ISMRM | ANZ CHAPTER

fMRI unpacked: A guided tour from raw data to functional analyses

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THE UNIVERSITY OF SYDNEY

shinelab

CIHR IRSC

Canadian Institutes of Health Research

Instituts de recherche en santé du Canada

ISMRM | ANZ CHAPTER

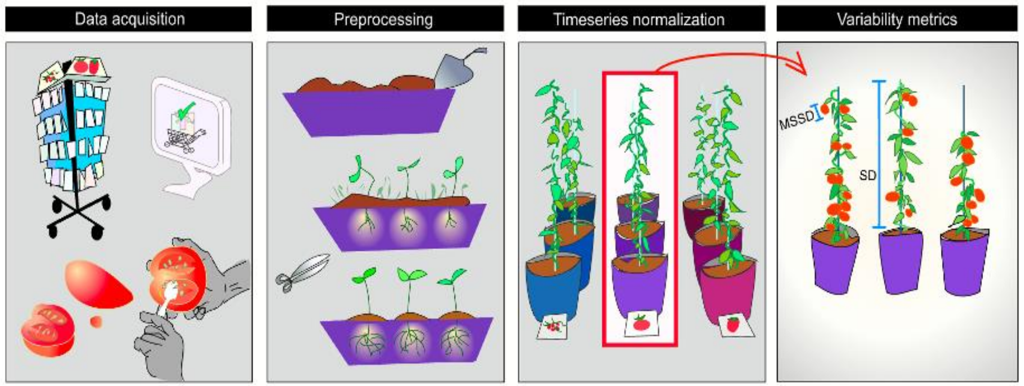
Declaration of
Financial Interests or Relationships

I have no financial interests or relationships to disclose with regard to the subject matter of this presentation.

Functional neuroimaging via fMRI

Theoretical concepts

- fMRI and the BOLD signal
- Intro to fMRI data acquisition, preprocessing & analyses



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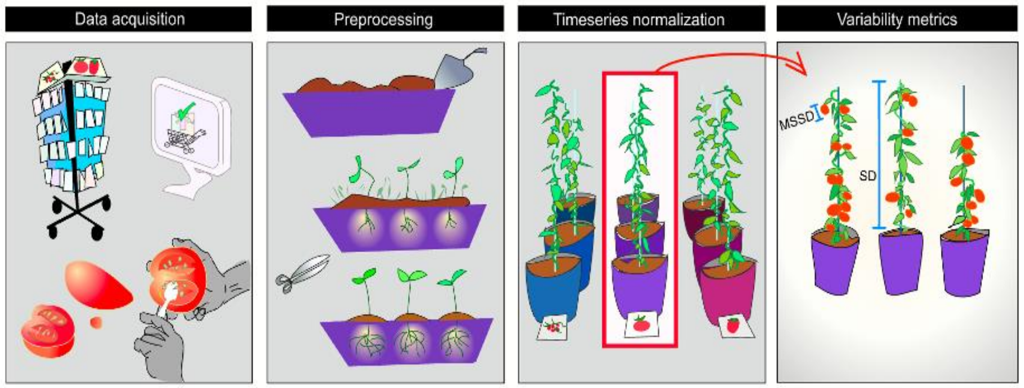
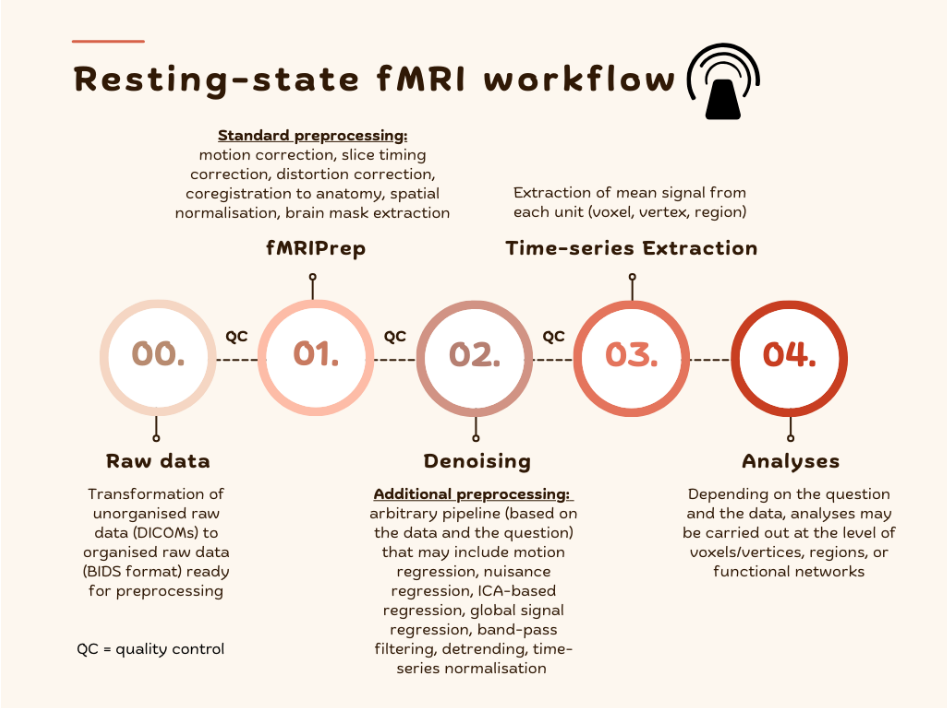


Tutorials & hands on analyses

Example: from raw data to analyses



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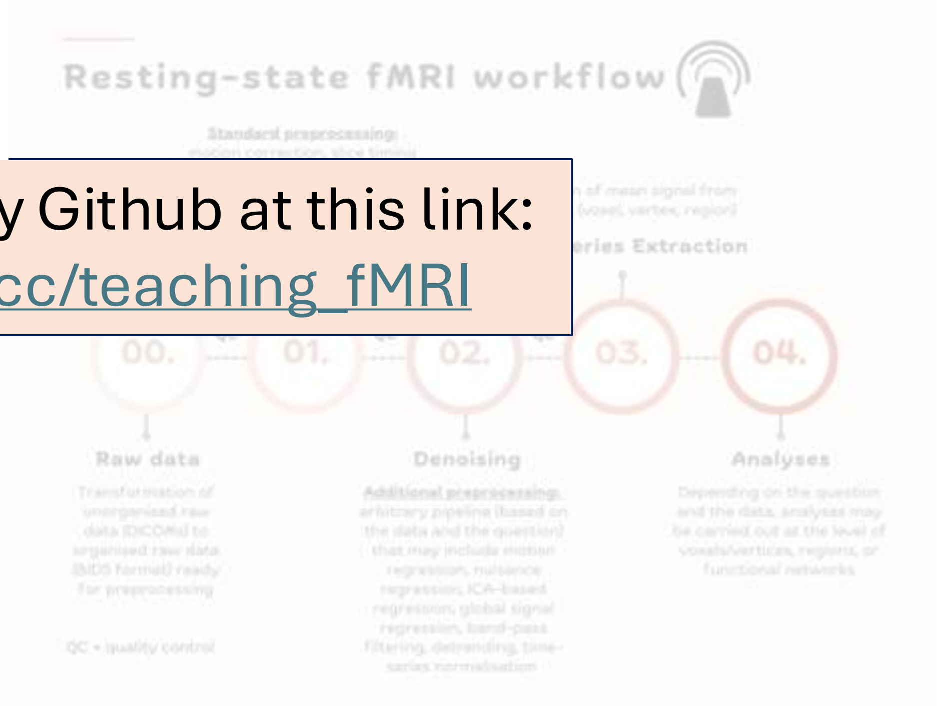
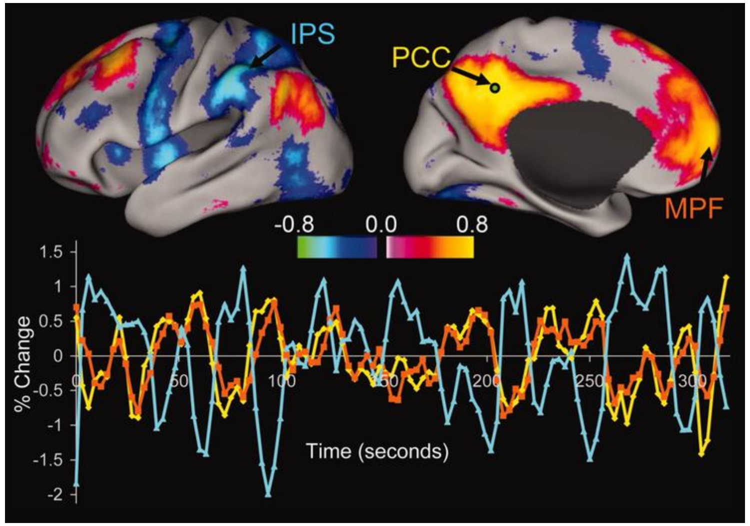
All materials can be found on my Github at this link:
https://github.com/giuliabaracc/teaching_fMRI



Quick Theoretical Recap: what is fMRI?

- Neuroimaging technique that allows us to image the brain at the macroscale:
  - at rest→ resting-state fMRI



Fox et al., 2005 *PNAS*

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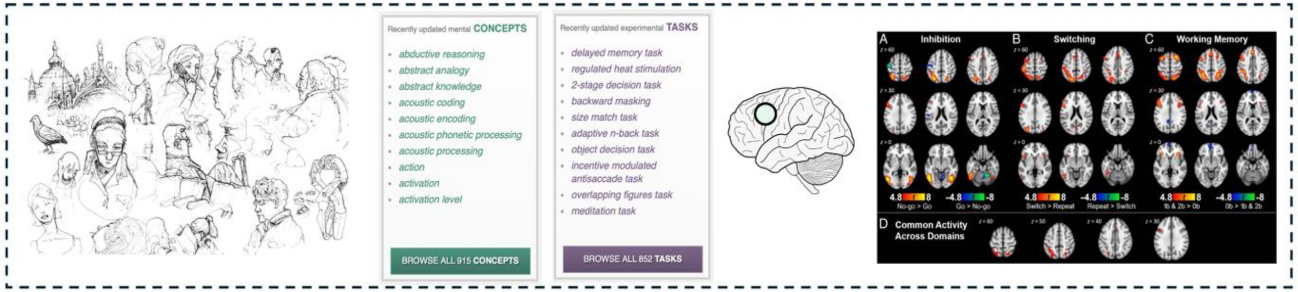
How the brain enables the mind = neural bases of human thought

cognitive psychology ⟶ neuroscience

Our current *modus operandi*



Poldrack et al., 2011 *Front. Neuroimform* Rieck, Baracchini & Grady, 2021 *JoCN*

phenomenological definitions of cognition | controlled environment | descriptive mapping analyses

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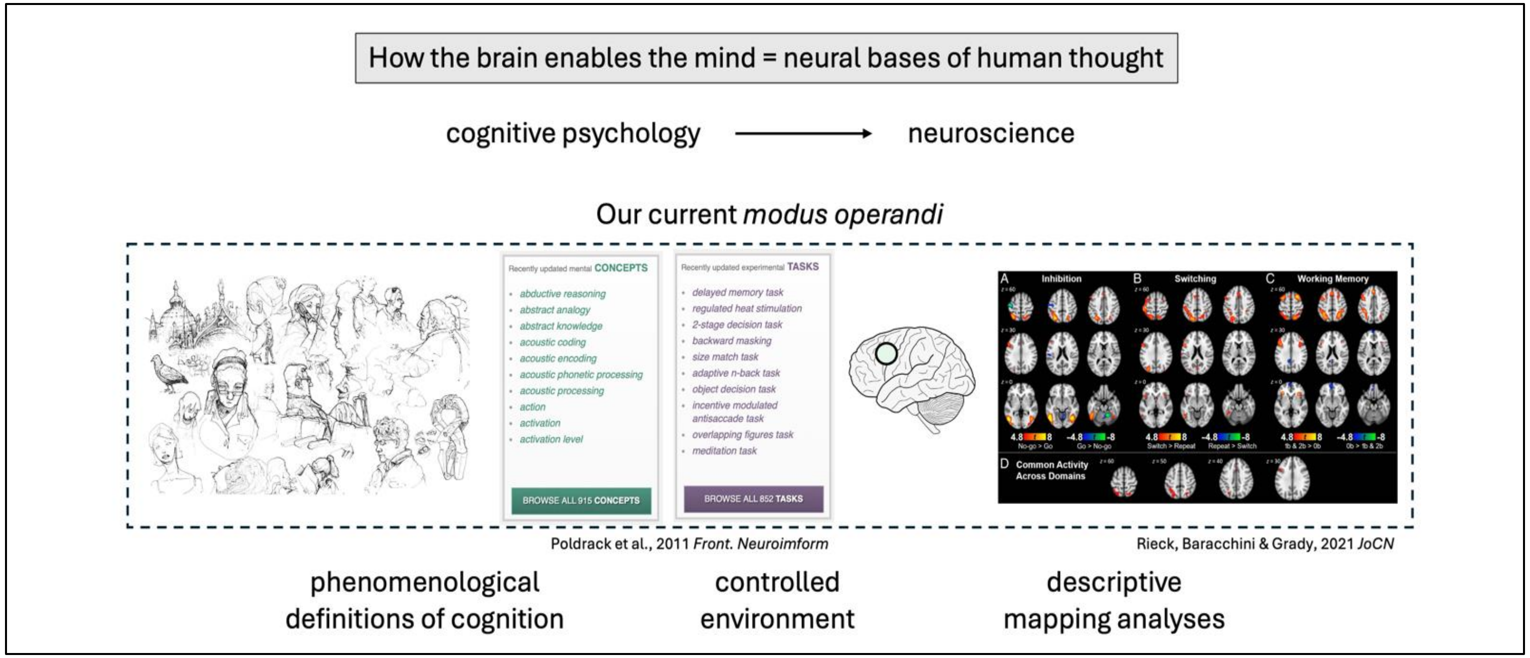
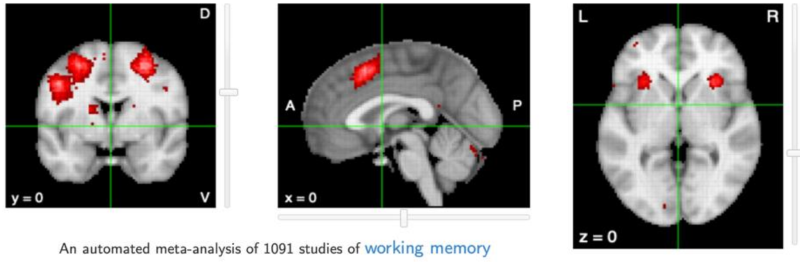
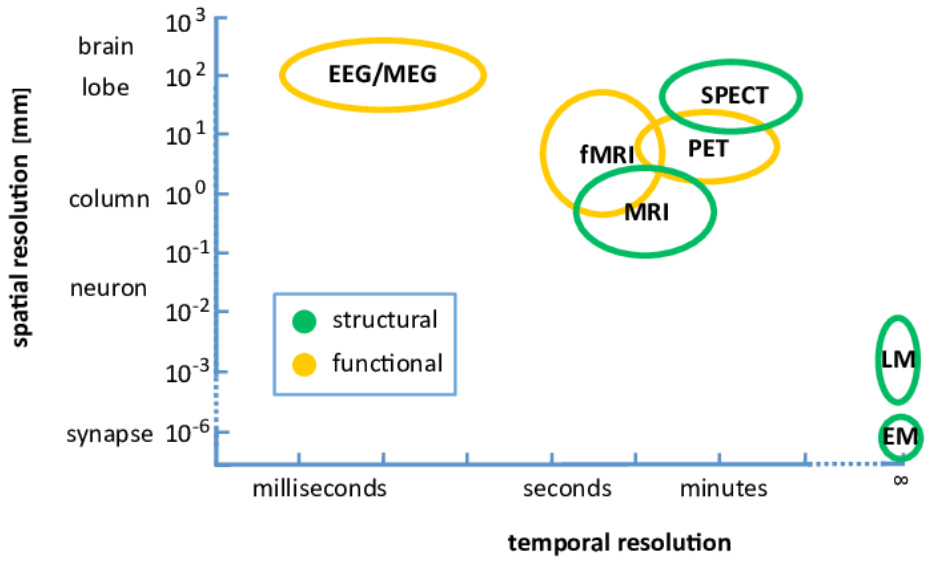




