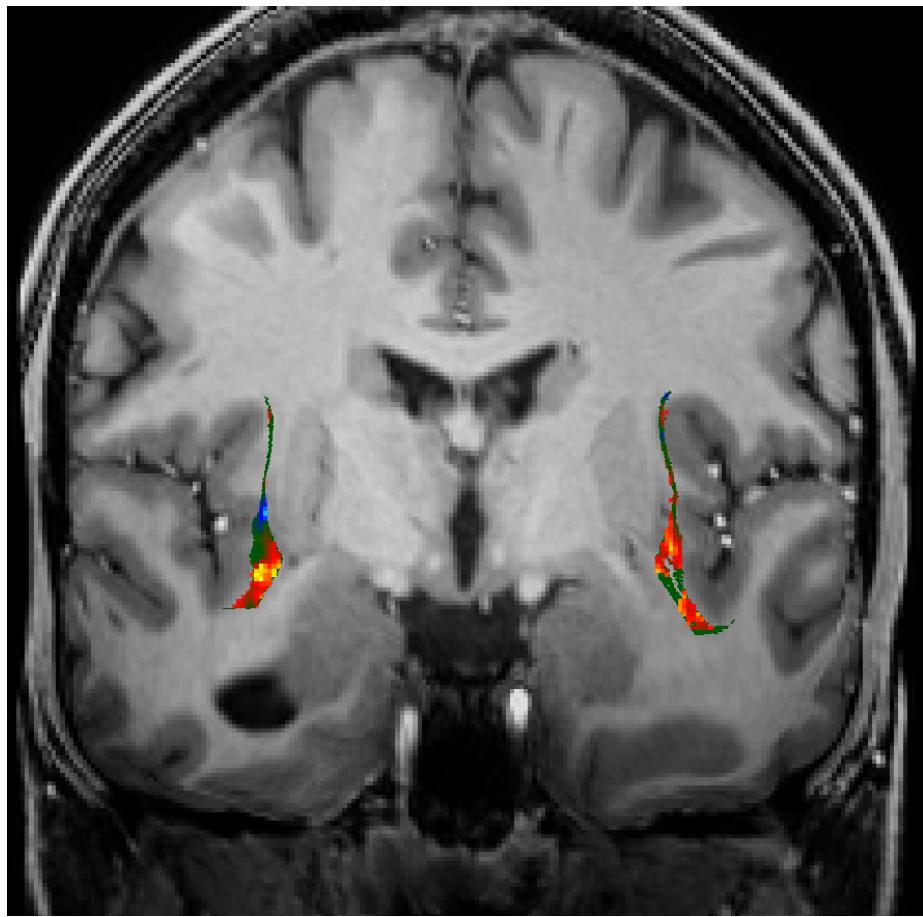


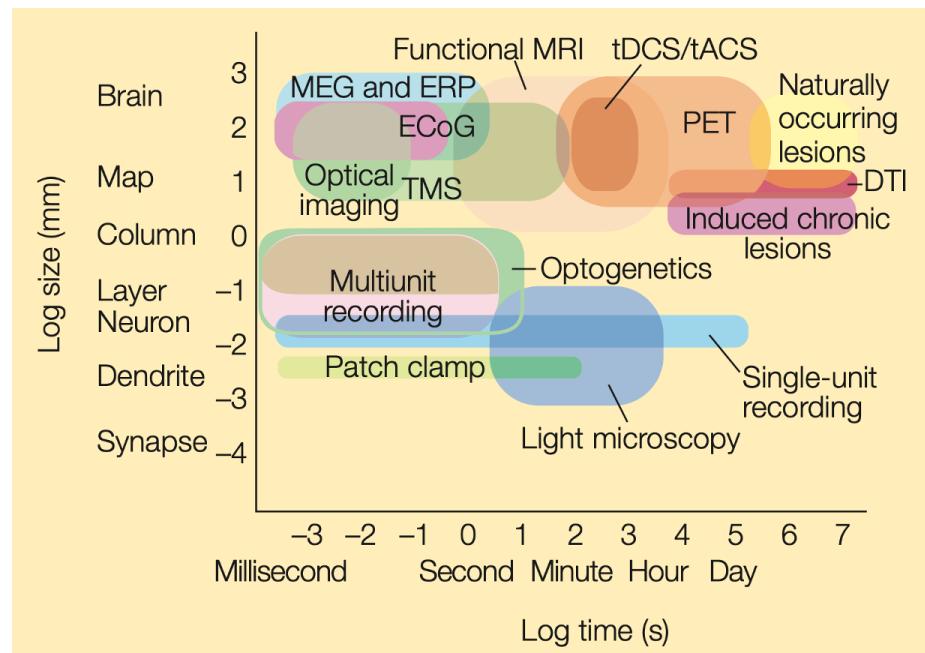
# Introduction to fMRI



Visual activity in the human claustrum (Coates et al. 2024)

fMRI is a method that allows to non-invasively measure brain activity.

## fMRI in comparison



Source: Gazzaniga 5th edition, Figure 3.45

## MRI scanner: 3 Tesla



<https://mri-lab.uni-graz.at/>

## MRI scanner: 7 Tesla



Image source: Medical University of Vienna

## MRI scanner: 9.4 Tesla



Image source: Max-Planck Institute for Biological Cybernetics, Tübingen

## Types of MRI contrasts

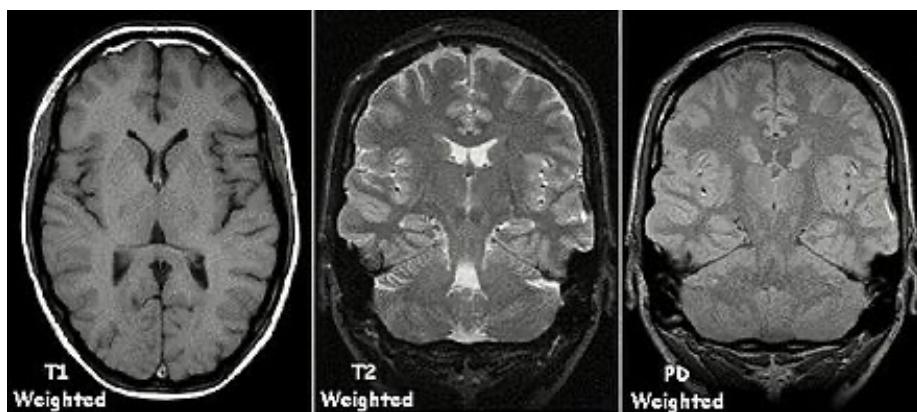
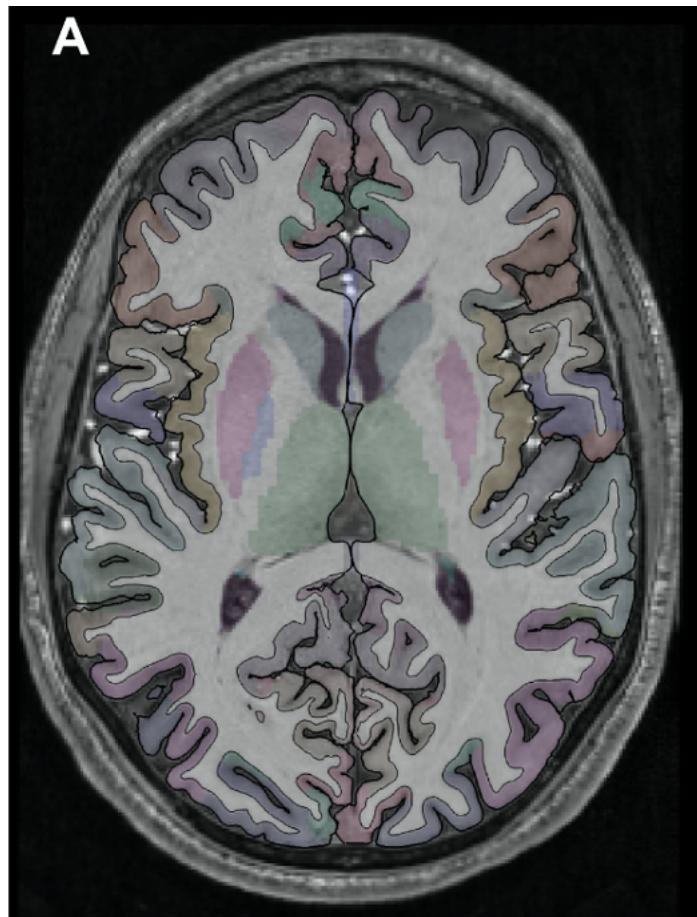


Image source: [https://en.wikipedia.org/wiki/Magnetic\\_resonance\\_imaging](https://en.wikipedia.org/wiki/Magnetic_resonance_imaging)

## T1w in cognitive neuroscience



Full Segmentation of a T1-weighted scan (Zaretskaya et al. 2018)

- Displaying activity on a high-resolution anatomical scan
- Quantitative morphometry
  - Aging
  - Neurodegeneration
  - Plasticity

