tens of thousands of euros
- Mobile devices are less accurate
- Participants with glasses or lenses often can't participate
  - Cut-off around ± 3 diopters
- Very time consuming (1 participant at a time)
- Many sources of 'noise'
  - Head movements
  - Make-up
  - Shape/color of glasses
  - Eye conditions (e.g., astigmatism, nystagmus, when the eyes keep drifting off, ...)

Eye tracking determines what the pupil is by determining what is the darkest part -> participants cannot wear make-up

The upside

Eye tracking: upside
Eye tracking is a widely applicable technique
- Can be used in many fields of social sciences (and beyond)

Eye tracking data is (often) easy interpretable
- Dozens of dependent measures can be studies

Suitable for paradigms that (closely) resemble daily life situations
- ! Converging evidence

Largest advantage: close to daily life situations (more ecologically valid paradigms)
They read more or less the same way they would do at home

EEG

1. Introduction

a. What it is

Interference: how invasive the technique is
EEG very precise temporally without being invasive (biggest advantages)

b. How it started

Started in 19th century, not with human participants (very invasive)
1875
Richard Caton: Electrical impulses from the rabbit and monkey brain
1890
Adolf Beck: Spontaneous electrical activity of the brain and rhythmic oscillations
Napoleon Cybuslki: Cortical activity in response to peripheral-nerve stimulation in dogs and monkeys
Beck & Cybulski collected a lot of knowledge
1914
Sabina Jeleńska-Macieszyna: first recording of epileptic seizures (in a dog), experimentally induced epileptic seizures from a dog's cortex
1924

Hans Berger: recording the electrical activity of the human brain from the surface of the head
Alpha wave: 'Berger' wave
1964
How to non-invasively record this in humans?
Hans Berger was first one to record from human subjects (often with his children)
First one to describe the rhythm of the brain waves, mostly interested in the alpha wave
He was very skeptical of his own findings, took 5 years to publish his first paper
Took years after someone replicated his study (field was also very skeptical)
William Grey Walter: described first ERP (event-related potential)
Contingent Negative Variation: An Electric Sign Of Sensorimotor Association And Expectancy In The Human Brain
Now we have very detailed knowledge of ERP's and oscillations
EEG is often used now to help with epileptic seizures

2. Neural basis of EEG: how it works

EEG reflectie voltages (mostly) generated by excitatory postsynaptic potentials from neocortical pyramidal cells.
Postsynaptic potentials
- Affect local ion concentrations surrounding the cell

Dipoles
- A difference of charge between two parts of the neuron

Summation
- Synchronous dipoles from aligned neurons summate

Scalp distribution
- Dipole potentials spread to scalp

EEG waveforms
- Electrodes measure changes in scalp voltage over time

Action potentials
- Building blocks of electrical signaling between neurons
- Rapid, transient, and all-or-none positive spikes in voltage that flow from the body to the axon terminal of a neuron
- Axcitatory
- NOT picked up by EEG

Post-synaptic potentials
- Long-lasting, slower post-synaptic potentials
- Graded responses, varying in amplitude depending in the strength of the signal
- Slower timing and larger span of post-synaptic potential is easier to detect with EEG

1. When an action potential reaches the axon terminal the neuron releases a neurotransmitter
2. The neurotransmitter binds to the receptor
3. The postsynaptic neuron gets depolarized (excitatory postsynaptic potential, EPSP) or hyperpolarised (inhibitory PSP, IPSP)
4. EPSP and IPSP summate temporally and spatially
5. If the postsynaptic neuron reaches a given depolarization threshold, an action potential is generated.

Dipoles
- Separation of charge over (small) distance
- Fundamental to measuring and recording activity

1. Excitatory signal causes depolarization of dendrites - an influx of sodium (Na+) ions into the cell
2. Local reduction in Na+ concentration - detected as a relatively more negative extracellular voltage
3. Extracellular voltage at the other end of the neuron becomes relatively more positive
4. Pair of equal and oppositely charged poles - a conduction medium for a dipole
5. Current flows from source (+) to sink (-)

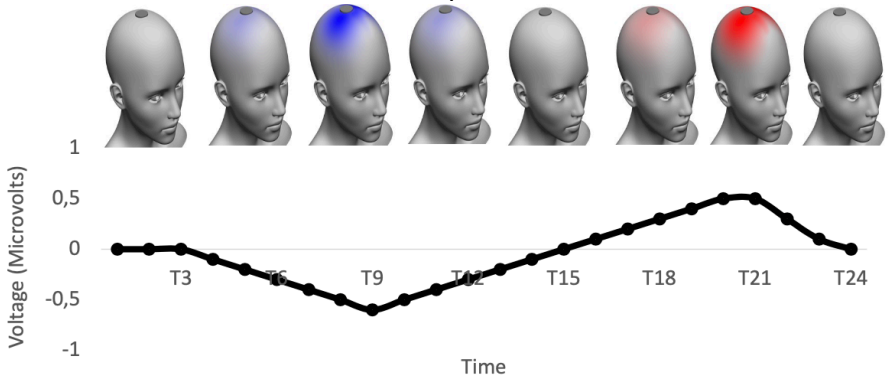
Dipoles: summation and orientation
- Electrodes measure the som of positive/negative charges in their vicinity

- Depending on the position of the neuron relative to the scalp, different changes in voltage will ben recorded

Radial dipole: perpendicular to the skull
Tangential dipole: parallel

Summation of dipoles
- Pyramidal neurons are ideal current generators:
  - Spatially aligned
  - Perpendicular to the cortical surface
  - They receive similar input (usually fire in synch)
- Many neurons (10 000 - 50 000) need to sum their activity to be detectable by EEG
- Dipoles need to be:
  - Arranged in parallel: orientation is crucial
  - Synchronously active: timing is crucial
- Summation of many such dipoles: can be modelled as one strong equivalent current dipole with three parameters:
  - Location
  - Orientation
  - Strength

Scalp distribution and EEG waveforms
This simplified model shows how a single electrode track changes in scalp voltage over time as the underlying brain activity changes.
The strength and polarity of the signal at the electrode depends on the:
- Orientation of the dipole
- Electrode location
- Electrode's distance from the dipole



What do we measure?
Scalp EEG signal is the result of the summation of excitatory and inhibitory PSPs at the dendrites of neurons in the cortex
We can measure:
- The synchronous activity of PSPs in (pyramidal) cells in the cortex (consisting of thousands of neurons)
- Excellent temporal resolution: can measure neural dynamics of cognitive processes in real time (vs. fMRI)
- Direct neural activity (vs. fMRI)

We can't measure:
- Action potentials
- Single neuronal events
- Any neuronal activity that is asynchronous (out of sync)
- Any dipole activity that is not organized in parallel, signals will cancel each other
- Activity in subcortical and deep brain structures (e.g., thalamus, basal ganglia, brainstem)

3. EEG data collection

How to do it
EEG measurements
- Voltage: the potential for current to move from one place to another

- EEG signal: a potential for current to pass between two electrodes
- Electrode: a metal disc that forms an electrical connection with a scalp
- Differential amplifiers: active electrode (A), ground electrode (G), and a reference electrode (R)
  - Ground - a common reference point for all voltages in the system
  - Voltage differences between A and R are recorded
  - Choice or 'neutral' R is important
    - Nose
    - Mastoid (left/right)
    - Earlobe (left/right)
    - Re-referencing offline

EEG setup
- EEG cap: different number of electrodes (32, 64, 128)
  - At least 32 electrodes, more common 64
  - Each has a specific location
  - Central electrode is on the vertex (center point of the head)
- Different electrode system montages:
  - 10-20 system is common (Jasper, 1958)
- Amplifier connected to recording PC and software

EEG recording: noise reduction
- Tiny voltage fluctiations of scalp EEG: amplification needed
- Electrical equipment in the laboratory: source of induced noise
  - Shielding is important: Faraday cage
  - AC line noise: 50/60 Hz needs to be filtered out
- Impedance: overall impediment to current flow measure in Ohms
  - Needs to be as low as possible <5kΩ
  - Increasing contact with the skin
- Physiological/muscle artefacts: need to be reduced
  - Heart potentials: electrocardiogram (ECG)
  - Blinks: eye-movement: horizontal and vertical oculogral (HEOG/VEOG)

Important to do recording when participant is as relaxed as possible
"Please, try not to blink excessively, relax, do not move your eyes or head..."
Especially when filtering low frequency ERPs you have to be careful

EEG recording
- EES is recorded continuously, in blocks
- Sampling rate: every 1/2/4 milliseconds (1000 Hz / 500 Hz / 250 Hz)
- 'Triggers'/markers of relevant events (e.g. stimulus on the screen/response)
- Combination of cortical activity, noises and physiological/muscle activity

Scale: microvolts (remember, this is a relative value to the reference)

EEG data

A. Event-related potentials (ERP)
- Stimulus-evoked changes in cortical activity, time-locked to stimulus
- Event-related potentials (Vaughan, 1969)
- "Reaction Tim" for 21st century: Reveals timing and organization of cognitive processes

B. Oscillations
- Alpha oscillations, but also beta, theta, delta
- "Rhythms" of the brain: repetitive patterns of brain activity
- How do cognitive states (wakefulness, attention) change the pattern of rhythmic activity?

Experimental design

How many trials?
- It depends on signal-to-noise ratio, which increases as a square root of number of trials
- No magic number, but ~50 trials is a good standard (some ERPs can be observed with 20 trials)

Inter-trial interval
- Events of interest should be sufficiently spaced (several hundreds milliseconds)
- This also depends on which components and frequencies you plan to analyse
- Stimulus offset also produces signal, so take this into account

How many electrodes?
- It depends on the purpose. If you plan to perform source reconstruction >100
- For ERP, common standard is 64 (unless there are other constraints, e.g., time)

Triggers
- Square-wave pulses sent from stimulus-computer to the EEG amplifier, recorded as a separate channel in the raw data. Amplitude of the pulse code for event-type
- Triggers are fundamental in the analysis, as they will be used to time-lock data offline
- Better to have triggers for everything that's important, i.e. condition, event, correct/incorrect response (It saves time later on)

Sampling Rate
- Nyquist theorem: at least twice the highest frequency we want to analyse
- In general 500-2000 Hz will be good for any analysis

4a. Event-related potentials (ERPs)

Data processing steps

1. Filtering (optional)
- Helps to clean out low (high-pass filter) and high (low-pass) frequency noise
- We need to be careful with filtering, as it can also introduce artefacts

2. Re-referencing (optional)
- Electric potentials are only defined with respect to a reference.
- Usually the linked mastoids, or an average of all scalp electrode

3. Segmentation/'epoching'
- Dividing up the continuous recording into epochs of interest
- Usually contains the trial length(stimulus and response), and a baseline period before the stimulus

4. Baseline correction
- Accounts for relative voltage differences between trails and conditions, by removing any slow 'drifts' in the data during recording

5. Artefact rejection/correction
- Identifying trials that are contaminated by artifacts
- Either based on a visual inspection of data (time-consuming, subjective) or automated procedure (e.g. treshold, spectral decomposition, kurtosis … depending on criteria, may miss out on noisy data)
- Trial rejected and/or correction of signal (eye-blink correction, Independent Component Analysis, ICA)
- Hansen's Axiom: There is no substitute for clean data!

6. Averaging

Averaging across epochs with identical events to increase signal-to-noise ratio
- Sometimes: averaging across 'clusters' of adjacent electrodes
- Averaging across participants: grandaverage

For all preprocessing steps, you can use different software: EEGlab, FieldTrip (MATLAB), MNE (Python)

- ERP waveform = changes in scalp-recorded voltage over time
- ERP peak = reliable local positive or negative maximum in the observed ERP waveform (not noise)
- ERP component = scalp-recorded voltage change, which reflects a neural or psychological process
- ERP components are typically labelled by their:
  - Polarity (negative or positive)
  - Peak latency (P100, N200) or order (P1, N1)
- Example: P1/P100 component is the first positive deflection
- Latency ~100 ms
- Amplitude: larger for attended/cued vs. unattended stimulus/uncued
  - Smaller alpha oscillation on the side contralateral to the stimuli

Data visualization
- Time domain plot: shows how electrical potentials change over time on an individual electrode or set of averaged electrodes
- NOTE: Positive polarity can be plotted up or down.

