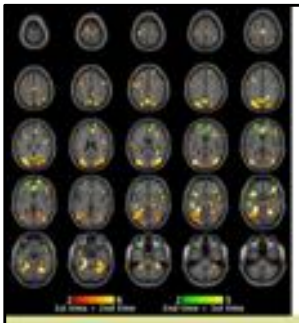
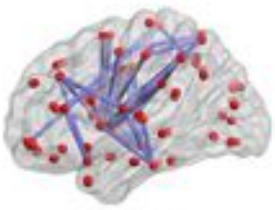
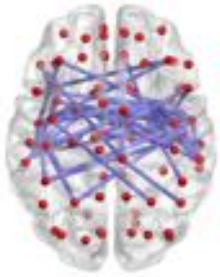




Side View



Top View

Functional MRI

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VUB

From class 1

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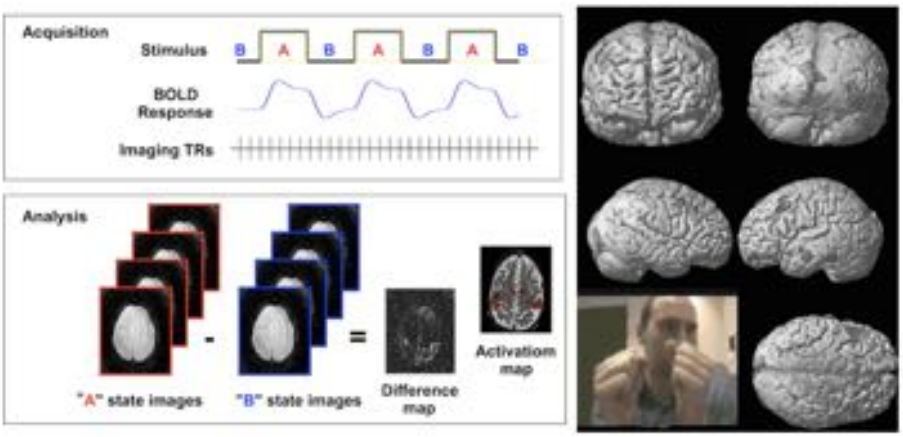
VUB

fMRI experiment



- To increase the sensitivity to detect contrast differences between task states, every 0.5-3s a full brain scan is taken while the participant/patient performs a task consisting of different conditions/states for at least 5 minutes
- During the analysis, the scans taken during the various task conditions are compared to come to activation maps.
- The resulting activation maps are overlaid on an anatomical scan

See also
Chen JE, Glover GH. Functional magnetic imaging methods. Neuropsychol Rev 2015; 25:289-313.
http://mriquestions.com/bold-pulse-sequences.html

Task paradigm: Audiovisual Valence Congruence

What you see has the same valence (emotion) as what you hear (congruent)
Versus
What you see has the opposite valence (emotion) as what you hear (incongruent)
for positive versus negative stimuli
↓
2x2 design: (congruent - incongruent) x (positive - negative)

Visual stimuli (video) + Auditory stimuli (music)
Stimulation as individual events (3s long)
4 conditions:
1. Visual positive + Auditory positive
2. Visual positive + Auditory Negative — Congruent trials
3. Visual negative + Auditory positive — Incongruent trials
4. Visual negative + Auditory negative

12 trials / run / condition -> 48 trials / run
8 catch trials (no stimulus, but responses recorded -> attention test)
20 subjects (all healthy adult volunteers)

Positive valence

Negative valence

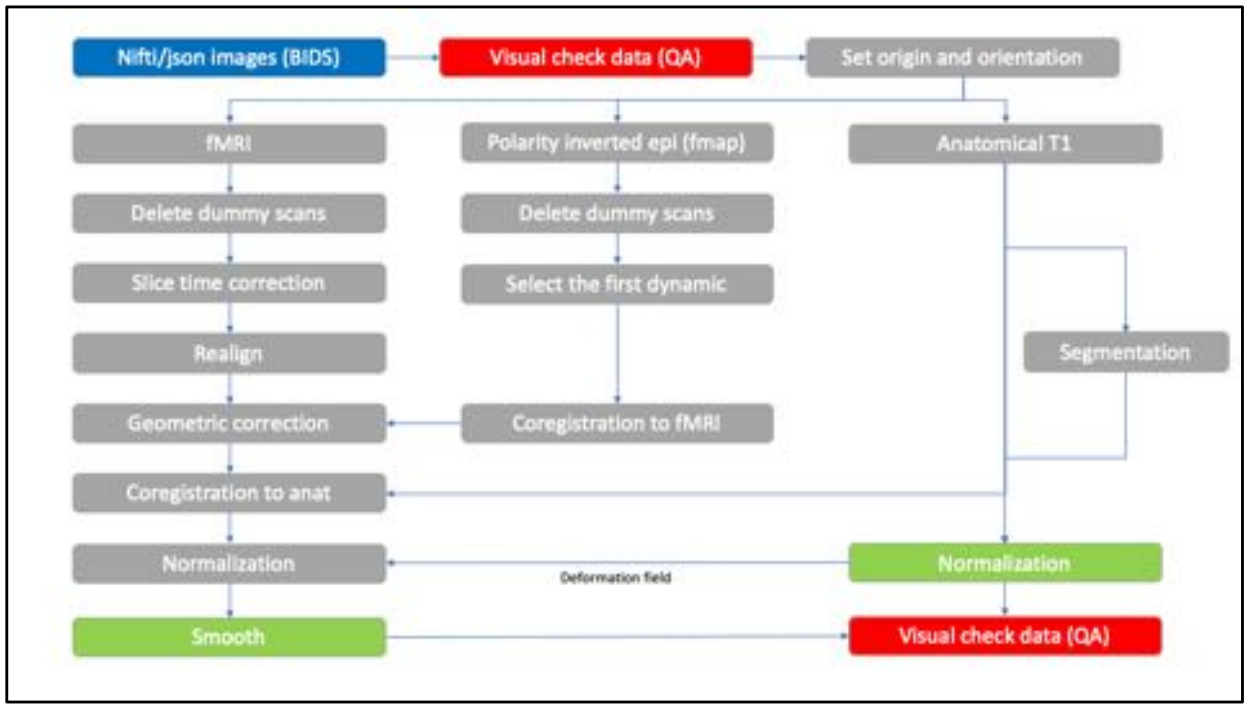
Gao, C., Weber, C. E., Wedell, D. H., & Shinkareva, S. V. (2020). An fMRI study of affective congruence across visual and auditory modalities. Journal of cognitive neuroscience, 32(7), 1251-1262
Chuanji Gao and Christine E. Weber and Douglas H. Wedell and Svetlana V. Shinkareva (2021). fMRI: Audiovisual Valence Congruence. OpenNeuro. [Dataset] doi: 10.18112/openneuro.ds003507.v1.0.1

We will process 1 individual fMRI run for 1 participant

We will perform group analyses based on the preprocessed group data

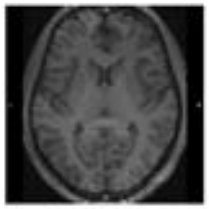
Task: indicate that the emotion in the music is the same or not as in the video by button presses

Catch trial = a trial within an experiment in which a stimulus is not present but the participants' responses nonetheless are recorded. For example, in an experiment in which participants identify auditory signals, catch trials are those in which no signal is given. The use of a catch trial may help to estimate the level at which a participant is guessing when no stimulus is present



Deformation field: contains the information for the non-linear transformation from subjects space to normalized (MNI) space

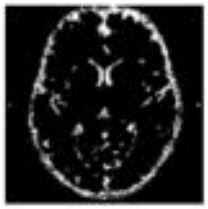
The preprocessing results



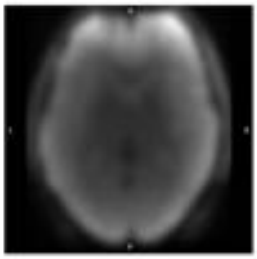
wosub-01_T1w

wc1osub-01_T1w

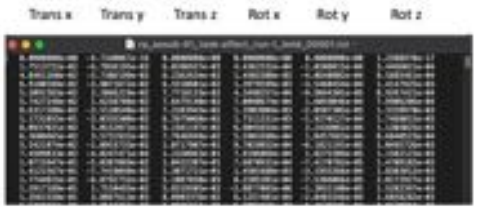
wc2osub-01_T1w



wc3osub-01_T1w



swuraosub-01_..._bold



Realignment parameters

First level analysis

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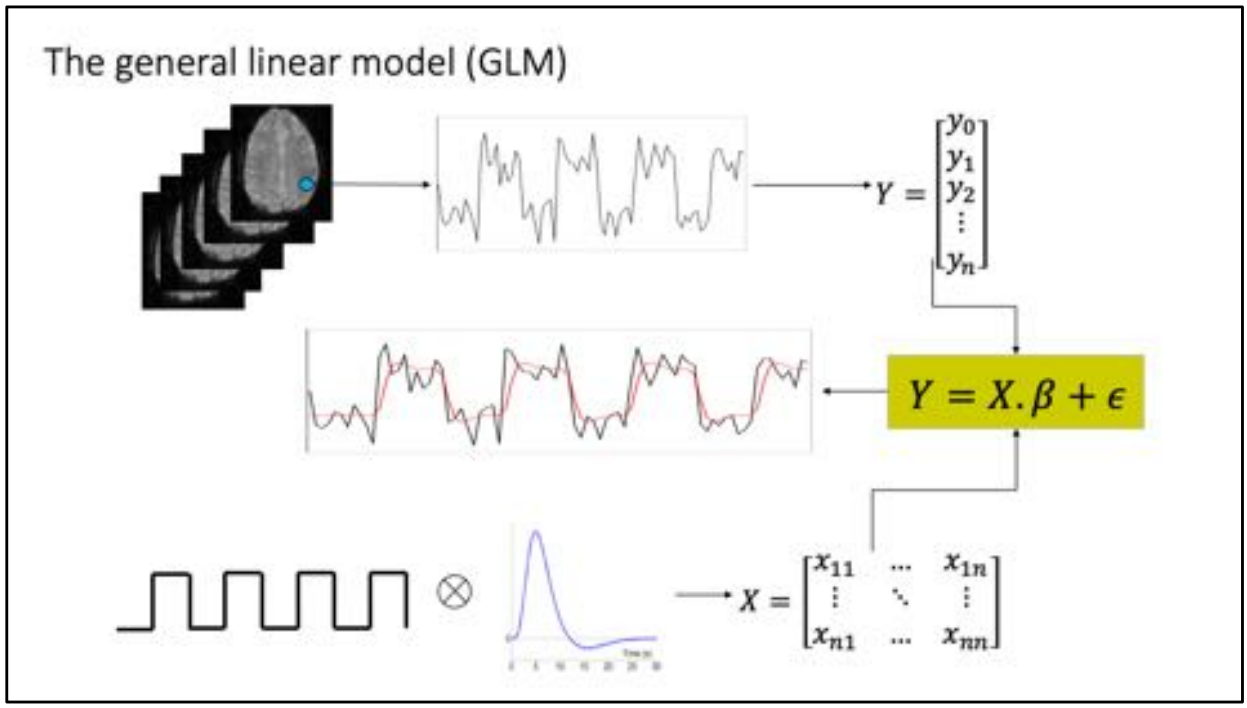
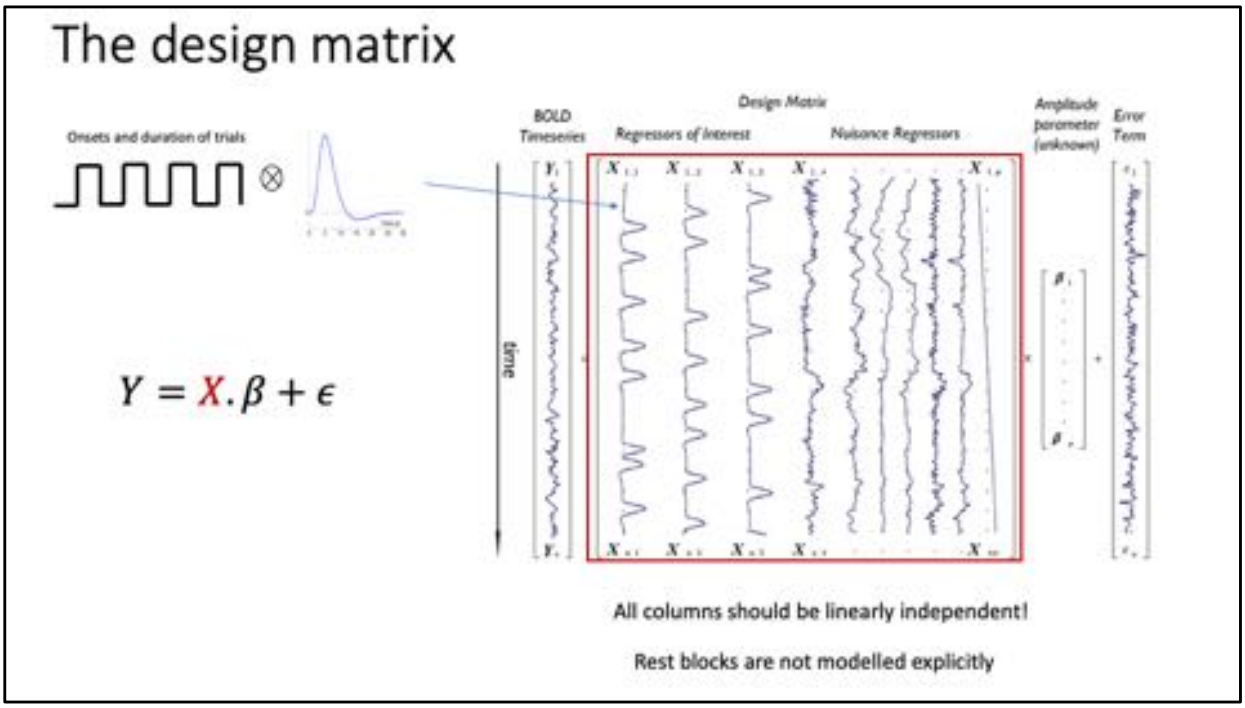


Using the general linear model approach (GLM)

- The measured signal -> column matrix Y
- The predicted BOLD response per task condition -> one column per condition in the design matrix X
- Additional regressors explaining some of the signal variations -> extra columns in the design matrix X
- Residual not-explained signal variations = random noise -> the column matrix ε
- Fitting the explanatory regressors to the measured data -> fitting parameters β