## The principle of fMRI

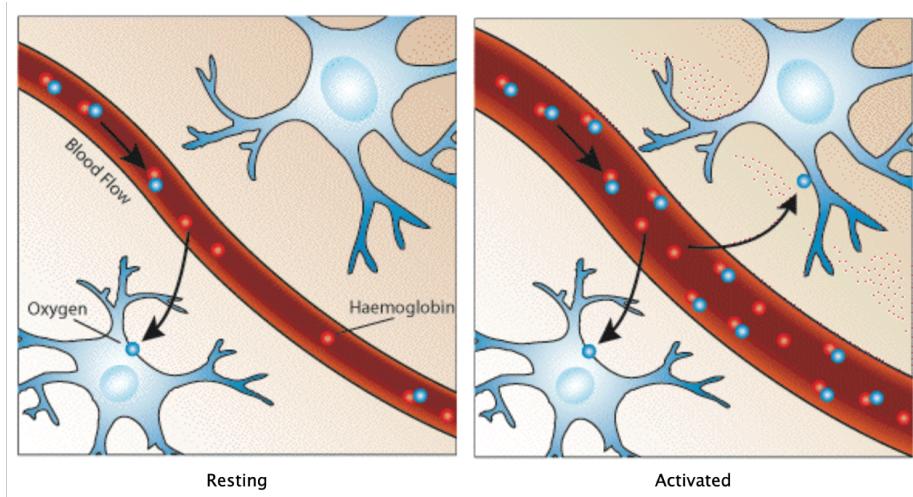
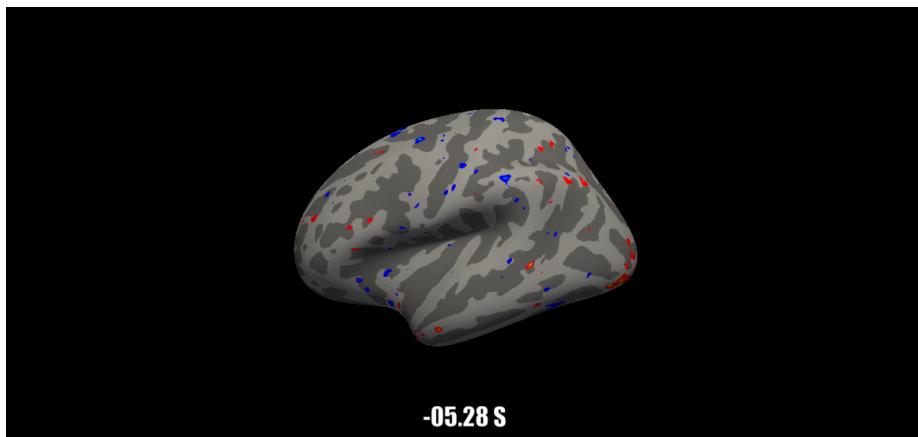


Image source: [https://www.nature.com/scitable/blog/brain-metrics/what\\_does\\_fmri\\_measure](https://www.nature.com/scitable/blog/brain-metrics/what_does_fmri_measure)

Hemodynamic changes: - increase in blood flow - increase in blood volume - increase in tissue CMRO<sub>2</sub>

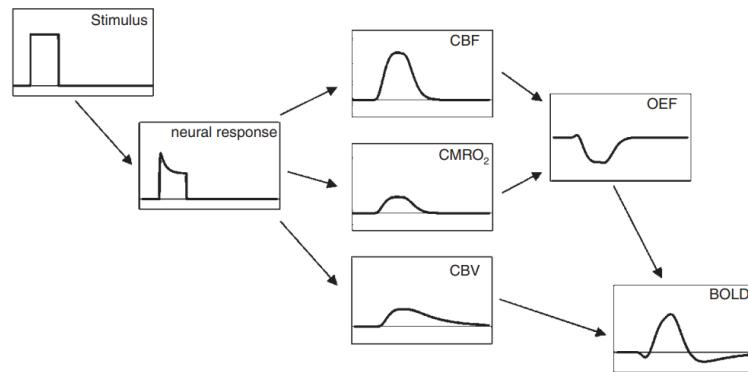
## Hemodynamic response



Source: Visual Neuroscience Lab

## BOLD signal

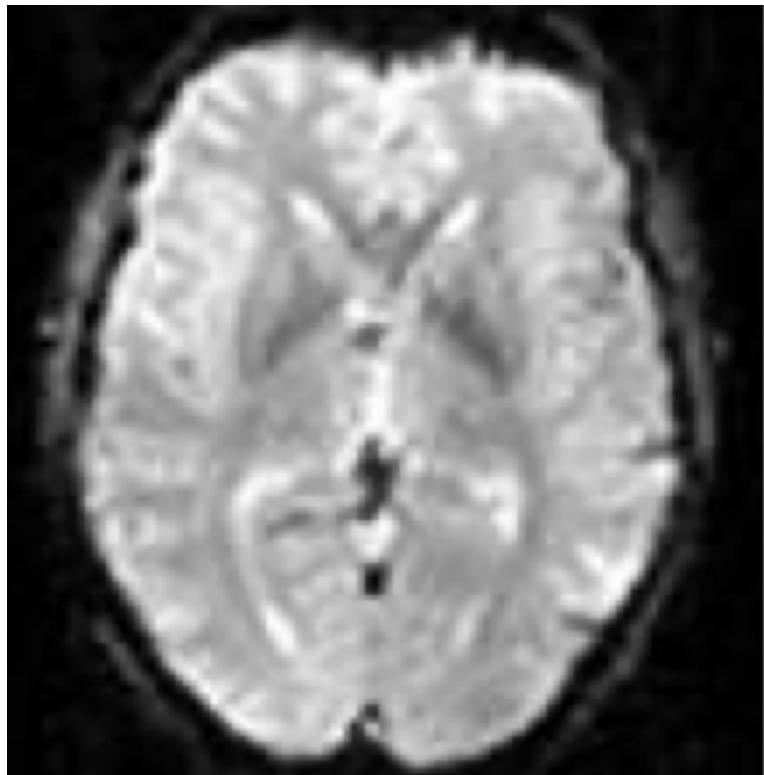
blood-oxygen-level-dependent signal



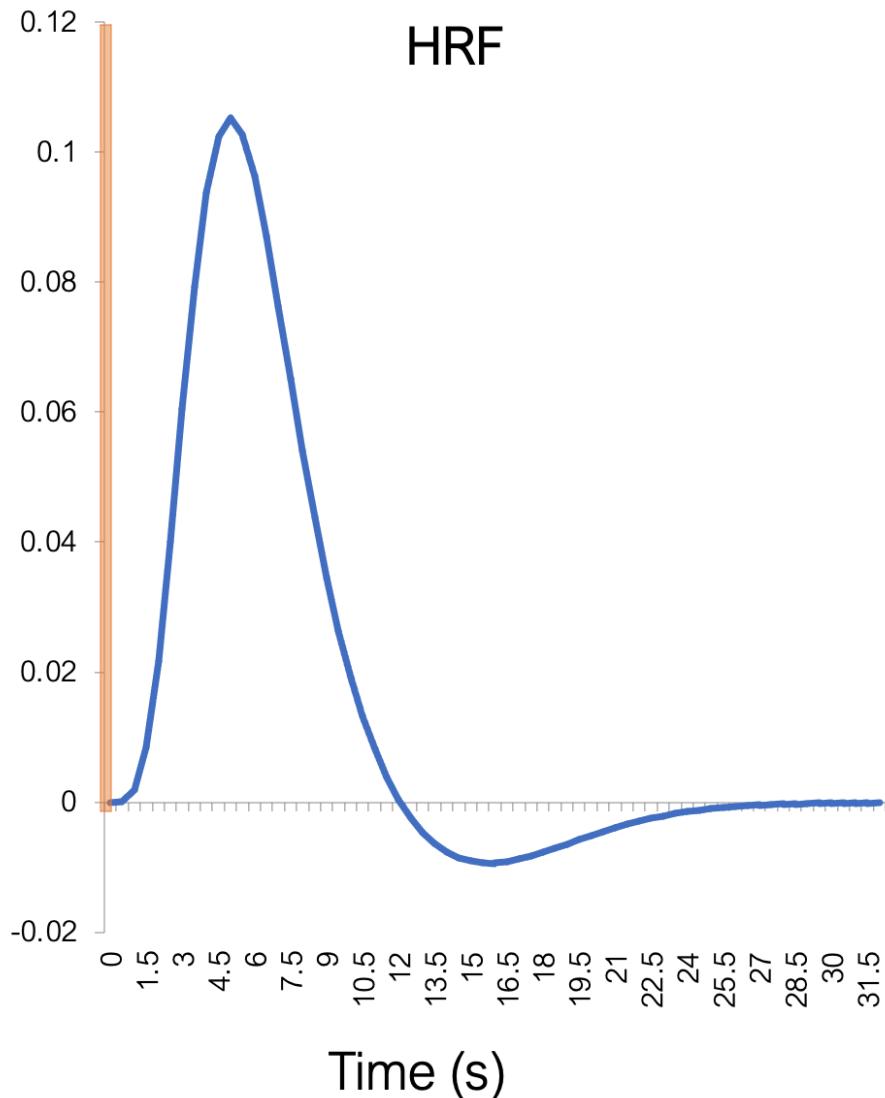
**Fig. 16.2.** The chain of events leading to the BOLD signal. A stimulus triggers local neural activity, which in turn triggers metabolic activity in the form of a large increase of cerebral blood flow (CBF), a small increase of cerebral metabolic rate of  $O_2$  (CMRO<sub>2</sub>), and a moderate increase of cerebral blood volume (CBV). The combined changes in CBF, CMRO<sub>2</sub> and CBV create the BOLD signal change. The response curves at each stage suggest ways in which the stimulus shape is altered in the progression to the BOLD response. A key aspect of this chain of events is that CBF and CMRO<sub>2</sub> are driven in parallel, rather than in series, and potentially by somewhat different aspects of the neural activity. OEF,  $O_2$  extraction fraction.