Neurosynth: https://neurosynth.org

Quick Theoretical Recap: what is fMRI?

- Key to obtaining *non-invasive whole-brain images at increased spatial resolution (compared to ephys)*



Pfister et al., 2012 **<u>*quite old</u>**

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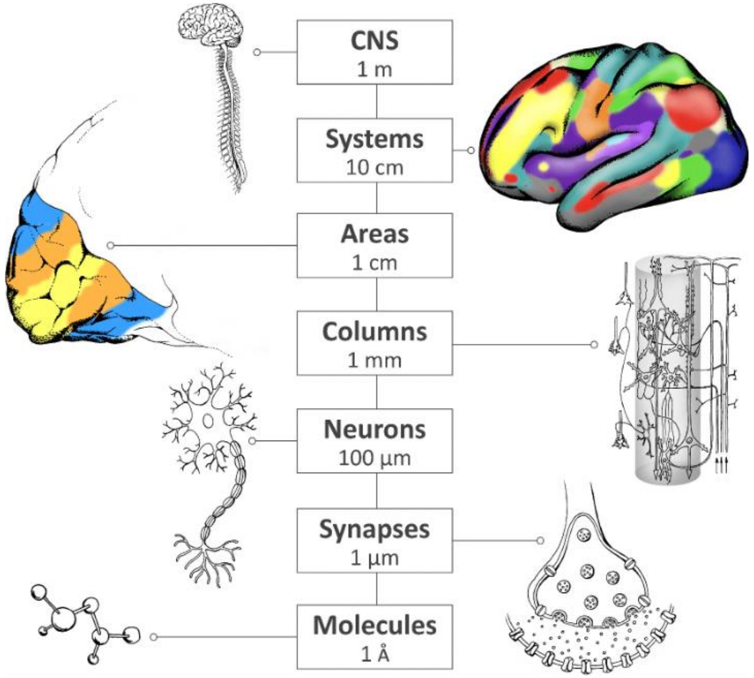

Petersen et al., 2024 *Neuron*

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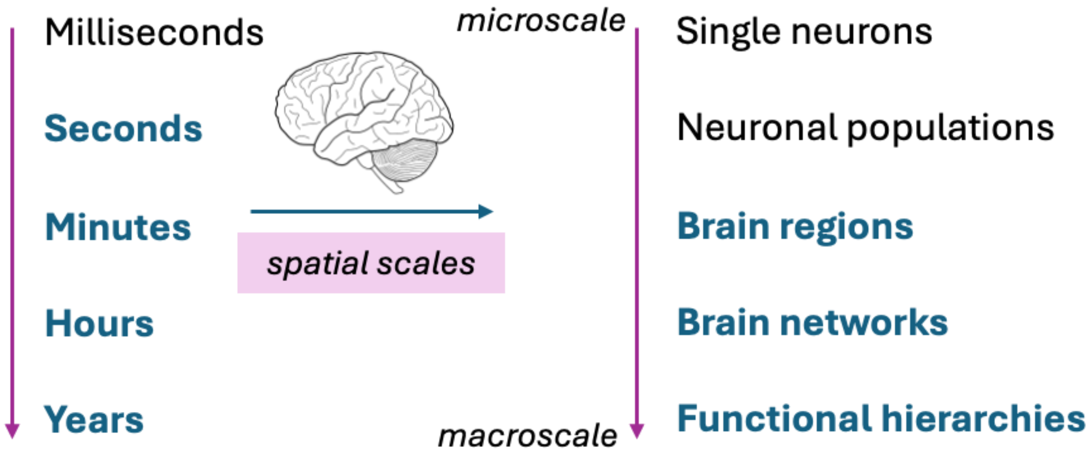
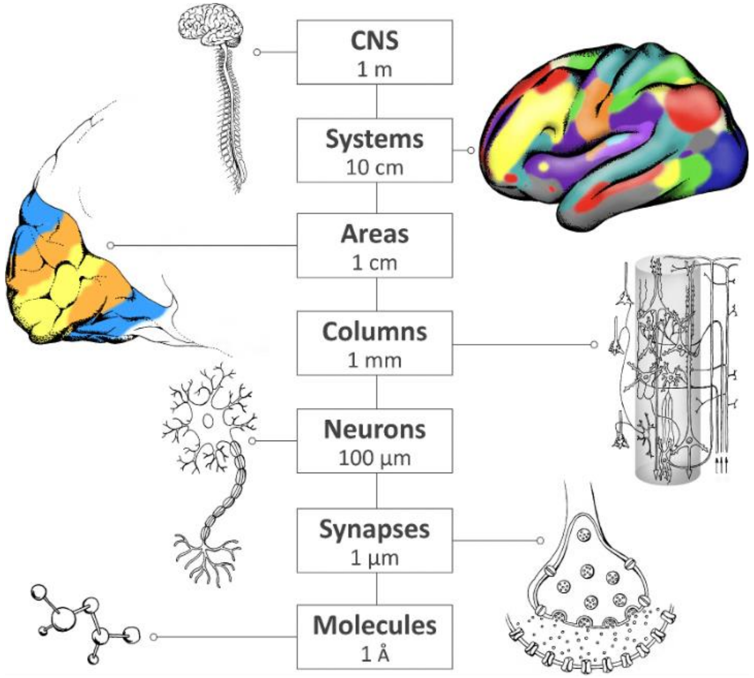
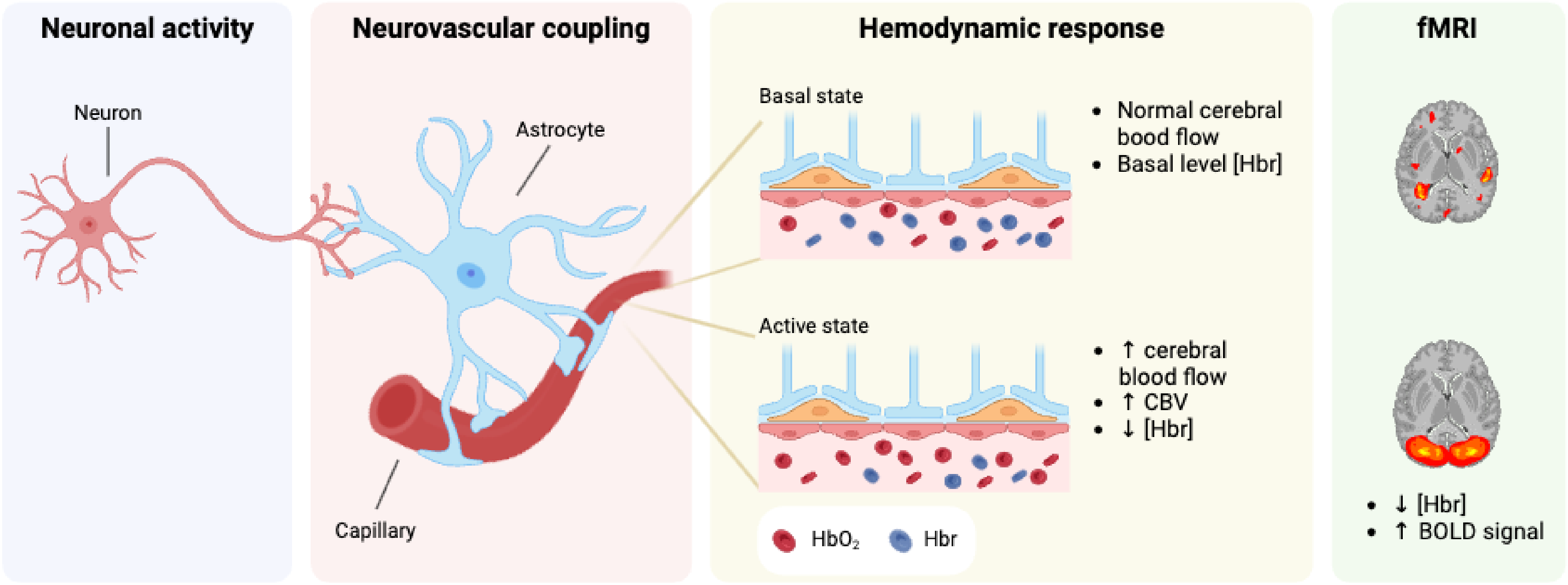




Petersen et al., 2024 *Neuron*

What is fMRI measuring?

BOLD signal

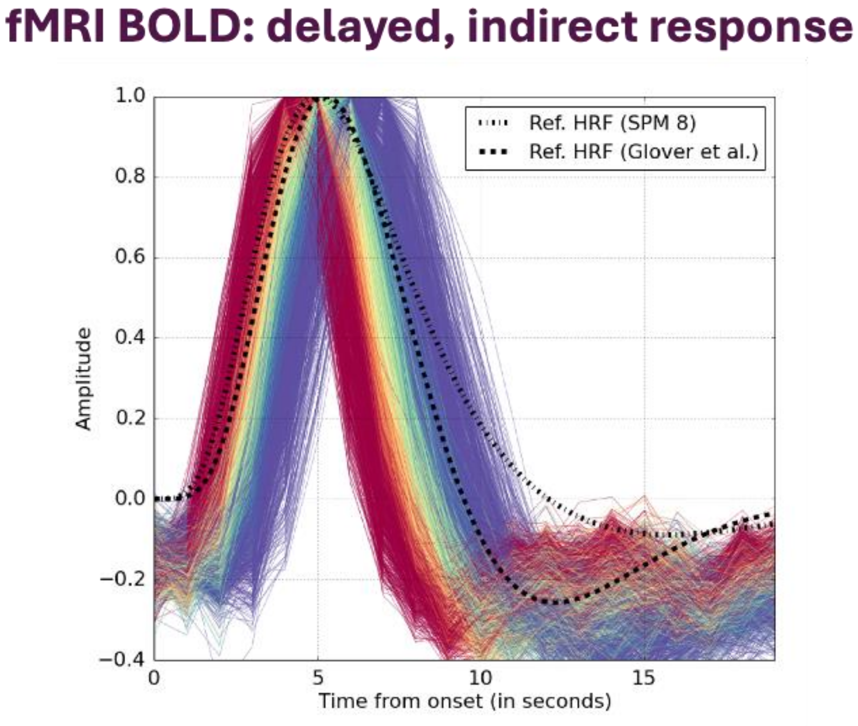


from https://www.biorender.com/template/bold-fmri-signal-overview

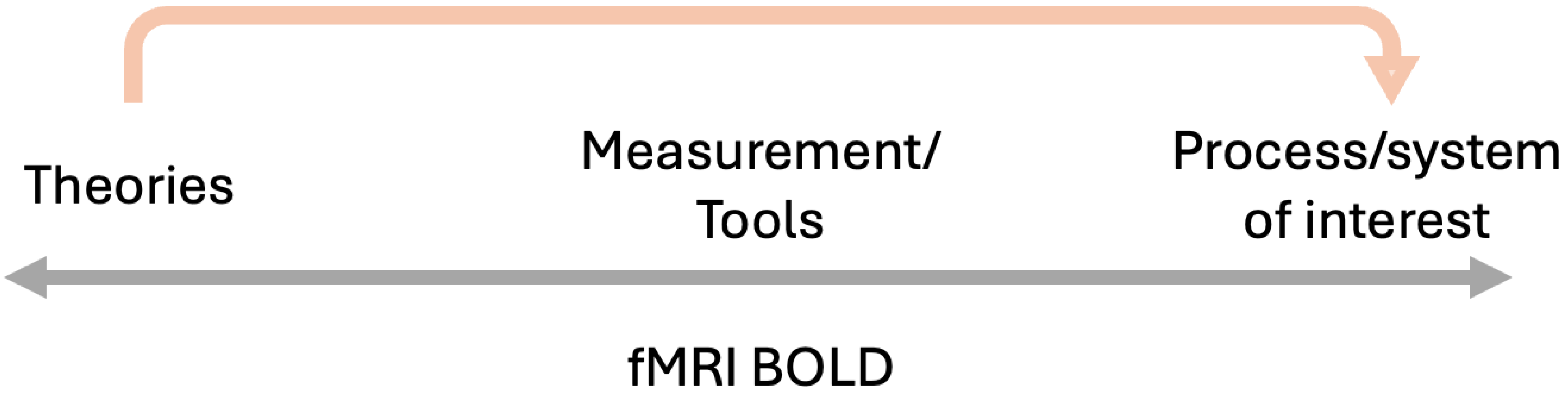
= local concentrations of deoxygenated/oxygenated Hb derived from neuronal activity