See also
http://mriquestions.com/fmri-statistical-analysis.html
http://mriquestions.com/general-linear-model.html
Poline and Brett. 2012. The general linear model and fMRI: does love last forever? NeuroImage 62(2):871-880
https://users.fmrib.ox.ac.uk/~stuart/thesis/chapter_6/section6_3.html
Pernet 2014. Misconceptions in the use of the general linear model applied to functional MRI: a tutorial for junior neuro-imagers. Front. Neurosci. 8:1
http://www.fil.ion.ucl.ac.uk/spm/doc/books/hbf2/pdfs/Ch7.pdf

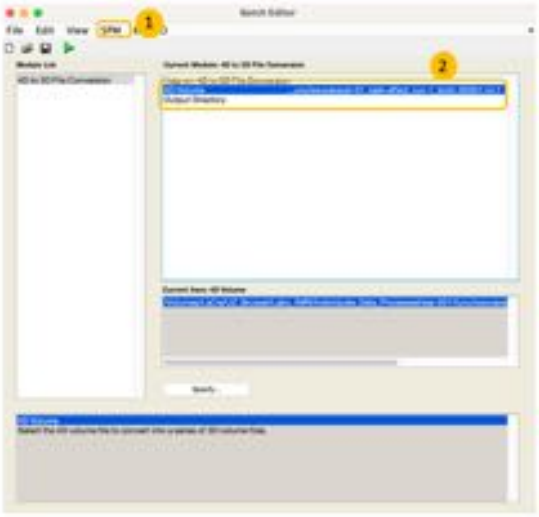


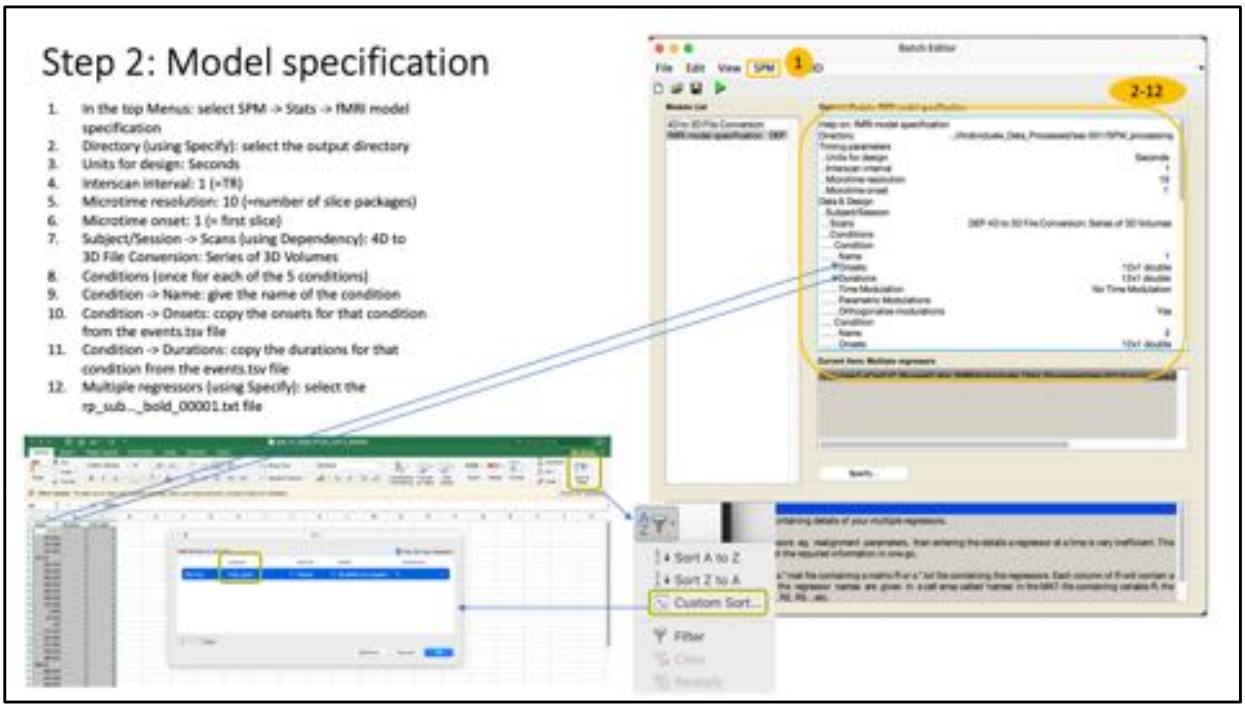
- The design matrix X -> Collection of explanatory time series
    - Regressors of interest: the expected signal evolution due to the various task conditions
        - For each task condition, the on-of time state convolved with a basic-function (e.g. the HRF mathematical model)
    - Nuisance regressors: all other known sources of signal variations (noise)
        - Motion (e.g. by include the 6 (3 translations, 3 rotations) parameters as determined during the regression step)
        - Flow pulsation curve as measured by the PPU
        - Breathing
        - …
    - All regressors included in the design matrix should be uncorrelated and orthogonalized

See also
http://mriquestions.com/general-linear-model.html

Step 1: Loading the preprocessed data

1. In the top Menus: select SPM -> Util -> 4D to 3D File Conversion
2. 4D volume (using Specify): select the preprocessed fMRI data (swurao... .nii file)
3. Make a folder to save the SPM processing results



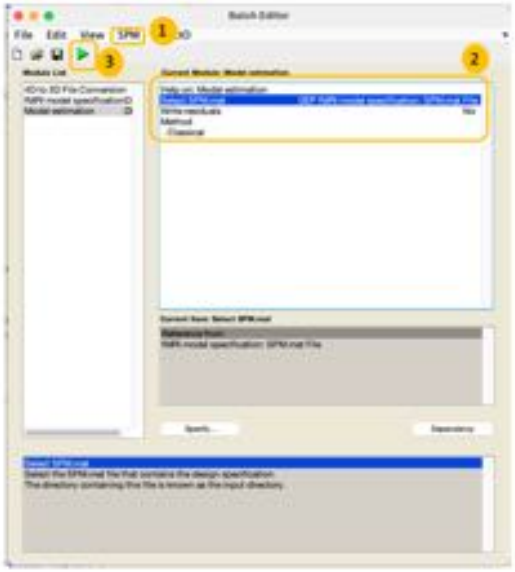


Be careful: the directory used to save the results will be emptied at the start of the analysis -> best to select an empty directory

It ’s easiest to open the events.tsv file as a spread sheet (e.g. excel or numbers) and sort the rows based on the trial_type column.

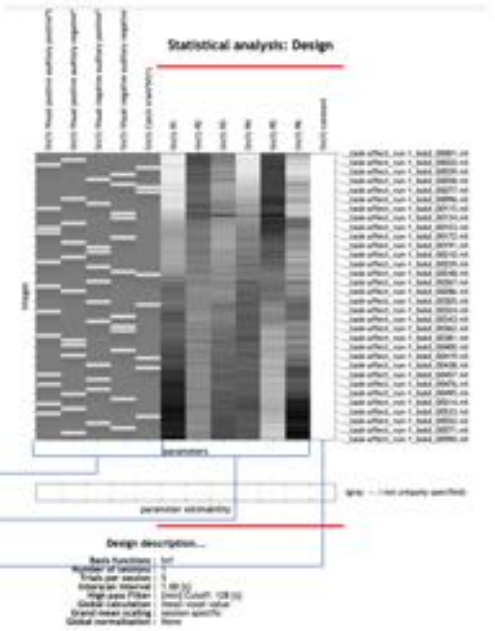
Step 3: Model estimation

1. In the top Menus: select SPM -> Stats -> fMRI model estimation
2. Select SPM.mat (using Dependency): fMRI model specification: SPM.mat File
3. Run batch



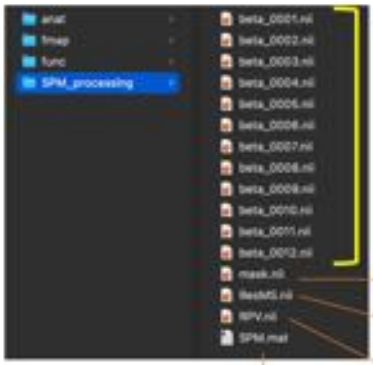
Step 3: Model review

1. In the Menu window: click Review
2. Select the SPM.mat file in the output directory



12 model regressors
- 5 task regressors
- 6 motion regressors
- 1 constant

The model estimation output



12 beta maps (1 per regressor)

A brain mask

The residue image: $ResMS = Y - \hat{Y}$ with $\hat{Y} = \beta.X$

Resels (resolution element) Per Voxel: measure for the smoothness of the data

Mat file containing the analysis parameter settings and file lists

Resels = size of image part that corresponds to the FWHM (full width half maximum) of the Gaussian convolution kernel that would have produced the observed image when applied to independent voxel values

The first level analysis results

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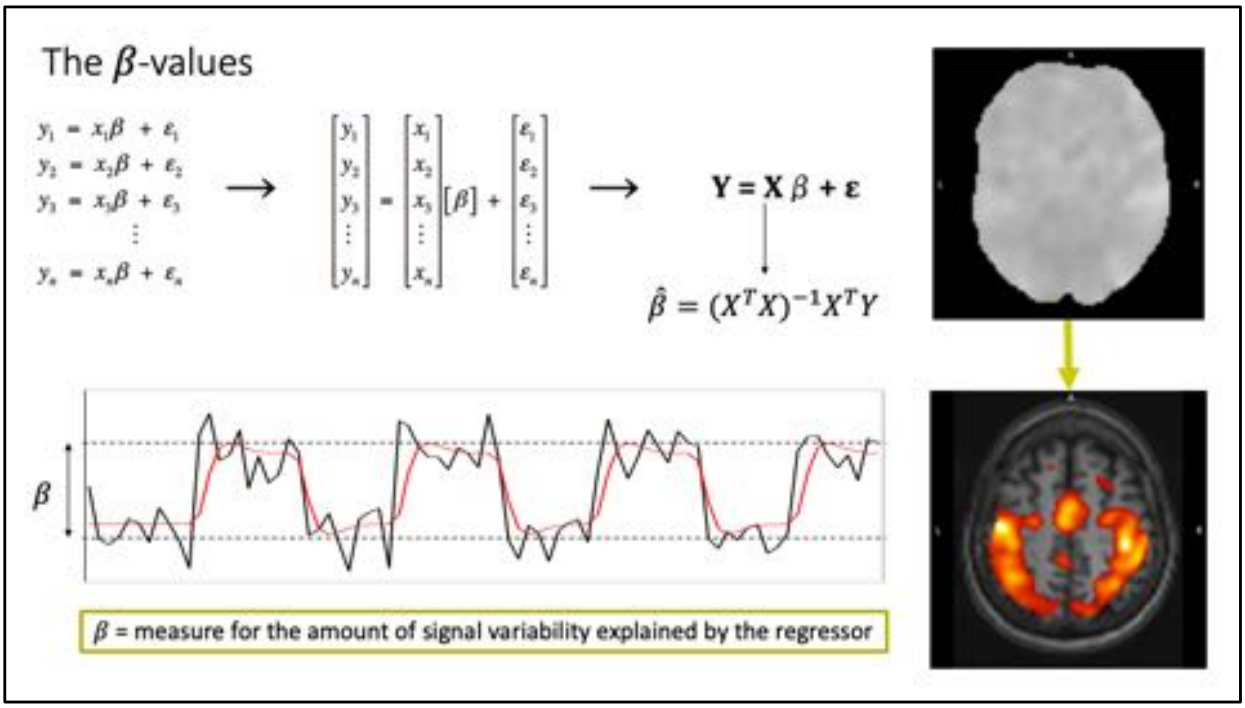
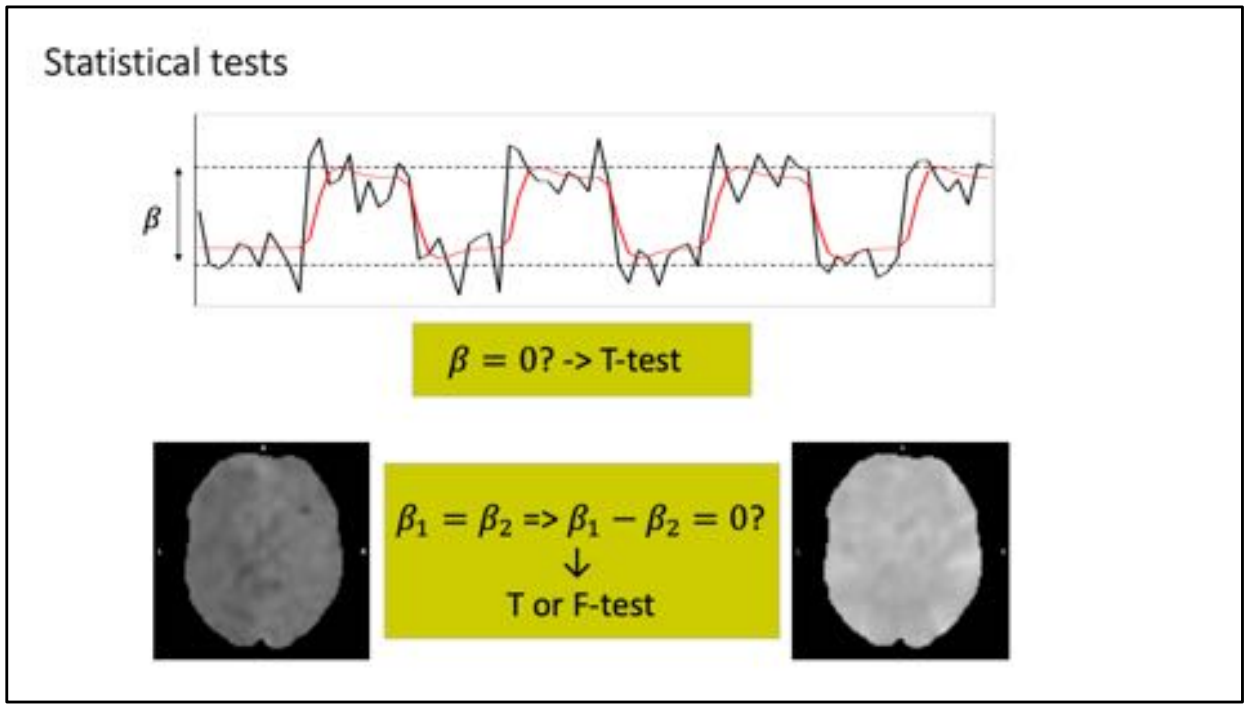
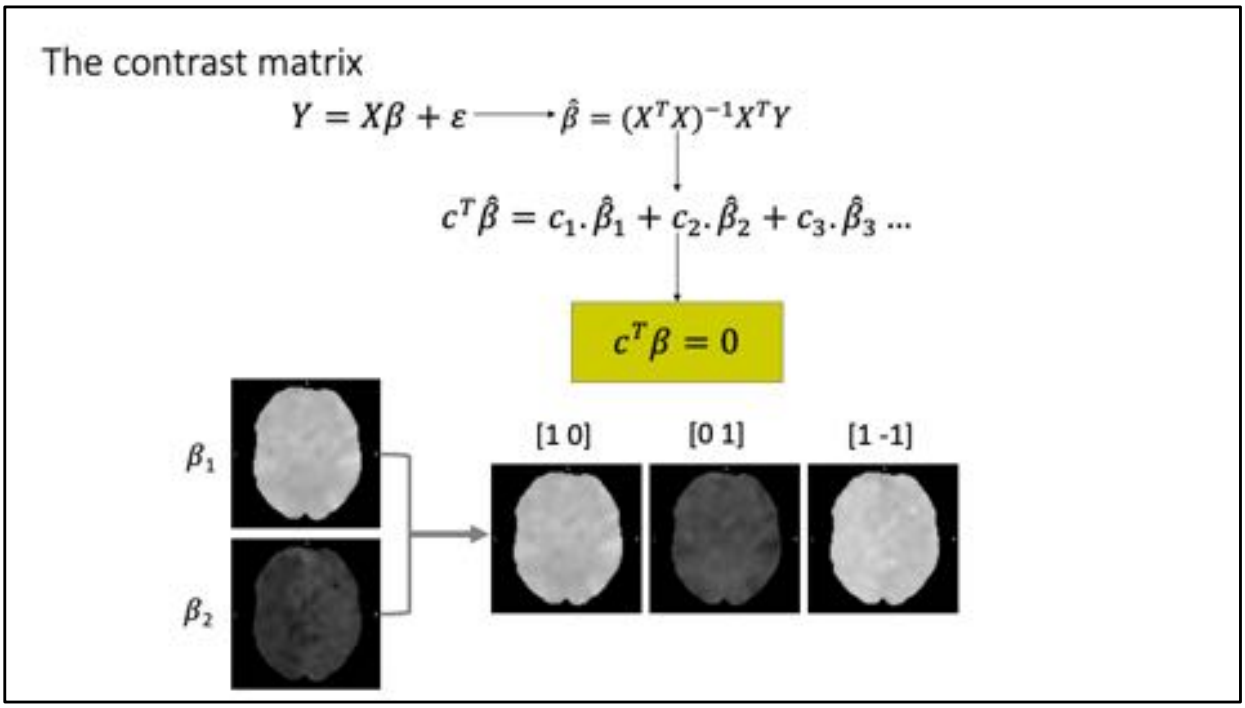