Hemodynamics leading to the BOLD signal (“Interpreting the BOLD Response” 2009)

## T2\*-weighted contrast



## Hemodynamic response function



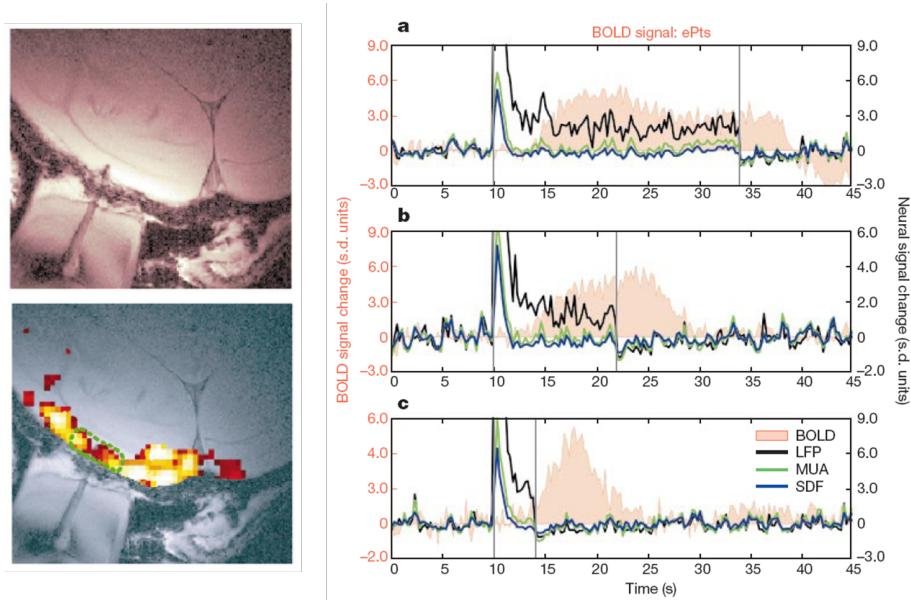
This curve describes the measured signal in response to a brief stimulus.

The response is heavily delayed, and has a relatively complex temporal structure, described by the so called hemodynamic response function (HRF).

After just a brief stimulus that lasts less than a second, the response lasts for seconds. It has a delayed rise phase, a peak, and a subsequent undershoot. The fact that the response is so slow is the main constraint of the temporal resolution

of fMRI. It constrains the kind of questions you can ask in your experiment and also puts some constraints on your experimental design.

## Neural activity and BOLD



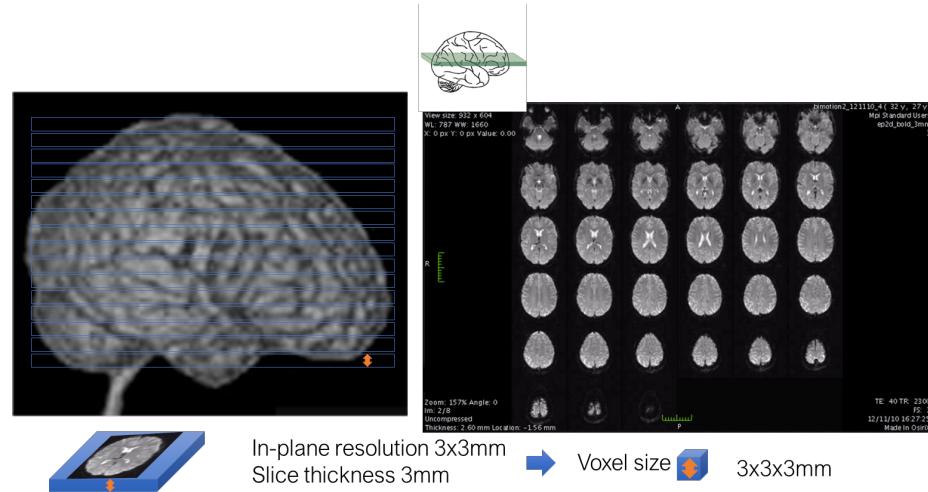
BOLD signal correlates better with LFP than with MUA (Logothetis et al. 2001)

LFP = Local Field Potentials (dendritic currents)

MUA = Multiunit Activity (action potentials)

SDF = Spike-density function (action potentials)

## Image acquisition



The functional volume is usually acquired slice-by-slice; this is why when you are acquiring the data you see an image like this on the monitor. A functional sequence is characterized by the in-plane matrix size and resolution, as well as by the slice thickness.

## Experimental design

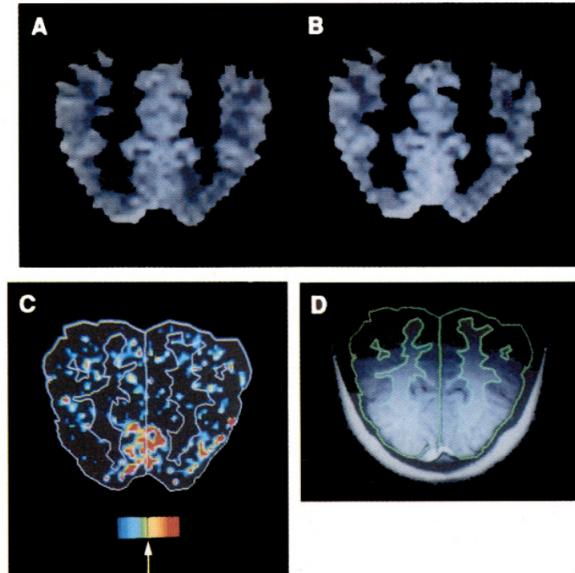
	Condition of interest	Control
Checkerboard		Gray background
Finger tapping		Rest
Faces		Houses
Cola		Pepsi

In contrast to e.g. fNIRS, the BOLD-fMRI does not measure the deoxyhemoglobin concentration directly. It is a measure that is weighted by the deoxyhemoglobin concentration, but depends on many other factors like scanner, sequence, participant, and brain area.

This is why any measure of fMRI experiment has to contain at least two conditions, a baseline, and condition of interest. The activation is always computed relative to some baseline. For instance a checkerboard vs gray background, finger tapping versus rest; faces versus houses, etc. And a typical analysis will compare the BOLD signal during condition of interest with rest.

## First fMRI experiment

**Fig. 3.** Magnetic resonance CBV maps of the brain during darkness (**A**) and during 7.8-Hz photic stimulation (**B**). Image intensity is proportional to CBV. All images are aligned along the calcarine fissure (Fig. 1), with the occipital pole at the bottom. (**C**) Subtraction image of changes in CBV induced by photic stimulation ( $C = B - A$ ). A linear color scale was used, with red equivalent to greatest activity. The arrow points to the  $+2$  SD threshold. (**D**) An anatomic (T1-weighted) image was used to segment the gray and white matter (20). This outline was applied to the CBV subtraction image. A marked area ( $\sim 600 \text{ mm}^2$ ) of increased blood volume ( $\sim 24\%$ ) is localized in the anatomically defined primary visual cortex (**C**). We acquired these CBV images using a  $3 \times 3$  by  $10 \text{ mm}$  voxel.



Brain images and statistical analysis (Belliveau et al. 1991)

The first experiment was not a BOLD, but a blood volume signal measurement.

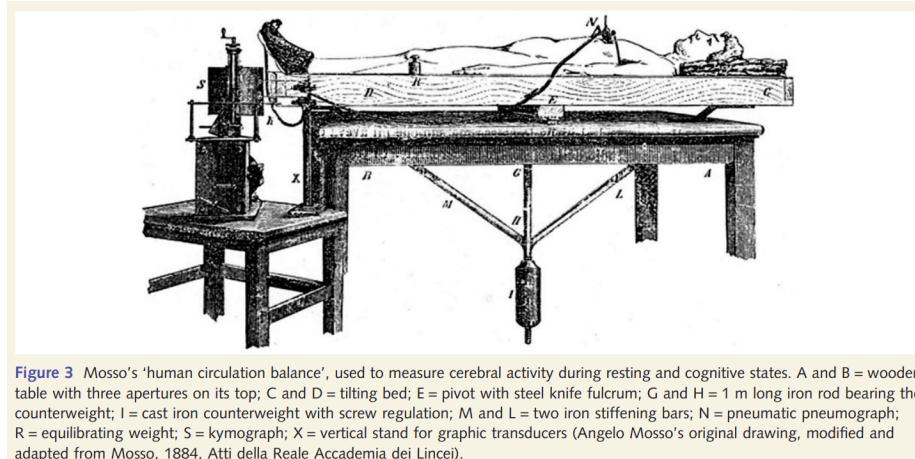
## First fMRI experiment



Cover of the science magazine where the paper was published (Belliveau et al. 1991)

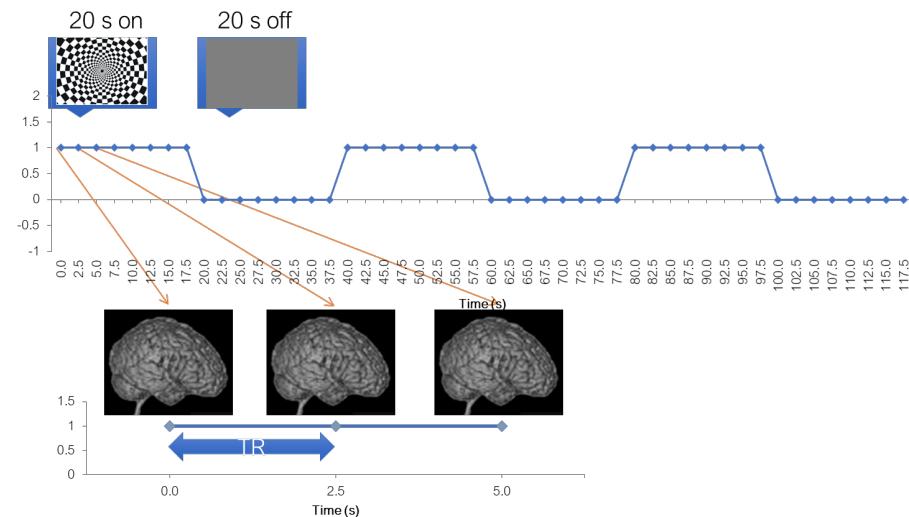
This rendering was produced specifically for the cover

## The truly first fMRI experiment



Experimental setup of Angelo Mosso (Sandrone et al. 2013)