- Topographical map: shows the distribution of electric potential over the scalp at a particular (period of) time

Types of ERP waveform
1. The electrode site
2. Event of interest
3. Stimulus modality

Example: Mismatch negativity (MMN)
Deviant - Standard: response in the brain to the change in stimuli and/or task

Other examples:
P1:
- Sensory component, elicited by visual stimuli (regardless of task)
- Strongly influenced by the stimulus parameters (e.g. luminance)
- Also influenced by attention: larger amplitude for cued/valid vs uncued/invalid stimuli

P3:
- Context-dependent: associated with attention allocation (higher-level cognitive process)
- Observed in oddball and reward paradigms
- Larger for targets vs. non-target stimuli

Error-related negativity (ERN):
- Being aware of an error
- May reflect activity of a response or conflict monitoring system

ERP changes in psychiatric disorders:
- Disorders of consciousness (MMN)
- Alcohol (N1, P2, N2, P3, …)
- Schizophrenia (N1, P2, N2, P3, MMN)
- Bipolar Disorder (P50, P3)
- Depression (P3)
- Phobia (P3)
- Panic disorder (P3)
- Generalized anxiety disorder (P3)
- OCD (P3, N2, ERN)
- Posttraumatic stress disorder (P50, P3)
- Dissociative disorder (P3)
- Personality disorder (N2, P3)

Statistical analysis
Lots of decision to make!
- Which component?
- Which time window?
- Which electrode of interest (or ROI)?
  - Literature/past research
  - “Neutral” or baseline condition (indépendant of your manipulation)

= Many degrees of freedom!
Need a solid hypothesis before making these choices OR use ‘data-driven’ methods

Statistics
- What is your dependent measure? ERP amplitude, latency, electrode site/cluster (ROI)…
  - Extract values from the ERP, then use appropriate statistics (e.g., t-test, ANOVA) to answer your question (e.g., group/task differences)
  - Need to correct for multiple comparisons if needed!
    - Most common: Bonferroni (divide p-value by the number of tests performed..
- Exploratory hypothesis without a-priori hypotheses:
- Cluster-based permutation test

Neural sources of ERP
- Volume conduction: recording electrical potentials at a distance from their source generator
- Spatial smearing: Instantaneous linear mixing of signals

- ERP signals contain multiple sources of neural activity that overlap in time.
- Amplitude and latency of ERP components may not perfectly reflect amplitude and latency of any one neural source.
- Topography maps reflect scalp voltage distributions, not brain locations
- Forward problem: We can predict the ERP waveform if we know the number, orientation, timing and strength of the neural sources/dipoles
- Inverse problem: An infinite number of latent/unobserved neural source configurations can produce the same observed/measured ERP wave
  - undetermined & ill-posed problem
- Solution 1: Well-designed experiments that systematically compare conditions allow some inferences about neural sources and cognitive associations
- Solution 2: Source reconstruction methods

Source reconstruction in a nutshell
Forward model: a well-defined, sensible solution to the forward problem constrains a solution to the inverse problem
1. head model: how the electric currents generated at the neural source spread through the volume conductor (head, borders between head and skull, skin and skull)
2. sensor description: exact location of sensors
3. source mode l: where are the neural sources
4. a lead field: weight matrix of how the sources and sensors connect to each other (this 'sums up' the points 1., 2., and 3.

It is helpful/desirable to perform:
- A structural MRI is helpful/desirable in forward modeling
- Digitization of electrode locations

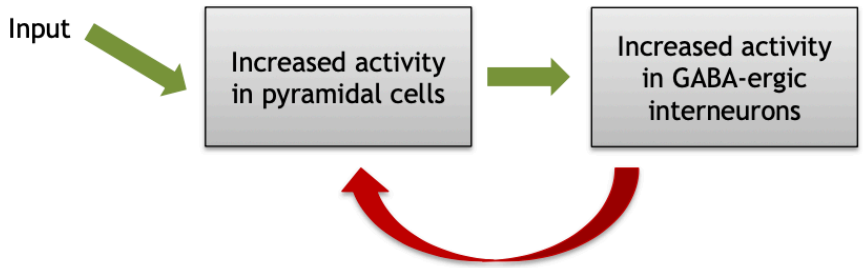
Source reconstruction in a nutshell
Solving the inverse problem: estimating the sources (dipoles) using forward model in combination with the actual EEG data.
Many different modelling techniques/solutions available:
- Equivalent current dipole modelling assumes a relatively small, fixed amount of dipoles:
  - Brain Electric Source Analysis (BESA)
- Distributed source methods
- Minimum Norm Estimate
- Low-Resolution Electromagnetic Tomography, LORETA

Essentially, all models are wrong, but some are useful. (G. Box, 1987)

4b. Oscillations

Classified according to the frequency of the oscillations
3 cycles per second: 3Hz

Neurophysiological basis:



Interneurons inhibit pyramidal cells.
As a consequence, activity in interneurons also decreases, and pyramidal cells' activity will increase again

Oscillations are characterized by their:
- Frequency: how fast?
- Phase: when?

- Power: how strong?

Brain rhythms are grouped into bands:
- Gamma (lower 30-80 Hz, upper 80-150 Hz)
- Beta 15-30 Hz
- Alpha 8-12 Hz
- Theta 4-8 Hz
- Delta 2-4 Hz

There are no precise boundaries defining the bands (theta might be referred to as 3-9 or 47 Hz).
Cognitive processes are associated with rhythms between 2 Hz and 150 Hz
- Note: gamma frequencies (30-80 Hz) are heavily attenuated in EEG, overshadowed by artefacts

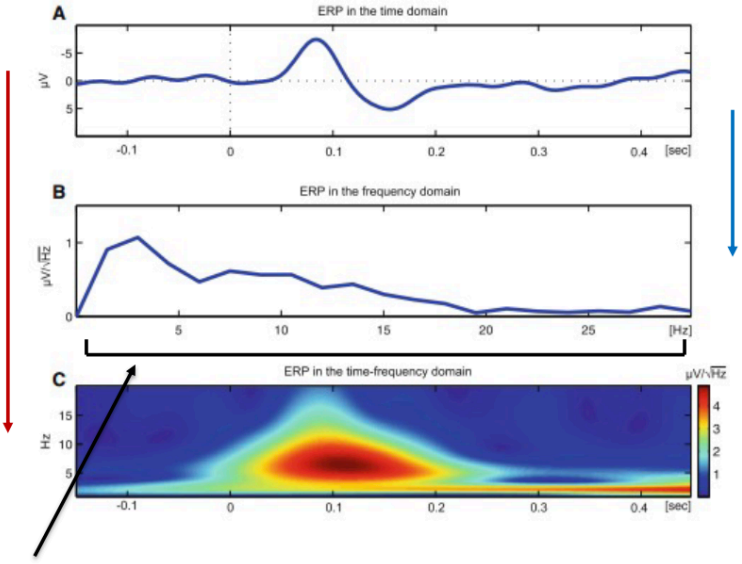
Basic time-frequency analysis:
1. Fourier series and transformation
- A Fourier series is a way to represent a wave-like function as the sum (linear combination) of simple waves.
- Decompose any periodic (repeating) signal into the sum of a set of sines and cosines.
- Can be represented in plots of frequency (x-axis) by amplitude (y-axis) = signal is now transformed from the time domain into the frequency domain
- requires the signal be stationary
- frequency structure hard to visualise over time

2. Wavelets

A wavelet is a complex sine wave that has been multiplied by a Gaussian (bell-shaped) distribution.
This wavelet is then convolved with the EEG data:
- It is slid along the EEG data to look at when and to what extent the data contain features that look like the wavelet.
- Repeated over the same EEG data by using wavelets of different frequencies
  - > time-frequency representation is formed

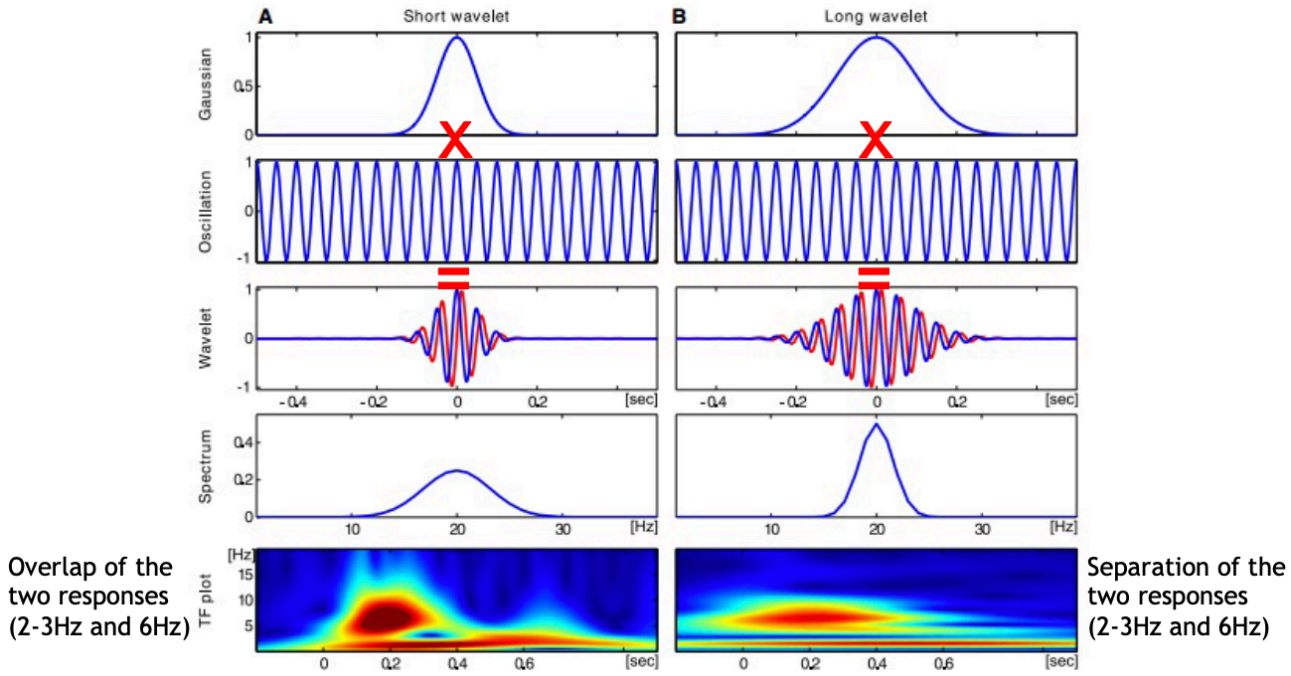
A quick re-cap
Use wavelet transformation to reveal the temporal evolution of components.
- Peaks at 6Hz as well as 2-3Hz.
- 6Hz peaks at 0.1s after stimulus onset (very short duration).

Use Fourier transformation to transform the signal into the frequency domain.
- Notice the peak around 2-3Hz.
- No info on how frequency components evolve over time.



Frequency spectrum was computed for one second. 6Hz duration too quick to be picked up; however, the 2-3Hz frequency has a longer duration, therefore can be observed.

Summary of wavelet analyses.



Short:
- Low frequency resolution
- High time resolution

Long:
- High frequency resolution
- Low time resolution

Example: Alpha oscillations
- Alpha oscillations decreased/desynchronized contralateral to cue direction
  - Desynchronized in preparation for stimuli (inhibition)
- functionally similar to P1 component
- Occipital electrode sites

5. ERPs vs Time-frequency

ERPs
- Easy to compute. Relatively few assumptions in the analysis.
- High temporal precision and accuracy (little or no filtering, ms precision).
- Extensive literature.
- Easy to look at the data (also for sanity check purposes).

Limitations:
- ERPs do not show non-phase locked activity (null result difficult to interpret).
- Non-linear neural activity patterns such as interregional synchronisation and cross-frequency coupling cannot be captured by ERPs.

Time-frequency (oscillations)
- Can be interpreted in terms of neural oscillations (well known neurophysiological bases).
- Can be linked to evidence from other species and methods (in vitro, in vivo, single cell in animals, intracranial recordings in humans, EEG, MEG).
- Extra information (on top of space and time).

Limitations:
- Time-frequency decomposition results in a decrease of temporal precision (especially at low frequency bands).
- Literature linking oscillations to congitive processes is growing (even booming) but still relatively limited as compared to ERPs

It depends on your hypothesis!

6. Summary: EEG pros and cons

Pros

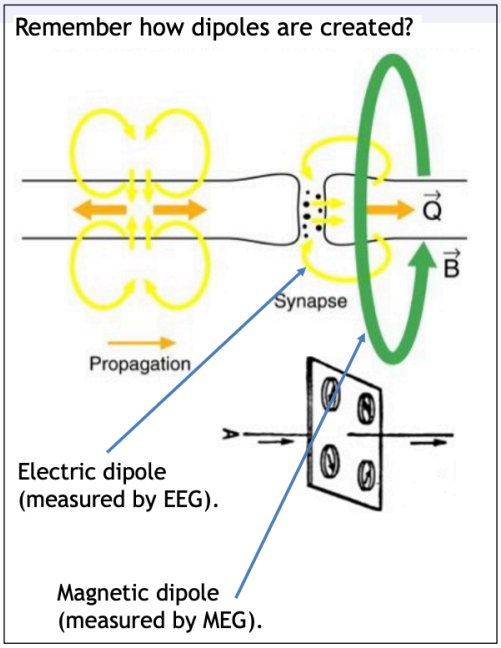
- EEG is cheap, non-invasive, has little effect on subjects' perception and behaviour.
- High temporal resolution enables assessing the temporal dynamics of cognitive processes non-invasively (same goes for MEG, but that's much more expensive) .
- Direct measure of neural activity (fMRI BOLD is indirect).
- Multidimensional (time, space, frequency, power).

Cons
- Low spatial resolution (volume conduction and inverse problem).
  - Impossible to localise with certainty and precision the real dipoles based on a certain scalp distribution.

Remember!
- High number of degrees of freedom (which components, frequencies, windows, electrodes?) -> leads to the multiple comparison problem.
- Needs a good theoretical a-priori hypothesis!