- The β-values are the results of the fitting of the explanatory model (defined in the design matrix X) to the measured time series
    - 1 β-value per regressor in the model
    - Each β-value is a measure for the amount of variability in the measured data, **after correction of all other regressors**, that is explained by the corresponding normalized regressor -> a change in the on-off state of the corresponding condition, induced a change in the MRI signal due to a change in the underlying neural activity
    - The β-value is a measure for the amplitude of the signal variations in relation to the corresponding regressor
        - If β is high -> change in neural activation
        - If β is small -> no change in neural activation
    - Especially in more complex task designs, the β-value is difficult to interpret as "activation-baseline"
- After correction of all other regressors means: if you subtract the variability explained by the other variables, still an amount in the residual variability can be explained by the regressor corresponding with the β.
- For each regressor in the design matrix, SPM calculates the corresponding β-value in each voxel -> β-maps
- The β-maps can be overlaid on the anatomical image and thresholded to see where a β is the largest -> where most variance is explained by the regressor -> activation map
- But …
    - The fitting will mostly result in a non-zero β, even if the regressor explains almost no variability in the measured time serries
    - What's the best threshold to discriminate real non-0 β's from accidental non-0 β's?
    - It tells nothing about signal differences between various conditions



- Questions:
  - Where in the brain were there changes in the neural activity related to a task condition?
    - A large part of the variability in the signal can be explained by the regressor
    - Is the β significantly different from 0? -> T-test with null condition: $\beta = 0$
    - T-test = comparing the variability explained to the residual noise
  - Where in the brain did the neural activity (signal) differ significantly during 2 task conditions?
    - We need to contrast the the changes in neural activity due to the 1st task condition to the changes in neural activity due to the 2nd task condition
    - Is the β from condition 1 different from the β from condition 2? -> T-test with null condition: $\beta_1 = \beta_2$ => $\beta_1 - \beta_2 = 0$



- From the GLM we estimated the β's -> $\hat{\beta}$
- To test a specific effect we make a linear combination of the estimated β's
    - The contrast parameters are defined in a contrast matrix c
    - The contrast matrix must contain as many elements as estimated β's
    - The resulted linear combination of β's are saved in contrast files -> con.nii files
    - The sum of all positive elements in the contrast matrix should equals 1 and the sum of all negative elements in the contrast matrix should equals -1
- Examples of contrast matrices
    - Linear increased response as predicted by the first regressor: $c^T = [1\ 0\ 0\ \ldots]$
    - Linear decreased response as predicted the first regressor: $c^T = [-1\ 0\ 0\ \ldots]$
    - Difference between the response predicted by the first regressor and the response predicted by the third regressor: $c^T = [1\ -1\ 0\ \ldots]$
- Null hypothesis to test: $c^T\beta = 0$
- Advantage of using a contrast matrix: the same approach can be used to test the significance of a β as to test the significance of any linear combination of β's

See also

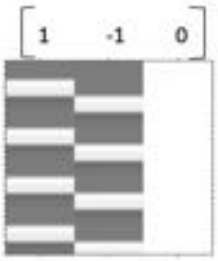
http://www.fil.ion.ucl.ac.uk/spm/doc/books/hbf2/pdfs/Ch8.pdf

http://mriquestions.com/general-linear-model.html

T-test

- Null hypothesis H_0: $c^T\hat{\beta} = 0$
- Alternative hypothesis H_1: $c^T\hat{\beta} > 0$

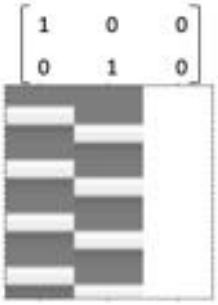
$$t_{df} = \frac{c^T\hat{\beta}}{sd(c^T\hat{\beta})}$$



F-Test

- Null hypothesis H_0: $\beta_1 = \beta_2 = \cdots = 0$
- Alternative hypothesis: H_1: existence of at least one $\beta \neq 0$

$$F = \frac{Explained\ variability}{Error\ estimated\ variance}$$



- To test the significance of the contrast, a T-test is done
  - Null hypothesis: H_0: $c^T\hat{\beta} = 0$
  - Alternative hypothesis: H_1: $c^T\hat{\beta} > 0$
  - The determined t: $t_{df} = \frac{contrast\ of\ estimated\ parameters}{\sqrt{estimated\ variance}}$
- Properties of the T-test:
  - Unidimensional: the contrast vector has only 1 row
  - Directional: the alternative hypothesis is that the contrast has a significant **positive** difference from 0
  - Possible to test the effect of 1 parameter or a combination of multiple parameters
- To test the existence of a response regardless of the sign of the effect or to which condition the area responded, an F-test can be performed
  - Null hypothesis H_0: $\beta_1 = \beta_2 = \cdots = 0$
  - Alternative hypothesis: H_1: existence of at least one $\beta \neq 0$
  - The determined F: $F = \frac{Explained\ variability}{Error\ estimated\ variance}$
- Properties of the F-test
  - The F-test contrast matrix = matrix of T-contrasts
  - Non-directional -> the F-test only state the existence of an effect
  - Not possible to attribute the obtained effect to a specific regressor

See also

http://www.fil.ion.ucl.ac.uk/spm/doc/books/hbf2/pdfs/Ch8.pdf

Step 4: Contrast estimation

1. In the top Menus: select SPM -> Stats -> Contrast Manager
2. Select SPM.mat (using Specify): fMRI model specification: SPM.mat File
3. Per T-contrast:
    1. Define contrast name
    2. Give the contrast weights (sum positive weights=1; sum negative weights=-1)

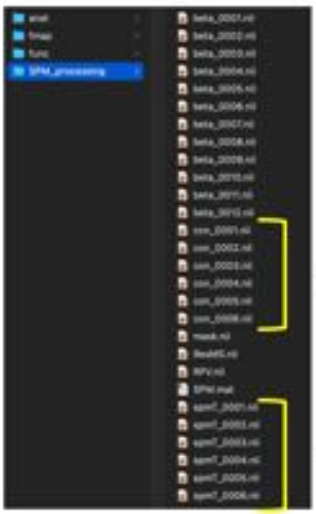
Trial conditions
1. Visual positive, Auditory positive
2. Visual positive, Auditory negative
3. Visual negative, Auditory positive
4. Visual negative, Auditory negative
5. Catch trials

2 task conditions: Congruent and Incongruent

Contrast matrix
1. Activations for congruent (Congruent>0 = (1+4)>0): c=[0.5 0 0 0.5]
2. Deactivations for congruent (Congruent<0 = (1+4)<0): c=[-0.5 0 0 -0.5]
3. Activations for incongruent (Incongruent>0 = (2+3)>0): c=[0 0.5 0.5 0]
4. Deactivations for incongruent (Incongruent>0 = (2+3)<0): c=[0 -0.5 -0.5 0]
5. Congruent>Incongruent ((1+4)-(2+3)>0): c=[0.5 -0.5 -0.5 0.5]
6. Congruent<Incongruent ((2+3)-(1+4)>0): c=[-0.5 0.5 0.5 -0.5]

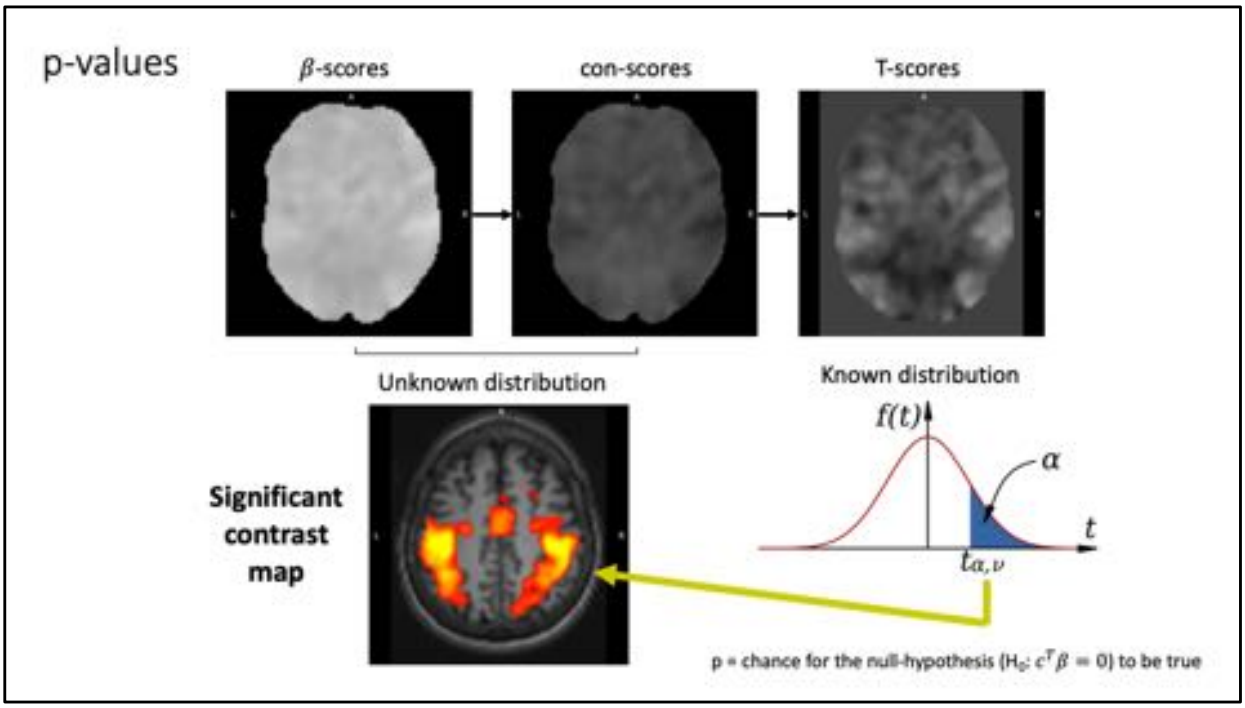
As a rule of thumb, the sum of all positive weights should equal 1 and the sum of all negative weights should equal -1 to avoid multiplication of the effect size (activation, deactivation or difference)

The contrast estimation output



6 contrast maps (the size of the calculated contrast)

6 T-maps (the t-value of the performed T-test per contrast)



- From the estimated β-scores, contrast values are calculated

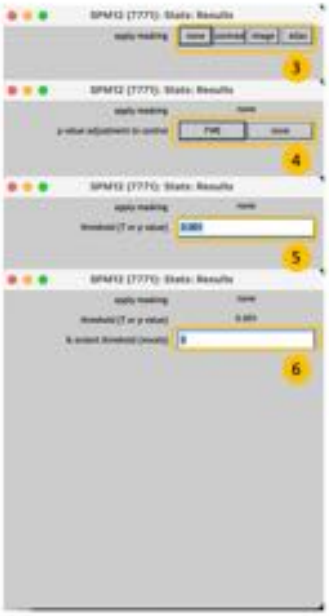
- These contrast values are according an unknown distribution (contrast specific mean and error) -> difficult to discriminate significant from non-significant values