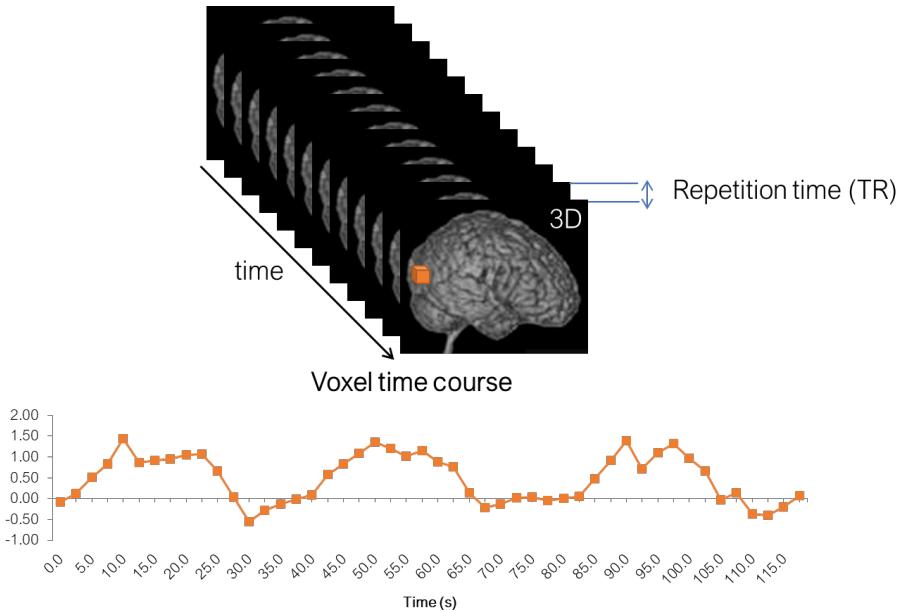
## A typical experiment



second interleaved with a 20 s rest, and we will measure a whole brain volume every 2 seconds, for e.g. to minutes or so.

```
Matlab code to generate stimulus, BOLD and shifted BOLD X = [1 1 1 1 1 1  
1 1 0 0 0 0 0 0 0 1 1 1 1 1 1 1 0 0 0 0 0 0 0 0 1 1 1 1 1 1 1 1 0 0 0  
0 0 0 0]; bf = spm_get_bf; % indicated a tr of 2.5 Y = conv(X,bf.bf); Ye =  
Y+randn(size(Y)).*0.2;
```

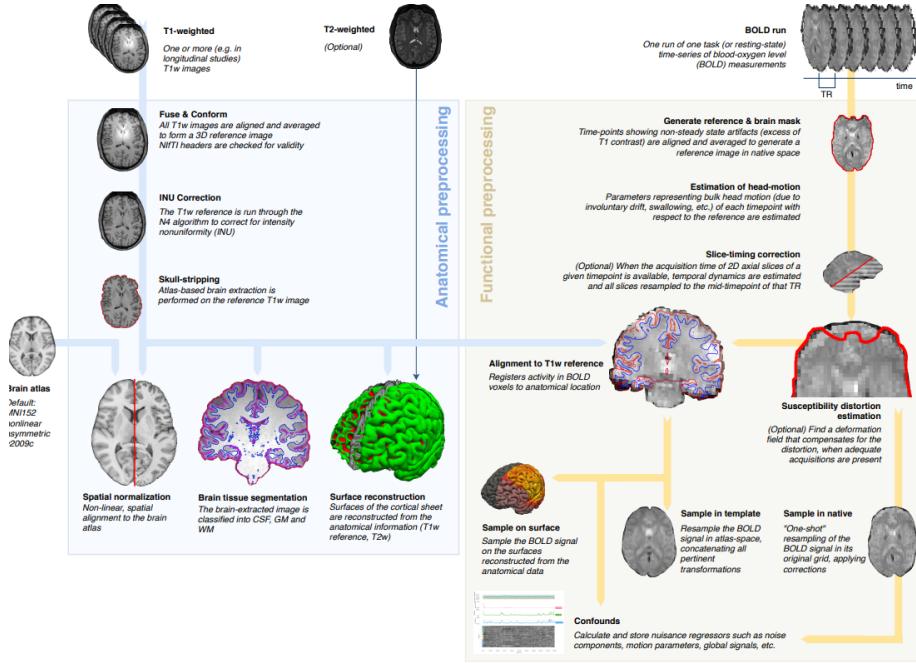
## 4D dataset



In a functional experiment we usually measure brain activity across the whole volume over extended periods of time, so it is comfortable to think about each functional dataset that we acquire as a 4D dataset, which consists of 3D brain volumes and time as a 4th dimension. This is how the data is typically stored.

A further important parameter is the repetition time (TR). It is the time passes between the acquisition times of two adjacent volumes.

# Preprocessing



Summary of preprocessing steps (Esteban et al. 2018)

The data quality of modern scanners is typically good enough to skip this step, if your subject is compliant. But it is nevertheless very much advisable and is ALWAYS performed. We will therefore dedicate a whole session to preprocessing. See [course schedule](#) for the date.

# Analysis

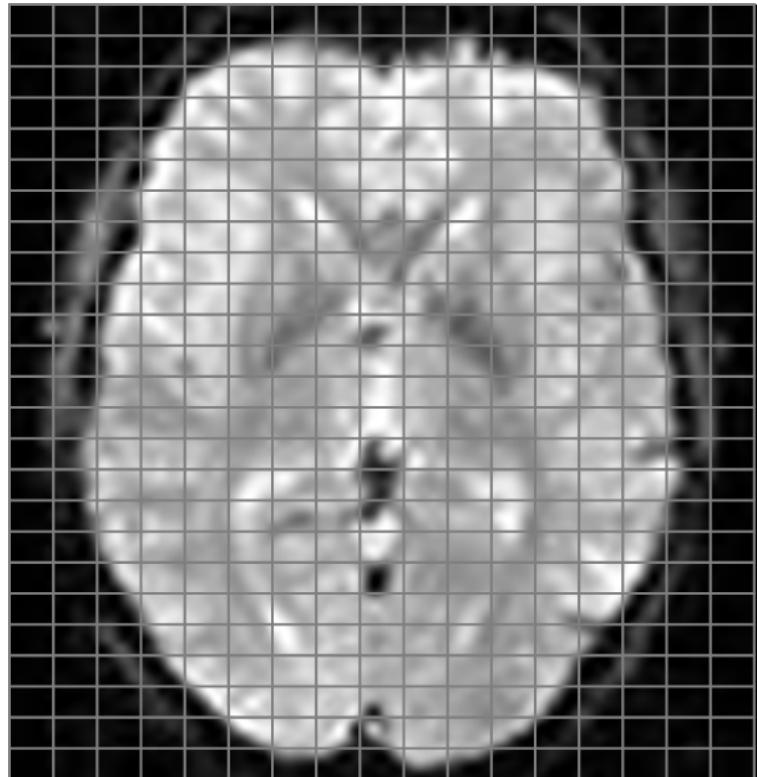
- Single-subject - first level - fixed effects analysis (FFX)
- Group - second-level - random effects analysis (RFX)

This is equivalent to e.g. conducting many trials per subject to measure reaction time, and then compute a subject-specific mean per condition, after which you would perform the actual statistical inference

Multilevel modelling is also possible (for small datasets), but less frequent.

## Single-subject (first-level) analysis

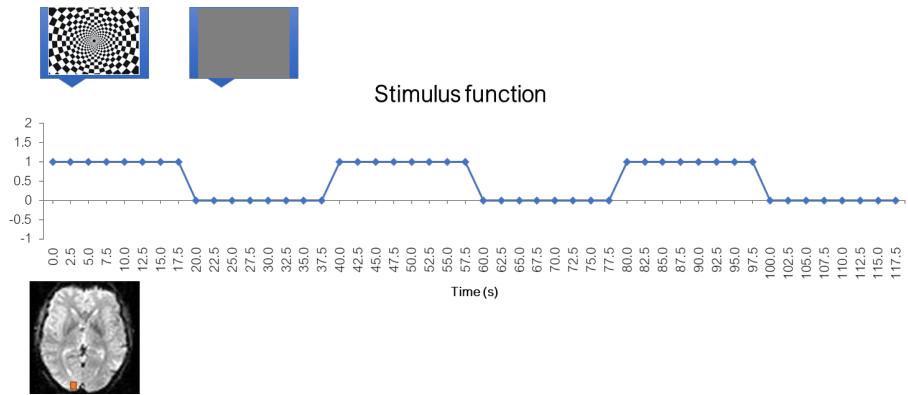
Univariate/voxel-wise analysis



Question: Which areas are activated by the flickering checkerboard?

Remember that an image is 4d and consists of little 3D cubes called voxels, with the 4th dimension being time. A classical analysis is done over time for every voxel in the brain independently.