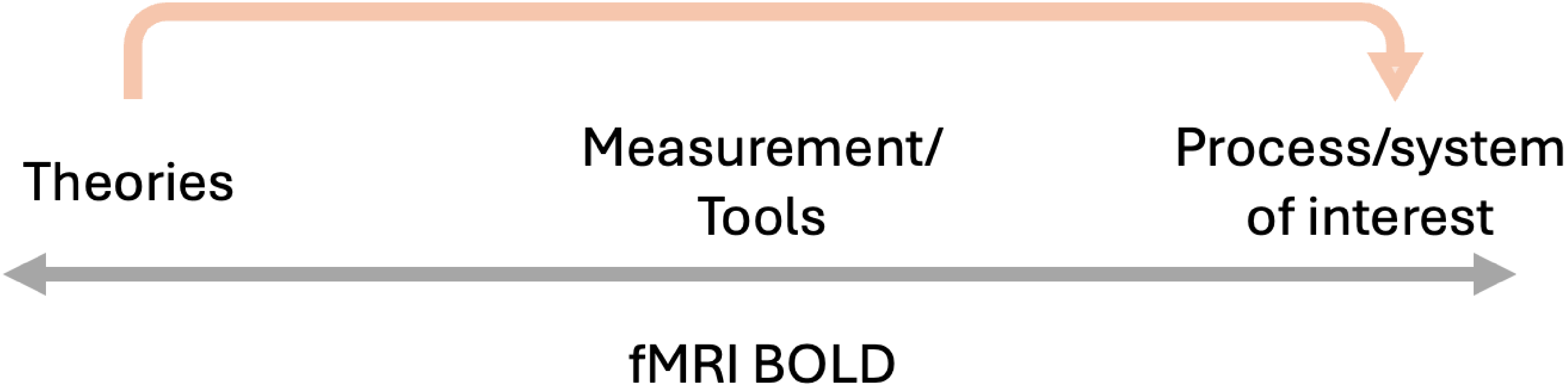
What is fMRI measuring?

BOLD signal



Pedregosa et al., 2015 *NeuroImage*
also Logothetis et al., 2001 *Nature*; Logothetis & Pfeuffer, 2004 *Magnetic Resonance Imaging*


Theories
Measurement/
Tools
Process/system
of interest
fMRI BOLD

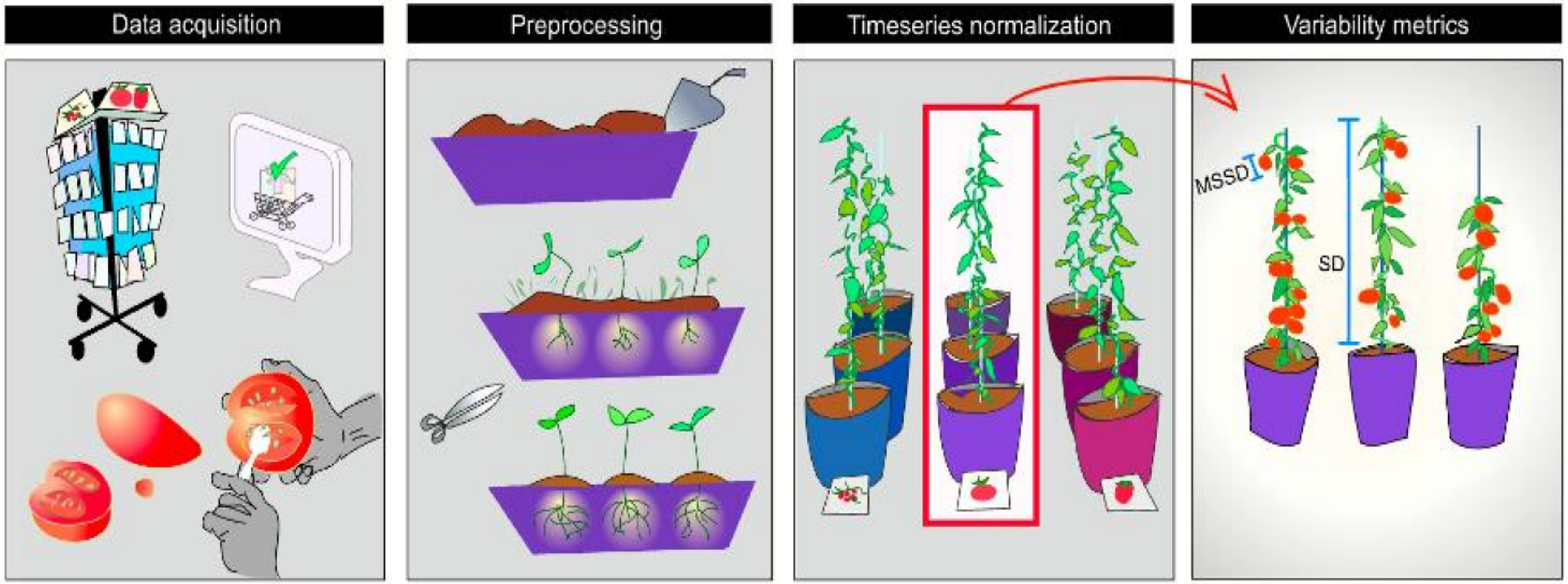


Key to have in mind a clear question:

good experimental design, data acquisition parameters, robust analyses*

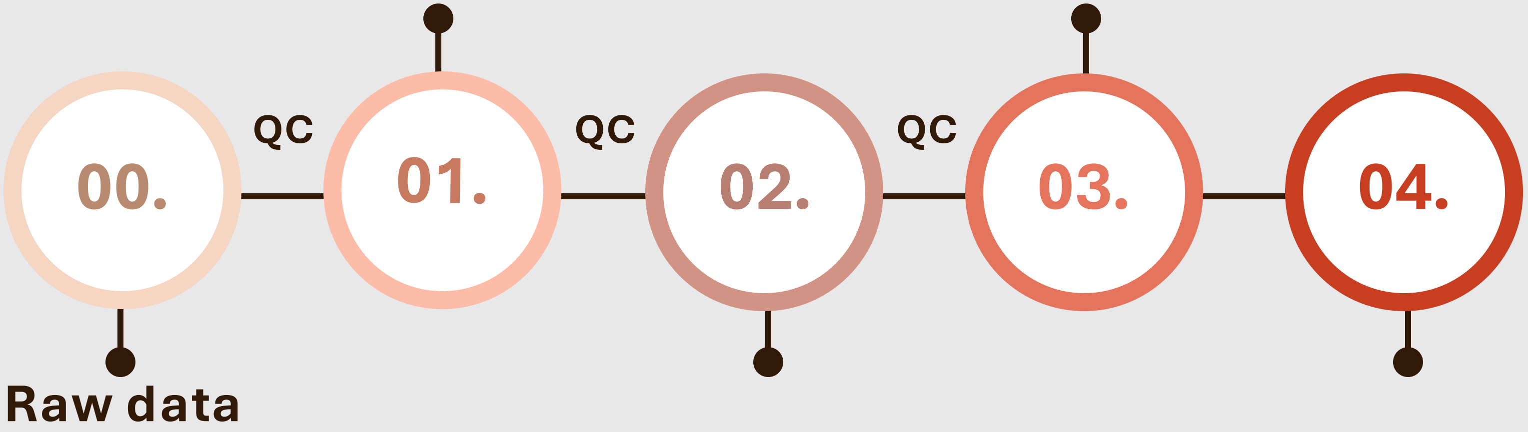
***not unlike any other technique in (neuro)science**

fMRI is like growing tomatoes ☺



*any fMRI-derived metric

Resting-state fMRI workflow

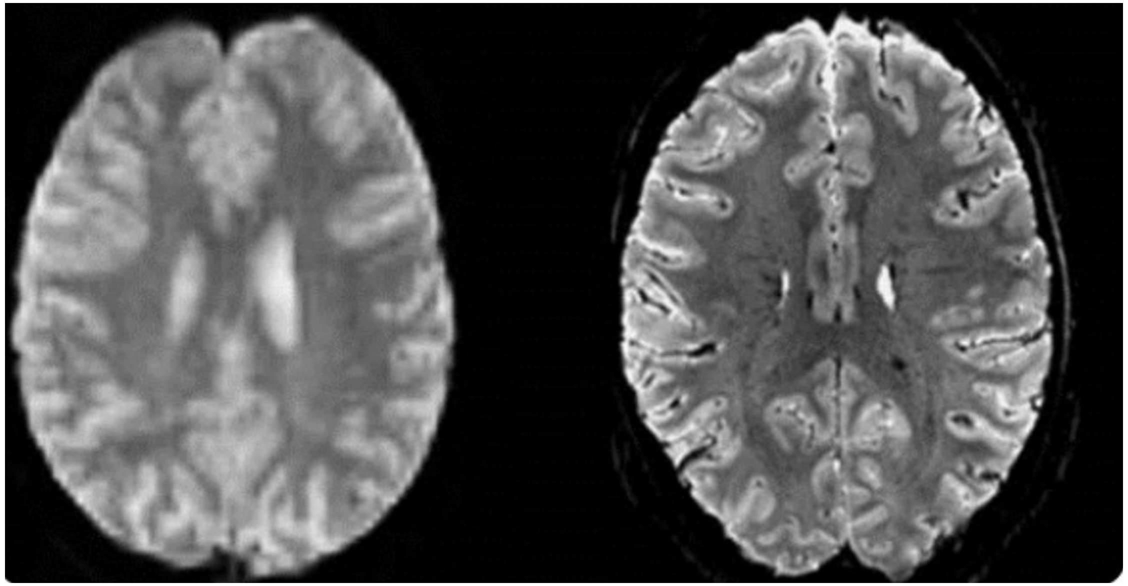


QC = quality control

00. Raw data (data acquisition)

3T; 3mm

7T; 0.75mm



Many flavours of fMRI data

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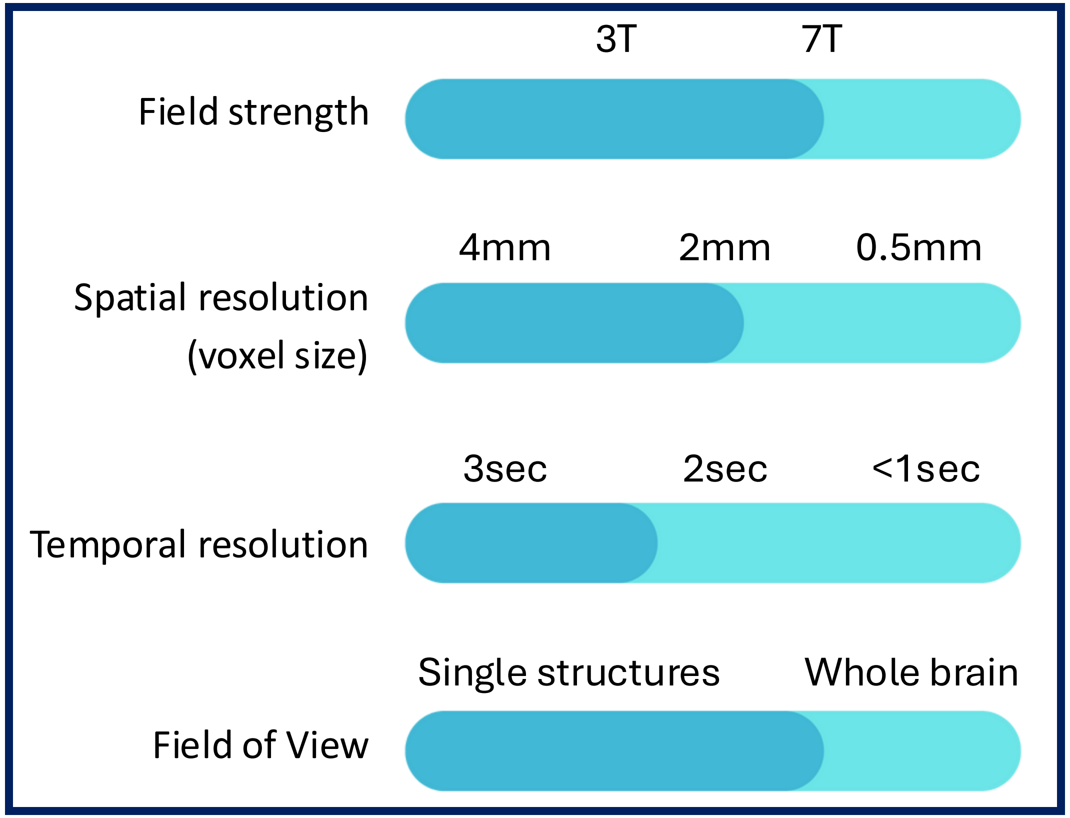