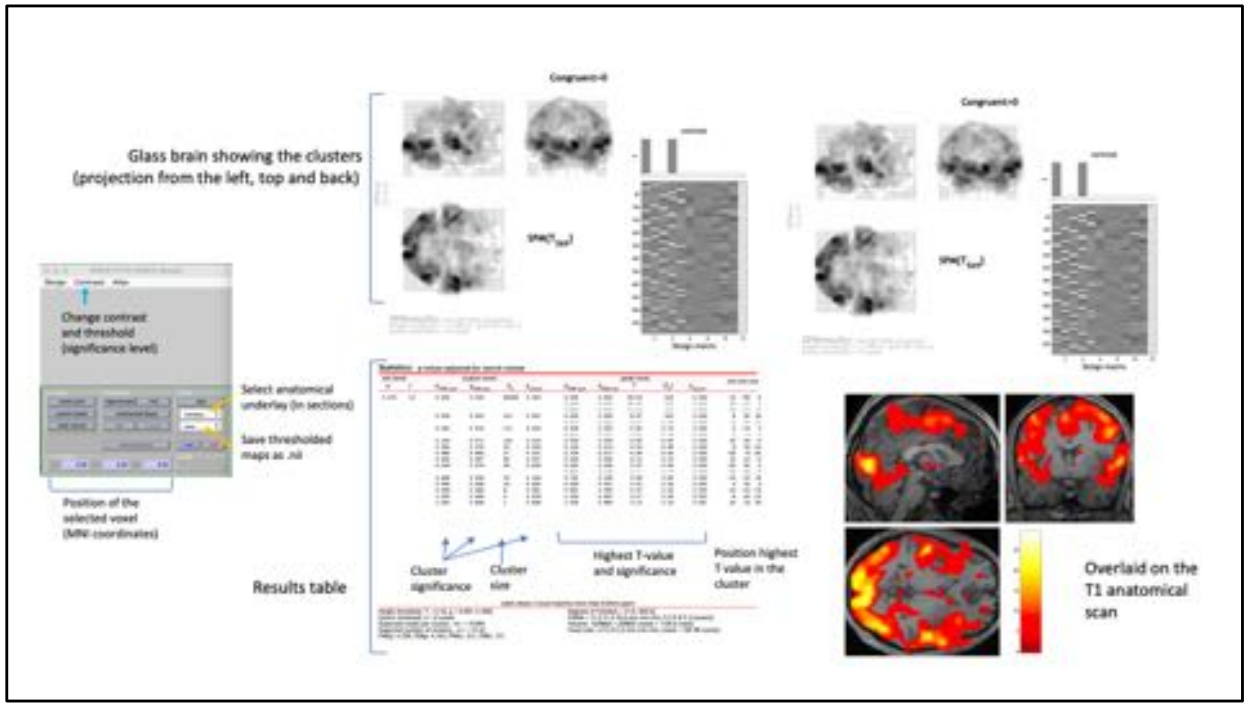
- The contrast values are transformed into T- or F-scores with a known distribution (mean and error for a specified degrees of freedom)
    - It is possible to determine the chance (p-value) that the null hypothesis (no significant effect) is true -> the smaller p, the more likely we can reject the null hypothesis
    - By using a significance threshold for p, we can discriminate those voxels at which it is the most likely that the null hypothesis is untrue -> means: the corresponding contrast is significantly different from 0

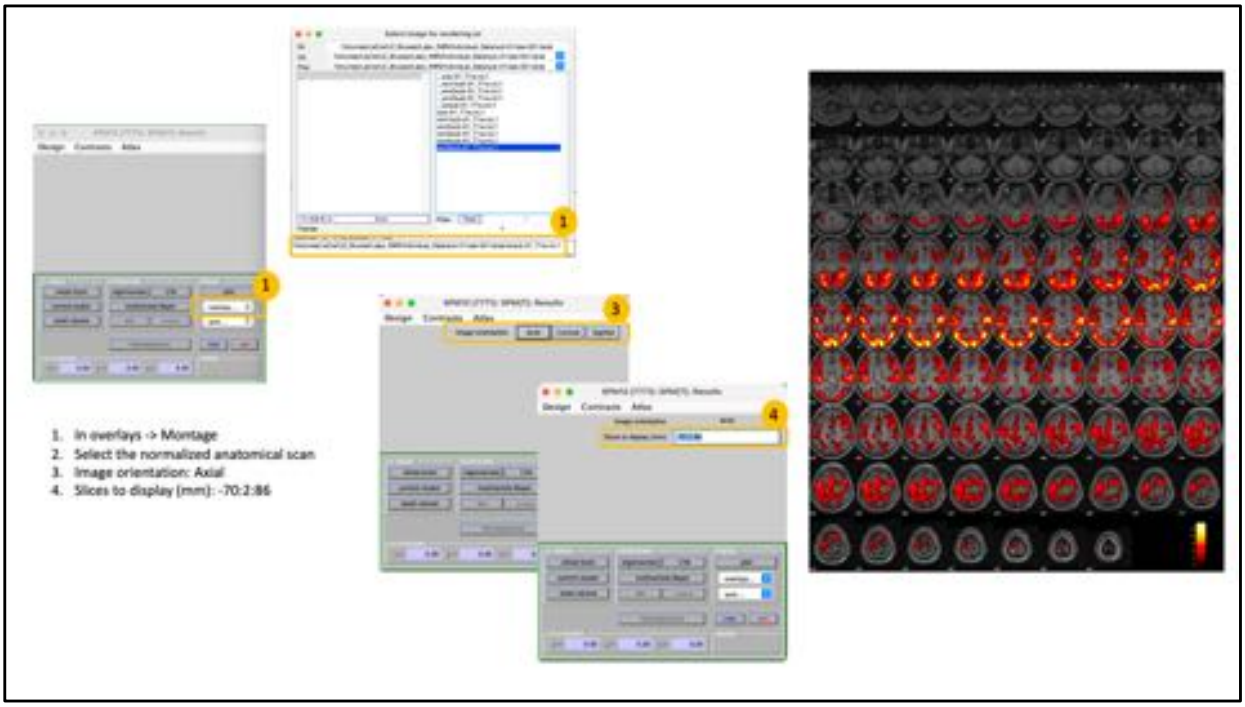
See also
http://mriquestions.com/activation-blobs.html

A look at the results

1. In the Menu window: click Results
2. In the contrast manager: select the contrast (e.g. 001 (T): Congruent>0)
3. Apply masking: select none
4. P value adjustment to control: select none
5. Threshold (T or p value): 0.001
6. & extent threshold (voxels): 0




Glass brain showing the clusters
(projection from the left, top and back)
Change contrast
and threshold
(significance level)
Select anatomical
underlay (in sections)
Save thresholded
maps as .nii
Position of the
selected voxel
(MNI-coordinates)
Results table
Cluster
significance
Cluster
size
Highest T-value
and significance
Position highest
T value in the
cluster
Overlaid on the
T1 anatomical
scan



1. In overlays -> Montage
2. Select the normalized anatomical scan
3. Image orientation: Axial
4. Slices to display (mm): -70:2:86

How do the results look like for the congruent contrasts?

To make the montage image:

- in the menu ‘overlays’ select ‘Montage’
- Select the T1 anatomical scan
- Image orientation: Axial
- Accept the default values for ‘Slices to display (mm)’

How do the results look like for the incongruent contrasts?

To make the montage image:

- in the menu ‘overlays’ select ‘Montage’
- Select the T1 anatomical scan
- Image orientation: Axial
- Accept the default values for ‘Slices to display (mm)’

How do the results look like for the congruent versus incongruent contrasts?

To make the montage image:

- in the menu ‘overlays’ select ‘Montage’
- Select the T1 anatomical scan
- Image orientation: Axial
- Accept the default values for ‘Slices to display (mm)’

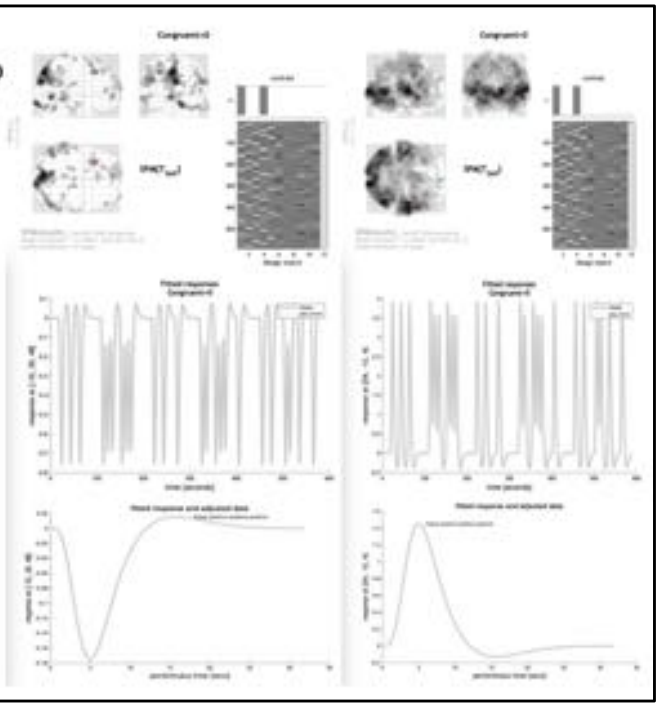
Negative contrast: deactivations?

Negative beta (e.g. $\beta_1 < 0$): $\hat{y}_j = \sum_{i=1}^{n} \beta_{ij}.x_j = \beta_1.x_j < 0$

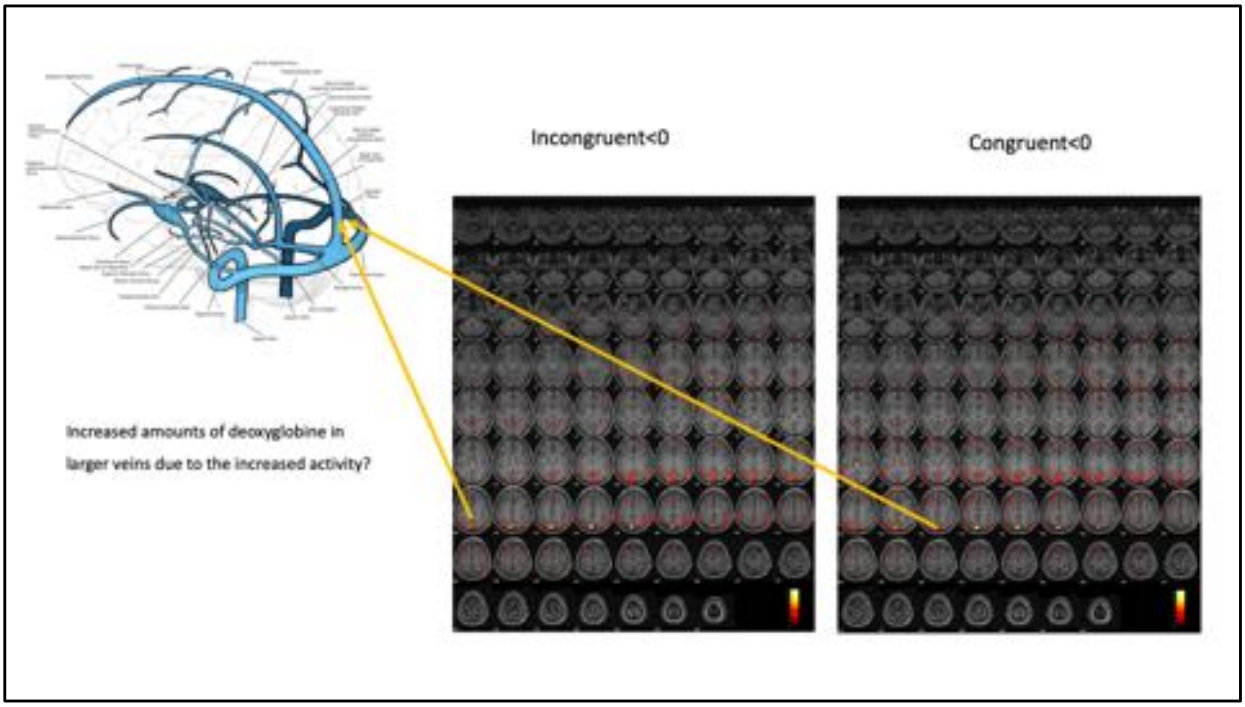
The inverse model fits the data

Possible interpretations:

- Could be an effect of the model (e.g. the noise regressors included) -> the results are model depended!
- Deactivations (negative BOLD response)
  - Inhibited neural activity compared to the default or rest state (ongoing neural activity)
  - Decreased CBF due to an increased CBF in a nearby area
  - Increased amounts of deoxyglobine due to nearby activity (e.g in larger veins)



Fitted model: $Y = \beta.X + \varepsilon$ => $\hat{Y} = \beta.X$ => $\hat{y}_j = \sum_{i=1}^{n} \beta_{ij}.x_j$ for n model regressors $j \in [1, T]$ (timepoint j)


Incongruent<0
Congruent<0
Increased amounts of deoxyglobine in
larger veins due to the increased activity?

Second level analysis

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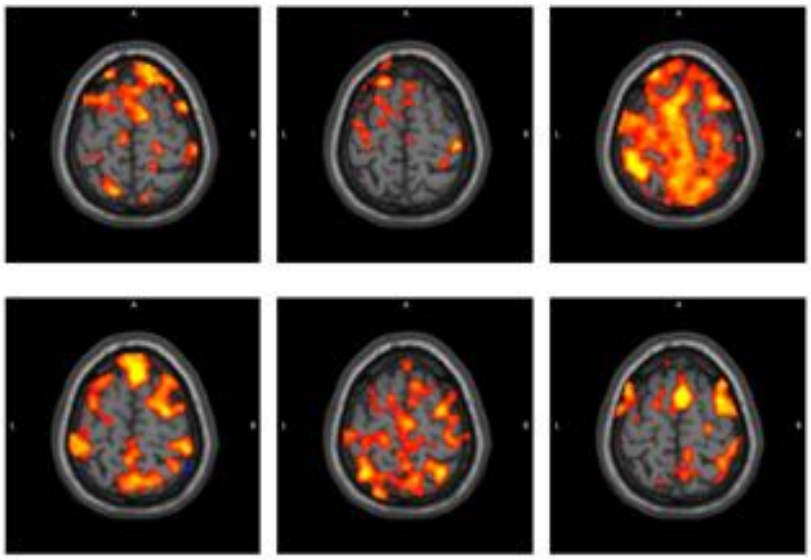
Individual results