## Stimulus function



Let's recall our checkerboard experiment where participants viewed either a checkerboard or a gray screen. Let's also create a function, called "stimulus function", which is one where the checkerboard was on, and zero where the checkerboard was off

## Side-note on terminology

### Trial

Continuous presentation of 1 experimental condition, usually 1-20 seconds

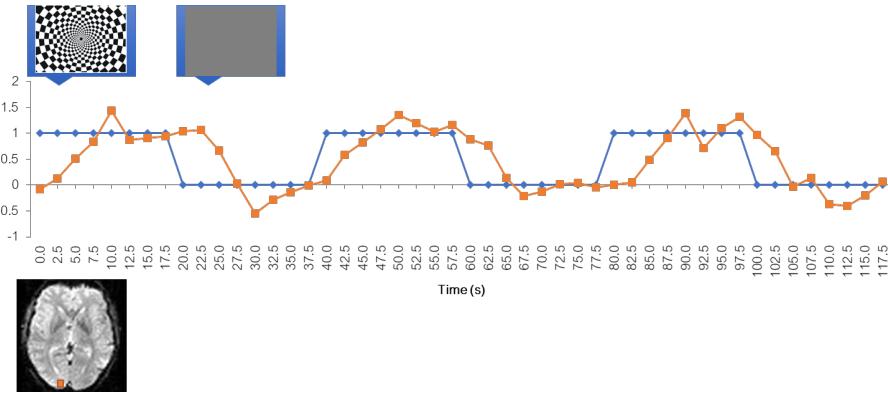
### Run

Block of trials separated by interruption of a scanner acquisition, usually 5-10 minutes

### Session

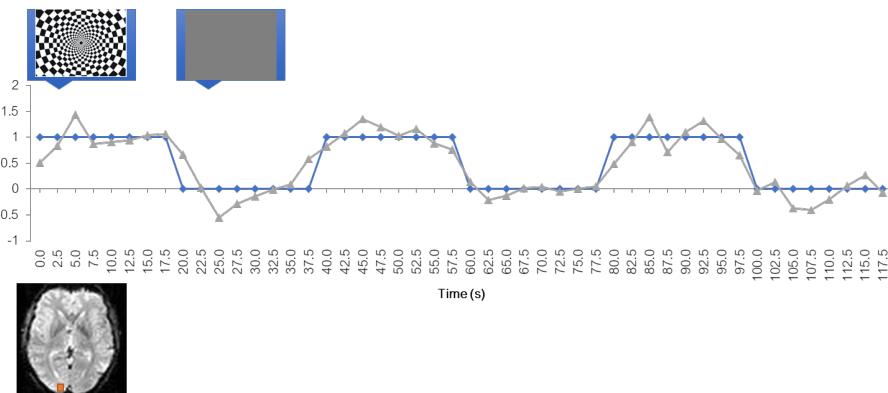
Block of runs, separated by subject going out of the scanner and going in again, usually at least one day

## Voxel time course



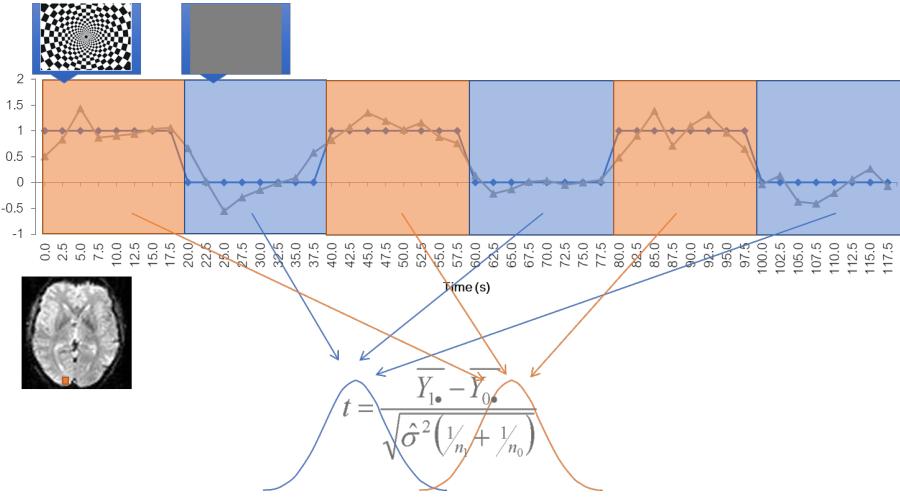
Let's now look at the time course of a voxel in the visual cortex. You see that it is noisy and delayed, but it kind of follows the stimulus.

## Simplest analysis: temporal alignment



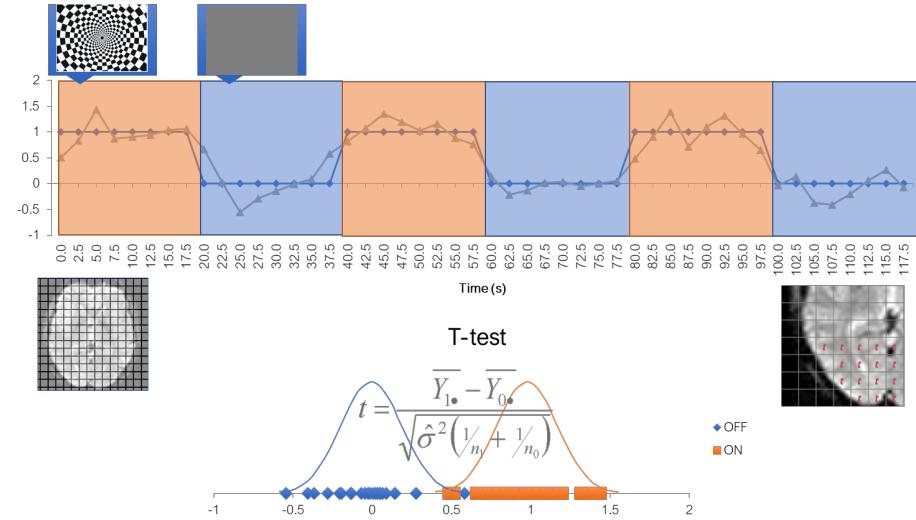
The simplest analysis would be to realign the BOLD time course with the stimulus time course

## Simplest analysis: statistical inference



Then label each value according to when it was acquired, stimulus or baseline  
Collect two types of data points into two big piles and do a statistical comparison  
via a t-test

## Simplest analysis: statistical map

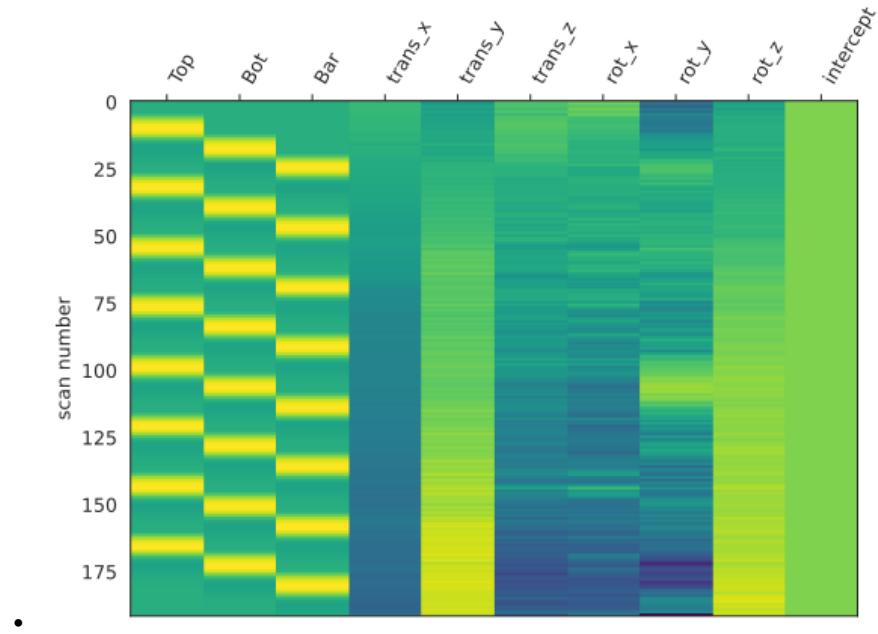


If you do it for every voxel, you can get a new volume, which consists of t-statistics

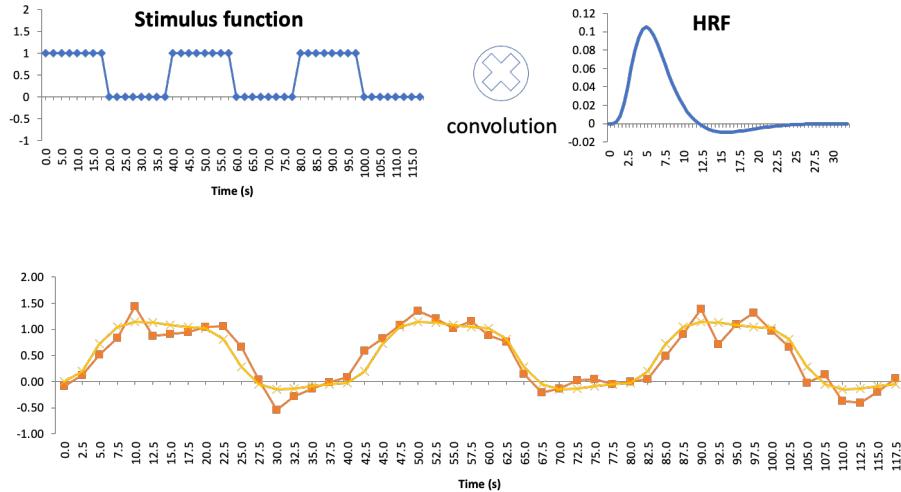
## General Linear Model (GLM)

Multiple regression with ingredients:

- Predictor(s): stimulus function convolved with the HRF (see [Building regressors](#))
- Nuissance regressors