7. Magnetoencaphalography (MEG)

What is it?
- A technique that measures the magnetic fields naturally generated by neuronal sources in the brain.
- Because these neuromagnetic signals are so small, MEG needs special equipment:
  - requires ' superconducting quantum interference devices' (SQUIDs).
  - these SQUIDs are bathed in a large liquid helium cooling unit (-269°C).
  - because of the low impedance at this temperature, SQUIDs can now detect and amplify magnetic fields generated by neurons a few centimetres away from the sensors.
- First measured by David Cohen in 1968 (without SQUIDS)
- Squids described in 1972 (D. Cohen in Science)
- Excellent temporal resolution.



How does it work?
Connection between electricity and magnetism first discovered by Orsted in 1820.
Electric current always generates a magnetic field.
Around 50,000 active neurons are needed to produce a detectable signal:
- pyramidal cells (similar orientations).

Advantages of using MEG
- Better spatial resolution - little skull/scalp effects.
- With most systems, it is possible to simultaneously record EEG.
- Participant preparation time reduced:
  - Sensors do not need to have direct contact to skin.
  - Supine or seated positioning.
- But MEG can't pick up radial dipoles

2 types of sensors:
- Magnetometer: magnetic field
- Gradiometer: gradient of the magnetic field

8. EEG vs. MEG

EEG
- Large signal (10mV).
- Sensitive to tangential and radial dipoles (neurons in sulci and on gyri).
- Signal distorted by skull/scalp:
- Limited spatial resolution.
- Spatial localisation ~1cm.
- Allows subject movement.
- Needs sensors to be directly attached to the head.

- Reference problem.
- Cheap and mobile.

MEG
- Tiny signal (10fT).
- Sensitive mostly to tangential dipoles (neurons in sulci).
- Signal unaffected by skull/scalp:
- Good spatial resolution.
- Spatial localisation ~1mm.
- Subjects must be still.
- Sensors in helmet (no direct attachment).
- Reference free.
- Very expensive.

In general, both have:
- Great temporal resolution (~1 ms)
- The inverse problem (Note: less signal attenuation by skull/scalp)
- Non-brain tissues/structures that can also affect the signal (eyes, heartbeat, muscles …).

fMRI basics

Before we begin …

X ray was first attempt at looking at the brain
Discovered on accident (accidentally photographed his wife's hand)
Pneumoencephalography: bubble of air in the bloodstream would travel to for example an infarct (but very painful)
Diffusion weighted imaging is made to look at white matter of the brain

How can we look at the function of the brain?
Positron mission tomography (PET)
Positron emission: Measuring radioactive decay with radioactive labelers
Attach radioactive material to glucose/dopamine molecule, this will move through the brain with the blood stream
fMRI has become a popular measure of brain activity, only over the last 20 years

Some terminology …

Image acquisition and analysis. We used a 1.5-T Siemens Sonata MRI scanner to acquire gradient-echo, T^*_2-weighted echo-planar images with blood-oxygen-level-dependent contrast. An additional T_1-weighted structural image was acquired for each subject.
Axial: if you look from the top
Coronal: if you look from the front (the face)
Sagittal: if you look from the side

How does it work?

How does the scanner work?
- Two ingredients:
  - Electromagnetic fields
  - Earth = 25 to 65 microtesla (μT)
    - Scanner = 1.5 to 7 tesla (= 1.500.000 to 7.000000 μT)
  - Electromagnetic (radio frequency) waves
- So what's all this other stuff then?
  - Mostly cooling for the scanner
    - The scanner uses an enormous amount of energy
    - > heats up: all would melt without the helium

Three components:
- Magnet
  - Very very long coil of wire
    - Superconductive (almost no resistance)
    - Current passes through
    - > creates a magnetic field
- Radio frequency coil (RF coil)

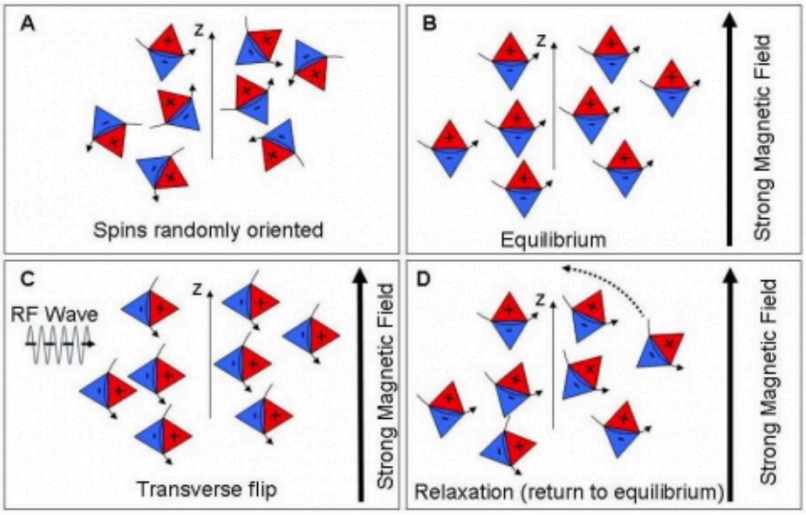
- Gradient coils

The magnet's main function:
- Magnetic field lines pass through bore of the scanner:
- Attracts ferromagnetic materials
- Also effects on molecules …
- Atoms with an uneven number of protons act as dipoles (like little magnets, with poles)
- Main magnetic field direction = B_0 direction

A lot of water in our body: H20, water molecules each have one proton and one electron
-> north and south pole
In scanner all protons line up

Radio frequency coil:
- The "antenna" of the MRI system:
  - Transmitting and detaching radio frequency waves
  - Reason why scanners are placed in Faraday cages
  - On top of the head

Protons spin at a slightly different speed



T1 time
- This time to get back in alignment with its surroundings (or "the lattice") is called the T1 time or spin-lattice relaxation time

T2 time
- Some protons start spinning a little earlier, some a little later, so after the 90° pulse, they start spreading out or dephasing (losing of phase alignment)
  - > the signal loses its "coherence"
- The time it takes to de phase is called T2 time or spin-spin relaxation time

T1 versus T2
In fatty substance T1 and T2 are fairly quick compared to water
Frequency is determined by the strength of the magnetic field

Recap:
- T1 time: how fast do nuclei align with the main magnetic field (slow, longitudinal relaxation)
- T2 time: how soon do nuclei fall out of phase and release energy (fast, transverse relaxation)

How does it work?
- Protons have an alignment and spin speed of the spin is related to the strength of the magnetic field)
- Radio frequency energy is being put in that matches the speed at which the proton spins (Larmor frequency: e.g., 3T = 127.7Mhz)

How do we get the T1 and T2 from different places?

- The Larmor frequency is determined by the strengthof the local magnetic field
  - 63.9 MHz at 1.5 Tesla
  - 127.7 MHz at 3 Tesla
- (!) If the strength of the magnetic field can be varied across locations, the spins will vary with it and different RF sensitivities can be expected

Vary the strength of the magnetic field -> You can vary the frequency

Gradient magnets
- Creates yet different varying magnetic fields:
- X - from left to right
- Y - from top to bottom
- Z - from head to toe

XYZ can vary in a magnetic field, only protons in XYZ will be affected by spinning
Protons will move in the transverse plane and start spinning, some faster, some slower

How do we get the T1 and T2 from different places?
- Frequency encoding
- Phase encoding

Gradient echo:
A rephasing gradient is applied (opposite in polarity to the dephasing gradient).
This reverses the phase shifts induced by the dephasing gradient
Extra gradient: try to get the proteins in the alignment
T2*: the difference between the theoretically perfectly aligned protons and the realignment of the protons during echo-time

Spin echo: Protons that were slower will start to spin faster -> catching up

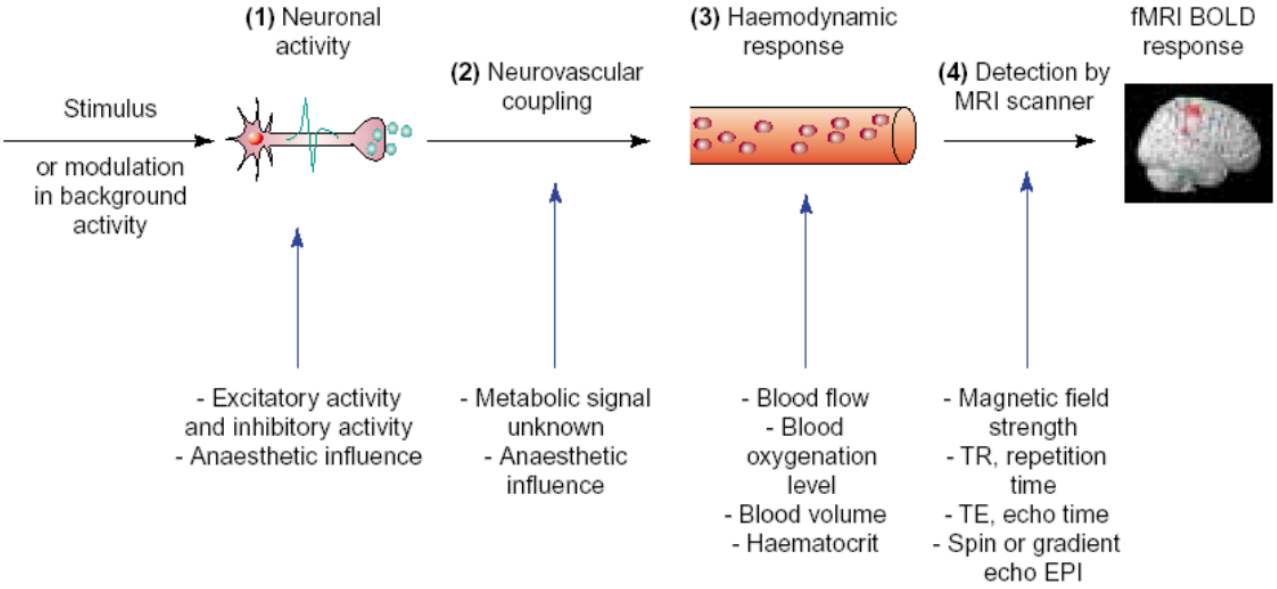
Where do these microscopic distortions that affect the rephrasing of protons come fromN
- Iron atoms can distort the magnetic field …
- Iron has slightly different magnetic properties depending or not wether it is bound (OxyHb) or unbound (DeoxyHb) to oxygen
- DeoxyHb: more distortions

BOLD signal (Blood-Oxygenation Level-Dependent signal)
- The BOLD signal takes advantage of the difference in T2* between oxygenated and deoxygenated hemoglobin
  DeOxyHb: decrease in signal; OxyHb: increase in signal
- Different brain states are associated with regional changes in the brain's oxygen utilization

Neurovascular coupling: the relationship between local neural activity and subsequent changes in cerebral blood flow
- fMRI signal (red) corresponds most closely to local fuel potentials (blue)

Hemodynamic response function: delayed peak

Recap
- Magnetic field alligns H_20 protons in direction with B_0
- An RF signal consistent with the Larmor frequency is sent
- The 90°pulse causes them to deflect
- Next, RF activity is measured:
  - T1 time: time to relax back in alignment with the B_0
  - T2 time: time it takes to dephase
  - > both are tissue-dependent!
- A gradient echo pulse causes the reverses the phase shift of the protons
  - T2* is the component that does not refocus: little microscopic distortions of the magnetic field that remain after the pulse, dependent on the level of oxygen in the blood
- Using various pulse sequences that orchestrate the gradient coils (who vary the magnetic field and hence the specific Larmor frequency) we can measure location specific activations



Field of view: area we want to cover
Smaller field of view -> higher resolution
Longer time -> higher resolution -> more noise
Image acquisition and analysis. We used a 1.5-T Siemens Sonata MRI scanner to acquire gradient-echo, T^*_2-weighted echo-planar images with blood-oxygenation-level-dependent contrast. An additional T_1-weighted structural image was acquired for each subject.

Designing and analyzing fMRI

Fourier transformed power spectra
Blocked design:
- One condition over a block of several seconds/minutes
- Statistically powerful
- Habituation

Power: measure of signal strength
Event related design:
- Fast trial-by-trial variation of conditions
- Completely randomized, unpredictable sequences
- Avoids order effects and habituation
- Lower signal/noise ratio

Fourier transformed power spectra: HRF favors designs with power at low temporal frequencies
Low frequency -> high signal strength
There is also increasing power in at low frequencies in noise
Hemodynamic response vs intrinsic noise (resting state)
Every 20 sec (compromise)
Best signal to noise ratio = block design

Sustained attention response task
Design: overlap and deconvolution
- Due to slow hemodynamic response, the signal in one trial can be influenced by the previous event at short ITIs (overlap)
- Can be reduced by using randomized sequences and ITIs, so that overlap does not systematically differ for conditions (deconvolution)
- Less powerful
- For analysis, either define parameters (conditioning/timing) a-priori, or extract from logfile post-hoc

Data acquisition
a) Localizer scan: a few low resolution scan (some seconds), Where's the head?
b) Anatomical scan: good overview of the anatomy of the brain (~5 minutes), One high-resolution T1 image, aligned on localizer scan

c) Functional scans (X times run-time): One T2* volume per repetition time (TR)
In each TR a different (2D) slice, put them together to make a 3D view
Different parameters based on your study
Functional scans defined by: TR, number of slices, slice thickness, voxel size, gap, in-plane resolution
High-resolution functional scans for small areas are possible (e.g. brainstem nuclei
-> However, often at the cost of brain-coverage!
Standard voxel: 3 x 3 x 3 mm
1 x 1 mm is very precise, high resolution
Too small: a lot of noise, at some places the blood vessels are very big so it doesn't make sense to go smaller

Data analysis:
1. Realignment: overlay all functional scans
- For the analysis it is essential that each voxel is in the same location across all scans
- No one can lie absolutely still, that's why all images have to be aligned post-hoc
- But still, the smaller the movement, the better the data!