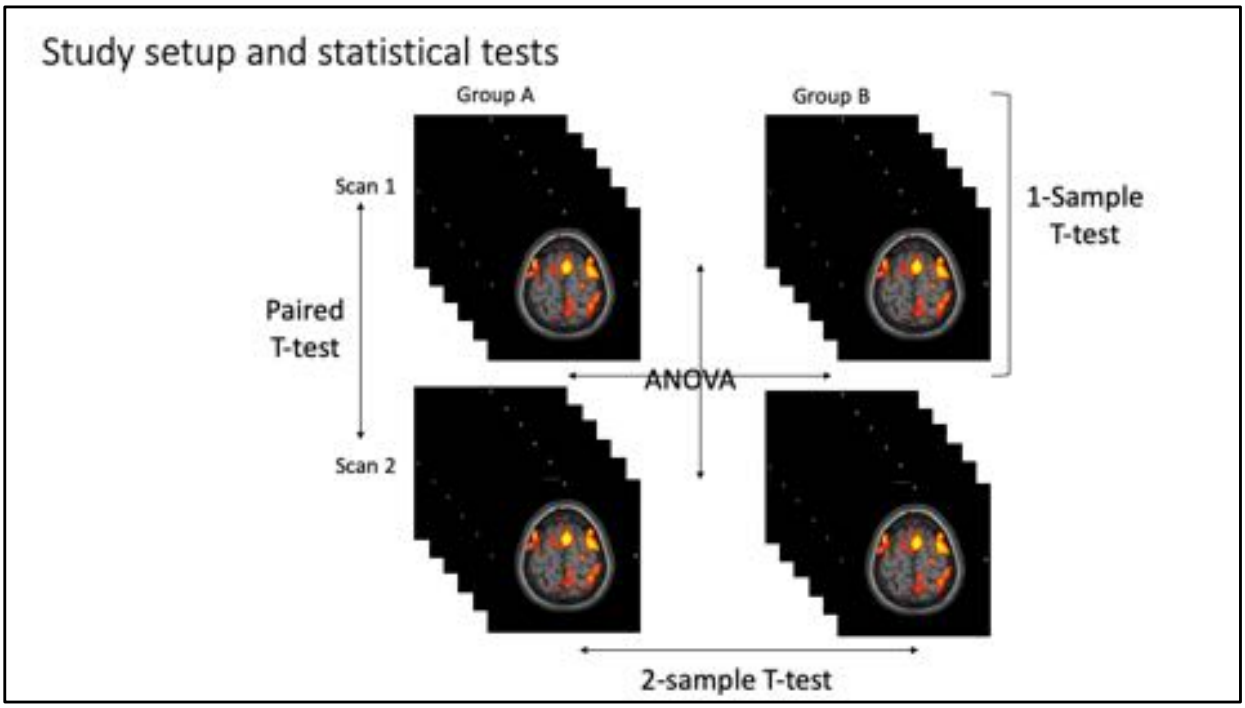
Variable
- Between subjects
- In time

Depending on
- Scanner
- Analysis settings
- Accidental activations
- Uncontrollable factors (fatigue, caffeine,…)

→ Low repeatability

- In a research studies, mostly more than 1 subject performed the fMRI experiment -> For each subject, an activation map is generated per contrast

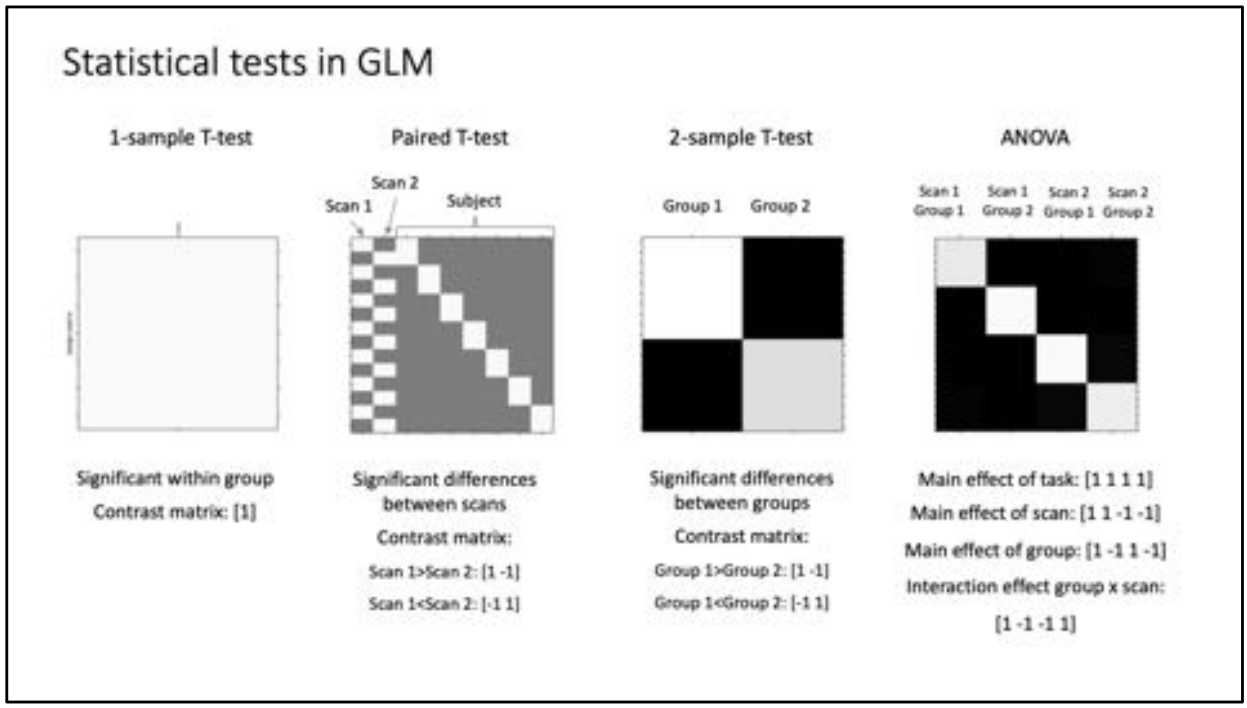
- High variability in the individual results
    - Small individual variations in the organization
    - Differences in task performance (attention, accuracy, …)
    - Secondary neural processes going on in the brain



- Kind of questions studied with fMRI
    - Where in the brain is a specific stimulus processed? -> wants to know what is the common activation in all subjects
        - Study design: 1 group of subjects, all performing the same test once
        - Statistical test: 1-sample T-test
    - What is the difference in neural activity between 2 scan moments within the same group? (e.g. at different ages)
        - Study design: 1 group of subjects, all performing the same task twice
        - Statistical test: Paired T-test
    - What is the difference in neural activity between 2 groups? (e.g. healthy subjects versus patients, boys versus girls, elderly versus younglings, …)
        - Study design: 2 different groups of subjects, all performing the same task once
        - Statistical test: 2-sample T-test
    - What is the effect of a specific treatment?
        - Study design:
            - 2 groups of subjects, each group receiving a different treatment (e.g. A real treatment versus a placebo, a new experimental treatment versus a reference treatment, …) and all subjects have performed the fMRI experiment before and after the treatment
            - 1 group of subjects once receiving one treatment and once receiving the other treatment and performing the fMRI experiment before and after each treatment
        - Statistical test: ANOVA
            - Main effects: comparison of the means along 1 dimension (e.g. Group A versus group B, scan 1 versus scan 2)
            - Interaction effect: comparison of the means along 2

dimensions (e.g. Difference in scan 1 and scan 2 for group A versus group B) -> cross effects

- Within subject variables : repeated scans from the same subject group
- Between subject variables: scans from different subject groups



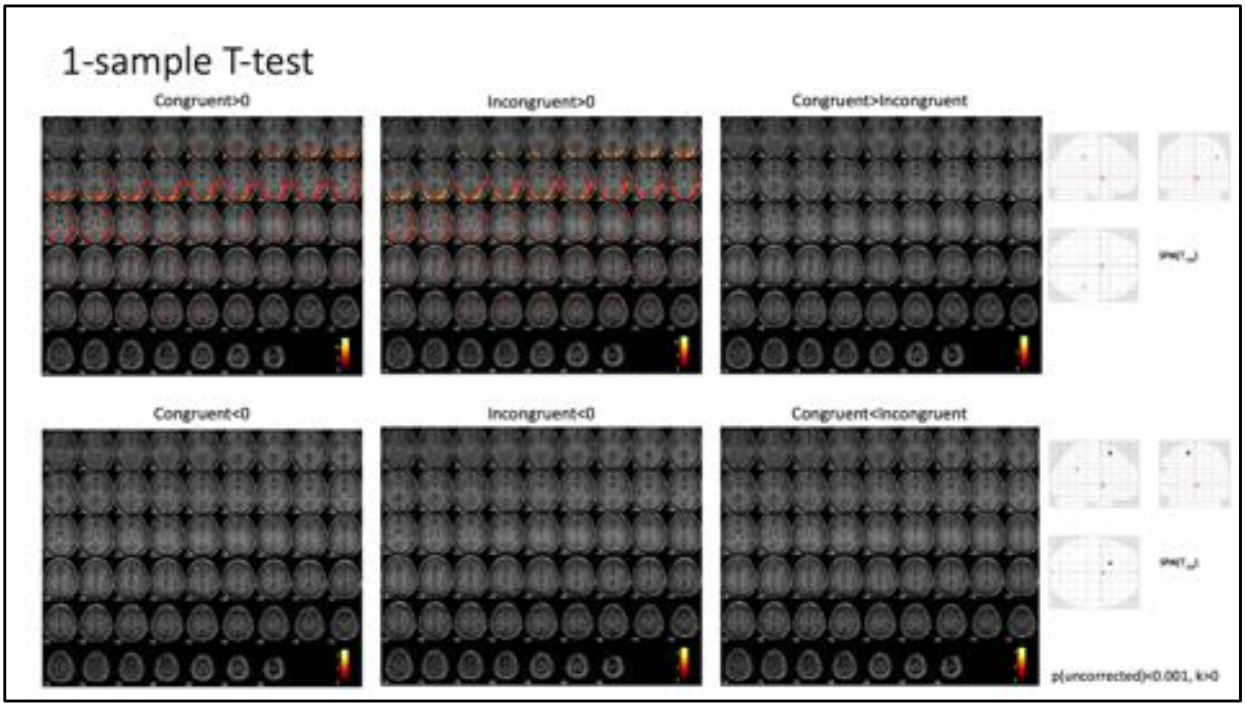
Comparative statistical tests are factorial designs that can be estimated using the GLM

The input data for the second level analyses are the contrast maps from the individual analyses.
The design matrixes represent

- 1 column per group (for paired T-tests, subject is added as covariate because the data from group 1 is not independent from the data of group 2)
- 1 if contrast map is from group and 0 is not (no convolution with HRF)
- Additional covariates (age, …) can be included in the design matrix

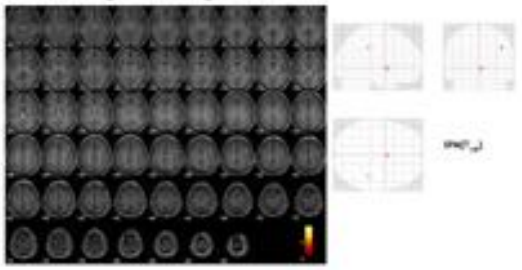
Statistical T- and F-tests are performed as for the individual analyses.

Contrast matrices are used to test for group differences

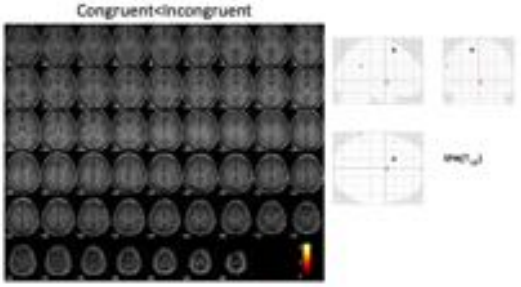

1-sample T-test
Congruent>0
Incongruent>0
Congruent>Incongruent
Congruent<0
Incongruent<0
Congruent<Incongruent
p(uncorrected)<0.001, k>0

Paired T-test: Congruent (con_0001) versus Incongruent (con_0003)

Congruent>Incongruent

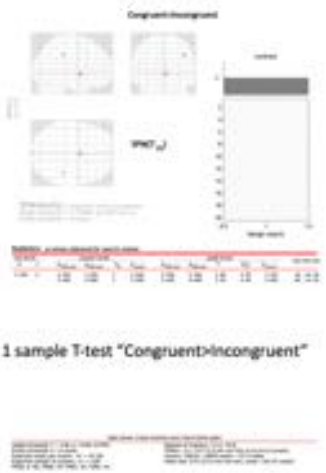


Congruent<Incongruent

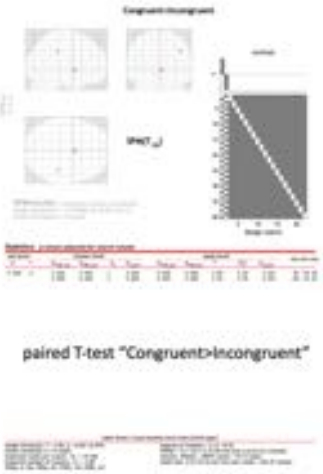


p(uncorrected)<0.001, k>0

Why are the result for the 1 sample T-test for the contrast "Congruent>Incongruent" the same as the results of the paired T-test "Congruent>Incongruent"



1 sample T-test "Congruent>Incongruent"



paired T-test "Congruent>Incongruent"

Statistical Thresholds

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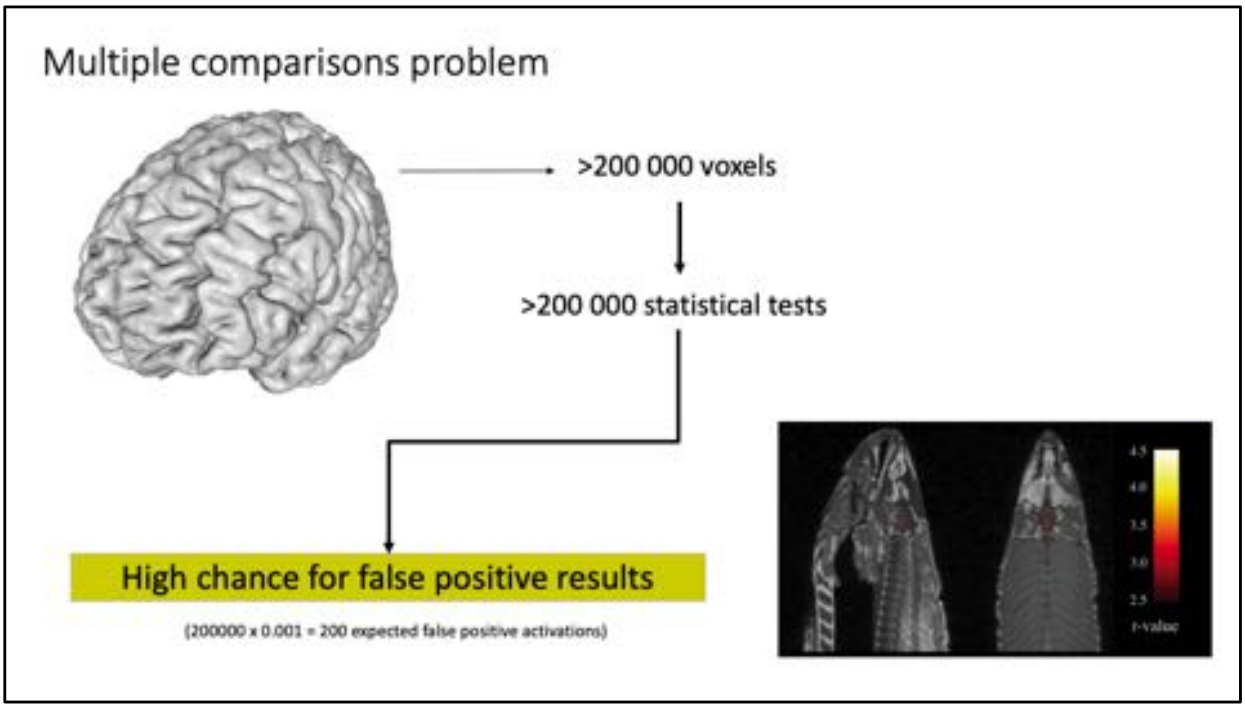