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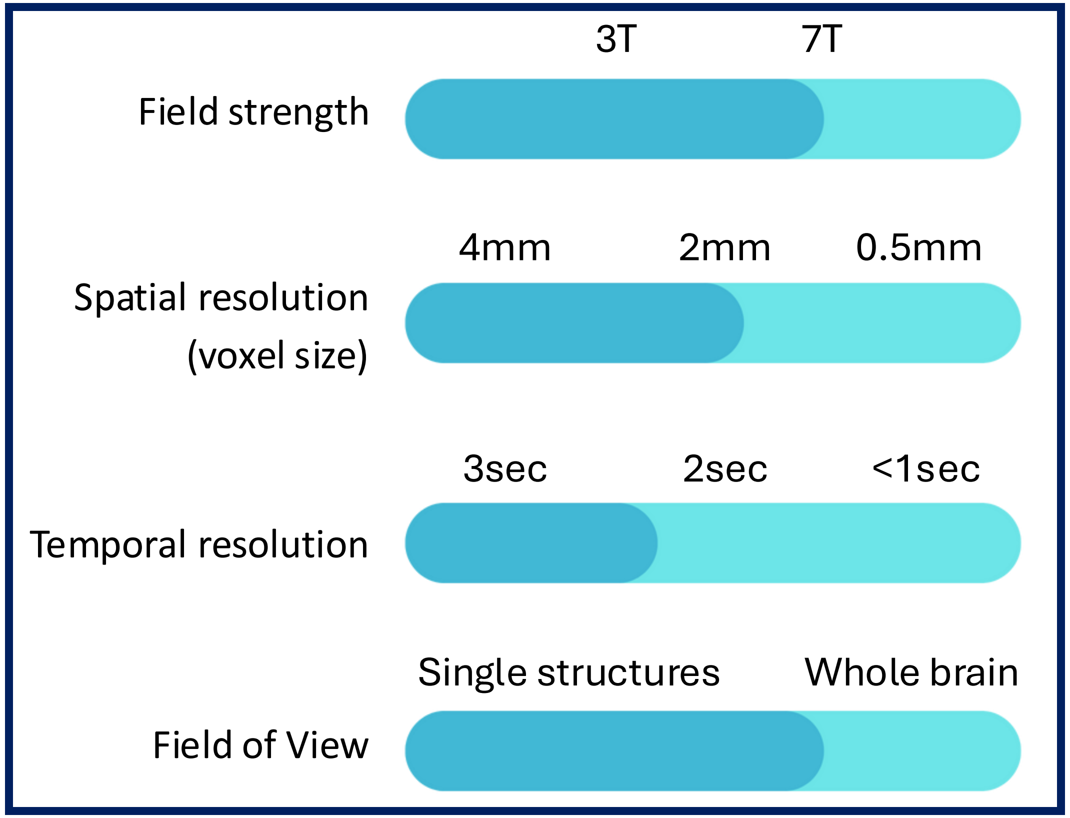

These user-dependent choices impact:

- ❖ Image quality (signal-to-noise ratio, SNR):
  - ❖ Good quality signal/lots of noise (non-biological)
- ❖ Ability to image smaller brain structures
- ❖ Ability to image fast dynamics
- ❖ Scanning time

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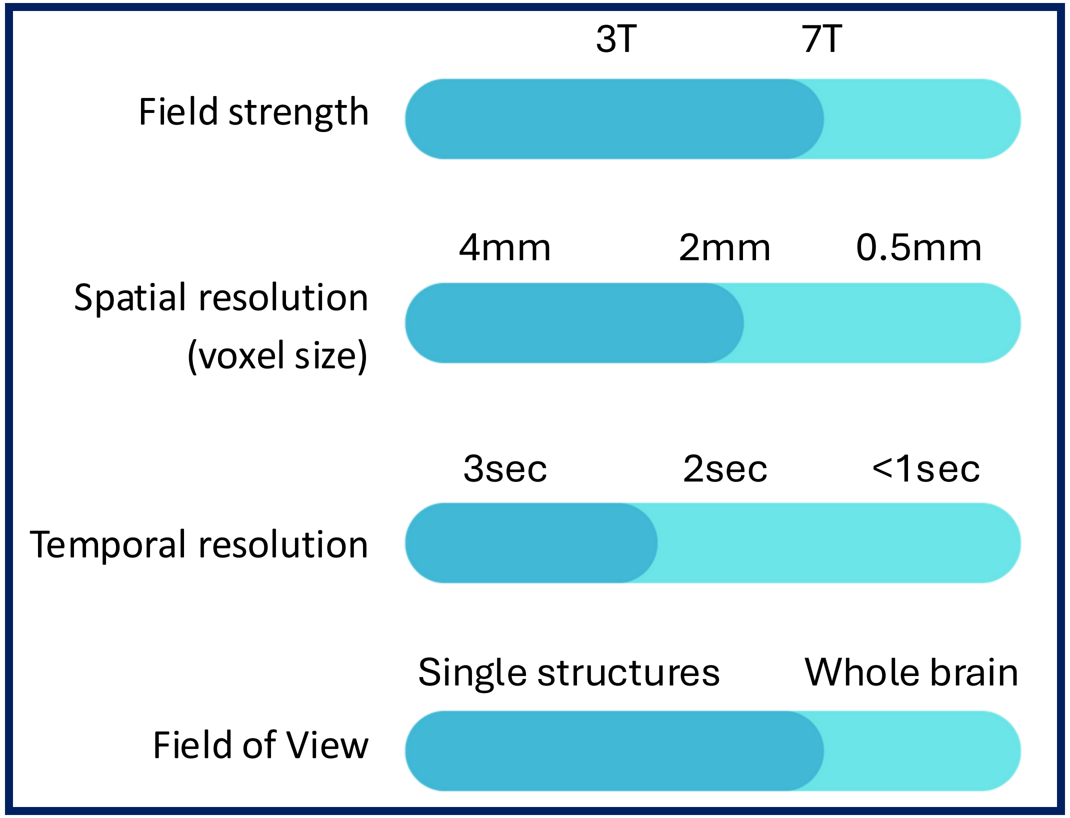
Generally, speaking:



7T:
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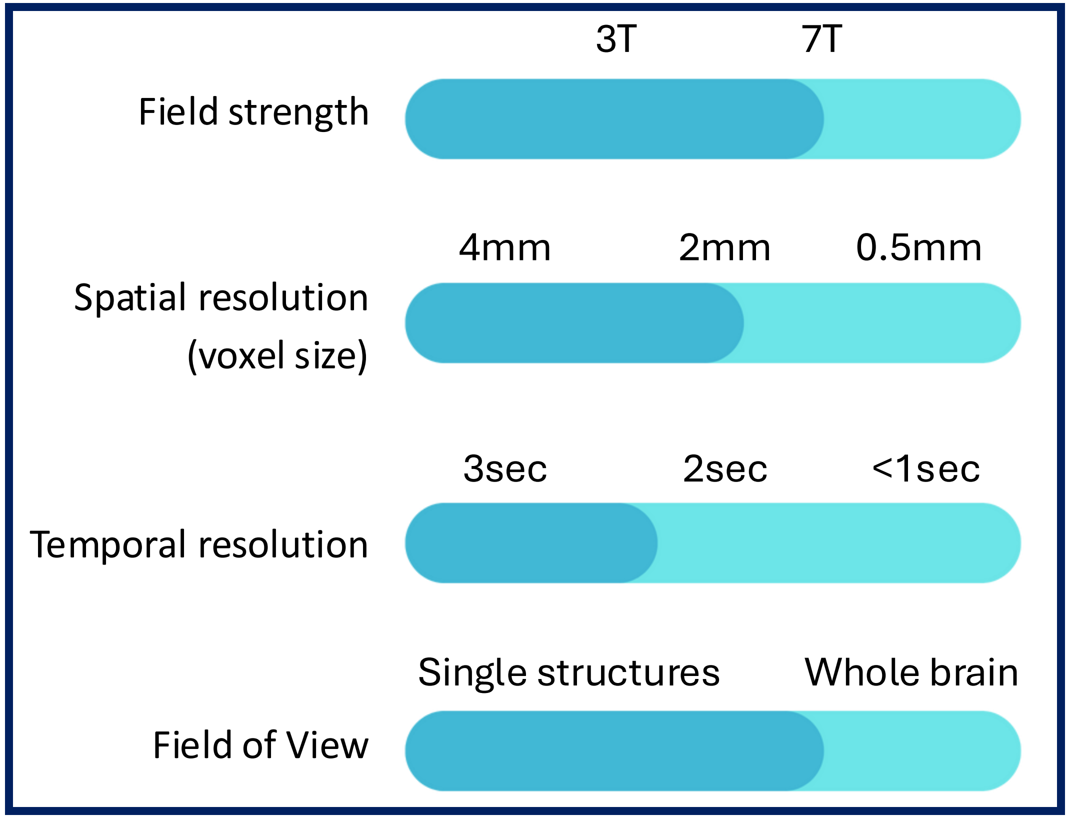

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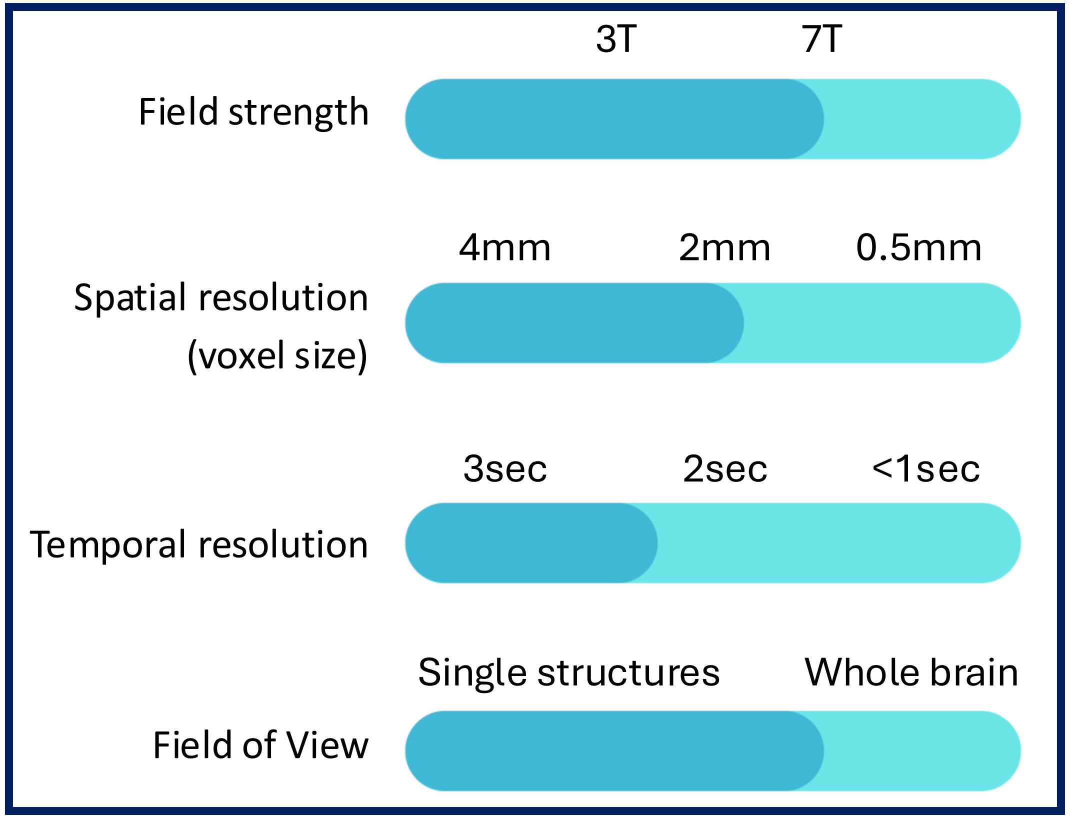

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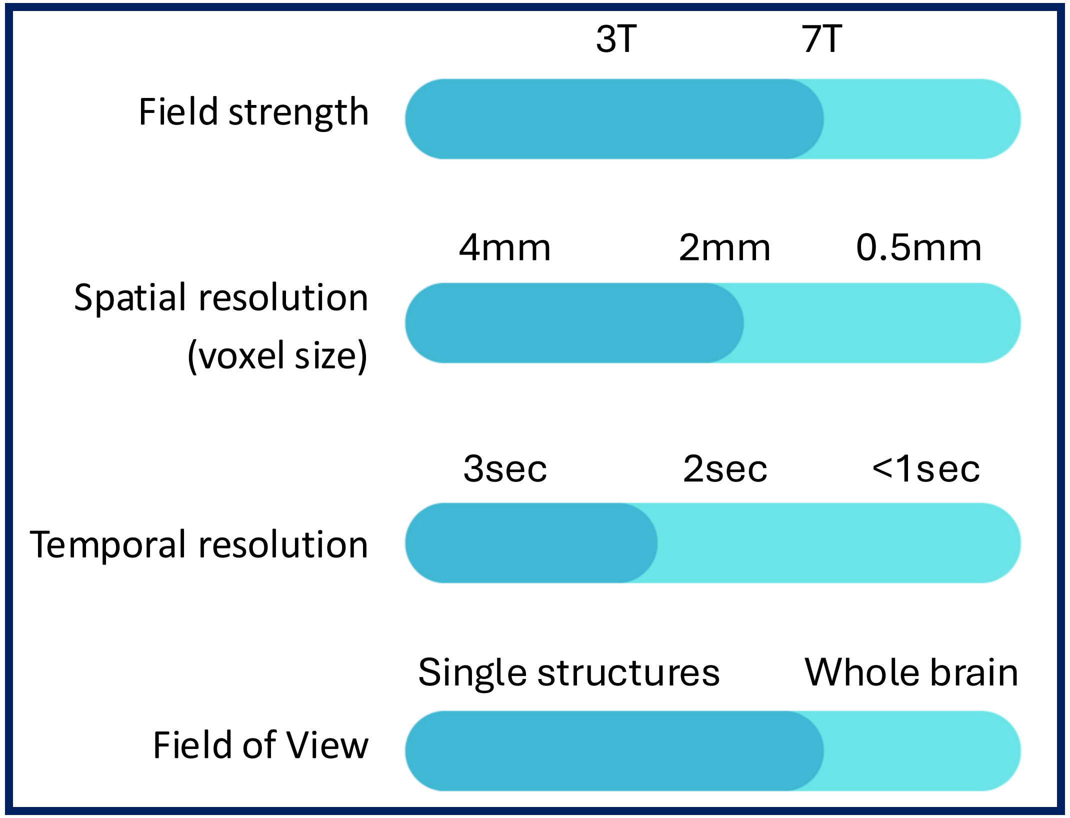

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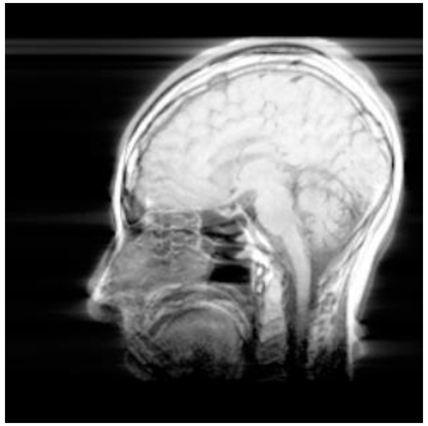
Structure-specific acquisition (eg laminar fMRI):
- >>> increased precision

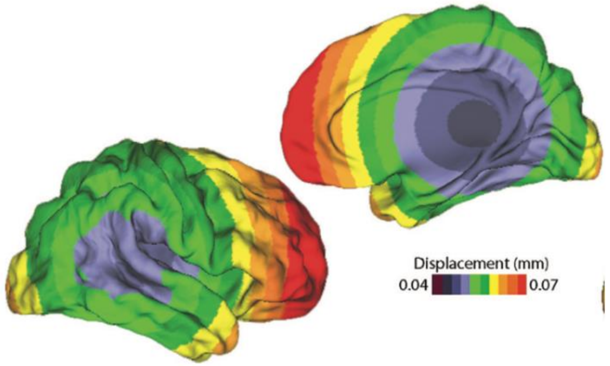
00. Raw data (data acquisition)

Yet, no matter the flavour of fMRI data:

Raw fMRI data are noisy due to...