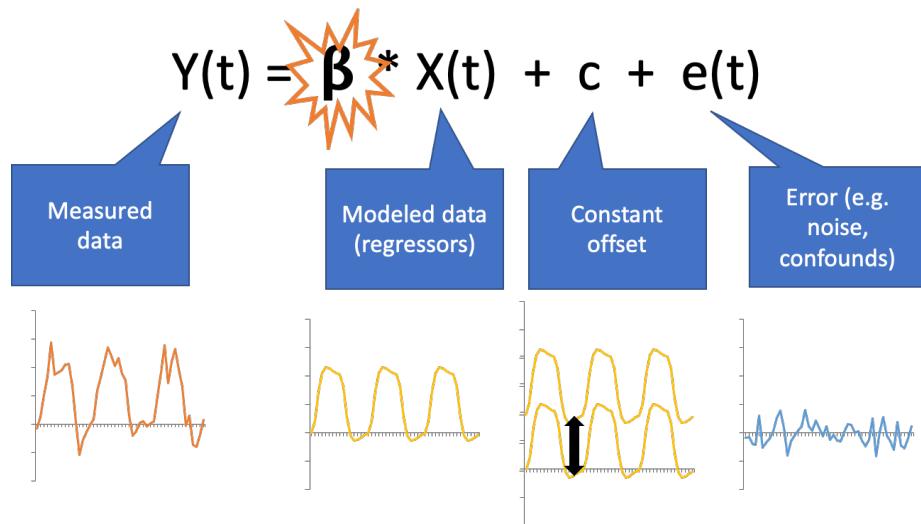


## Buidling regressors

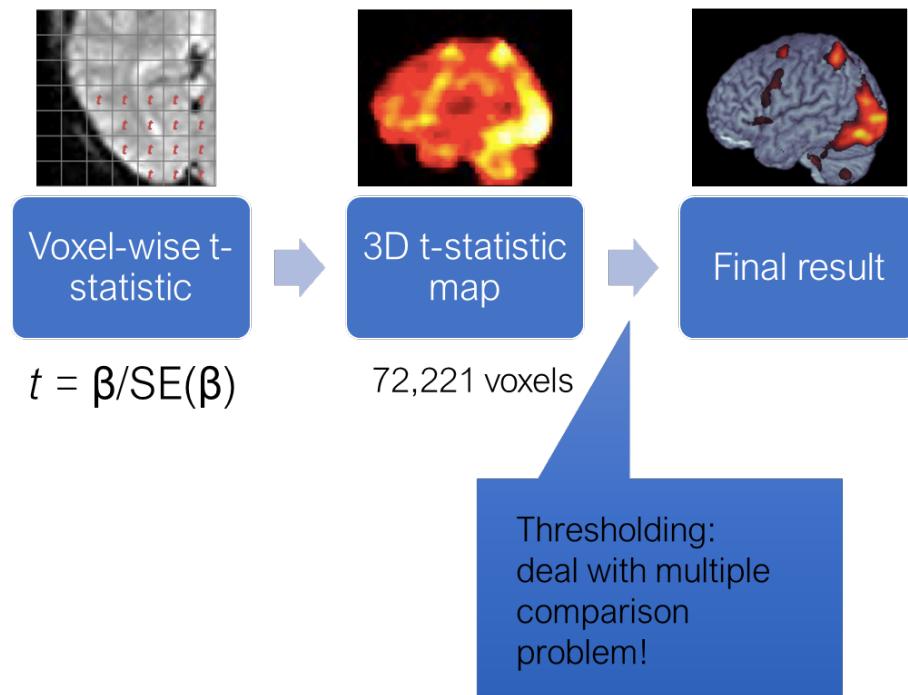


Some convolution examples can be found here (Lindquist 2008)

## General linear model



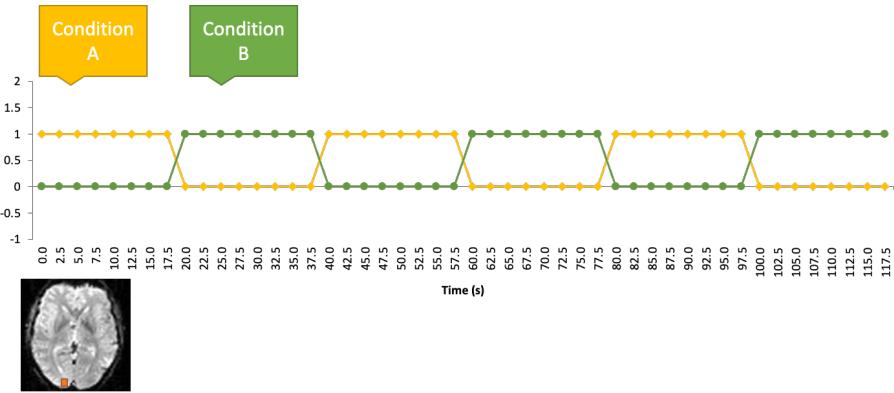
## Statistical inference in whole-brain analysis



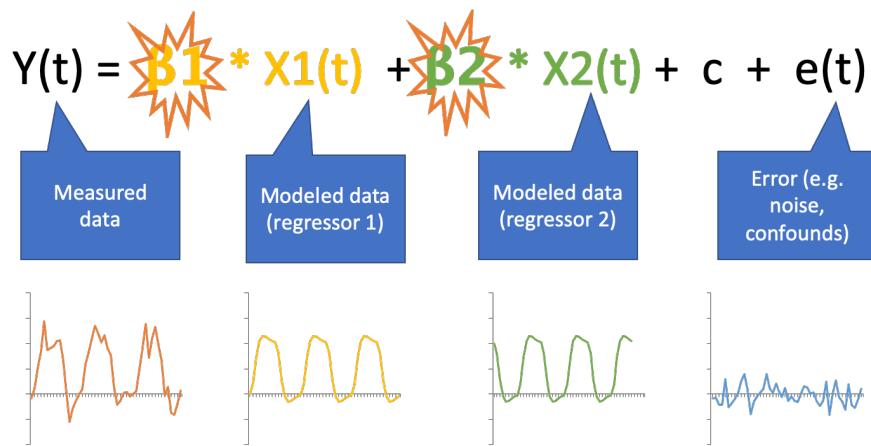
How do we get from beta estimates to making statistical inference? As in a typical regression, beta estimate divided by its standard error is a t-statistic with a t-distribution. So to know whether an activation in one voxel is significant is relatively straightforward.

If we had just one voxel, we would have computed the t-statistic, and then depending on the degrees of freedom determined if it exceeds the critical value. However, we are doing the same statistical test for MANY voxels. SO the probability that we find a significant voxel simply by chance increases. This is the multiple comparison problem that is encountered anywhere in statistics. In fMRI it is particularly prominent, because the number of single tests is enormous. There are several ways and philosophies for dealing with it, I won't go into details right now. The important thing is that any voxel-wise analysis MUST deal with this problem in some way.