- The normalized brain contains more than 200 000 voxels
- In each voxel, a statistical test is performed -> >200 000 statistical tests performed

=> High chance that some tests give a significant result by accident -> false positive results (Type I errors)

- We need to correct for multiple corrections to reduce this chance for type I errors
- This problem was nicely illustrated in a study revealing brain activity in a dead salmon (http://prefrontal.org/files/posters/Bennett-Salmon-2009.pdf)

See also
Advanced discussion and papers cited at http://mriquestions.com/activation-blobs.html

Family-Wise Error (FWE) correction

Family-wise null-hypothesis: no activation in any voxel

Family-wise error: a false positive activation somewhere in the image

Family-wise error rate (α): p-value corrected for the chance to get a false positive result in at least 1 voxel

Adjusting the rejecting threshold for multiple comparisons: $p = \frac{\alpha}{N}$

If α=0.05 and N=200 000 the corrected p=$2.5.10^{-7}$

↓

TO CONSERVATIVE

- The Family-wise error (FWE) correction -> comparable to the Bonferroni correction
    - Corrects the significance threshold to reject the null hypothesis
    - For a predefined significance threshold (α) and N voxels
    - For a very large N (as in fMRI: N>200 000) the corrected p-value becomes extremely small -> almost all tests will be rejected

    => the FWE-correction is to conservative -> inflated chance for false negative results (type II errors)

See also
http://www.brainvoyager.com/bvqx/doc/UsersGuide/StatisticalAnalysis/TheMultipleComparisonsProblem.html
Lieberman and Cunningham 2009. Type I and type II error concerns in fMRI research: re-balancing the scale. Soc. Cogn. Affect. Neurosci. 4(4):423-428
https://en.wikipedia.org/wiki/Family-wise_error_rate

Random field theory

FWE correction assumes N independent voxels <- not true, contrast maps are smooth

↓

Spatial correlation between neighboring voxels

↓

Number of independent observations n_i<N

↓

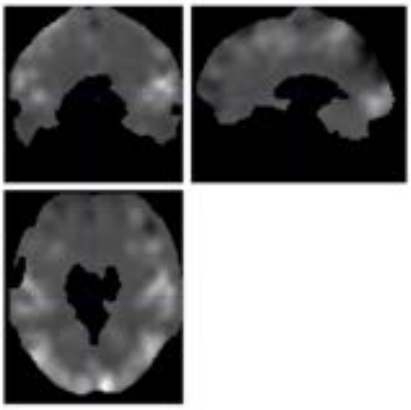
FWE corrected $p = \frac{\alpha}{n_i}$

↓

But ... n_i = ?

Resels (resolution element) = number of independent observations in the image

$$R = \frac{Image\ Volume}{FWHM_x . FWHM_y . FWHM_z}$$



The smoothness in an image is not equal to the applied smoothing kernel, but is also defined by smoothing effects from the realignment and normalization steps.

See also: https://www.fil.ion.ucl.ac.uk/spm/doc/books/hbf2/pdfs/Ch14.pdf

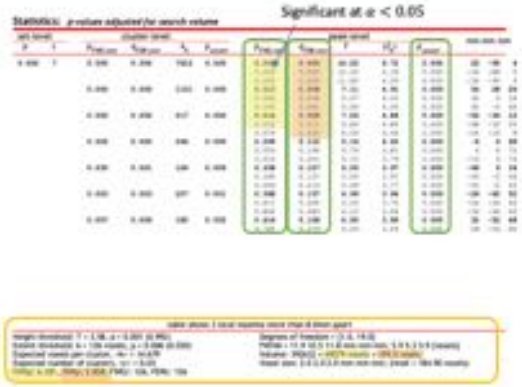
Random field theory

FWE correction: $\alpha = E[EC]$

E[EC] = expected EC = chance to have at least 1 significant activation voxels above threshold

(null hypothesis: no significant activation voxel above the threshold)

Significant at $\alpha < 0.05$

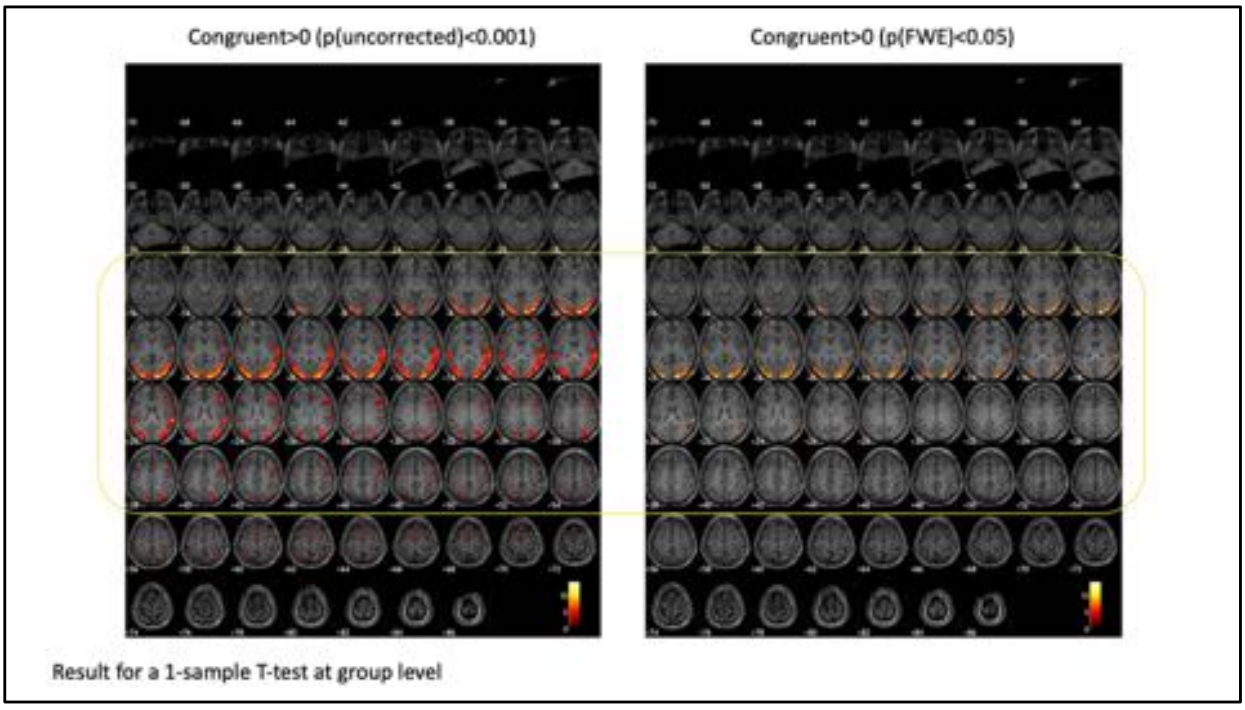


Euler Characteristic (EC) = number of voxel found after thresholding

Result for a 1-sample T-test at group level

In 3D, the formula to calculate E[EC] is complex, but still only depends on R and the threshold Z_T

See also: https://www.fil.ion.ucl.ac.uk/spm/doc/books/hbf2/pdfs/Ch14.pdf



Result for a 1-sample T-test at group level

In our single subject analysis, for the results of congruent>0 with the threshold set at p(FWE)<0.05

The number of resulting clusters is =

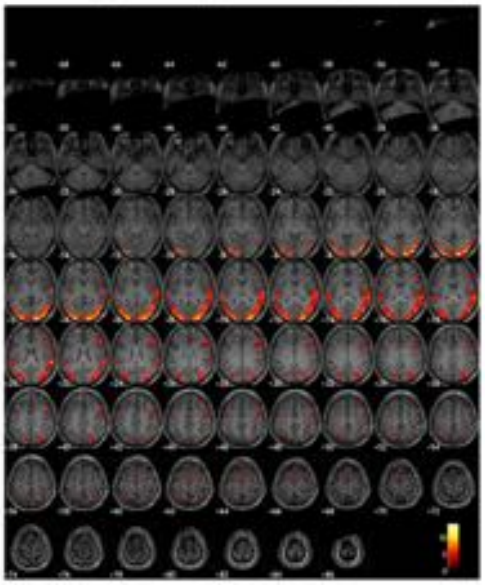
The smallest cluster size =

The largest cluster size =

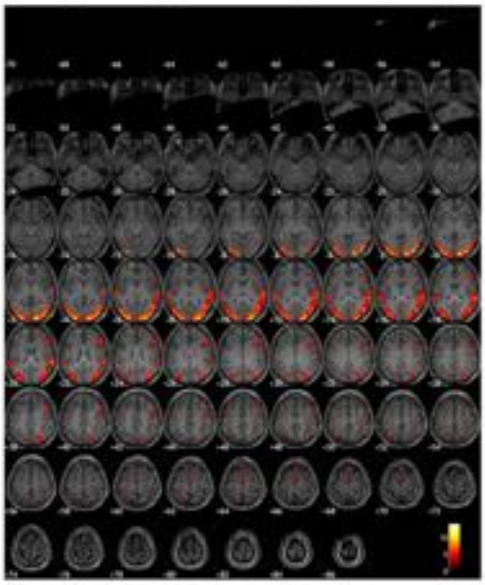
Significance threshold at cluster level

Result for a 1-sample T-test at group level

Congruent>0 (p(uncorrected)<0.00, k=0)



Congruent>0 (p(uncorrected)<0.001, k>126)



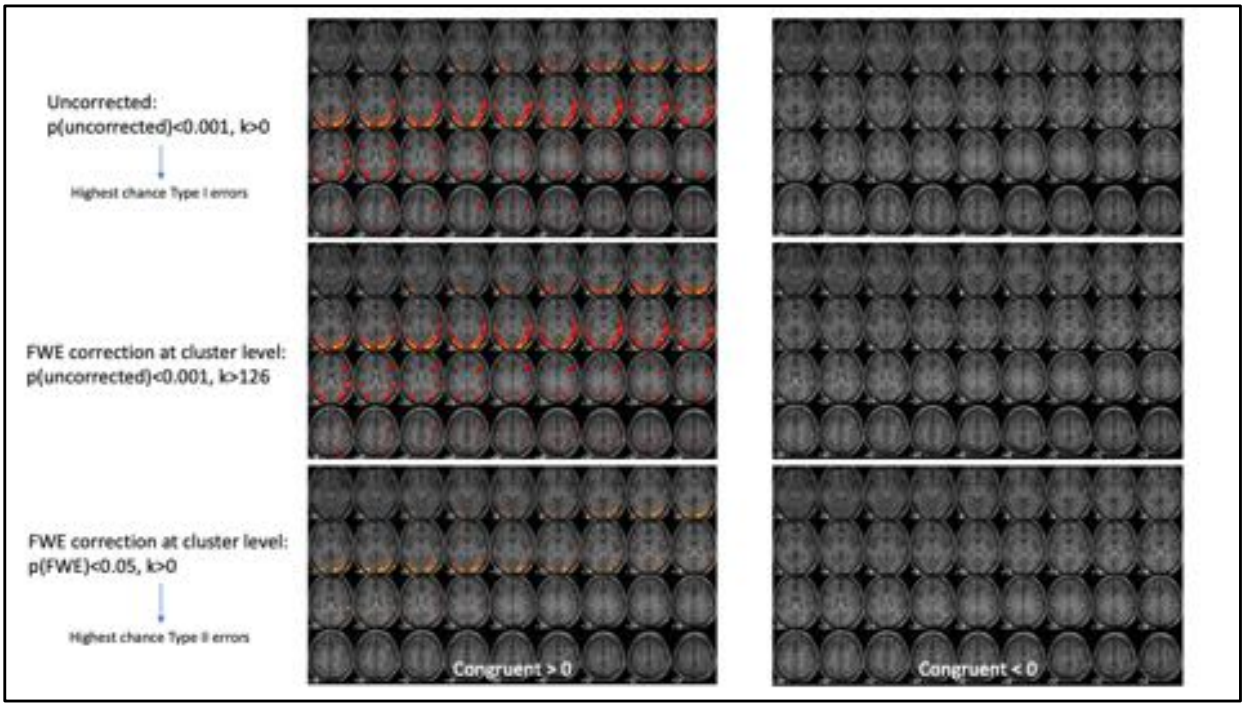
Result for a 1-sample T-test at group level

In our single subject analysis, for the results of congruent>0 with the voxel significance threshold set at p(uncorected)<0.001 and the cluster significance set at p(FWE)<0.05

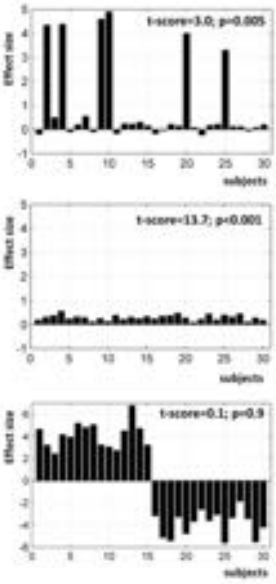
The minimum cluster size is =

The number of resulting clusters is =

The largest cluster size =


Uncorrected:
p(uncorrected)<0.001, k>0
Highest chance Type I errors
FWE correction at cluster level:
p(uncorrected)<0.001, k>126
FWE correction at cluster level:
p(FWE)<0.05, k>0
Highest chance Type II errors
Congruent > 0
Congruent < 0

Remarque: Significant ≠ Large or important



Significant:
$mean(effect) \neq 0$

Significant effect

But … only a large effect in a few subjects

Significant effect

But … it's a small effect in all subjects

No significant effect

But … a large positive effect in one halve of the subjects

and a large negative effect in the other halve

Seghier M.L., Price C., 2016. Visualising inter-subject variability in fMRI using threshold-weighted overlap maps. Sci Rep 6: 20170