Within 6 seconds it could be the participant moved a little bit, it could happen that scans are slightly mismatched -> stack brain regions perfectly on top of each other

2. Slice-time correction
- Slice-time correction: "as if all were acquired at the same time"
- Interleaved slice order to avoid influences from previous slice acquisition (skip one)

Build a stack starting at the brain stem -> correct as if slices were taken at the same time
Interleaved: influence from fMRI could have noise on the next slice

3. Co-registration: overlay anatomical on functional scans
Anatomical before functional scan -> anatomical scan is more precise

4. Normalization: transform anatomical scan to match template brain
Normalize: when we do fMRI you don't look at one subject but a group, but everyone had a different sized brain

5. Smoothing: blurring the picture
- Reduces uncorrected noise across voxels
- The wider the smoothing, the more neighboring voxels are considered
- The resulting "blurry" picture is less sensitive to anatomical differences between subjects

There is always movement -> average out noise with the other surrounding slices

Ingredients for analysis:
- What is the best design for my research question?
- Taking into account limitations of the BOLD response (SNR)
- Amplitude may vary as a function of the experimental condition (e.g. famous vs. non-famous)
- Can be measured based on regional changes in blood oxygen utilization (BOLD)

fMRI analyses
1. fMRI data structures
1 volume (image) = ~ 100 000 voxels
2 key features
- Spatial location (X, Y, Z coordinates)
- Signal intensity

Repetition time (TR): time it takes to acquire a single volume (often ~2s)
Time series = signal intensity in a single voxel across volumes / time

Key concepts
- Voxel
- Volume
- TR
- Time series
- BOLD

- HRF

2. First level

Input & output
First level: building a model
Build a statistical model
Time x signal intensity

First level = single subject model
Regression approach
Mass univariate model = each voxel is modelled separately and independently
Main output = one beta weight for each regressor in the model

we are measuring how much oxygen, fairly slow response

$$Y = X \times \beta + E$$

Data	Independent variable, manipulated in the experiment	Relative contribution, to be estimated	Error

Y: the observed fMRI time course in a specific voxel e.g. dependent variable
X: A set of specified predictors / regressors. Each of which has a unique expected signal time course.
Beta: Quantifies how much each predictor contributes to the observed data (I.e. the voxels' time course, Y)
E: Variance in the data not explained by the model. Represents the mismatch between the observed data and the described model.

Repetition: convolution
- BOLD contrast (Blood Oxygenation Level Dependent) = ratio of oxygenated to deoxygenated haemoglobin
- HRF (Haemodynamic Response Function) = typical shape of BOLD signal after neuronal activity. Much slower than neural events.
- Convolution = averaging of two functions across time

X1 = constant signal
X2 = present human faces
X3 = present houses

Each of these predictors get beta-weight.
Model of the true signal = lineair sum of all predictors, multiplied by their respective beta weights.
For every point in time: signal(t) - model(t) = error(t)
Errors = whatever our model cannot explain
Their will always be some degree of error
Mass univariate approach: you repeat this for every voxel

General Lineair Model (GLM):
$Y = X \cdot b + E$
BOLD signal = model + error
BOLD signal = explained variance + unexplained variance
GLM solves this equation
What we know:
- Y: collected data
- X: design matrix

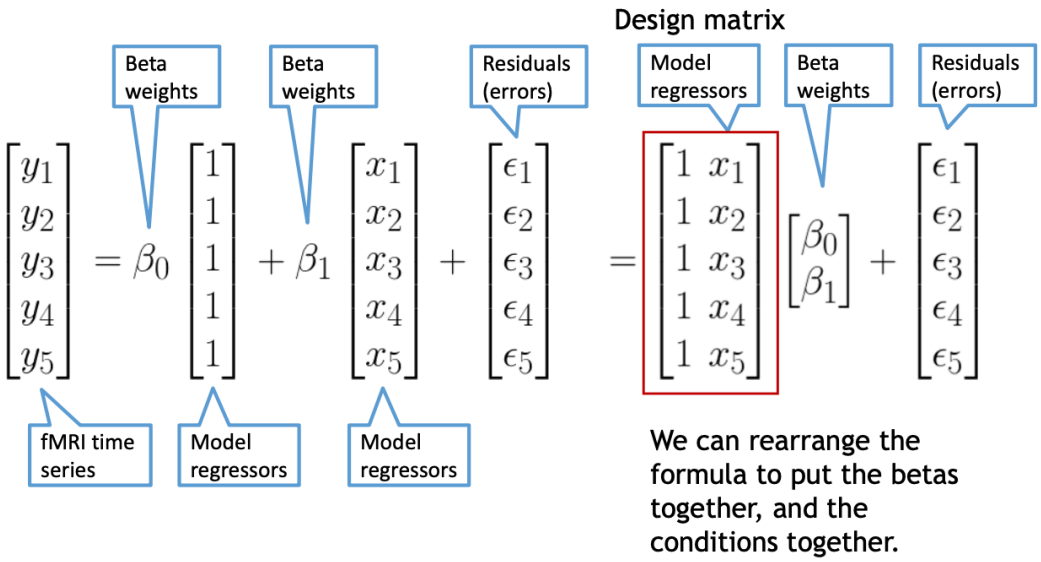
What we want to find:
- b: vector of beta-weights that give the best approximation of the BOLD signal.

How we find it:
- By minimizing the sum of squared errors.

- In practice, the GLM has a formula that finds these beta-weights.

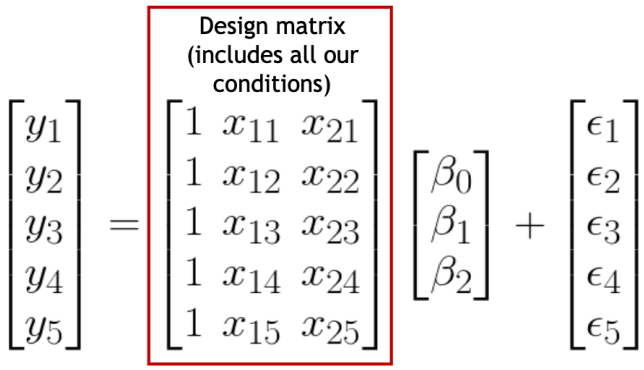
$$
\begin{aligned}
y_1 &= b_0 + b_1X_{11} + \cdots\ \cdots\ \cdots + b_pX_{1p} + e_1 \\
y_2 &= b_0 + b_1X_{21} + \cdots\ \cdots\ \cdots + b_pX_{2p} + e_2 \\
y_3 &= b_0 + b_1X_{31} + \cdots\ \cdots\ \cdots + b_pX_{3p} + e_3 \\
&\vdots \qquad\qquad\qquad\quad \vdots \qquad\quad \vdots \qquad\ \vdots \\
y_n &= b_0 + b_1X_{n1} + \cdots\ \cdots\ \cdots + b_pX_{np} + e_n
\end{aligned}
$$

y = measured single voxel time course for n vowels
b_0: constant (signal baseline)
b: weights associated with each predictor
X: predictor time courses (convolved with HRF) for p predictors
e: residual variance (error term)



The GLM at the first-level in fMRI analysis:

$$Y = X \bullet \beta + E$$



Effects of Interest
- Each individual condition (X1, X2 …)
- A 'constant' predictor (X0)

Effects of No Interest (i.e. confounds):
- Each physiological confounds (head movement)
- Each psychological confounds (stress, attention)

Design matrix
Effects of interest:
- Faces
- Houses
- Trees

(Include temporal+ dispersion derivatives)

Effects of non-interest:
- Head movement
- Constant

Output: one beta weight per column per voxel

Beta maps = all betas for a single predictor across the whole brain
Fitting a GLM will produce a beta image for every estimated regressor.
3D image of beta values (vs baseline)
Express strength of the effect in each voxel
Beta images are used to compute contrasts.
SPM: statistical parameter mapping
306 volumes
1 predictor for face, 1 for houses, …
3 dimensions in to move in and to rotate in -> 6 dimensions of head movement

Contrasts
- Statistical tests performed on beta estimates.
- Performed at both first level (within subject) and second level (group).

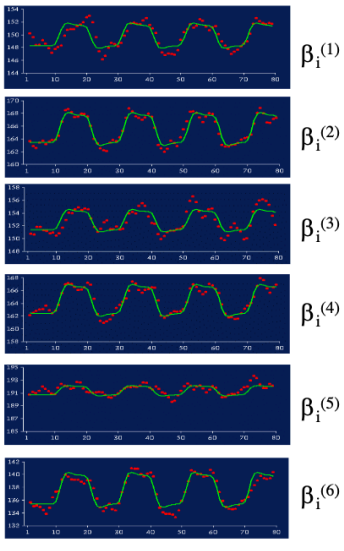
T-contrast: beta 1 of subject 1 - beta 2 of subject 2
- Can test wether H1 ≠ H0
  - Can be against baseline, or another condition
- Can see if the effect of one regressor is smaller or greater than another
- Directional: looks at the lineair combination (i.e. subtraction) of two conditions.
- Simple to compute and interpret

F-contrast: beta 1 of subject 1 or beta 2 of subject 1 or beta 3 of subject 1
- Can test the main effect of a regressor (both positive or negative)
- Can look at several conditions simultaneously (more than two).
- Non-directional: tests for any differences between conditions, looks for an overall effect.
- Can look at several parameters / conditions simultaneously (but need to follow up with t-tests).

Key concepts:
- Mass-univariate analysis
- Predictors
- Beta-weights
- Error-term
- GLM
- Design matrix
- Beta map
- Contrasts

3. Second level analyses

Second level analysis test the experimental hypothesis in a group of subjects
This is called the Random Effect analysis (RFX):
- We compare the effect at the group level to between-subject variability
- We look for a consistent effect across subjects, on which we can draw conclusion on the population.



Moving from first level to second level …
First level (within subject)
Time series in red, prediction in green
Set up a contrast for the design matrix: [1 -1]

Second level (between subject)
Second level needs contrasts too! We just want the mean of the difference between Cond 1 and Cond 2 (already calculated at first

level) so … [1]
Mean signal per person, one row per participant
FDR: false disovery rate

T-tests
Second level (one sample t-test)
Have to specify a second level contrast:
- Already have first level contrasts calculated for the difference between Cond 1 and 2.

-> Is this effect significant across participants?
[1] (give me the mean of the differences already worked out from first level)

Second level (two sample t-test)
Have to specify a second level contrast:
- Already have the first level contrasts, but now need to contrast the two groups

-> Significant difference between two groups (e.g. this region is activated more strongly for the control group compared ro the patient group).
[1 -1] (give me the difference between the two groups)

Factorial designs
Group comparisons
2 by 2 ANOVA (looking at main effects):
4 conditions:
- Rew & novel (A1)
- NoRew & novel (A2)
- Rew & familiar (B1)
- NoRew & familiar (B2)
- Main effect of Reward: (A1+B1) - (A2+B2)
- Main effect Novelty: (A1+A2) - (B1+B2)

Main effect of reward: is activity of reward (novel and familiar) bigger than no reward
Interaction of Reward and Novelty: (A1-B1) - (A2-B2)
- An interaction occurs when one factor effects the results differently depending on a second factor.
- Difference between columns depending on the rows (or vice versa).

The multiple comparison problem
The GLM approach is a mass-univariate approach
- thousands of tests are performed (~ 100 000)
  - For n>1 tests, cumulative alpha > single-test alpha
  - High chance of false positive findings
  - Need to adjust the single-test alpha to control cumulative alpha (False Discovery Rate FDR, Family-Wise Error Rate FWE)

Key concepts
- Random effect analysis
- Contrast vector
- Factorial design
- Multiple comparison correction

4. Region-of-interest (ROI) approach

ROI analysis
What is ROI analysis?
- Selecting a cluster of voxels (or a brain region) to specifically examine for effects.

You are most likely not interested in all of the brain

Why do ROI analysis?
1. For statistical control - by preselecting, you can reduce the amount of corrections (correct only for the number of ROIs, not the whole brain
2. For functional specification - to see if a specific religion plays a role in other manipulations.

Selecting the part of the brain you are interested in (you can pretty likely say which parts will be affected based on theory) -> gives you more power to detect effects

How define regions of interests?
Individual structural images to outline ROI
- Time-consuming
- Most precise

Go through every slide of 3D map and handpick areas -> often in clinical settings, because every individual brain is different

Co-ordinates from literature: create a sphere (or box) ROI around that co-ordinate
- Fast and easy
- Anatomically inaccurate

Pre-existing atlas
- Fast and easy
- Easily combine different ROIs

Most people take a predefined atlas
Standardized space for a healthy brain, other people have already put in the work of selecting where brain regions are, you just select the ones you need

Use past literature, use the same regions they found
E.g. a meta-analysis of fMRI studies looking at reward processing.
Single study is not as reliable as a meta-analyses
Functional ROI's: need good quality data, meta-analyses

Localiser task
Run a separate fMRI experiment that activates the ROI. Use that in your main experiment.
-> extra part in experiment for each individual participant, localize the voxels that significantly respond
Cave: double-dipping
"Double-dipping": if you select only the parts that respond and you exclude everything else, you are biasing results in favor of finding a response
E.g. Show participants alternating blocks of pictures (faces vs. houses)
"Double dipping"
- Biasing an ROI towards certain regressors.
  - Need to make sure that the ROI and the contrast you are interested in share no common regressors
  - Example: Three regressors (face, house and tree). The contrast face-house is used to define a functional ROI. With this ROI, the experimenter tests the contrast face-tree. This is not good! ROI it's already biased towards face (as it includes voxels that are more active for face), so face-tree will probably also show something significant.