## Two conditions

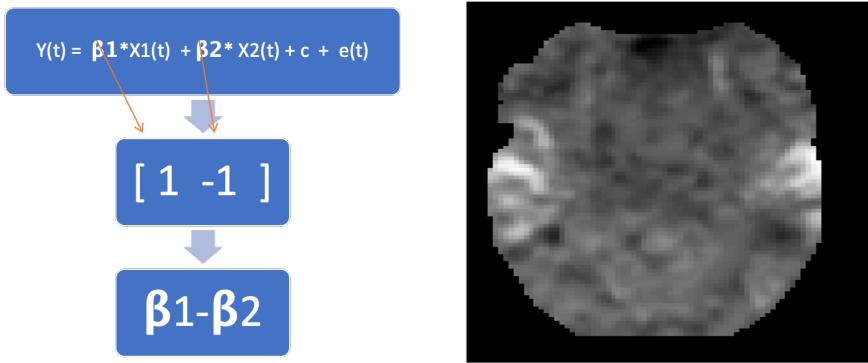


## General linear model with two conditions

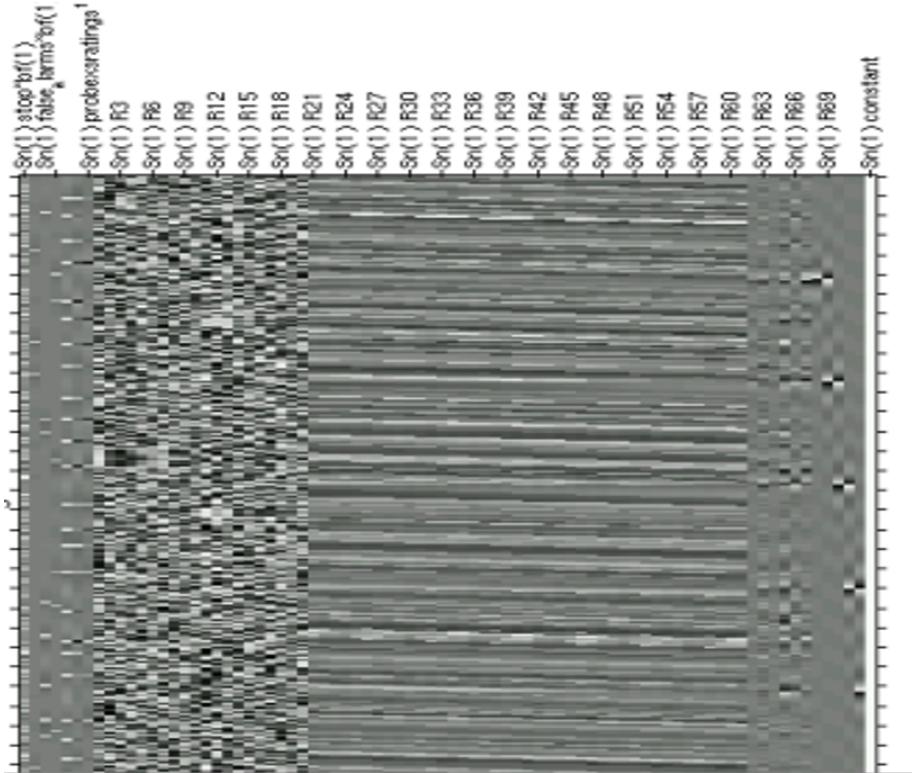


## Contrast

A linear combination of beta estimates

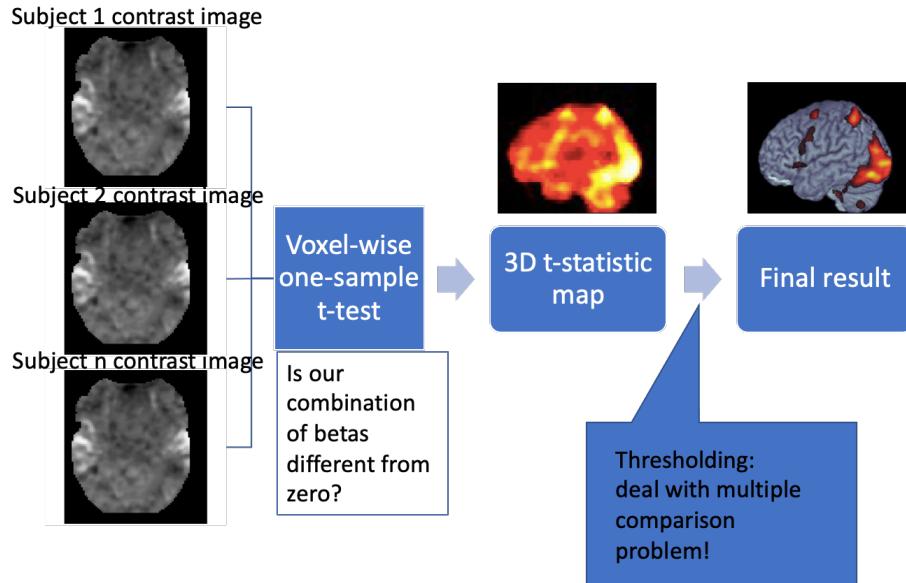


## GLM advantages

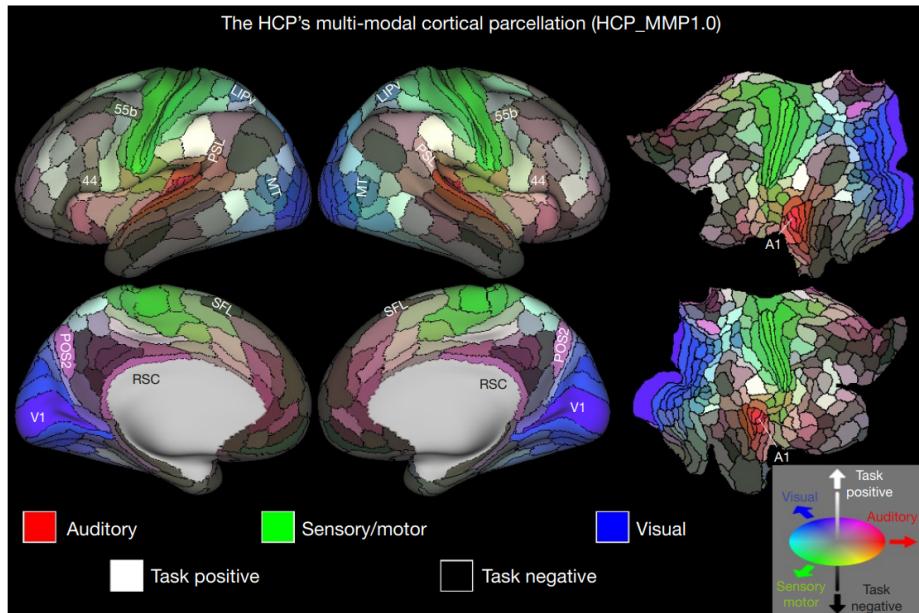


- Complex experimental designs
- Discounting uninteresting effects/confounds
- HRF shape estimation

## Group analysis



## Region-of-interest (ROI) analysis



Question: Does the primary visual cortex respond to flickering checkerboards?

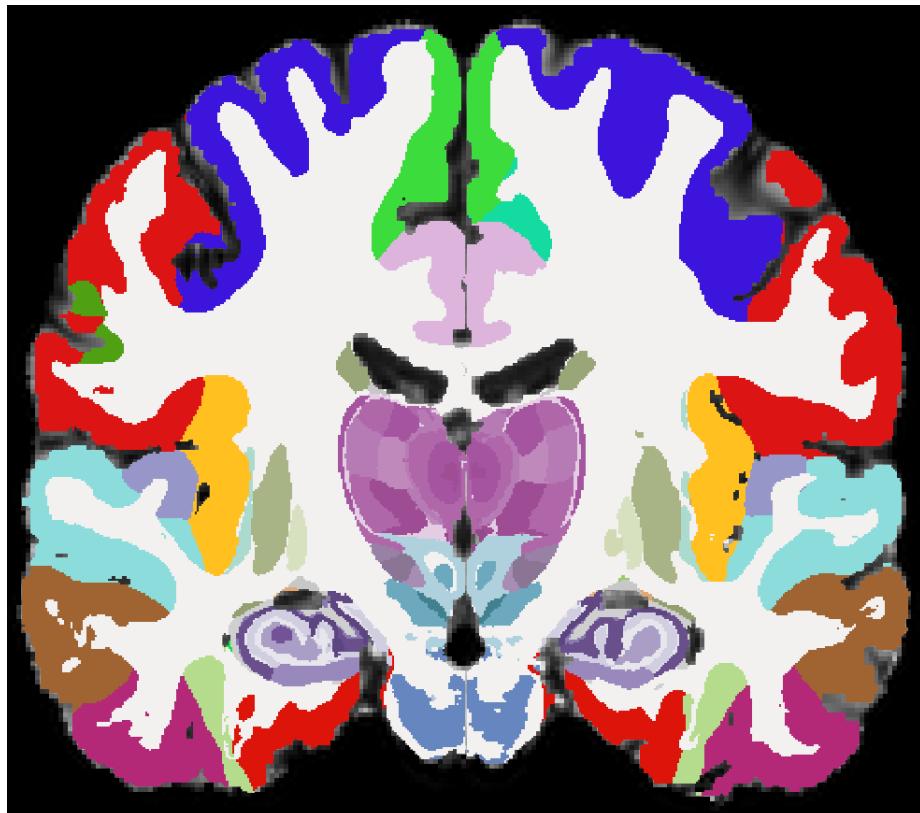
HCP cortical parcellation (“Glasser atlas”) (Glasser et al. 2016)

ROI analysis is a way to deal with multiple comparison problem. But it requires an a priori and independent definition of an ROI.

There are two ways to define ROIs:

- From an anatomical scan
- From an additional (separate) functional experiment

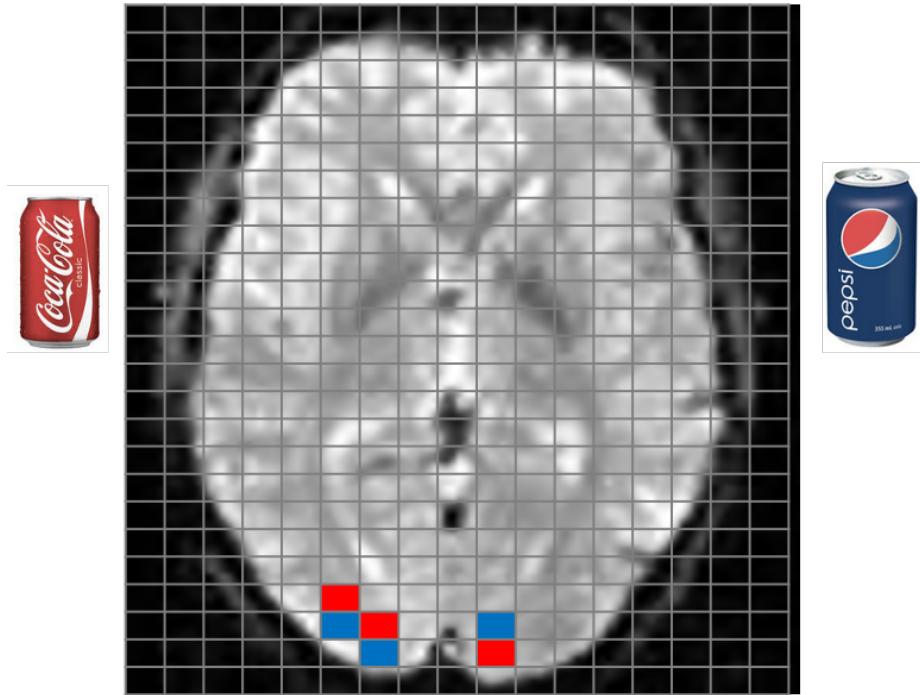
## Subcortical ROIs



NextBrain parcellation containing 333 anatomical structures (Casamitjana et al. 2024)

This is the state-of-the-art for anatomically-based ROI definition based on deep learning

## Multivariate pattern analysis

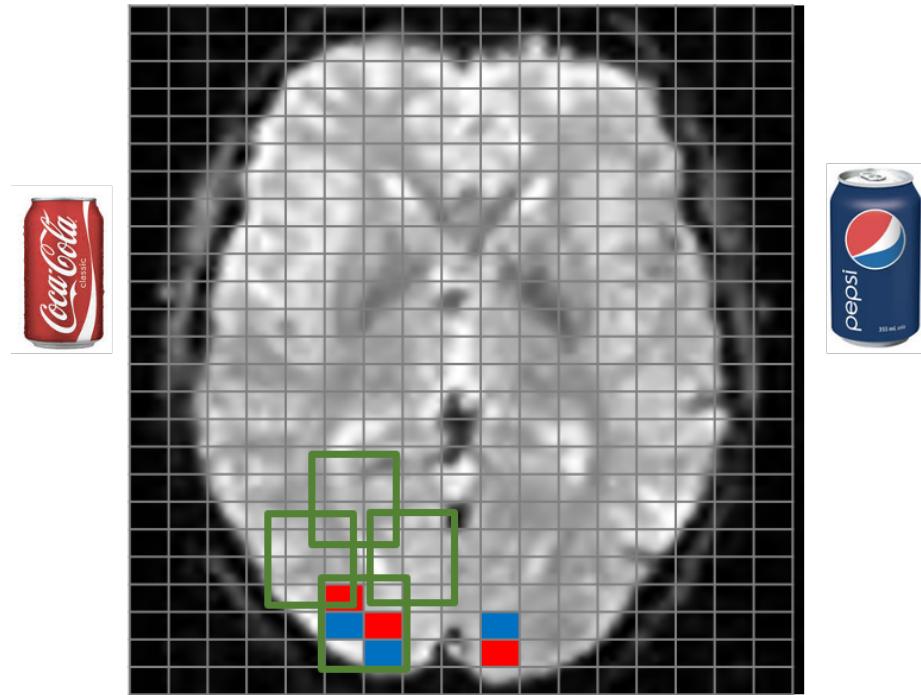


Question: Is the voxel response *pattern* different in condition A and B?