Anatomical labeling

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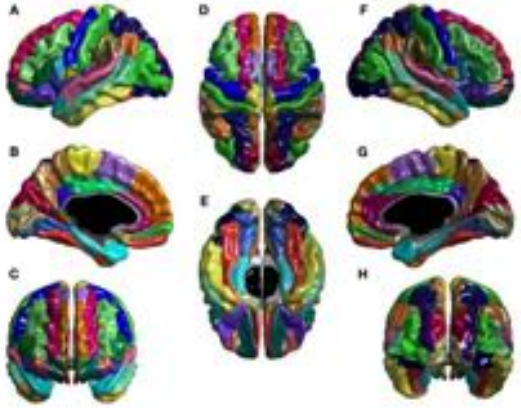
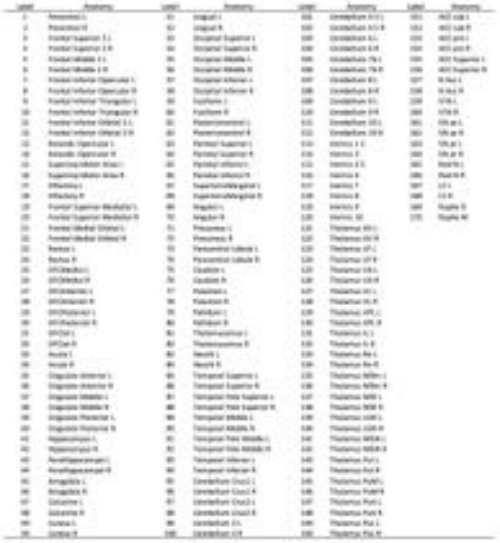
VUB

AAL: Automated Anatomical Labeling atlas

170 anatomical brain areas

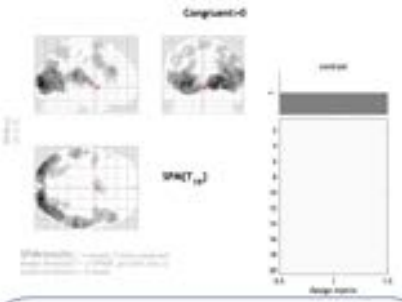
Locations defined in MNI template space





AAL: Automated Anatomical Labeling atlas

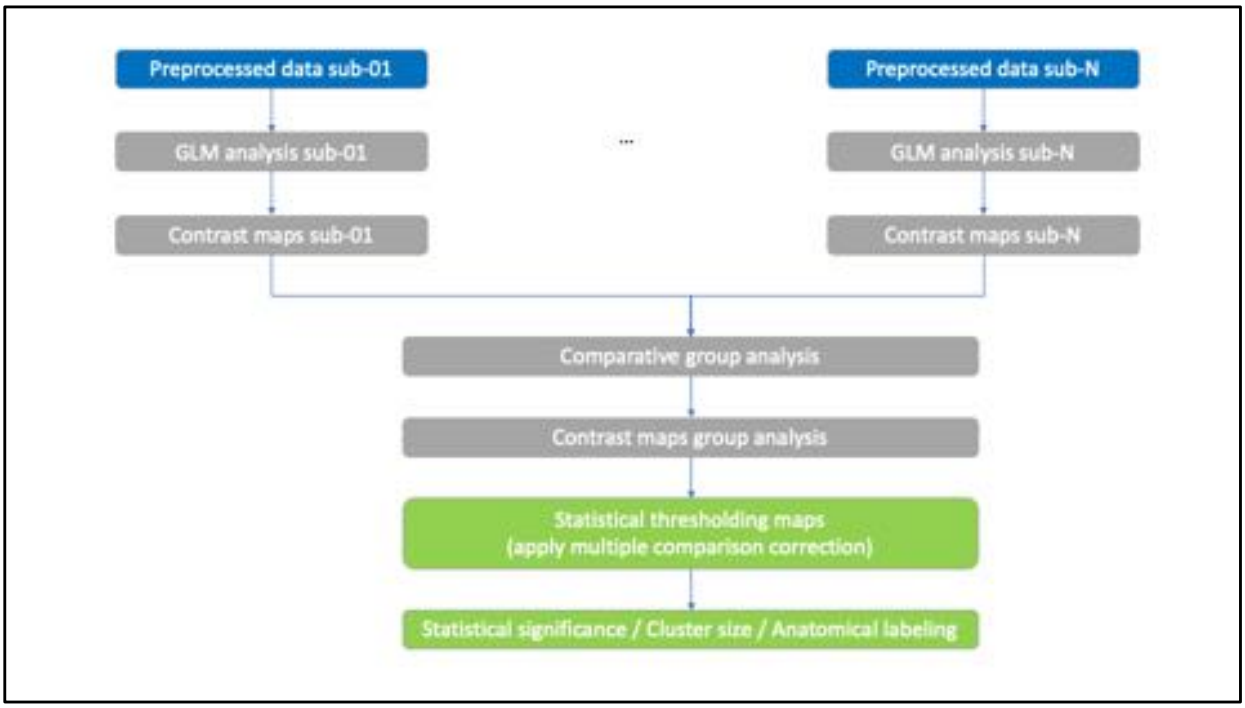
1. Open the results first
2. In the Menus Window: Toolbox: -> AAL3
3. In the AAL window -> Local Maxima Labeling
4. Select an labeling atlas: ROI_MNI_V7.nii file



Result for a 1-sample T-test at group level

In our single subject analysis, for the results of congruent>0 with the voxel significance threshold set at p(uncorected)<0.001.
What is the AAL label for the cluster at

1. [12, -98, 6]:
2. [-30, -6, -40]:
3. [20, -10, -18]:
4. [6, 62, 30]:


Preprocessed data sub-01
Preprocessed data sub-N
...
GLM analysis sub-01
GLM analysis sub-N
Contrast maps sub-01
Contrast maps sub-N
Comparative group analysis
Contrast maps group analysis
Statistical thresholding maps
(apply multiple comparison correction)
Statistical significance / Cluster size / Anatomical labeling

Resting state fMRI

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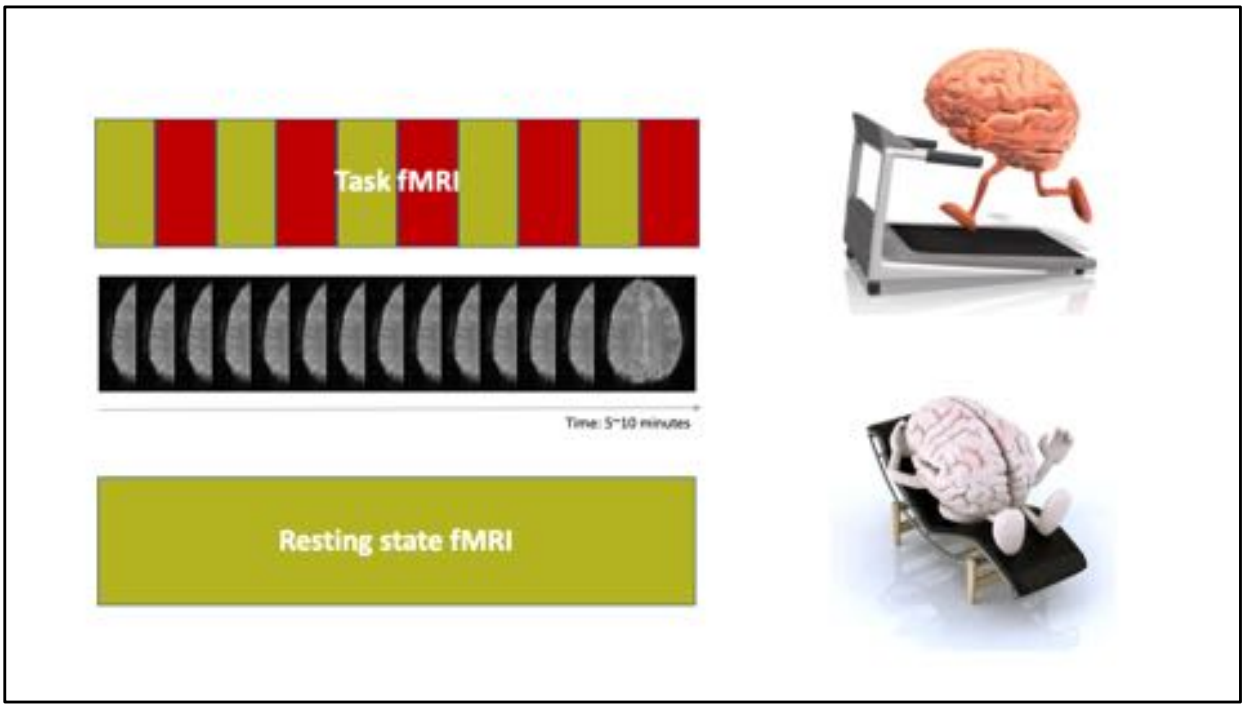


Task fMRI: the participants have to do a task during the fMRI scan -> the brain is in an active state

Resting state fMRI: the participants have to relax, think of nothing and keep their eyes closed during the fMRI scan -> the brain is in a resting state

Table 1.

Presents a brief comparison between task based fMRI and Resting state fMRI.

Sl no.	Task-based fMRI	Rs-fMRI
I	Analyses of the spontaneous modulations in the BOLD signal in the presence of a particular activity (e.g. finger-tapping, eye-blinking, naming, memorizing, etc.)	Analyses of the spontaneous BOLD signal in the absence of any explicit task or an input
II	Task-related increase in neuronal metabolism are less than 5%	60–80% of brain's energy is consumed during resting state
III	During task-based activity the focus is only on a very small fraction of the brain's overall activity	In terms of overall brain function, the resting state brain activity is far more significant than task-related activity
IV	The signal during a task-related activity is very small compared to the noise, i.e. 80% of the BOLD modulation is discarded as noise	The signals which are discarded as noise in task fMRI is taken as signals in rs-fMRI as they are the low frequency spontaneous fluctuations in the BOLD signal
V	Due to discarding of signal as noise, task fMRI has a low SNR	Have improved SNR since it takes the overall spontaneous low frequency fluctuations
VI	For the interpretation of results, a large number of trials are required in task fMRI	No need of more trials like task fMRI
VII	If one wants to analyse the motor function and language function, a separate task may be required to analyse each function in task-based fMRI	In rs-fMRI, the acquired may be used to analyse one or more functions
IX	Patient cooperation is essential to do task fMRI	Paediatric patients, patients with low IQ and even patients in the vegetative and coma state are able to do rs-fMRI
X	Repeated sessions of task-based activity to assess the disease prognosis, treatment effect etc. will result in familiarity with the task which will affect the output adversely	In rs-fMRI even we are taking different sessions, due to the absence of task, we are able to avoid the task-related confusions and uncertainties faced by task fMRI

fMRI: functional magnetic resonance imaging; rs-fMRI: resting state functional magnetic resonance imaging; BOLD: blood oxygenation level-dependent; SNR: signal to noise ratio.

From Smith et al. Resting state fMRI: A review on methods in resting state connectivity analysis and resting state networks. Neuroradiol J. 2017 30(4): 305–317

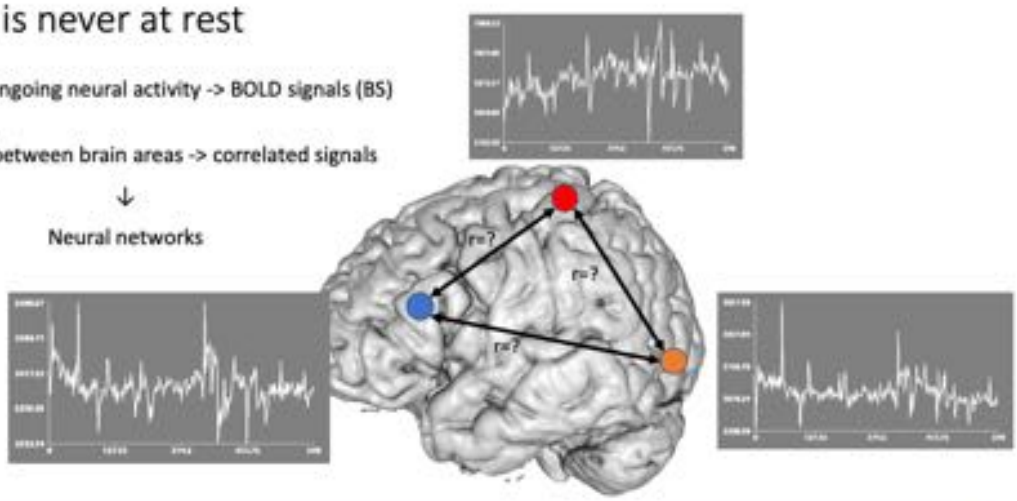
The brain is never at rest

Spontaneous ongoing neural activity -> BOLD signals (BS)

Interactions between brain areas -> correlated signals

↓

Neural networks



Resting-state functional connectivity measures **temporal correlation** of spontaneous BOLD signal among spatially distributed brain regions, with the assumption that regions with correlated activity form functional networks.

Why didn't we look at connectivity (correlations between the neural activity in different brain areas) in our task-fMRI experiment?