Localizer task needs to be independent for example showing scrambled pictures vs completed ones
-> activity in voxels when picture is complete-> these voxels respond to any type of complete image, not just houses and faces = independent

Correct for multiple ROIs
- If >1 ROI
  - Each ROI is independent -> correct for multiple comparisons.
  - Bonferri correction: alpha/number of ROI tests.
  - To reduce the number of corrections, have a good a priori hypothesis about your ROI, and use only few ROIs

If you have fewer ROI's, you have to correct for less -> easier to find something

5. Summary: fMRI pros and cons

Pros
- Excellent spatial resolution (mm).
- Harmless and non-invasive
- Allows testing of precise hypotheses about neuro-cognitive architecture of brain and behavior (see advanced analysis methods)

Cons

- Low temporal resolution (~ 2s), due to physiological constraint.
- Measures hemodynamic changes, not neural effects
- Expensive (scanner, maintenance, rent, consumption)

Remember!
- High number of degrees of freedom (lots of voxels, brain regions) -> leads to the multiple comparison problem.
- Need a good theoretic a-priori hypothesis!

Key concepts
- Anatomical vs functional ROIs
- Double dipping
- Temporal vs spatial resolution

Advanced fMRI analyses

Regular fMRI analysis
- Single subject GLM (1st level)
- Single subject contrasts (1st level)
- Group analysis e.g. ANOVA (2nd level)

-> interpretation: regions more or less active for different categories

1. Multivariate pattern analyses

Zooming in to observe differential voxel patterns
- Mass univariate analysis: neighboring voxels behave similarly
- Does not see information encoded across multiple neighboring voxels

1.1 Representational Similarity Analysis

2 data sets
Correlate voxel pattern within and between conditions (across data sets)
Not one dependent variable, but many
r(mouse1, mouse2) > r(mouse1, banana2)
9 different voxels, 4 are active, but different for mouse and banana
-> if you average out you will see no effect
Correlate for same stimuli in different blocks
Category selective region: some stimuli correlate more then others -> region is activated by that stimulus (highest correlating)
Look at more than one variable at a time -> get more out of your data

1.2 Decoding

2 datasets
Train classification algorithm to discriminate conditions in training data set
Present data from test data to assess performance -> accuracy in %

Cross validation: 'leave-one-out': use n-1 runs to train, the last run to test. Rotate through all runs.
Average performance across folds.
Increases generalizability of results.
same concept as double dipping, leave one out for cross-validation
Distribution of data in one voxel is very much overlapping
-> higher dimension of space, more voxels, you can separate more
You can separate using straight line, also with a squiggle, but with a squiggle you are probably over-fitting your data (too many regressors)
Don't fit the data too tightly -> lineair line is better than curve

Summary
- Flexible tool
- Representation vs activation
- Increased sensitivity

But: not all research questions and designs are suitable for MVPA!
- Increased sensitivity = increased susceptibility to confounds

- Needs optimized experimental designs with large number of trials
- Complex statistical analyses (e.g.) cross-validation) might be incompatible with research question

Key concepts:
- Multivariate voxel patterns
- Representational Similarity
- Decoding
- Cross-validation

2. Functional connectivity analysis

Functional segregation (localizationism)
- Assumption: specific brain regions represent specific functions
- Analysis: regionally specific affects
- Examples: Univariate analyses, GLM, MVPA

Functional integration (connectionism)
- Assumption: Functions rely on / arise from connectivity between regions
- Analysis: inter-regional effects (regions interact)
- Examples
  - Functional connectivity
    - Correlations between separate areas
    - Whole-brain connectivity
    - Model-free
    - Exploratory
    - No causation
  - Effective connectivity
    - Influence of one region over another
    - Selected set of regions
    - Model-based
    - Confirmatory
    - Causal

2.1 Resting-state connectivity

Task-free: participants rest in scanner
Correlate time-courses across all voxels
-> Several functionally linked networks (e.g. default mode network, attention network)

Seed-region approach:
- Pick a region of interest (seed)
- Correlate its time-course with other voxels throughout brain
- Does seed region correlate positively/negatively with other regions?

same idea, slightly different approach
Orange: high correlation with interested region (yellow)
Bleu has negative correlation

Graph analyses to assess network properties
- Possible parameters: Global network strength, local clustering vs. hubs, average path length (processing speed), etc.
- Graph analysis can be used for functional and structural data (see DTI)

Correlate each of the interested regions
Try to identify connector hubs -> only one region connected to both

2.2 Dynamic causal modelling

Assumptions
- Brain is set of interconnected neural nodes that interact all the time
- Experimental manipulations change network dynamics (“how does my manipulation propagate through the network?”)
- Example: attention to motion
- Model-selection framework: which model describes our data the best?

  - Generative model: assumes input stimuli and a neural architecture, then generates data.
  - Neural model: how would neural activity look like under model assumptions?
  - Heamodynamic model: How would BOLD response look like given the neural model?

-> Bayesian model comparison
-> compute the likelihood of each model
Pick the one that is closest to data

Critical issues DCM
- Region selection
- A priori definition of the neural model (relevant functions, existing anatomical connections, functional relation between regions) and hypothesis about modulating factors.

Summary
Resting-state connectivity
- Pro: task-free, investigating large-scale differences in connectivity, interesting for clinical context
- Con: no direct relation to task manipulation

Effective connectivity: DCM
- Pro: model-based conclusions regarding directionality and causation possible; flexible (not limited to one model)
- Con: requires good models based on anatomy and function; sophisticated implementation

Key concepts:
- Functional vs effective connectivity
- Seed region
- Generative model

3. Structural connectivity: Diffusion Tensor Imaging
- Imaging of white matter tracts ('fibre tracking') reflects actual structural connectivity between regions
- Directed diffusion of water molecules (along axons)
  - Water molecules can easily move along the axon, but not in and out of the axon
  - See how it travels trough the brain (fibre bundles)
- Estimate direction of diffusion in 3D
  - Each voxel gets a different direction
- Functional relevance derived by correlating with fMRI or behavioural data

Example:
- Resting-state connectivity
- DTI
- White-matter integrity and bundles connecting putamen and dorsal attention network was predictive of improved executive function in aging
  - The better the bundles stay intact, the better they are on different tasks

Graph analysis to assess network properties:
- Possible parameters: Global network strength, local clustering vs. hubs, average path length (processing speed), etc.
- Graph analysis can be used for functional data and structural data

4. Volume differences: Voxel-Based Morphometry
- Structural images are separated into grey/white matter
- Tissue volume: Count gray matter voxels in ROI
- "how big is region X?"
- Functional relevance established through correlations with fMRI or behaviour

Example:
- Fronto-temporal atrophy (volume reduction) was observed in three dementia groups (mild, medium, severe)
- The degree of atrophy was predictive of cognitive deficits

Key concepts:
- Fibre tracking
- Diffusion direction
- Tissue volume

Neurotransmitter-based methods

1. Neurotransmitter systems

All of the lecturers have a lot of expertise with fMRI
Neurons and synapses
Remember the action potential: Discrete, rapid voltage spikes, generated by special types of voltage-gated channels embedded in a cell membrane
AP passes along axon

Synaptic cleft
Neurotransmitters released into the synaptic cleft lead to postsynaptic potentials that will turn initiate to inhibit action potentials
The type of neurotransmitter depends on the cell type, e.g. a dopaminergic neural will release dopamine at the synapses
If neurotransmitters fit the receptor, it will start a new cascade of events in the next neuron

Important neurotransmitters in the brain
Excitatory:
- Acetylcholine (Ach)
- Glutamate (amino acid)
- Aspartate (amino acid)

Inhibitory:
- Y-aminobutryic acid (GABA) (amino acid)
- Glycine (amino acid)

Monoamines:
- Dopamine (DA)
- Noradrenaline (NE)
- Serotonin (5-HT)

-> While amino acids (and ACh) are always either excitatory or inhibitory, the effects of monoamines depends on the receptors of the postsynaptic cell!

3 monoamines in cognition and behavior:
- Dopamine
  - Reward, learning, cognitive control
  - Memory formation, motor control
  - Main pathways mesolimbic/mesocortical (“cognition”), nigrostriatal (“motor”), tuberoinfundibular (prolactin release)
  - Additional projections: hippocampus, amygdala, cingulate
- Noradrenaline
  - Arousal, vigilance, attention
  - Memory formation
  - Widespread projections
- Serotonin
  - Mood, emotion processing
  - Impulsivity
  - Widespread projections

Monoamines are not always widely distributed in the brain, but more specific
Don’t have to remember the pathways, only that it always originates from the mid-brain
Adrenaline: fight-flight, but also other functions
Originates in brain-stem (locus coerelus)
Serotonin also originates in brain stem
In depression serotonin is too low

Imaging brain activity at the “chemical level”
- Transmitter receptor binding (e.g. dopamine D2 receptor PET)

- Transmitter transporter binding (e.g. dopamine transporter PET): how many go back into the presynaptic neuron
- Metabolic spectrum (e.g. GABA MRS)

Versus:
- fMRI = hemodynamic level
- EEG/single-cell recordings = electrophysiological level

We don't have good spatial resolution (can't pinpoint a synaps)

2. Positron Emission Tomography (PET)

Principles of PET
Transmitter-specific PET: radio-labeled ligands (tracers), most common: Carbon (C) and Fluorine (F)
We inject people with material that has trace radioactive elements that bind to a certain receptor types
Procedure in brief:
Tracer injection -> decay of radioisotope in the tissue -> emission of a positron that collides with the next best electron, releasing 2 gamma-ray photons -> detected and localized by the PET camera -> 3D image construction based on detection of a significant number of events
-> tracer binding potential maps are the inverse of the binding of the transmitter of interest! I.E. higher tracing binding means lower actual transmitter binding
Side note: Transmitter-unspecific PET: cerebral blood flow (CBF) and glucose metabolism (FDR) has been largely replaced by fMRI
PET: Measures how much blood is going around -> bad temporal resolution -> fMRI took over

Receptor and transporter PET
- Dopamine PET
  - [11C] Raclopride (D2 receptors)
  - [18F] Fallypride (D2 receptors)
  - [11C] PE21 (DAT)
- Noradrenaline PET
  - [11C] Despiramine (NET)
  - [18F] FMeNER-D2 (NET)
- Serotonin PET
  - [11C] P943 (1B receptors)
  - [11C] DASB (5-HTT)

Don't need to remember the chemical composition
D2: striatum dopamine receptors
Fallypride is less specific (also D2 but wider)

2. Single Photon Emission Computed Tomography (SPECT)

Sometimes Spect is used, but no need to know specifics for the exam
Spect is quite blurry
Receptor and transporter SPECT
- Dopamine SPECT:
  - [123I] IBZM (D2 receptors)
  - [123I] FP-CIT (DAT)
- Noradrenaline SPECT:
  - [123I] INER (NET)
- Serotonin SPECT:
  - [123I] ADAM (5-HTT)
- Others:
  - Ach receptors (nicotinic/muscarinic)
  - GABA receptors (benzodiazepine)

Others are almost everywhere in the brain (regionally unspecific)

3. Magnetic Resonance Spectroscopy (MRS)

Principle of MRS
- MR-based method to assess the regional metabolic spectrum of brain tissue

- Principles similar to MRI (radio-frequency waves affect the spin of nuclei in magnetic field), but while MRI is about the spin of H in H2O molecules, MRS reflects the resonance of other molecules
- The resulting spectrum reflects particle resonance (ppm, parts per million) of metabolites that are associated with specific neurotransmitters or other substances in the brain tissue.