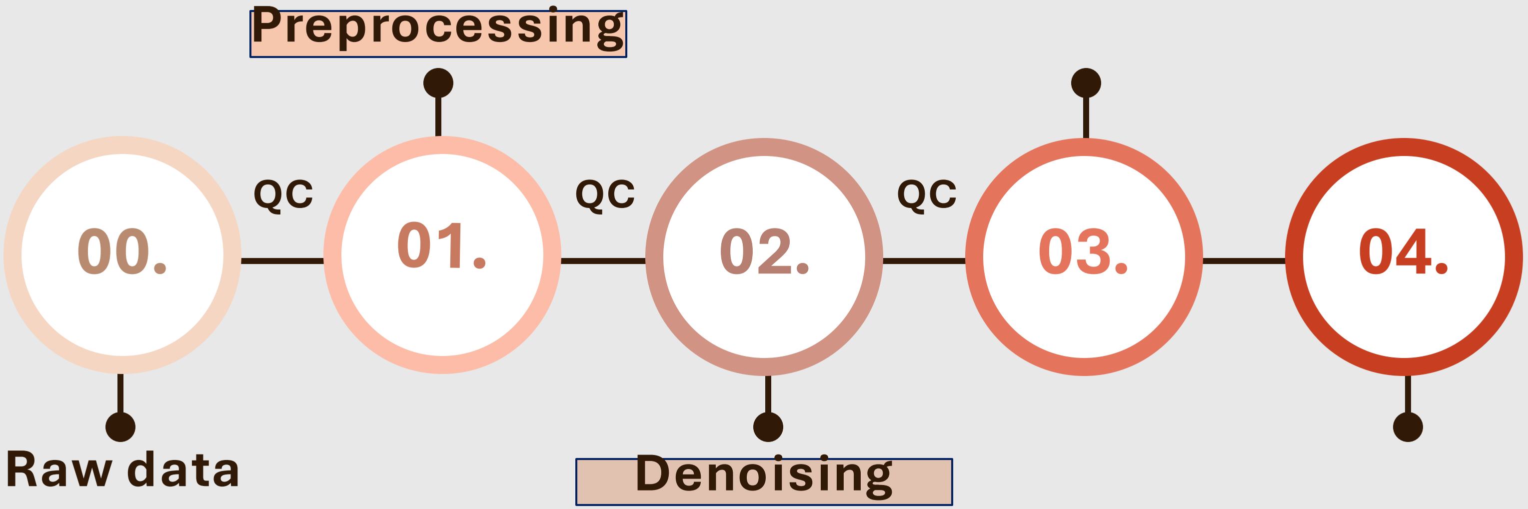
- scanner artefacts (inhomogeneities, signal drop outs)
- participant (motion, breathing)
- non-neuronal sources (yet biological)





Satterthwaite et al., 2019 *Human Brain Mapping*

Resting-state fMRI workflow



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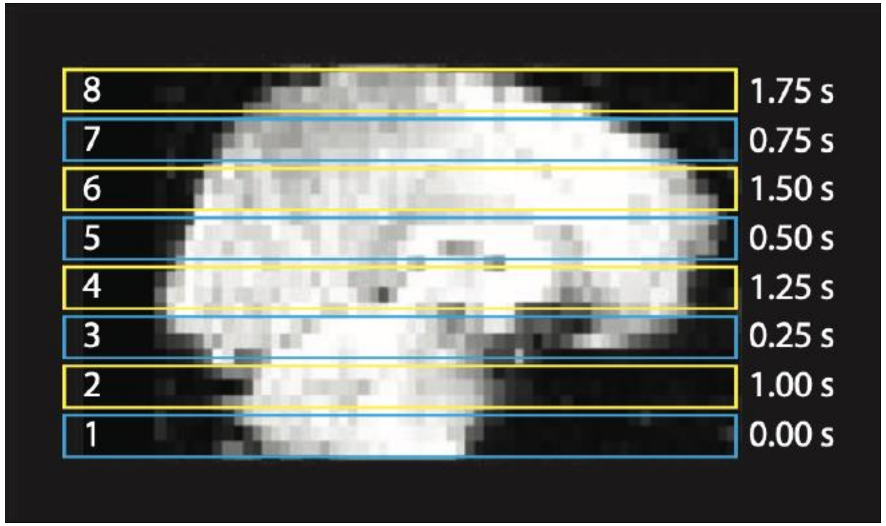

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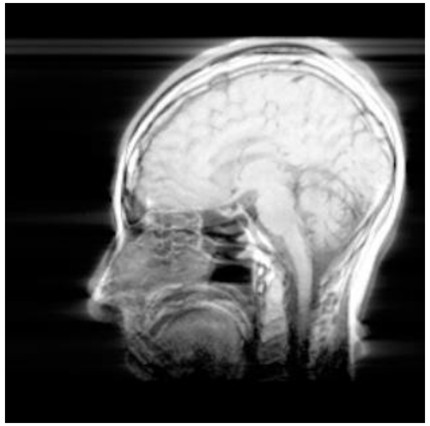

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Raw data are noisy + **underlying assumptions are UNTRUE**:

- *For each TR, whole-brain data acquired simultaneously*
- *Data constant across time*
- *Data constant spatially across people*

01. Data preprocessing

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standardized, aligned data with noise

common across acquisition protocols and labs

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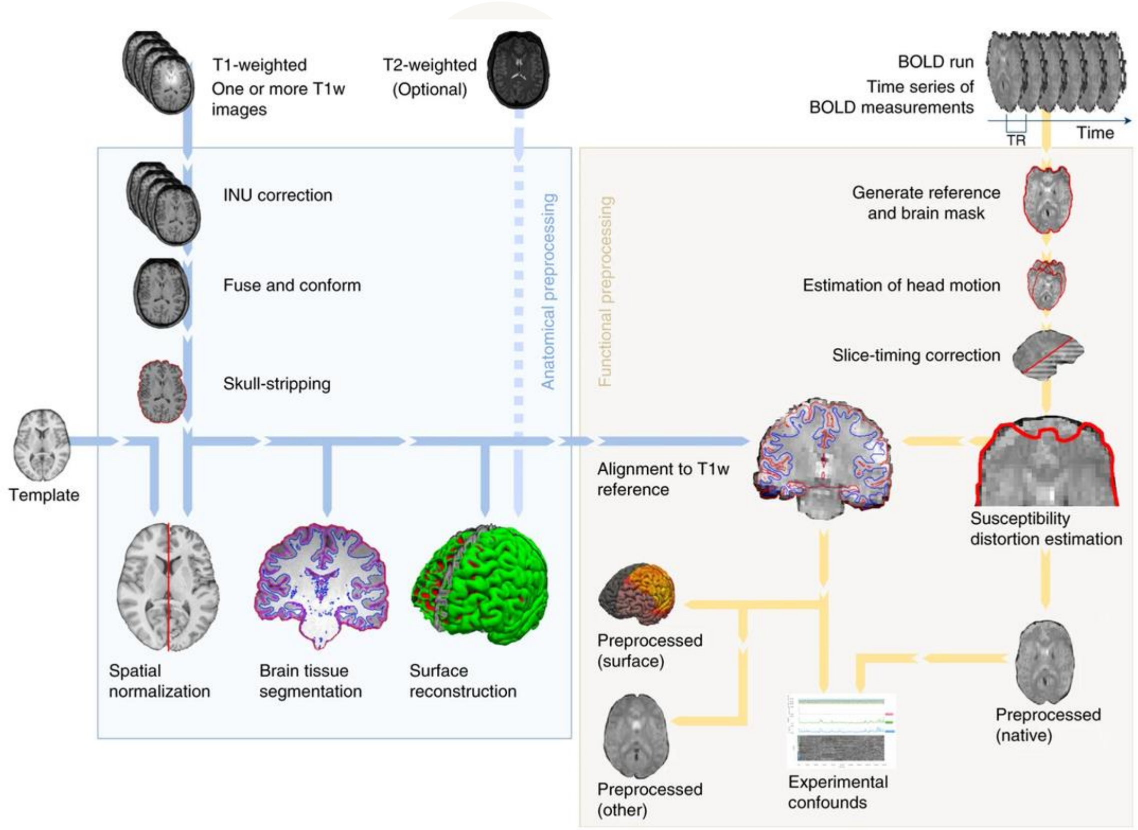
fMRIPrep



Esteban et al., 2019 *Nature Methods*

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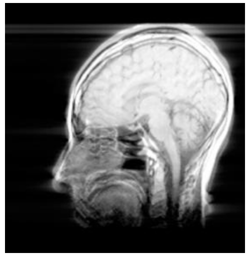
motion correction
slice timing correction
distortion correction
coregistration to anatomy
spatial normalisation
brain mask extraction

common across acquisition protocols and labs

Motion Correction
Corrects head movement by aligning volumes



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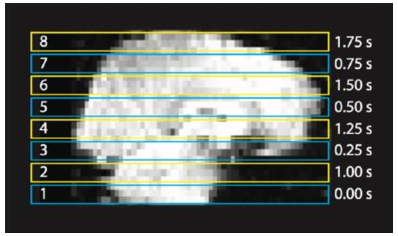

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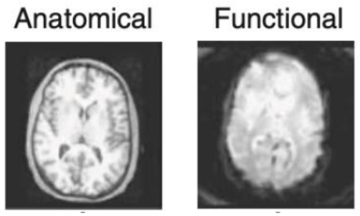

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Brain Mask Extraction
Includes only brain voxels for analysis

01. Data preprocessing

Exemplar script to run fmriprep: /code/fMRIprep.sh

In your own time: /fmriprep_outputs/*.html

(to open the html you need to download the respective sub figures folder)

Summary

- Subject ID: 003
- Structural images: 1 T1-weighted
- Functional series: 1
  - Task: rest (1 run)
- Standard output spaces: MNI152NLin6Asym, MNI152NLin2009cAsym
- Non-standard output spaces:
- FreeSurfer reconstruction: Not run

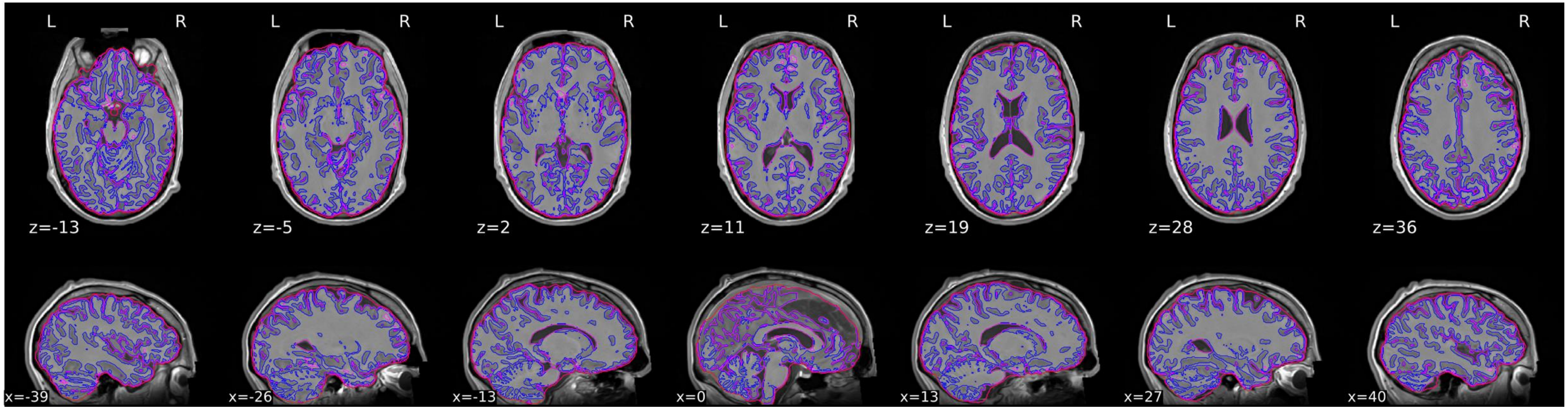
Anatomical

Anatomical Conformation

- Input T1w images: 1
- Output orientation: RAS
- Output dimensions: 192x256x160
- Output voxel size: 1mm x 1mm x 1mm
- Discarded images: 0

Brain mask and brain tissue segmentation of the T1w

This panel shows the template T1-weighted image (if several T1w images were found), with contours delineating the detected brain mask and brain tissue segmentations.