Remember that an image is 4d and consists of little 3d cubes called voxels, with the 4th dimension being time. A classical analysis is done over time for every voxel in the brain independently. In a multivariate analysis we first perform a univariate analysis and then look at response patterns of multiple voxels at a time.

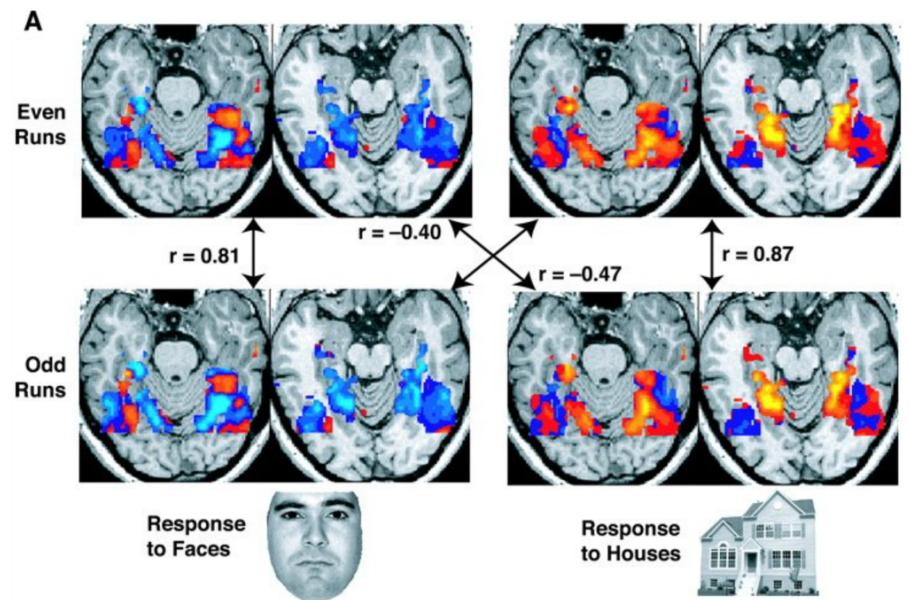
In this example, both coca cola and pepsi activate the same 6 voxels in the visual cortex. But the red ones are activated more by cola than by pepsi, and the blue ones the other way around. If you just look at the average response over 6 voxels, you will not see a difference.

## Searchlight analysis



MVPA can be done for a region of interest, or for the entire brain. In the latter case, the brain is subdivided into multiple ROIs, and the relevant analysis is performed for each of them. This procedure is called “searchlight”. In this case, MCC is as relevant as it is in a univariate analysis.

## First MVPA paper



Representation similarity analysis (Haxby et al. 2001)

## Machine learning

nature  
neuroscience

ARTICLES

## Decoding the visual and subjective contents of the human brain

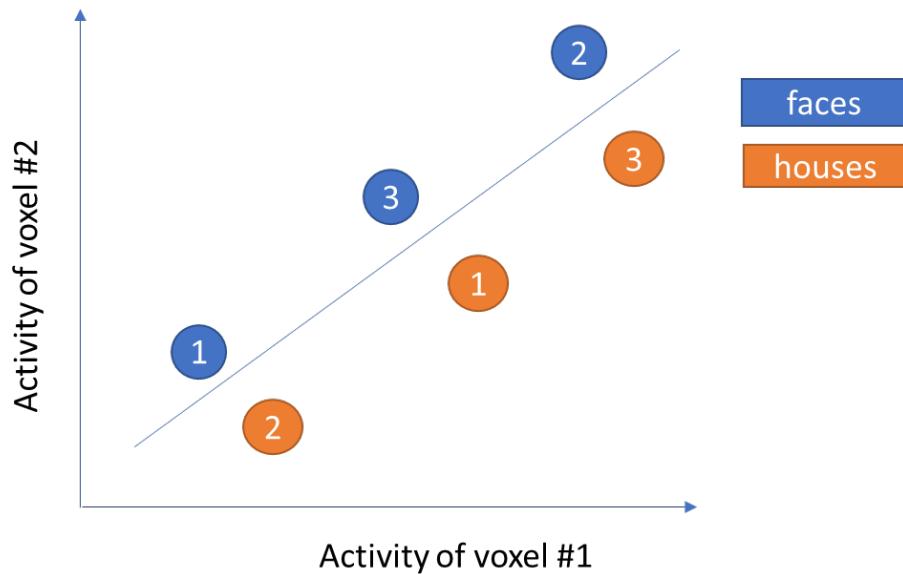
Yukiyasu Kamitani<sup>1</sup> & Frank Tong<sup>2,3</sup>

## Predicting the orientation of invisible stimuli from activity in human primary visual cortex

John-Dylan Haynes<sup>1,2</sup> & Geraint Rees<sup>1,2</sup>

First MVPA papers, both appeared in 2005 (Haynes and Rees 2005; Kamitani and Tong 2005)

### SVM classifier



Toy example for 2D feature space. Further reading in e.g. Cohen et al. (2017)

### References

Belliveau, J. W., D. N. Kennedy, R. C. McKinstry, B. R. Buchbinder, R. M. Weisskoff, M. S. Cohen, J. M. Vevea, T. J. Brady, and B. R. Rosen. 1991.

- “Functional Mapping of the Human Visual Cortex by Magnetic Resonance Imaging.” *Science* 254 (5032): 716–19. <https://doi.org/10.1126/science.1948051>.
- Casamitjana, Adrià, Matteo Mancini, Eleanor Robinson, Loïc Peter, Roberto Annunziata, Juri Althonayan, Shauna Crampsie, et al. 2024. “A Next-Generation, Histological Atlas of the Human Brain and Its Application to Automated Brain MRI Segmentation.” <http://dx.doi.org/10.1101/2024.02.05.579016>.
- Coates, Adam, David Linhardt, Christian Windischberger, Anja Ischebeck, and Natalia Zaretskaya. 2024. “High-Resolution 7T fMRI Reveals the Visual Zone of the Human Claustrum.” *Imaging Neuroscience* 2: 1–15. [https://doi.org/10.1162/imag\\_a\\_00327](https://doi.org/10.1162/imag_a_00327).
- Cohen, Jonathan D, Nathaniel Daw, Barbara Engelhardt, Uri Hasson, Kai Li, Yael Niv, Kenneth A Norman, et al. 2017. “Computational Approaches to fMRI Analysis.” *Nature Neuroscience* 20 (3): 304–13. <https://doi.org/10.1038/nn.4499>.
- Esteban, Oscar, Christopher J. Markiewicz, Ross W. Blair, Craig A. Moodie, A. Ilkay Isik, Asier Erramuzpe, James D. Kent, et al. 2018. “fMRIprep: A Robust Preprocessing Pipeline for Functional MRI.” *Nature Methods* 16 (1): 111–16. <https://doi.org/10.1038/s41592-018-0235-4>.
- Glasser, Matthew F., Timothy S. Coalson, Emma C. Robinson, Carl D. Hacker, John Harwell, Essa Yacoub, Kamil Ugurbil, et al. 2016. “A Multi-Modal Parcellation of Human Cerebral Cortex.” *Nature* 536 (7615): 171–78. <https://doi.org/10.1038/nature18933>.
- Haxby, J V, M I Gobbini, M L Furey, A Ishai, J L Schouten, and P Pietrini. 2001. “Distributed and Overlapping Representations of Faces and Objects in Ventral Temporal Cortex.” *Science (New York, N.Y.)* 293 (5539): 2425–30. <https://doi.org/10.1126/science.1063736>.
- Haynes, John-Dylan, and Geraint Rees. 2005. “Predicting the Orientation of Invisible Stimuli from Activity in Human Primary Visual Cortex.” *Nature Neuroscience* 8 (5): 686–91. <https://doi.org/10.1038/nn1445>.
- “Interpreting the BOLD Response.” 2009. In, 400–424. Cambridge University Press. <https://doi.org/10.1017/cbo9780511605505.020>.
- Kamitani, Yukiyasu, and Frank Tong. 2005. “Decoding the Visual and Subjective Contents of the Human Brain.” *Nature Neuroscience* 8 (5): 679–85. <https://doi.org/10.1038/nn1444>.
- Lindquist, Martin A. 2008. “The Statistical Analysis of fMRI Data.” *Statistical Science* 23 (4). <https://doi.org/10.1214/09-sts282>.
- Logothetis, Nikos K., Jon Pauls, Mark Augath, Torsten Trinath, and Axel Oeltermann. 2001. “Neurophysiological Investigation of the Basis of the fMRI Signal.” *Nature* 412 (6843): 150–57. <https://doi.org/10.1038/35084005>.
- Sandrone, Stefano, Marco Bacigaluppi, Marco R. Galloni, Stefano F. Cappa, Andrea Moro, Marco Catani, Massimo Filippi, Martin M. Monti, Daniela Perani, and Gianvito Martino. 2013. “Weighing Brain Activity with the Balance: Angelo Mosso’s Original Manuscripts Come to Light.” *Brain* 137 (2): 621–33. <https://doi.org/10.1093/brain/awt091>.

Zaretskaya, Natalia, Bruce Fischl, Martin Reuter, Ville Renvall, and Jonathan R Polimeni. 2018. “Advantages of Cortical Surface Reconstruction Using Submillimeter 7 t MEMPRAGE.” *NeuroImage* 165 (January): 11–26. <https://doi.org/10.1016/j.neuroimage.2017.09.060>.