Main MRQ metabolites:
- N-Acetul Aspertate (NAA): index for neuron/axon integrity, decrease indicates tissue loss or damage
- Choline (Cho): related to membrane turnover
- Creatine (Cre): related to energy metabolism
- Glutamate (Glu): exitatory neurotransmitter
- GABA: inhibitory neurotransmitter

-> Measure on average how much GABA is in a small part of tissue

Most often used in clinical contexts (e.g. tumors, Alzheimer's, Parkinson's disease), neurotransmitter metabolites (Glu, GABA)
Can also be interesting for cognitive neuroscience (covariate for behavior, fMRI, EEG data)
Not functional/event related, but global, generally -> assume that it doesn't fluctuate a lot, more an individual measure, structure of the brain
Gaba has become more interesting because it has a link to inhibitory control

Example: motor response inhibition
- Automatic motor control has been associated with activity in the Supplementary Motor Area (SMA)
- Automatic response tendencies were probed by a subliminal priming task
- MRS was acquired from an SMA ROI (fMRI localizer)
- Higher GABA levels in the SMA were associated with smaller behavioral priming effects (NCE), indicating that SMA GABA counteracts automatic responses triggered by subliminal primes
- ROI selection is essential, here, fMRI is used to define the ROI that is then tested with MRS
- Compared to control regions where no relationship between GABA and priming is expected

Flanker task with masked prime stimuli that's sometimes in the opposite direction (conflict)
People make mistakes on the incongruent trials
Collected data of brain region, measured GABA
Higher GABA levels ~ smaller priming effect, counteracts automatic response
Gaba is global inhibitor, helps with motor control
Used fMRI to localize this region (activation during the task)
Gaba in 4 other regions did not correlate, only the one activated during task

4. Pharmalogical manipulations

Transmitter agonist, antagonist, and modulators (e.g. reuptake inhibitors) are mainly used to develop and validate treatments for neuro-psychological disorders
Mostly coming from clinical studies
In basic psychology, mild manipulations are used to study relationships between neurotransmission and cognitive functions and personality (low-dose drugs or via specific diet, e.g. tryptophan depletion)
Agonist: binds to receptor and takes role of transmitter over -> increase activity
Antagonists (often in psychiatry): binds to receptor and blocks it -> reduce activity
Diet: certain substance in high dose changes how much neurotransmitter is synthesized in the brain
For example tryptophan is needed to build serotonin

Example: dopamine receptor binding of different antipsychotic drugs
- PET/SPECT study of striatal dopamine receptor binding in schizophrenic patients and healthy controls
- And healthy volunteers, tracer competes only with endogenous ("natural") dopamine
- In patients, tracer binding is weaker since receptors are blocked by the drug (DA antagonist)
- Risperidone has the strongest effect on striata dopamine (benefit vs. side effect of a drug)
- Side finding: similar results for PET and SPECT, but PET has better spatial resolution

Antagonist is main way of manipulating neurotransmitter activity

Healthy: no drug, normal dopamine
Different people have more/less dopamine, this is an average
Reflects receptor availability
Olanzapine is dopamine antagonist -> receptors are blocked
PET assesses how much is blocked/how much goes trough
Clozapine: more Pet is binding -> less is blocked -> least effective drug
Risperidone is strongest drug -> no PET is binding
Strong schizophrenic medication has big side effects -> important to compare efficacy of the drugs
Risperidone: dopamine is important for movement and cognitive function, makes sense that when people take this, they are drowsy
Both PET and dopamine are in the system, you assume about half of the available receptors are taken by PET

Example: effects of serotonin on motivated behavior
- Reward anticipation task (you get paid in this task) with different feedback probabilities
- Low serotonin levels (dietary tryptophan depletion) were compared to normal levels (baseline)
- With normal serotonin levels, high reward probability (90%) leads to faster but less accurate responses
- Serotonin depletion leads to slower and more accurate responses, reflecting reduced impulsivity
- Relevant for depression: some antidepressant drugs can increase suicide risk before the antidepressant effect unfolds (still feeling bad, but more impulsive due to higher serotonin levels)

TRP - is depletion, black is normal
In 90% condition response time is decreased, faster but less accurate responses (impulsivity)
Depleting serotonin: a bit slower, but higher accuracy
Depressive people have too little serotonin -> but with drugs they increase the suicide risk (impulsivity)
Effect on mood takes longer than impulsivity effect

Conclusion
Benefits
- These approaches relate to actual neural transmission while fMRI is only an indirect measure of these processes (based on hemodynamics)
- Valuable for clinical conditions that are related to neurotransmitter disturbances
- Combining neurotransmitter-based methods with other neuroimaging methods can reveal interesting relationships between neurotransmission, cognition, and behaviour

Limitations
- No or low temporal resolution; mostly used as ‘snapshot’ (state) measures, but basic block designs are possible with PET
- Lower spatial resolution compared to fMRI, often limited to certain regions
- Access to PET is limited, and short half life of certain tracers requires on-site production (cyclotron)
- Except for MRS, these methods are more invasive than fMRI, EEG, TMS (exposure to radiation, injections, medication) and require medical supervision

Combining methods
1. Combine complementary methods: fMRI-EEG
Combine complementary methods:
- fMRI-EEG
- fMRI-PET

Add trans cranial magnetic stimulation:
- TMS-EEG
- TMS-fMRI
- TMS-PET

Add pharmalogical manipulations: e.g. pharmaco-fMRI
Add computational modeling: e.g. model-based fMRI

fMRI-EEG

Recap
fMRI
+ High spatial resolution (mm), including subcortical areas
+ Non-invasive
- Low temporal resolution (sec), but event-related designs are possible
EEG
+ High temporal resolution (ms), online monitoring of cognitive processes
+ Non-invasive
- Low spatial resolution
- Limited to cortical surface

EEG recording in the scanner
Most important aspects:
- Use certified MR-compatible EEG equipment
- Optimize paradigm to fit both modalities
- Synchronize fMRI and EEG acquisition (essential for artefact correction)
- Reduce any noise that could increase artefacts:
  - Type of fMRI sequences, type of head coil
  - Straight cable routing, isolate from MR table, use tape and sandbags to reduce cable vibrations
  - Turn off helium pumps during scanning
  - Minimize head movements of the participants

Complex to combine everything, don't need to know the full image
If EEG cap warms up the metal will cause burn marks -> caps without metal
Reduce noise (EEG is small signal, influenced by lots of noise)

Dealing with artefacts
- Technical noise (scanner) -> big, but OK to remove based on time-locked acquisition (correction template-based)
- Physiological noise (mostly heartbeat) -> much harder to remove because inconsistent (manual correction, template-based, ICA-based)
- You often still see residual cardio signal in the data - still biggest challenge for fMRI-EEG recordings!

Additional noise is bursts -> we can subtract these
If you lie down the heart has a bigger impact -> more noise from the heart, inconsistent -> try to subtract them manually
EEG in scanner is not as good as outside the scanner

Main options of analysis
a. Two parallel data sets, analyze separately and compare, correlate etc
b. Use fMRI localizer for EEG source reconstruction
c. Use EEG single-trial amplitudes as parametric modulator in GLM

Sequential inhibitory control processes assessed through simultaneous EEG – fMRI
Paradigm: Flanker task with noGo trials
Include EEG amplitudes as single-trial parametric modulator in GLM
- N2: decrease default/motor activity (before inhibition)
- P3: increased insula/inferior frontal activity (after inhibition)

-> this method creates a 'temporal tag' for the fMRI signal
You can derive temporal resolution from the ERP

Pro:
- fMRI and EEG are complementary in terms of spatial and temporal resolution; and both can handle event-related designs (e.g. not the case for PET)
- Simultaneous fMRI – EEG is favourable compared to separate data sets from the same participant: no between-subject variance (obvious); no order and practice effects; identical situation with respect to task performance, stimulus perception, body position, noise, instruction/experimenter effects
- > these aspects increase statistical power and ensure that differences between conditions in one measure are not due to differences between fMRI and EEG session

- Allows trial-by-trial covariation of spatial and temporal signatures of condition-specific brain states, exceeding across-participant approaches (of course only when EEG signal is clean enough)

Con:
- Compromises regarding study design (e.g. timing) and technical limitations (e.g. fMRI sequences)
- More time consuming to set up, slightly more uncomfortable for participants
- Elaborate methods are needed for artefact correction of the EEG data (MR and cardio-ballistic artefact)
- Even more degrees of freedom in the analysis than with one method alone

fMRI-PET
Recap
fMRI
+ High spatial resolution (mm), including subcortical areas
+ Non-invasive
- Low temporal resolution (sec), but event-related designs are possible

PET
+ Neurotransmitter binding (synaptic level)
+ OK spatial resolution (mm-cm)
- Very low temporal resolution (min), no event-related designs (only between-session)
- Rather invasive
- Difficult logistics

Mesolimbic functional magnetic resonance imaging activations during reward anticipation correlate with reward-related ventral striatal dopamine release
Design: 2 fMRI and 2 PET sessions in each participants; reward vs. no-reward “session” (block manipulation) rather than actual event-related design due to low resolution of PET

PET tracer binding (inverse of dopamine level: If you have lots of drugs in the system, the tracer cannot bind)
- The red blobs indicate average tracer binding in the entire striatum (which, again, is the inverse of dopamine levels)
- The line graph indicates tracer binding over time: reward versus neutral block, and left/right nucleus accumbens (NAcc)
- Lower tracer binding in reward versus neutral condition = more dopamine in reward versus neutral condition
- Additional check: total binding needs to be corrected for any specific binding, unrelated to the task (here, cerebellum)
- Positive correlation between PET dopamine release (reward > neutral) and activity in fMRI activity in VTA/SN and NAcc
- Link between hemodynamic activity and dopaminergic regions (fMRI) and actual dopamine transmission (PET)

TMS-EEG
Recap
TMS
+ Stimulation of regions affects ongoing processes (interference, virtual lesions)
+ Repetitive TMS can result in long-lasting changes (‘restructuring’)
- Limited to cortical surface
- No direct measure via cortical activity, (indirect via behavioral changes and MEPs)
- Somewhat invasive

EEG
+ High temporal resolution (ms), online monitoring of cognitive processes
+ Non-invasive
- Low spatial resolution
- Limited to cortical surface

Subsecond changes in top-down control exerted by human medial frontal cortex during conflict and action selection: a combined transcranial magnetic stimulation electroencephalography study

Paradigm: Flanker task
LRPs: lateralized readiness potentials
- Lateralized readiness potentials (LRPs) over left and right motor cortex reveal asymmetry between congruent and incongruent trials (initial activation of the wrong hand in incongruent trials)
- rTMS over dorso medial frontal cortex (dmPFC) increases this LRP difference and is associated with more errors
- Reveals “causal” role of dmPFC in conflict resolution: top-down control to inhibit/overrule wrong response tendency to enable correct response
- Side note: TMS and EEG can also be combined in an event-related fashion (single-pulse TMS)

More errors under TMS pulse
More causal view on a certain function

TMS-fMRI
Recap
TMS
+ Stimulation of regions affects ongoing processes (interference, virtual lesions)
+ Repetitive TMS can result in long-lasting changes (‘restructuring’)
- Limited to cortical surface
- No direct measure via cortical activity, (indirect via behavioral changes and MEPs)
- Somewhat invasive

fMRI
+ High spatial resolution (mm), including subcortical areas
+ Non-invasive
- Low temporal resolution (sec), but event-related designs are possible

Studying the role of human parietal cortex in visuospatial with concurrent TMS – fMRI
- Typical contralateral activity increase in visual cortex when attending to left and right visual field
- High- vs low-intensity TMS bursts over parietal cortex enhance these attention effects, suggesting causal role for parietal cortex in directing attention
- Paired-pulse TMS increases functional connectivity between ventral premotor (PMv) and primary motor (M1) cortices
- Evidence for plasticity changes, but also valuable for understanding the effects of TMS on cortical activity in general

Apply bursts with TMS: enhanced attention effect
Repetitive has a longer lasting effect

Paired pulse: don’t have to remember the experimental design
2 coils over 2 sides, alternate the pulses
-> improve communication between those regions

TMS-PET
Recap
TMS
+ Stimulation of regions affects ongoing processes (interference, virtual lesions)
+ Repetitive TMS can result in long-lasting changes (‘restructuring’)
- Limited to cortical surface
- No direct measure via cortical activity, (indirect via behavioral changes and MEPs)
- Somewhat invasive

PET
+ Neurotransmitter binding (synaptic level)
+ OK spatial resolution (mm-cm)
- Very low temporal resolution (min), no event-related designs (only between-session)
- Rather invasive
- Difficult logistics

TMS-PET makes most sense with repetitive TMS, which induces long lasting changes (due to the low temporal resolution of PET, no event related designs possible)
Striatal dopamine release inducing by repetitive transcranial magnetic stimulation of the human motor cortex

- In this example, rTMS over motor cortex leads to reduced tracer binding in the striatum, which reflects increased striatal dopamine release
- Occipital cortex is used as control region (subtracted as unspecific change in binding potential)
- Evidence for controlling dopamine levels via cortical efferents in humans

TMS seemed to increase dopamine in striatum -> intresting for example for Parkinson

3. Add pharmalogical manipulation

Pharmalogical manipulations can be combined with any methadone under strict ethical guidelines
Can create confounds (e.g. global changes of fMRI haemodynamics beyond the effects of interest)
If you give a drug, it can change the metabolism of the brain -> be aware

pharmaco-fMRI
L-DOPA disrupts the activity in the nucleus accumbens during reversal learning in Parkinson's disease

- Dopaminergic medication to improve motor functions in Parkinson's patients disrupts reversal learning and nucleus accumbens activity
- Evidence for inverted U-shape of dopaminergic medication

L-DOPA is precursor of dopamine
Task is related with activation in striatum
L-DOPA improves motor function but makes them worse at this task (cognitive function)
Inverted U-shape of dopamine levels: overdose of dopamine impares cognitive functions

4. Add computational modeling

Behavioral models are already applied for a long time (e.g. reinforcement learning; perceptual decision making); now they are extended to neural data

Model-based fMRI
Model-based approaches to neuroimaging: combining reinforcement learning theory with fMRI data

- Here, ventral striatum activity is correlated with reward prediction errors derived from simulations of reinforcement learning model

Conclusions: combining methods

- Powerful approach: integrating information about space, time, neurotransmission, causality, …
- Additional equipment in the fMRI scanner cannot contain ferrometal (e.g. special EEG caps)
- The study design has to fit both methods (e.g. PET can only be used as one shot or block design)
- This leads to compromises in the design, and many degrees of freedom in the analysis: a priori hypothesis and adequate corrections for multiple comparisons are even more important here!