01. Data preprocessing

Section	What to Check
Overall Summary	- Sub ID correct? Freesurfer? All spaces there?
Anatomical Summary	- Conformation: one T1w image? Dimensions consistent across subs? No volumes discarded?
	- Brain mask and tissue segmentation: T1w and segmentation look okay? No distortion/cutoff?
	segmentation fits gray matter (red line) and white matter (white area surrounded by blue line)
	- Normalisation: alignment okay (grey matter, white matter, ventricles)
Functional Summary	- Check that the report is accurate (TR, sequence, slice timing/susceptibility correction, non-steady volumes)
	- Alignment func/struct: do the two (anatomical is skull stripped vs functional is not) correspond okay?
	- Check alignment based on ventricles, cingulate cortex, cerebellum
	- Brain mask and CompCor ROIs: main thing is red line includes whole BOLD image (okay if a bit cutoff)
	not okay if red line excludes cerebellum, brainstem and other cortical regions
	- BOLD Summary: okay if movement still there (will be dealt with later). Note if DVARS max > 4 and FD max > 1.
	- Carpet plot may still have some spikes/motion. If noise is regular, take note.
Overall errors	- Critical errors or warnings?

02. Data denoising

1

standardized, aligned data with noise

common across acquisition protocols and labs

2

removal of noise from prepared data

custom pipeline based on acquisition, question

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02. Data denoising

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nuisance regression
ICA-based regression
global signal regression
band-pass filtering
detrending
time-series normalisation

custom pipeline based on acquisition, question

Nuisance regression
Physiological noise
(white matter, CSF)

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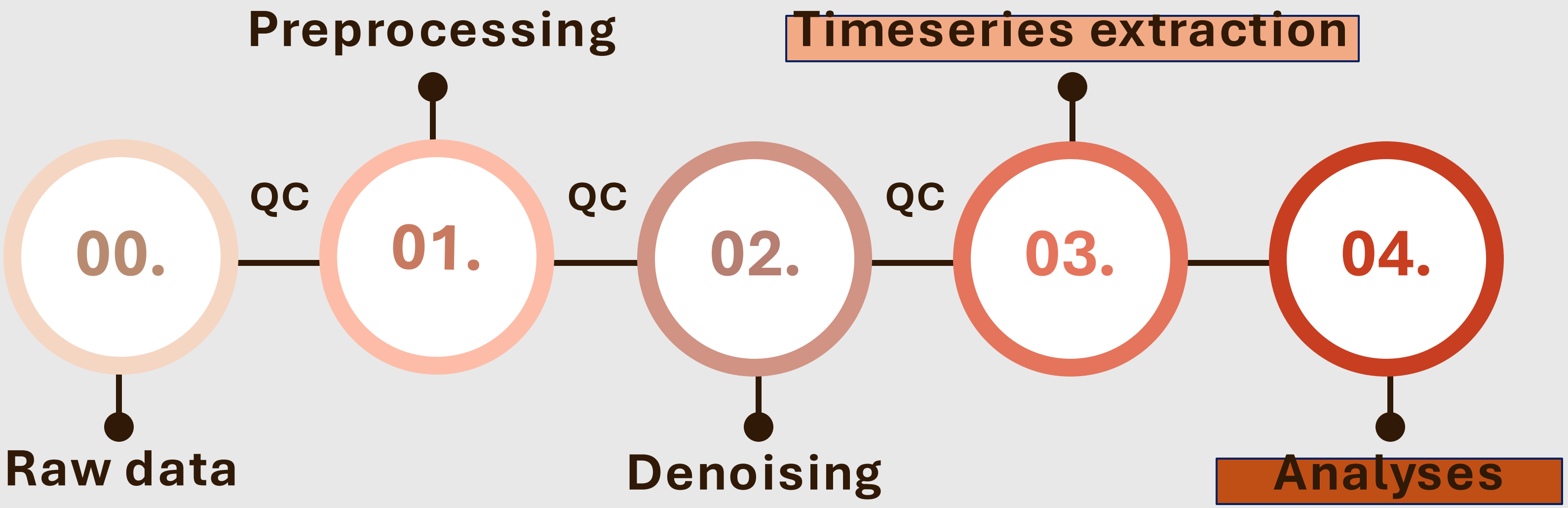
TS normalisation
To enable comparison amongst voxel/vertices/regions

Resting-state fMRI workflow

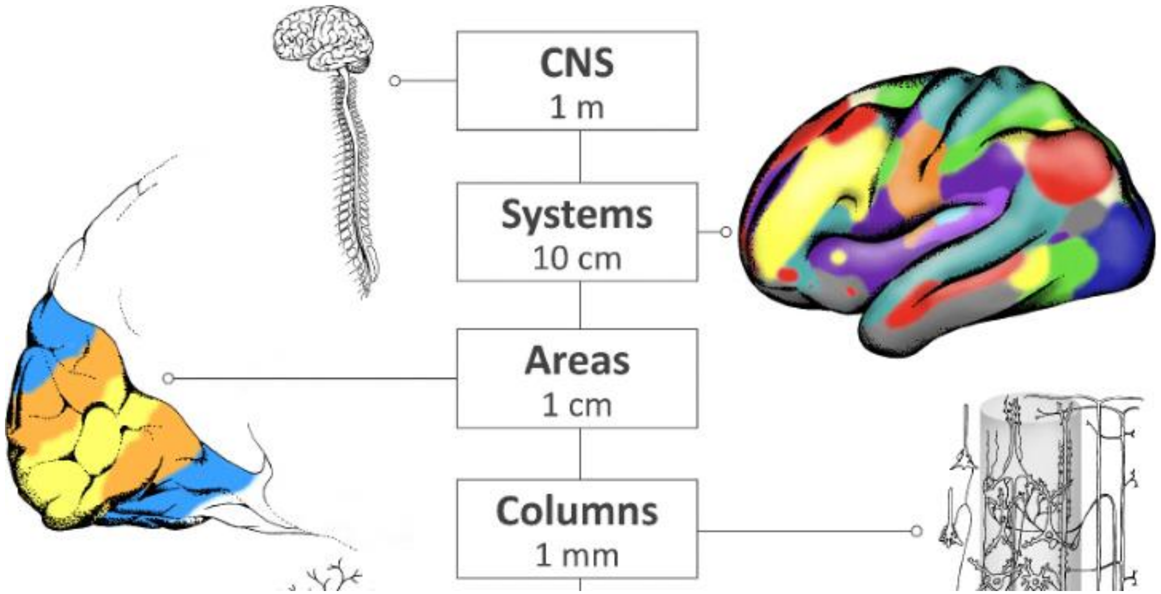


QC = quality control

03. + 04. TS Extraction + Analyses

What is my unit of interest?

Voxels? Regions? Networks? Systems?



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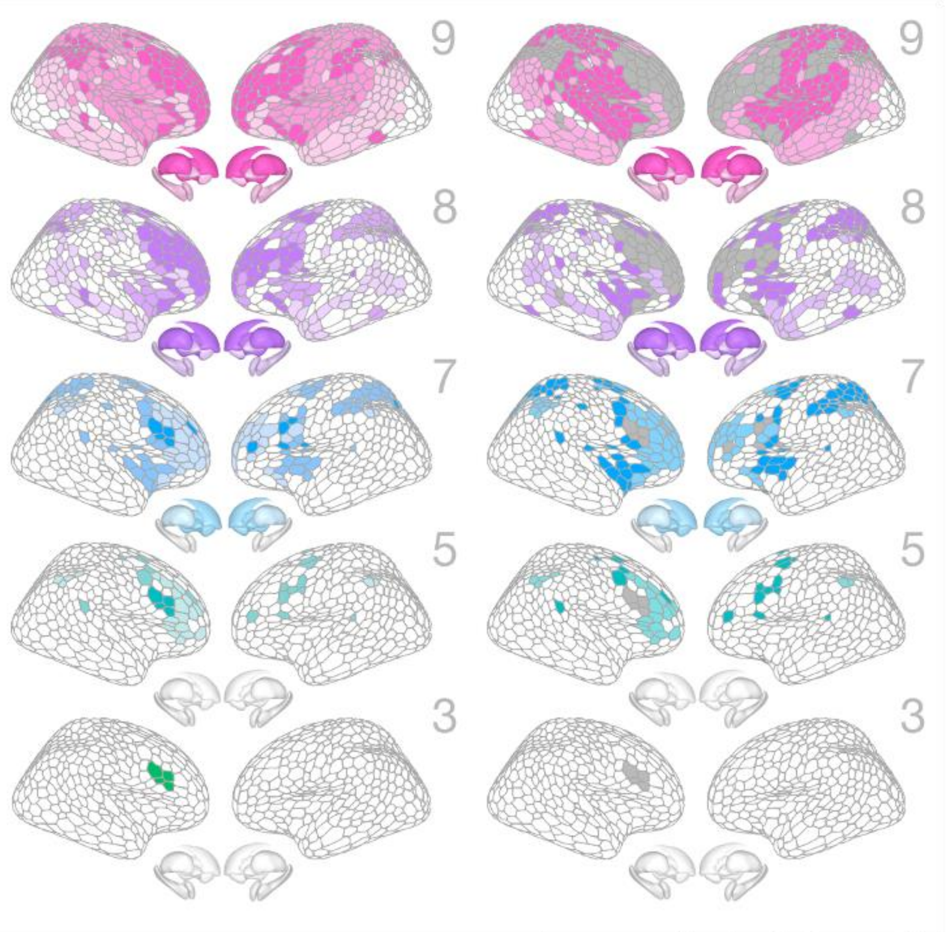


Image credit: Annie Bryant ☺

Hierarchy/gradient

Network

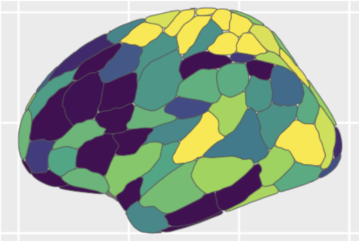
similar function (functional connectivity)

Region/Area

average contiguous

Voxel

03. Timeseries Extraction



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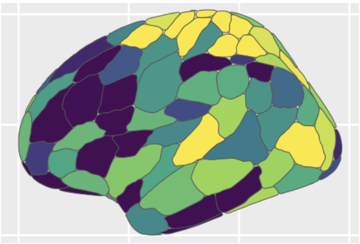

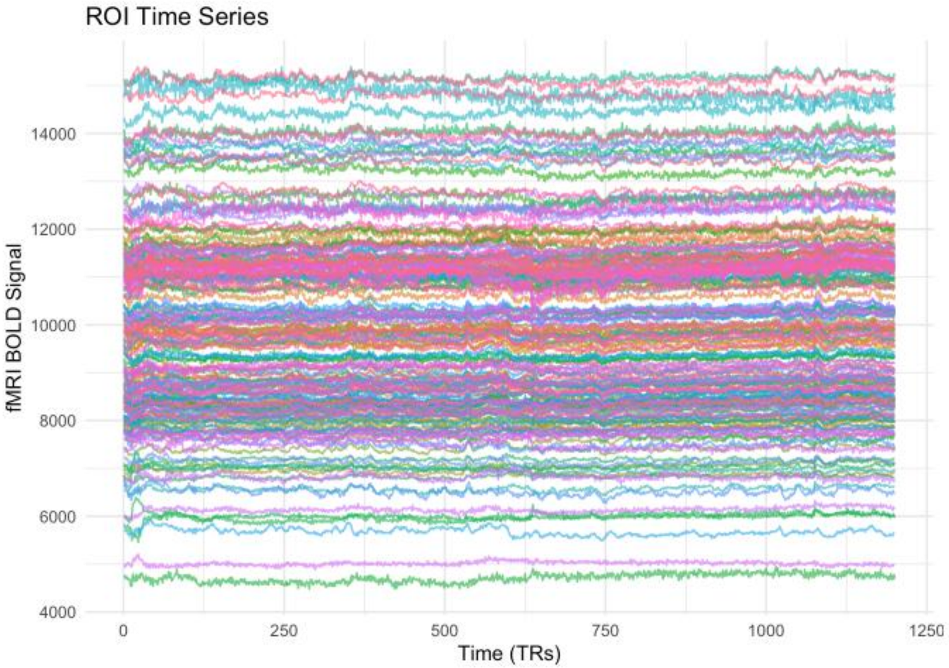

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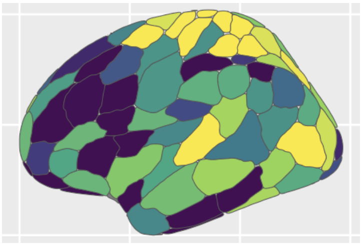
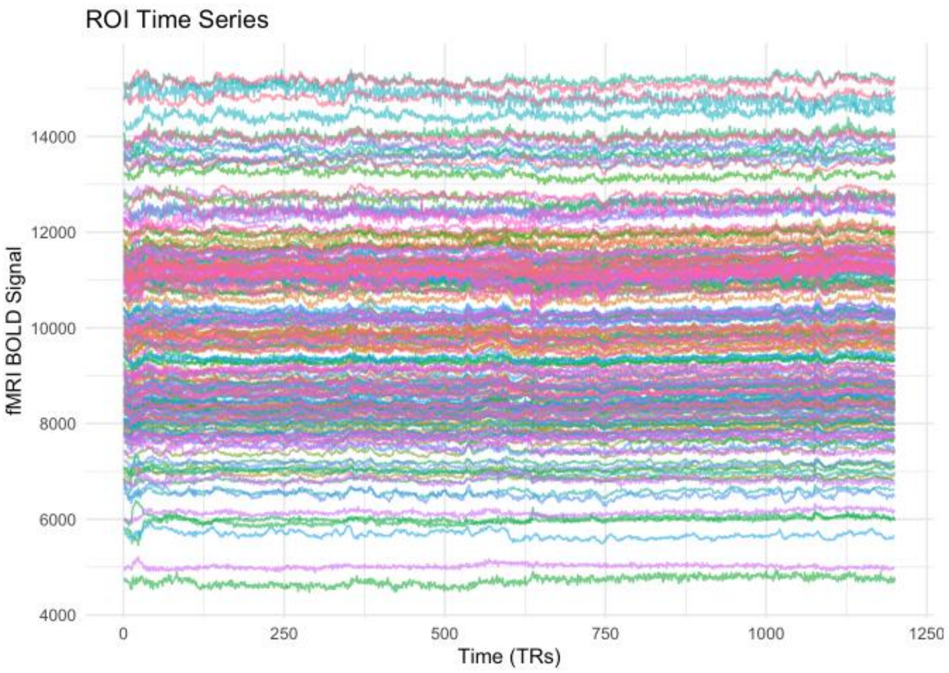
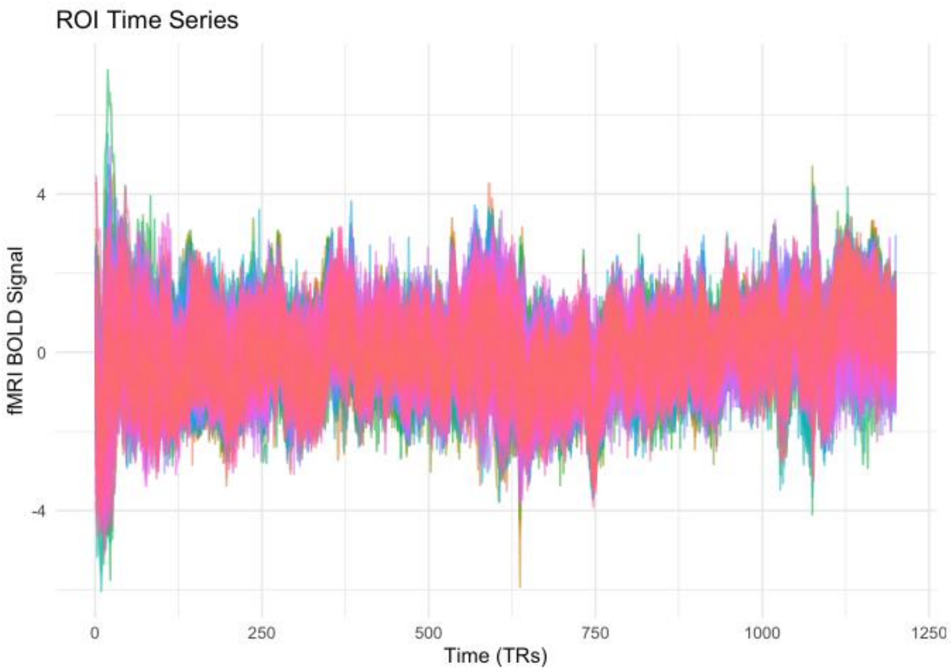