Combining methods always leads to more chance at false positives

Genetic polymorphisms and genetic imaging

What are genetic polymorphisms?

Genes have the information to build proteins
4 different bases

- Cytosine
- Guanine
- Adenine
- Uracil/Thymine

Room for messenger RNA to copy one strand of DNA
-> build a new strand of DNA, similar to original
Ribosome attaches to messenger RNA and reads it and builds different amino acids that will fold into a protein
Ribosomes read the mRNA sequences three nucleotides (= a codon) at a time. This is translated into specific combinations of amino acids, which we call proteins
3 nucleons at a time = 1 codon
Ecg codon (64 possibilities) codes for an amino acid (25 possibilities)
Different codons can code for the same amino acid

The human genome project (1990-2003):
- A complete set of genetic information for humans
- 23 pairs of chromosomes, over 3 billion base pairs
- 20 500 human genes

A lot of junk DNA, but it most likely has some function

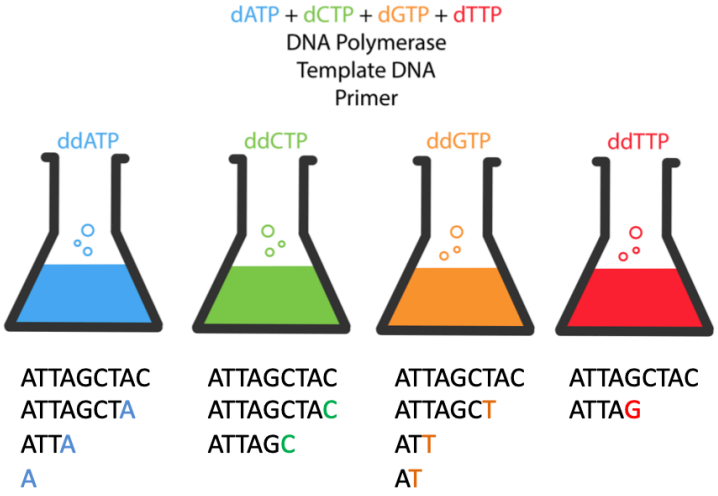
Now, what are genetic polymorphisms?
- Genetic polymorphisms: genetic variety
- Poly = many, morph = types
- A type of gene that varies across individual within a single species (allowing for diversity)
- > 1% of the population
  - If it is with less than 1% of human population, we do not call it polymorphism but a mutation
- Allele: a specific variation of a gene (resulting from polymorphisms)
  - They can be similar (homozygotes), or different (heterozygotes)
- Single nucleotide polymorphism = positions in a genome where one nucleotide differs

How do we measure them?

How is DNA measured?
- Polymerase chain reaction (multiply DNA strands)
- Gel electrophoresis (order DNA strands)
- DNA sequencing (mark and read replication stoppers)

Polymerase Chin Reaction (PCR):
- A process of DNA amplification (replication)
- Ingredients: DNA, heat, primers nucleotides
- Method: thermal cycling: rapid cycles of cooling & heating
  - All phases happen at different temperatures

Gel electrophoresis: ordering segments according to their length
- Lots of different copies of the same DNA strands, but they have different lengths
- Nucleotides are negatively charged, move from negative to positive pole
- Shorter strands move much faster
- We also throw in four different replication-stopping nucleotides with different fluorescent markers …
- Stopping molecules are marked with a certain color
- Different fragments all have a different stopping molecule
- Blue: we know at the beginning of the strand there's an A, also at the fourth place
- Different lengths of DNA and we only know the last letter
- First strand must only contain one nucleotide, last letter is A -> we know which nucleotide this is
- Last to arrive is longest segment and must be a T



How (and why) do we study them?

Once we have an. Individual's gene info …

- We can start looking for (cor)relations with phenotypes
  - GGC vs. TGC
  - Might cause a protein to be slightly different
  - Left: produces a neurotransmitter in the brain, right: less of that neurotransmitter/brain region is active
- We can focus on neurotransmitter or brain functions (sometimes called intermediate phenotypes)
- Study the relationship between a specific polymorphism and brain data during a task
- Studying polymorphisms is a less invasive alternative to form a logical manipulation studies
  - Alternative to pharmaceutical, time consuming but less invasive
  - Popular way of doing studies
- Single nucleotide polymorphisms: e.g. the COMT gene
  - The COMT gene breaks down extracellular dopamine in the prefrontal cortex
  - Val158Met variant: an SNP from G to A, results in an amino acid change from valine (val) to methionine (met)
  - COMT gene is one of most studied
  - Different variations of this gene, determined by only one nucleotide in the DNA (G->A)
  - Both break down dopamine, but VAL does that 4x faster -> less dopamine
  - Example: the attentional networks test
    - A task designed to dissociate three attentional networks: orienting, alerting, executive functioning
    - Fixation cross, cue: alerting or orienting
    - Target consists of different arrows, congruent or incongruent (you have to inhibit the flanker arrows)
    - Single snip can have an effect on cognitive functioning
- Choose your polymorphism of interest witch care:
  - Is it functional? (i.e. does it generate proteins)
  - Only 1.5% has instructions for proteins -> only this part should be studied
  - Is there a reason to believe it may impact activity in the region of interest or affect the cognitive function?
  - Alleles can be dominant or recessive
  - People could be heterozygotes -> study both the dominant and recessive effects
- Choose your imaging task with care:
  - Is there evidence that the cognitive process being measured is heritable?
  - Does the task have high reliability? Does it reliably (across individuals) activate the region of interest?

When setting up a study:

- Genotyping prior or post the imaging study?
  - Prior is best for matching samples, but a large number of volunteers need to be screened
  - Post guarantees experimenter blindness
- Multiple comparisons (avoiding false positives):
  - Naturally, be cautious when testing relations between many different polymorphisms and phenotypes
  - If you study the full genome you will get lots of false positives, way too much to compare

A systems approach

- Pleiotropy: a single gene can be involved in multiple processes
- Polygenicity: mental functions involve the products of different genes

There's more:

- Genome-wide association studies
  - Study correlations with SNPs across the entire genome between two groups
  - A substantial number of methodological challenges (e.g. multiple comparisons
- Commercial companies -> competition -> different techniques
  - Lack of consistent definitions
  - Popular genome tests were banned for a while
- Epigenetics

- Alterations in gene expression caused by the environment

Clinical groups

Between groups

Two or more groups of subjects each being tested by a different testing factor
OR
Two or more groups of subjects that differ in e.g. diagnosis/age/ … and each being tested by a similar testing factor.

- Insight in the disorder
- Knowledge about certain neurobiological conditions allow for inferences about brain functions
- Simply by looking at comorbidity might tell you something about overlapping functions
  - One mechanism may underlie the coexistence of 2 or more conditions

Pharmalogical manipulations
- Between subjects: one group treatment A, other group B
- Within-subjects design: whole group gets A, then B
  - You can alternate, medication first, next no medication or the other way around

Testing patients on and off their medication
- Test de novo patients before and after their first medication
  - Allows dor clean off-medication condition
  - But, problem with order effects …
- Ask patients to do a day without their medication …
  - Now we can control for order effects
- Test new medications

PD patients ON/OFF dopamine
- PD: a marked depletion of dopamine in the basal ganglia
  - Impaired control over a range of motor behaviors
    - But patients (on medications) show minor impairments in cognitive control
- Dopaminergic medication improves motor control
- Dopaminergic overdose in prefrontal cortex functions? (Cools, 2005) (inverted U-shape)

Depletion in dopamine -> loss in motor function, …
Cells in ventral temperamental area get an overdose of dopamine on medication for Parkinson -> loss of cognitive function

A speciaal role for serotonin in episodic memory? (Levkovitz et al., 2002)
- Prozac (serotonine) and Deprexan (noradrenaline) are equally effective in treating depression.
- However, serotonine is also thought to have additional effects on episodic memory …

Possible COVID-19 treatment: combination of hydro chloroquine and azithromycin
80 patients involved, after 14 days all are cured
Only 15% had fever -> weren't really sick, those that had fever weren't also very sick
No control group

Lesion studies

Studying patients with particular brain lesions
"when damage to a specific brain region is followed by an impairment of a cognitive function, this region must be responsible for this cognitive function."
Method:
- No ethical problems as the damage occurred naturally
- Keep the group as homogenous as possible, lesion is in same regio and comparable size, same amount of time since trauma, …
- Include a control group
- If possible, look for double dissociations
  - Lesion 1: function A impaired, B intact
  - Lesion 2: function B impaired, A intact
  - A and B are independent processes (e.g. Wernicke & Broca aphasia)

For example Wernicke and Broca area -> double dissociation

Compare multiple lesion groups (Stuss et al., 2000)
Participant have to match number with for example color or shape

Look for converging evidence with “TMS lesions” (or animal lesion studies)
For example lesion followed up by a TMS study that replicates the lesion

More detailed information using post-mortem measures
If people had a lesion and donate their brain, you can do an imaging study on the donated brain (post-mortem) -> more done on animals

Uncommon but not unseen in humans: remember patient HM?
Induce lesions mostly in people with epilepsy
Famous patient: removed part of hippocampus, couldn’t make any new memories

Disadvantages
- Necessarily largely data-driven (which lesion patients are available to you …)
- Lack of precision
  - Damage is not controllable
  - Often follows structural boundaries (e.g. vascularization), rather than functional boundaries
- Problems with comparison;
  - How did the person behave before the trauma?
- Often small sample sized

-> Less used since the invention of TMS (however, subcortical lesion studies remain popular)

Problems with interpretation
Lesion studies may “examine the capability of other cortical circuits in the absence of removed tissue and not the true functions of the removed tissue” (Lomber, 1999)
- Brain networks:
  - Regions are interconnected and complex behavior often relies in a combination of regions or brain networks (systems approach)
- Neural plasticity:
  - Soon after a disease or trauma that the rest of the brain adapts and is over certain functions of the lesion
  - Areas surrounding can compensate for the lesion
- Behavioral plasticity
  - The organism adapts to its new impairments (and often new environments, learns new routes to survive, there’s certain malfunctions …)

Intercranial recordings

Certain medical procedures involve the placement of intracranial electrodes (intracranial EEG, iEEG)
- Electrocorticography (ECoG) refers to the use of the electrodes placed directly in the exposed surface of the brain to record electrical activity from the cortex
- Stereoelectroencephalography (SEEG) is the practice of recording electroencephalographic signals via depth electrodes (electrodes surgically implanted into the brain tissue)

Intra-operative recordings STN during foot-tapping task
During surgery participants move feet on pedals like they are walking
Incongruence: person has to stop
Feeling that people suddenly can’t walk anymore: caused by overload of the system, …

Disadvantages
- You cannot pick your subjects
- You are time constraint: within the short period of time that electrodes are present (~ a week)
- Currently the gold standard for defining epileptogenic zones. However, remains risky and highly invasive …

Intracranial electrodes can also be used to stimulate the brain:
- Deep brain stimulation (DBS): electrodes are implanted more permanently and controlled by a “brain pacemaker” sending electrical impulses

Deep brain stimulation and rewarding learning in PD (Frank et al., 2007)
- DBS stimulates the STN, thereby improving motor symptoms in PD patients. A model of the basal ganglia also predicts increased impulsivity with STN stimulation
- Indeed, patients on DBS showed speeded responses to conflicting choice conditions (i.e. increased impulsivity), independent of their medication

Vagus nerve stimulation (VNS) stimulates the vagus nerve (parasympathetic nervous system)
Vagus nerve is connected with locus coereleous
Vagus Nerve stimulation and the P300 (De Taeye et al., 2014)
- VNS is believed to influence locus coeruleus and noradrenaline activity, thought to be responsible for the P300, a well-known late ERP component following surprising stimuli (e.g. oddballs)

Functional Near-Infrared Spectroscopy (fNIRS)

1. Working principle of fNRIS

Shine near-infrared light on skull -> light penetrates skull (±3cm) and bends -> attenuation of light depends on amount of oxy/deoxyhemoglobine (~ brain activity) -> Intensity of reflected light is measured as a proxy of brain activity

Equipment: NIR-emitting and detecting optodes
Red: sources
Green: detectors
Sources emit the light detectors detect it, distance between the two is crucial
Much more compact than fMRI, also possible while sitting, standing, …

Light penetration
Short waves immediately get absorbed
Longer wavelengths go deeper in the skull until light is able to reach the blood vessels
Too close: you would only detect the light in the skin, hasn't actually reached the activity that is deeper
The further away, the deeper the activity
Too far: the deeper, the more light is absorbed, light still has to be detectable
-> you can't measure deep brain activity (basal ganglia etc)

Light attenuation
Senders need to emit at least two NIR-signals with different wave lengths (< 800 nm and >800 nm)
Concentrations of oxy- and deocyhemoglobine can be derived using fancy equations.