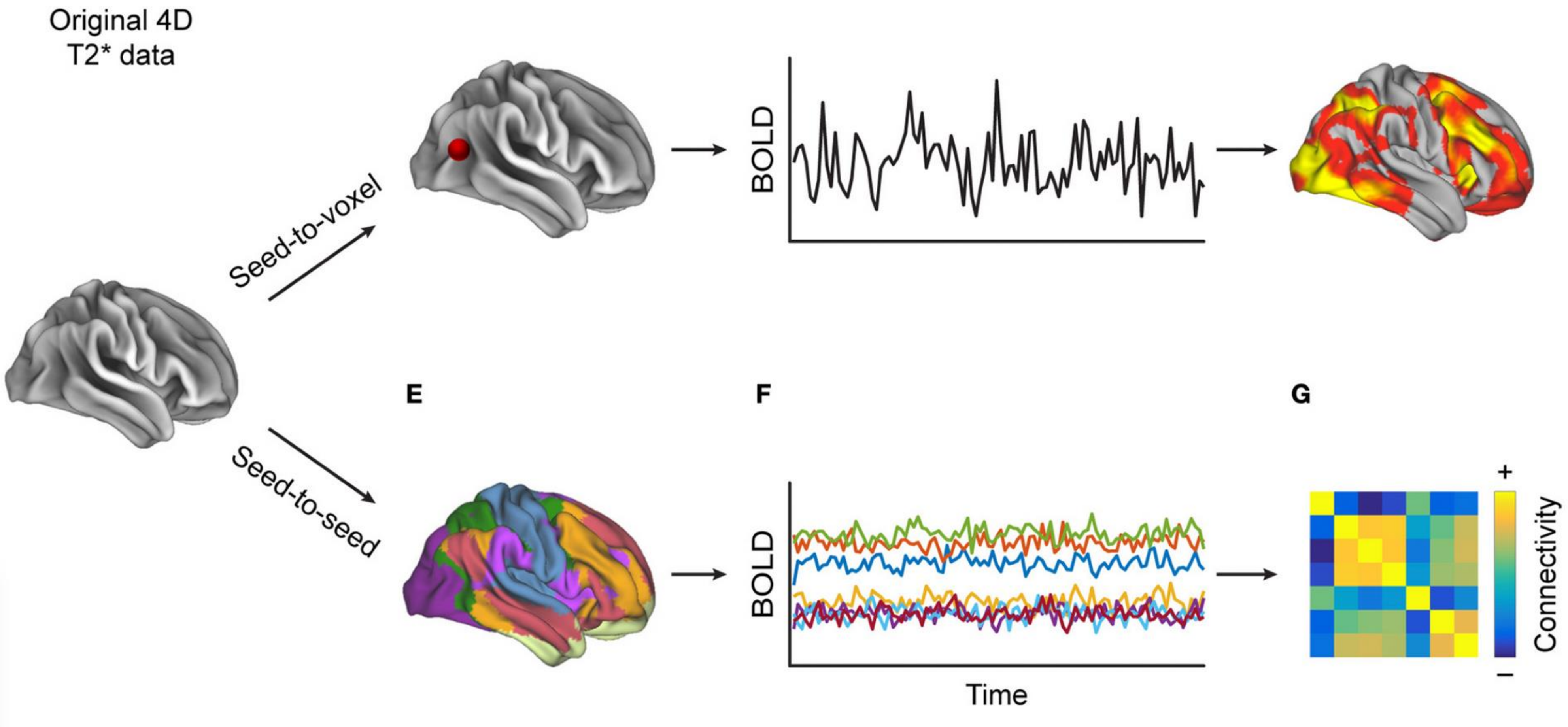
Timeseries normalization (zscoring)



04. Functional connectivity

statistical construct (Pearson's correlation)

= similarity in the activity of two units over time



Tahedl et al., 2018

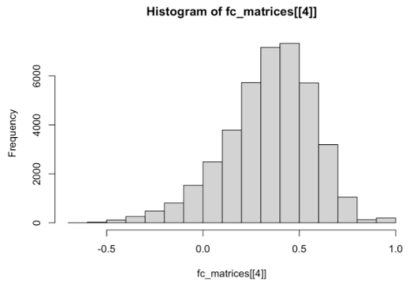
04. Functional connectivity

```r
# Since we have multiple subjects, we are going to calculate FC and store these matrices in a list
fc_matrices <- lapply(files, function(file) {
  ts <- as.matrix(read.table(file)) #convert to matrix format to do calculations

  # Pearson's correlation
  cor_mat <- cor(ts)

  return(cor_mat)
})

# let's look at the distribution of these FC values: let's look at subject 4
hist(fc_matrices[[4]])
```



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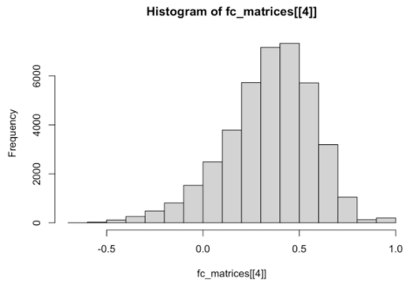


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# For group analyses, we need to normalise these FC values: Fisher-z transformation
z_matrices <- lapply(fc_matrices, function(correlation) {
  z_mat <- atanh(correlation)

  # Clean matrix: replace Inf values that come from Fisher z-transform
  z_mat[!is.finite(z_mat)] <- NA

  return(z_mat)
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hist(z_matrices[[4]]) #let's check if normalisation worked
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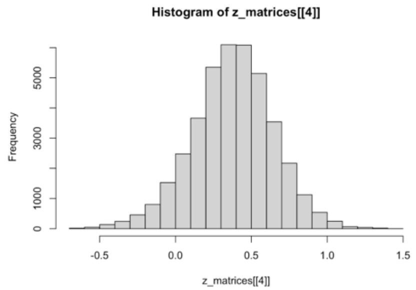

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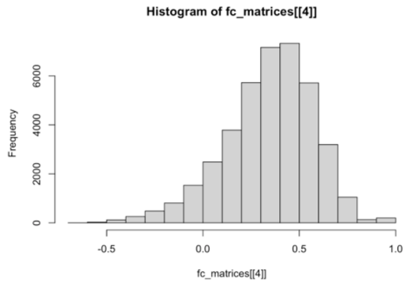


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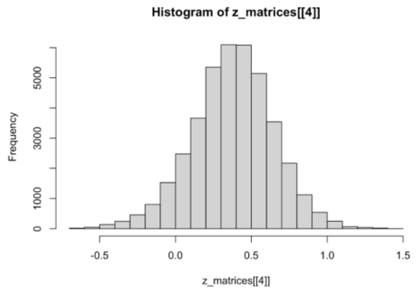
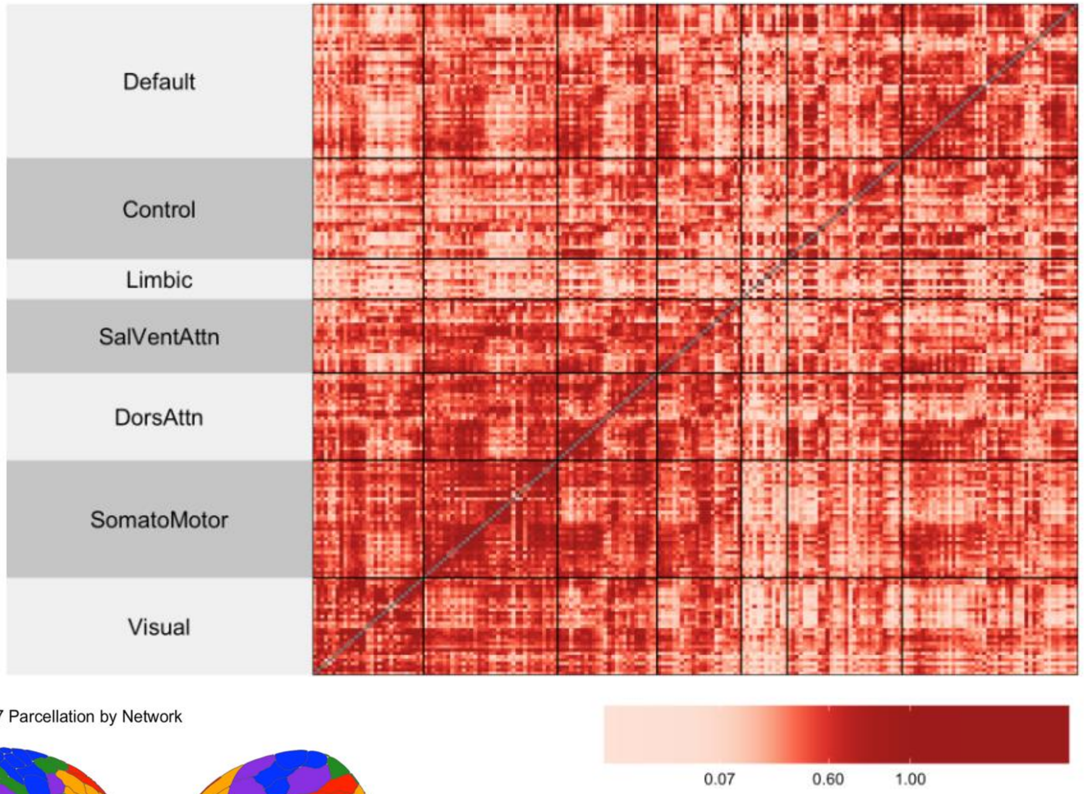
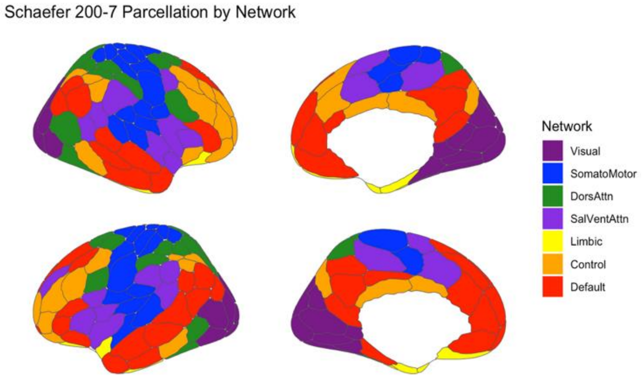




Fisher z values



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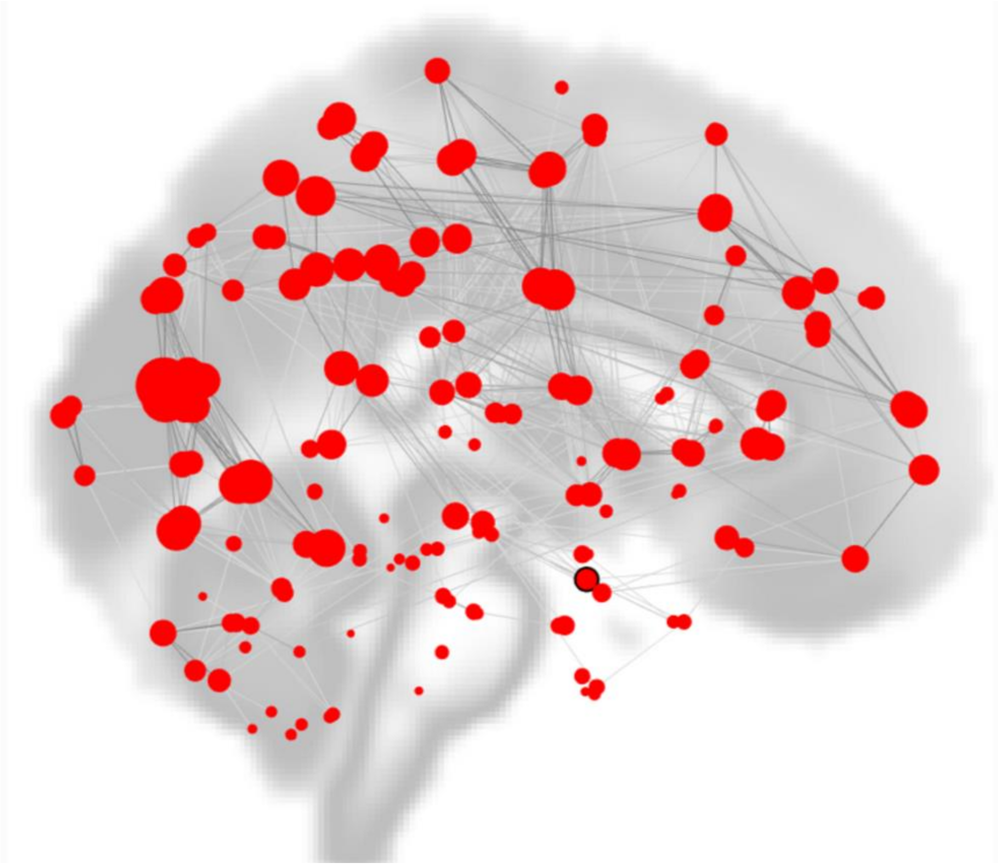