A proxy of brain activity
Oxy-deoxy concentrations depend on brain activity: BOLD-response

2. Advantages and disadvantages of fNIRS

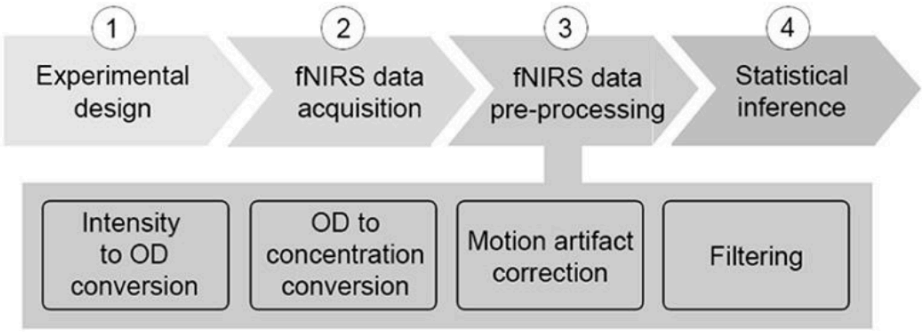
Advantages
- Better temporal resolution (± 125 ms) and cheaper than fMRI
- Better spatial resolution than EEG
- Less sensitive to motion compared to fMRI
- More inclusive than fMRI (no issues with claustrophobic participants, metal implants, …)

Disadvantages
- Inferior spatial resolution (3cm) compared to fMRI (1,5 to 3 mm^3)
- Inferior temporal resolution compared to EEG (order of ms)
- Sometimes tricky to get good quality signal if participants have dark/thick hair
- Low(er) signal-to-noise ratio (e.g. compared to fMRI)
- Can measure superficial activity only

Either best of both worlds, or worst of both worlds

3. fNRIS experiment design considerations

fNRIS experiment pipeline

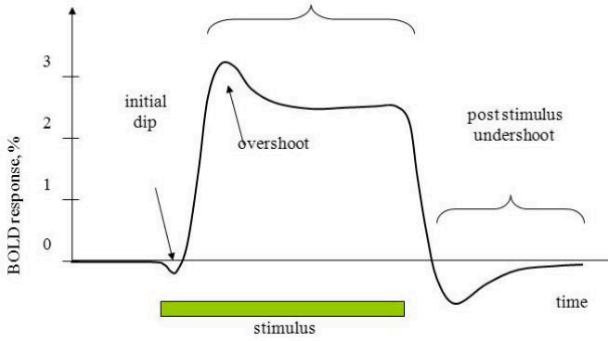


Experimental design
Decisions
- Optode template ('montage')
  - Optodes are time consuming (brush hair aside, refitting, …)
  - The more optodes, the lower the sampling frequency
  - Too many -> light patterns will interact with each other
  - Trade-off: number of optodes and sampling frequency
  - > Good a-priori idea of where activation will occur
- Design: block vs event-related
- Duration
- Number of participants

Longer duration and more participants compared to fMRI (cf. low SNR)

4. Preprocessing the fNRIS signal

Expectations
Desired signal: Initially very short dip, after huge increase which falls back to baseline
Reality: a lot of noise



A. Intensity to OD and OD to concentration conversion

$$OD_{channel} = \frac{Intensity_{detector}}{Intensity_{sender}}$$

Concentration of foxy and deoxyhemoglobine derived from OD using scary formula (Beert-Lambert law)

B. Noise removal
1) Instrumental
Other light sources in the room (dark room)

2) Experimental errors
Put an extra cap for pressure: some participants can't stand the pressure and want to stop
You want to avoid the cables from moving too much
If participants moves their head it is visible in the data, but very hard to correct

3) Physiological
Heart rate, perspiration rate spikes oxyhemoglobin
Speaking also has impact on breathing
Short-distance measures for noise removal: only pick up in skin and skull, not deeper
-> regress that out of deeper measurement
Filtering and algorithmic approaches to remove noise

5. Statistical inference

Block averaging
Perform t-test/ANOVA on average/maximum/slope
-> multiple comparison issues (cf. fMRI)
Be careful with multiple comparisons -> more comparing -> more false positives

General lineair model
Function convolve with hemodynamic function, put it on top of brain activity

6. Applications of fNIRS

- Populations less suitable for fMRI, e.g. infants/young children
- Measures online verbal responses
  - Verbal: when you speak you are very likely to move the head -> bad for fMRI
- More ecologica test environment
  - Seated testing
  - Portable fNIRS
- Experiments involving a lot of movement (e.g. throwing) or relying on subtle auditory manipulations
- Large N-studies (fMRI might be too expensive)
- Method to pilot experiments prior to fMRI scanning

Example: development of language lateralization
Very young children to adults
Online recordings, at very young age, people are already lateralized for language

Example: fNIRS during simulated driving
Can fNRIS differentiate between exhausted and rested drivers? Yes

Example: Hyperscanning
does brain activity synchronize when people perform the same task simultaneously?

Critical views on neuroscience

Neuroenchantment

People (researchers included) tend to believe studies more (or consider them more important) when Neuroimaging is involved (also termed “seductive allure” of neuroscience)

- Weisberg et al. (2009): laymen judge bad explanations as more satisfying in the context of irrelevant neuroscience information
- McCabe & castel (2008) asked people to rate scientific studies and presented these abstracts either with or without a bar graph and with or without a map of brain activation
  - Participants rated the findings higher when they were accompanied by a brain image
- Ali et al. (2014) found that (neuroscience) students could be easily convinced that a fake neuroimaging instrument (recycled salon hair dryer) could predict their thoughts
  - The students deemed the technique highly plausible and were hardly skeptical

But: this view has been challenged by other studies (Farah & Hook 2013; Michael et al. 2013)

These examples show that much depends on interpretation, how data is analyzed, published, and communicated to the community
Neuroscience is still a very young science: naturally, the limits and possibilities of most techniques are still unknown and parameter settings are often arbitrary
For example, Horvath et al. (2015): “evidence that transcranial direct current stimulation (tDCS) generates little to no reliable neurophysiological effect.” & “our quantitative review does not support the idea that tDCS generates a reliable effect on cognition.”

- Be critical when reading but also when doing your own research
- Don't feel threatened by complex terms
- Consult or collaborate with experts

Neuroenchantment is already less popular at the moment

Blobology

The practice of singling out fMRI blobs and attributing them to a single function
Hyper criticized paper try to find a “blob” for very complex phenomena
Cingulate cortex is often activated, but not directly related to everything
The Cingulate Cortex Does Everything
“By the early 21st century the cingulate cortex had been found to be involved in loneliness (Eisenberger et al., 2004), religious experiences(Beauregard and Paquette, 2006), political leanings (Amodio et al., 2007), stimulus-reward associations (Takenouchi et al., ; 1999; Cardinal et al.,

2003), motor planning (Shima and Tanji, 1998), error detection (Devinsky et al.,1995), pain perception (Harris et al., 2007), social exclusion (Eisenberger et al., 2004), reward expectancy (Shidara and Richmond, 2002), sleep (Rolls et al., 2003), the placebo effect (Wager et al., 2004), optimism (Sharot et al.,2007), political liberalism (Amodio et al., 2007) and work from our group on neuroprosthetic models (Marzullo et al., 2006a)."

Reverse inference (related to blobology)

The practice of attributing a psychological construct to a participants behaviour because of the activation of certain brain region.
Insular cortex is activated so it must be love

But: reverse inference can be informative in certain contexts (Poldrack, 2006, 2011)

- Multi-variate pattern analysis (MVPA)
  Instead of reverse inference based on activations and assumptions about cognitive functions, MVPA infers mental states from data (unbiased).
- Large scale literature search via neursynth.org
  Using reverse inference in a different way: instead of asking how predictive an activation map is for particular cognitive process, it asks how well one can predict the presence of a particular term (word) in the paper given activation in a particular region.

Caution when using Neurosynth:
"The claim of pain selectivity is based on a statistical preference in dACC activation studies for the use of pain related words, compared with a modest number of alternatives (e.g. "salience). Neurosynth analyses are based on word frequencies and published papers. They may not reflect the actual processes studied, and are not linked specifically to particular brain locations. Neurosynth is useful for exploring structure to function mappings across a large literature, but it cannot provide definite of inferences about specific brain regions."
People used it slightly wrong -> biasing of results
Careful because you can make mistakes

Interim Conclusion

How to get past blobology and reverse inference?

- Most activations are relative effects
  - Knowing the baseline or comparison condition is crucial!
- There is often a "lower-level" explanation for an activation
  - E.g. anterior cingulate is involved in many processes which have a common ground (General saliency and behavioural relevance)
- A cognitive process is typically not only supported by one blob/region
  - Consider other regions of the network (connectivity analysis)
- Is the activation relevant/instrumental for observed behaviour
  - Correlational approaches, computational modeling

Statistical issues

- Multiple comparisons problem
- Circular analysis ("double dipping")
- "Voodoo correlations"
- Related issues: p-hacking, excess succes, publication bias

General problem: only reflect data indirectly
We need to acknowledge that the results only reflect the true data indirectly due to complex analyses and researchers assumptions

- Especially when combining neuroimaging methods, the researchers' degrees of freedom are very high
- Another general issue in research is the low level of transparency regarding data collection, analysis, and reporting of results

Assumptions are so circular that they already affect the data
Might not be as problematic if you have full disclosure, which is more often not the case
That said, we should try to avoid obvious pitfalls

Multiple comparison problem

Neural correlates of inter-species perspective taking in the post Mortem Atlantic salmon: an argument for multiple comparisons correction
Even in a dead salmon they found activity = random noise = false positive
The size of this cluster was 81 mm^3 with a cluster-level significance of p = 0.001. Out of a search volume of 8064 voxels a total of 16 voxels were significant.
Why?
- When testing thousands of voxels, random noise in the fMRI data can create false positive results
- Standard statistical tresholding at p<.001 is not sufficient, especially at small cluster sizes
- Algorithms to define significance thresholds for a given cluster size: FDR & FWZ in SPM, or 3dClustStim (whole-brain Bonferri correction would be too conservative: voxels are not independent and belong to a region)
- Another option is to reduce the data space, i.e. define ROIs, and then correct for number of ROIs (Bonferroni)

-> Not only a problem for fMRI but for any method that involves multiple tests of the same difference (multiple fMRI voxels, EEG channels, Genetic imaging, correlations with personality scales, etc.)

Circular analyses (double dipping)
Some analysis can be highly circular, example fMRI ROI analyses
Example 1: Voxel-wise comparison A>B reveals activity in a region (ROI-defining), then the same difference (A-B) is tested in this ROI
The analysis is entirely based by the ROI-defining contrast (A>B), both regressors are non-independent. You will find the exact same result as in the voxel-wise analysis just with more statistical power.

Example 2: Voxel-wise comparison A>B reveals activity in a region (ROI-defining), then the difference A>C is tested in this ROI
not completely circular
The analysis is biased in favour of condition A over C because you are looking at a region that was defined by A (one none-independent regressor). You are not only biasing the result; you will also miss out on differences elsewhere in the brain between A and C.

42% FMRI papers in 2008 (Nature, Science, Nature Neuroscience, Neuron, and J of Neuroscience) contained at least one non-independent or circular analysis, which could lead to invalid inference
Solutions for ROI analysis
- For both examples: extract the data from an independent ROI (literature, anatomical, functional localizer), perform statistical test A>B or A>C within this predefined ROI
- For example 1: Define the ROI based on the voxel-wise comparison A>B from all subjects except one, perform statistical test A>B within this ROI for the left-out subject (repeat for each subject)

“Voodoo correlations”
Puzzling high correlations in fMRI studies of emotion, personality, and social cognition
- fMRI studies frequently report correlations with personality data that are too high (r >.8) given the reliability of the two measures, which provides an upper bound for correlations
- Inflated correlations in 53% of the evaluated 55 fMRI studies due to non-independent analysis
- Common “error” (among others): computing separate correlations for individual voxels and reporting the mean of only those voxels that reached selected significance thresholds

-> To avoid this, extract fMRI activity from a data independent ROI and correlate the main activity across all voxels in this ROI with the personality (or other) measure of interest
-> But note that the strong statement by Vul was criticized in several reply papers
Separate correlations for each voxel, some are significant, some aren’t
If you take the average of the significant correlations, you are increasing correlation (throwing away the lowest scores)

Related issues: p-hacking, excess success, publication bias
P-hacking: collecting and selecting data or a statistical analyses until non-significant results become significant (e.g. include participants step-wise; exclude participants, trials, or conditions;

including covariates etc). Not all of these cases are problematic by definition, especially if the choices have been made a priori.
Selecting voxels is an example of p-hacking

Excess success: reported findings are often too good to be true as revealed by tests for excess significance (compare power estimates based on sample and effect size with the reported findings). This excess success is based on suppression of parts of the results within a paper and improper or selective analyses (all of the above).
bit related to voodoo correlations, but broader
Correlations above the a priori power
Tried a lot of things and only revealed the nice effect

Publication bias: Studies with significant results are more likely to be published (and published earlier) then studies with null results, creating a bias for findings that are in line with previous research (circularity across studies)
studies with less convincing results don't get published
Something might only work 1/50 times, but it will only get published when it does work

Critical views on Neuroimaging
Be aware!
- These issues result in a skewed picture of results in neuroscientific research
- Almost everyone taps into this at some point - and we are often not aware
- There is growing awareness in the research community

Increase transparency and integrity!
- Researchers: Share data/code, preregistration (register hypotheses and analyses prior to study), open access publications, publish/share null results
- Journals/Editors/Reviewers: don't favor confirmatory results over null results or controversial results; be more critical regarding statistics and overly successful replications, ask for data/code sharing