Brain Connectivity Toolbox
CONN Toolbox
Great guide: Andy's Brain Book

Graph Theory

Node = region

Edge = connection

many metrics: e.g., nodal strength

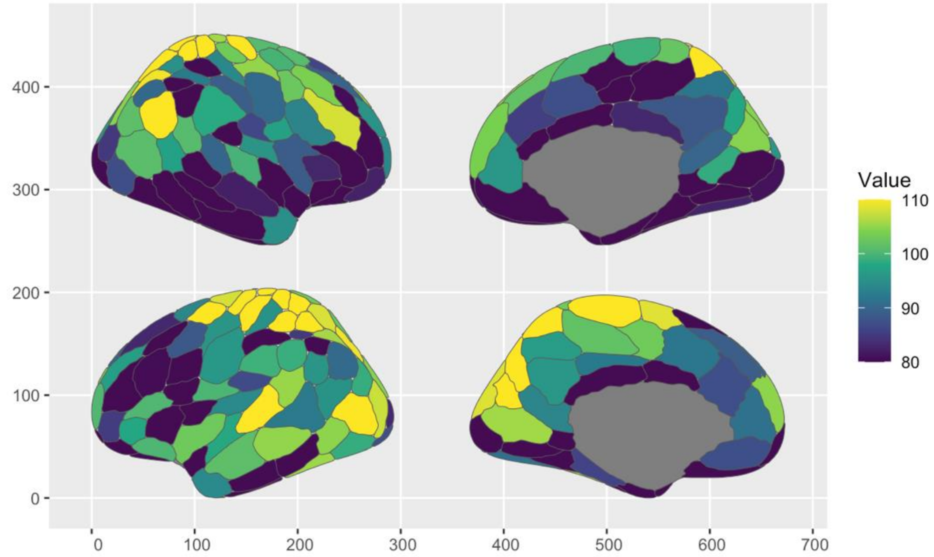
Highly recommend checking out the work (books, articles) of Olaf Sporns; e.g., Networks of the brain, 2010

04. Functional connectivity

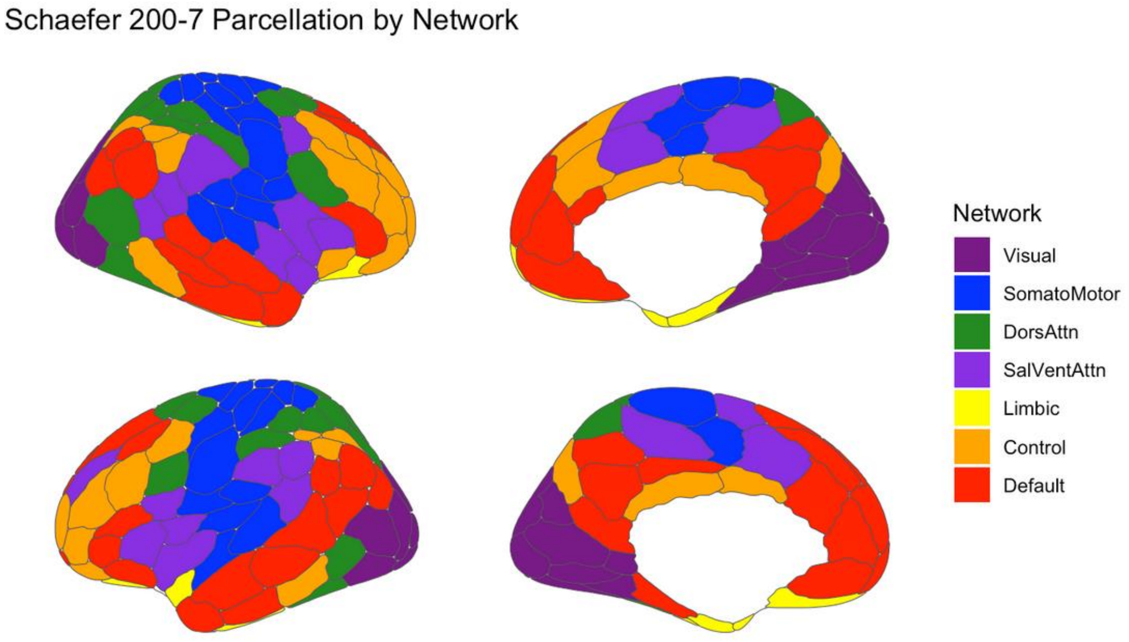
statistical construct (Pearson's correlation)

= similarity in the activity of two units over time

```
node_strength <- rowSums(group_fc, na.rm = TRUE)

node_strength <- as.data.frame(node_strength)
rownames(node_strength) <- seq(1:200)

region <- atlas$Region

node_strength[,2] <- region

colnames(node_strength)[1] <- "Value"
colnames(node_strength)[2] <- "region"

node_strength %>%
  ggplot() +
  geom_brain(atlas = schaefer7_200,
             position = position_brain(hemi ~ side),
             mapping = aes(fill=Value, geometry=geometry)) +
  scale_fill_viridis_c(limits = c(80, 110), oob = scales::squish)
```

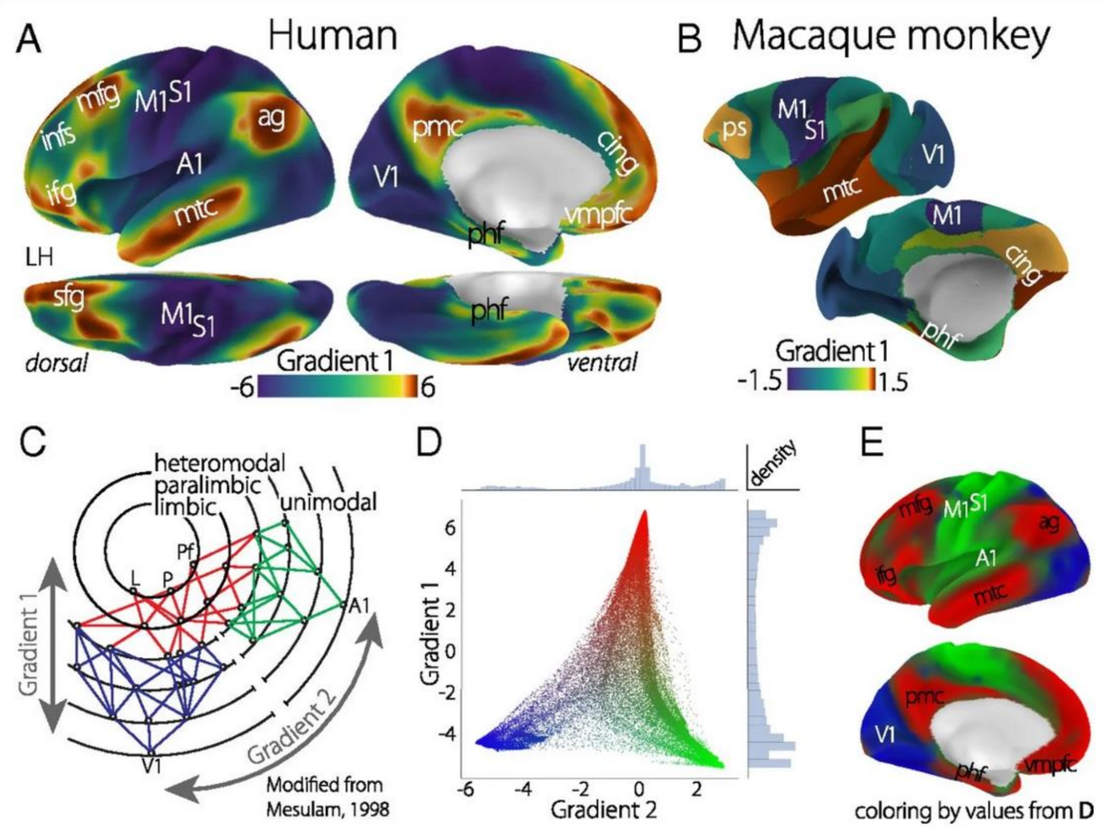


Canonical resting-state networks



generated in R based on Schaefer et al., 2018
also check out Yeo et al., 2011

Principal gradient of brain organisation



Margulies et al., 2016 *PNAS*
based on Mesulam, 1998 *Brain*

03. + 04. TS Extraction + Analyses

All materials can be found on my Github at this link:
https://github.com/giuliabaracc/teaching_fMRI

Tutorial from step 01 to 04:
fMRI_restpreprocnet.html

Your turn to create brain maps:
/code/Tutorial_fMRI_forstudents.R

Other useful resources (+ applications)

- Some news articles with the latest about fMRI:
  - The Transmitter is a good place: here and here
- Past, present, future of fMRI:
  - Finn, Poldrack & Shine, 2023 *Nature*
  - Biswal & Uddin, 2025 *Nature*
- Some cool methods/applications:
  - Shine et al., 2019 *Neuron*
  - Markello et al., 2022 *Nature Methods*
  - Baracchini et al., 2024 *Aperture Neuro*

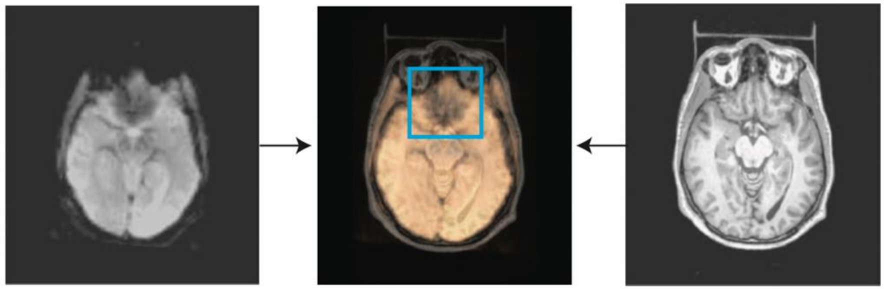
00. Example: Multi-echo, multi-band fMRI

Great standard sequence for whole-brain coverage

Multi-echo

Multi-band

Signal drop out



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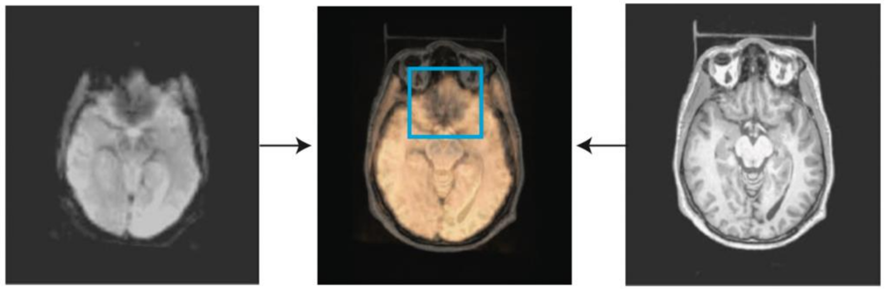

Acquisition of multiple echo images per slice = T2 decay can be modelled at every voxel at every time point -> increased spatial resolution*

Kundu et al., 2012 & 2017 *NeuroImage*

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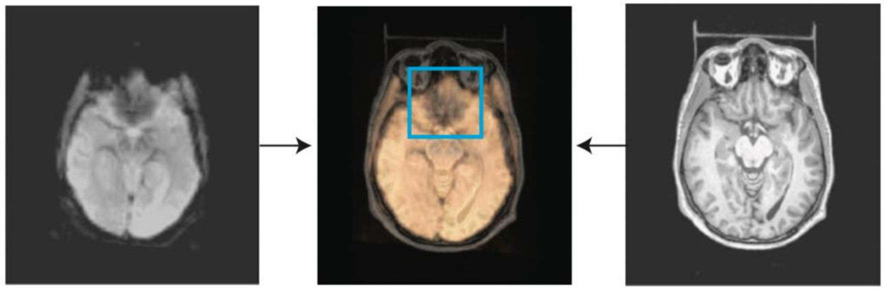

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+ cleaner signal cause ME uses TE-dependent preprocessing strategies:
– Neuronally induced fluctuations depend on the selected TE
– Nuisance signal fluctuations are non-TE dependent

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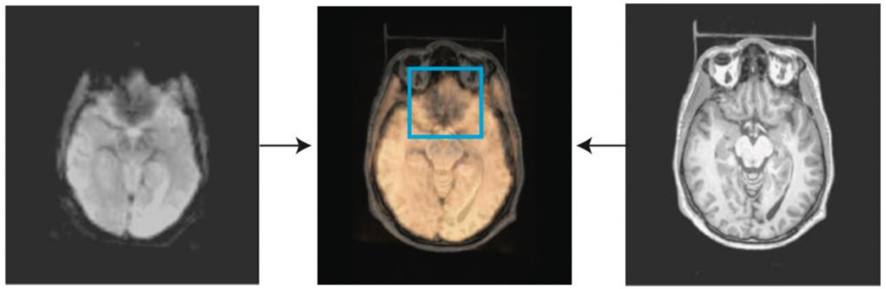

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Wall, 2023 *Aperture Neuro*

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- *Faster acquisition time*
- *Larger field of view or smaller voxel size*

BUT at risk of greater noise + greater signal drop out

Wall, 2023 *Aperture Neuro*