1. There is no connectivity between brain areas during processing a task
2. The neural activity in all brain areas correlate with the task
3. The correlations between the neural activity and the task lead to correlations between brain areas
4. All areas involved in processing the task are connected to each other

Resting state fMRI paradigm

GE-EPI at temporal resolution of 2s

Instructions:

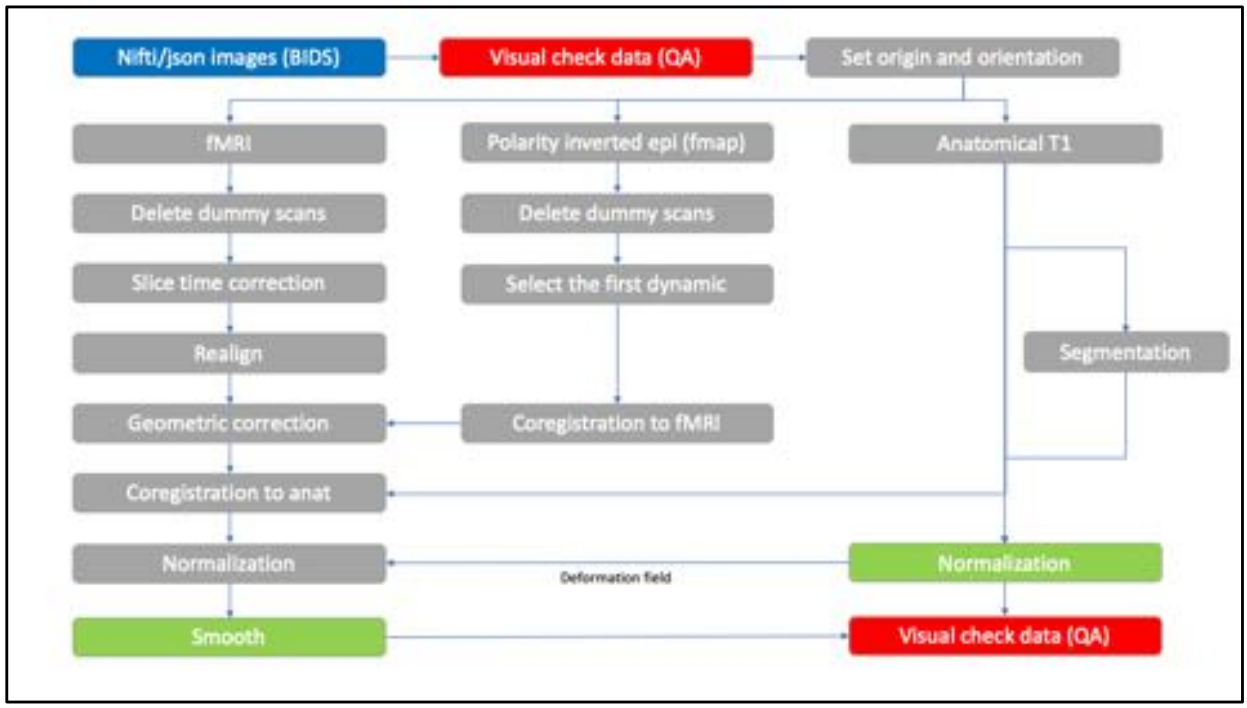
- Relax but don't fall asleep
- Try not to move
- Try not to think of anything
- Keep eyes open

20 healthy subjects

- Resting state fMRI (RS-fMRI) studies the brain at rest -> no fMRI task involved
    - The brain is never truly at rest
    - All kind of neural processes are continuously going on -> default activation -> variations in BOLD signal
- Rest state ≠ sleep
    - Rest:
        - Eyes closed
        - Not doing anything nor thinking at anything
        - Awake
    - Sleep: contains various sleep states
- Scan:
    - Standard fMRI SE-EPI or GE-EPI sequence
    - Duration of the RS-fMRI scan: minimal 5 minutes and maximal 10 minutes
    - Temporal resolution: 2s

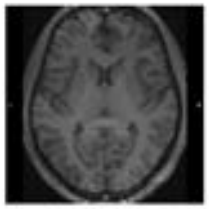
See also

Van den Heuvel and Hulshoff Pol 2010. Exploring the brain network: A review on resting-state fMRI functional connectivity. Europ. Neuropsychopharm. 20:519-534
Margulies et al. 2010. Resting developments: a review of fMRI post-processing methodologies for spontaneous rain activity. Magn. Reson. Mater Phy. 23:289-307
Cole et al. 2010. Advances and pifalls in the analysis and interpretation of resting-state fMRI data. Front. Syst. Neurosci. https://doi.org/10.3389/fnsys.2010.00008
https://en.wikipedia.org/wiki/Resting_state_fMRI
http://mriquestions.com/resting-state-fmri.html



Deformation field: contains the information for the non-linear transformation from subjects space to normalized (MNI) space

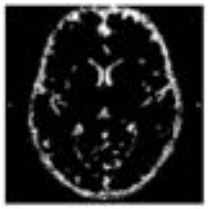
The preprocessing results



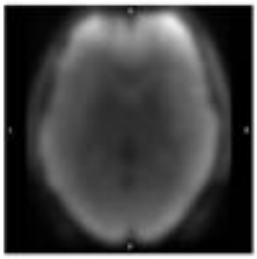
wosub-01_T1w

wc1osub-01_T1w

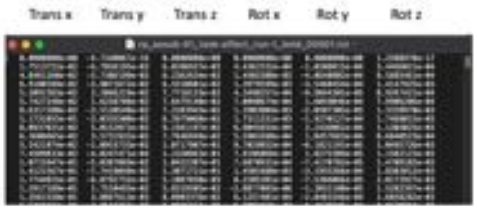
wc2osub-01_T1w



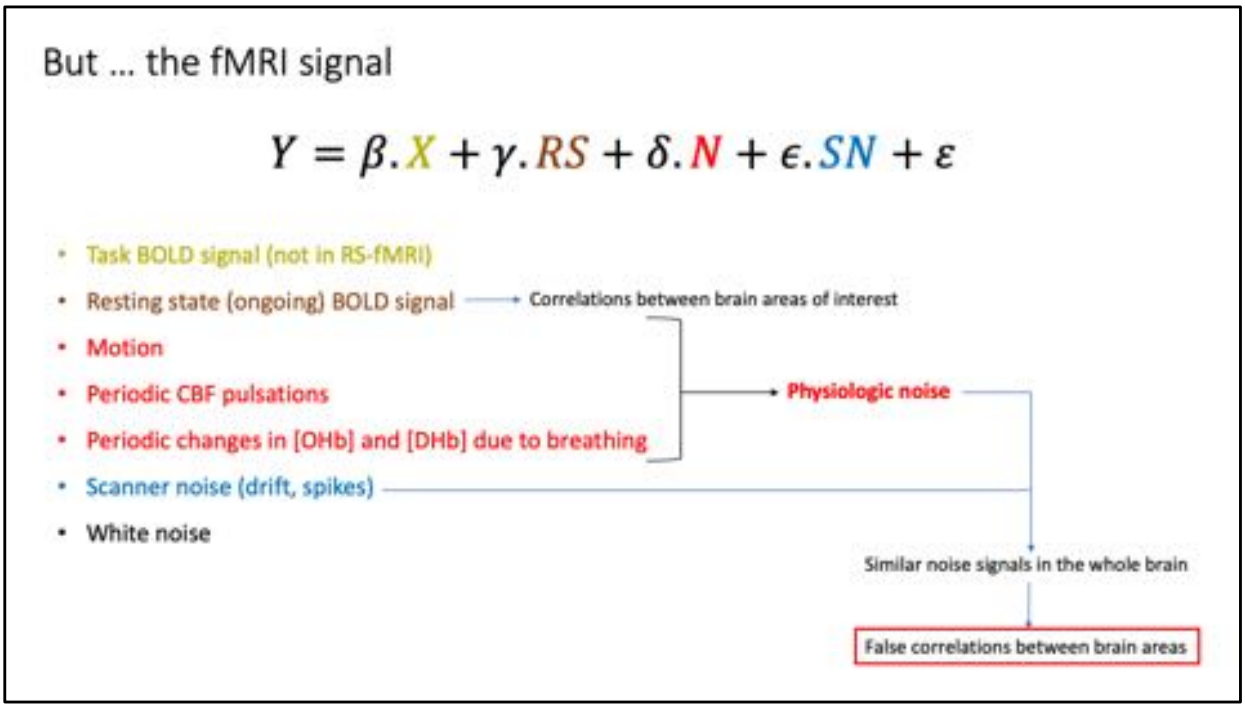
wc3osub-01_T1w



swuraosub-01_..._bold



Realignment parameters



- By the GLM we try to explain the variations in the temporal signals based on the performed task timings convolved with the HRF. All other signal variations are considered as noise.
- But … there are many other sources of signal variations present in the fMRI signal that are not the BOLD effect of the task nor white noise
    - Motion (head movements, eye movements, blinking)
    - Periodic CBF pulsations -> from the beating heart
    - Periodic changes in the oxyhemoglobin and deoxyhemoglobin concentrations due to breathing
    - Scanner drift
        - Due to the fast gradient switching in EPI the scanner hardware heats
        - Slow, continue, change in the baseline signal
    - Other ongoing neural activity
    - …
- The BOLD signal of interest disappears in the noise
    - Leads to false positive results (type I errors)
    - Reduces the power -> increased the chance for false negative results (type II errors)

    ⇒ **prior to and during the analysis we will try to reduce the noise in the data = denoising**

- A first reduction is done during the preprocessing steps by realigning and smoothing the data (spatial noise) to reduce the noise

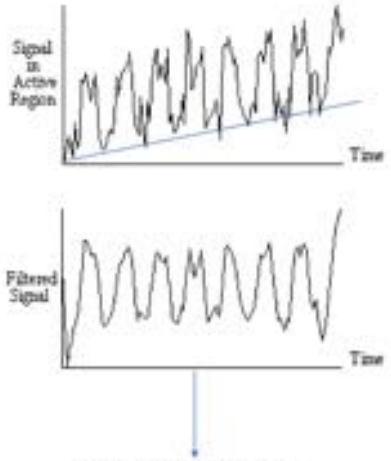
See also
Cabalero-Gaudes and Reynolds 2017. Methods for cleaning the BOLD fMRI signal. NeuroImage 154:128-149

Denoising

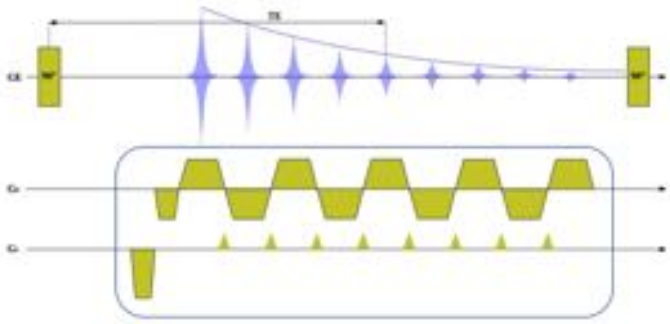
Universitair Ziekenhuis Brussel

VUB

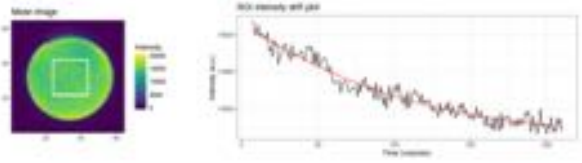
fMRI signal drift

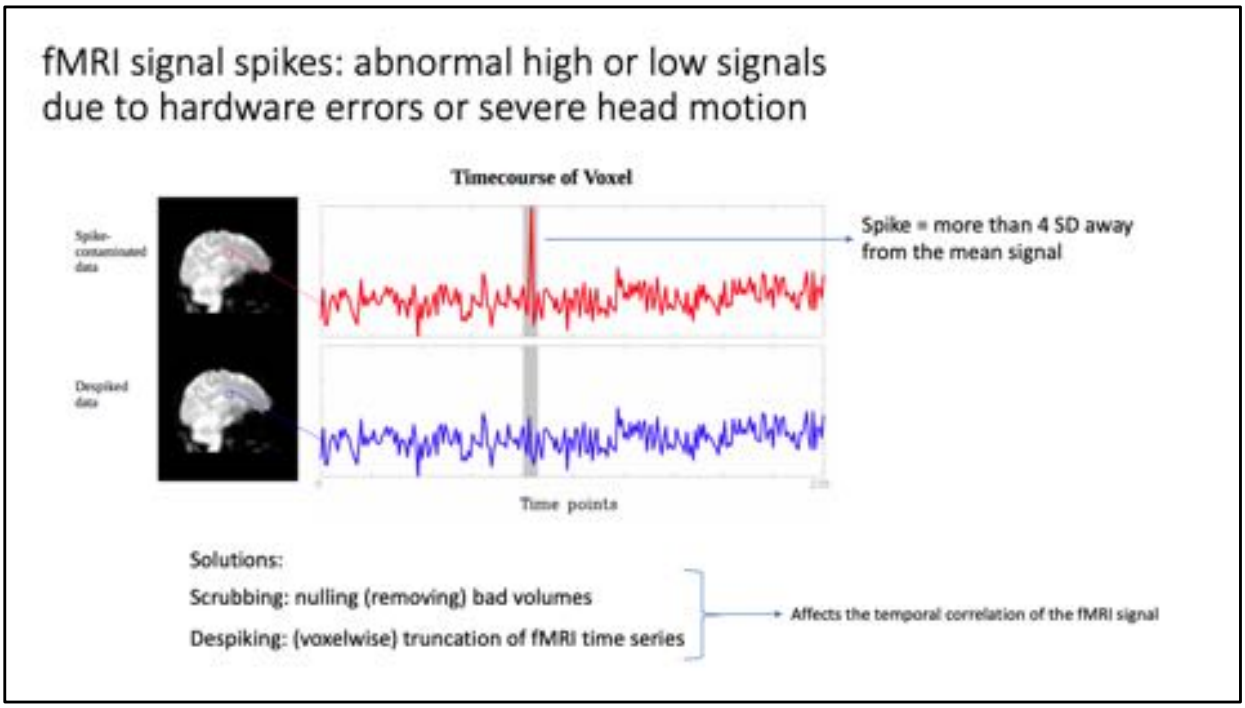


Subtracting a trendline
or by doing a high pass filter (done in SPM)



Repeated fast gradient switching -> heating of the gradient coil



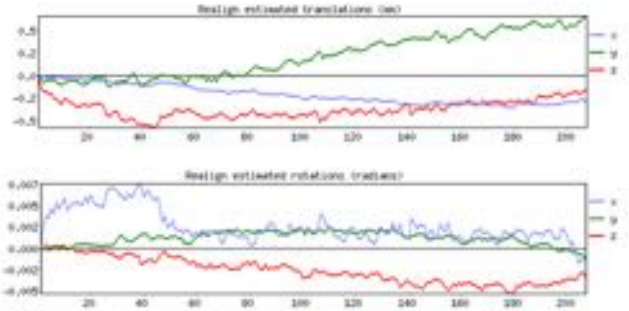


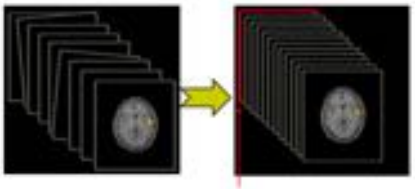
- Outliers can occur in the data due to severe head movements
- Each time volume is compared to the mean to detect outliers (outlier test)
- The found “bad” volumes are
    - corrected by interpolation from neighboring volumes (censoring the time series)
    - replaced by zero images (scrubbing the time series)

See also

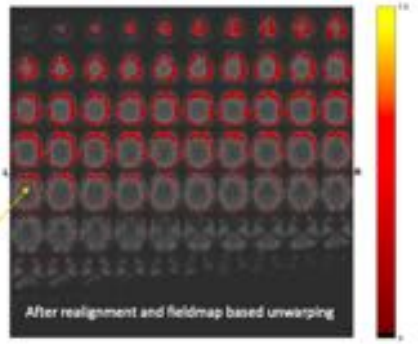
http://cibsr.stanford.edu/tools/human-brain-project/artrepair-software.html

Head motion



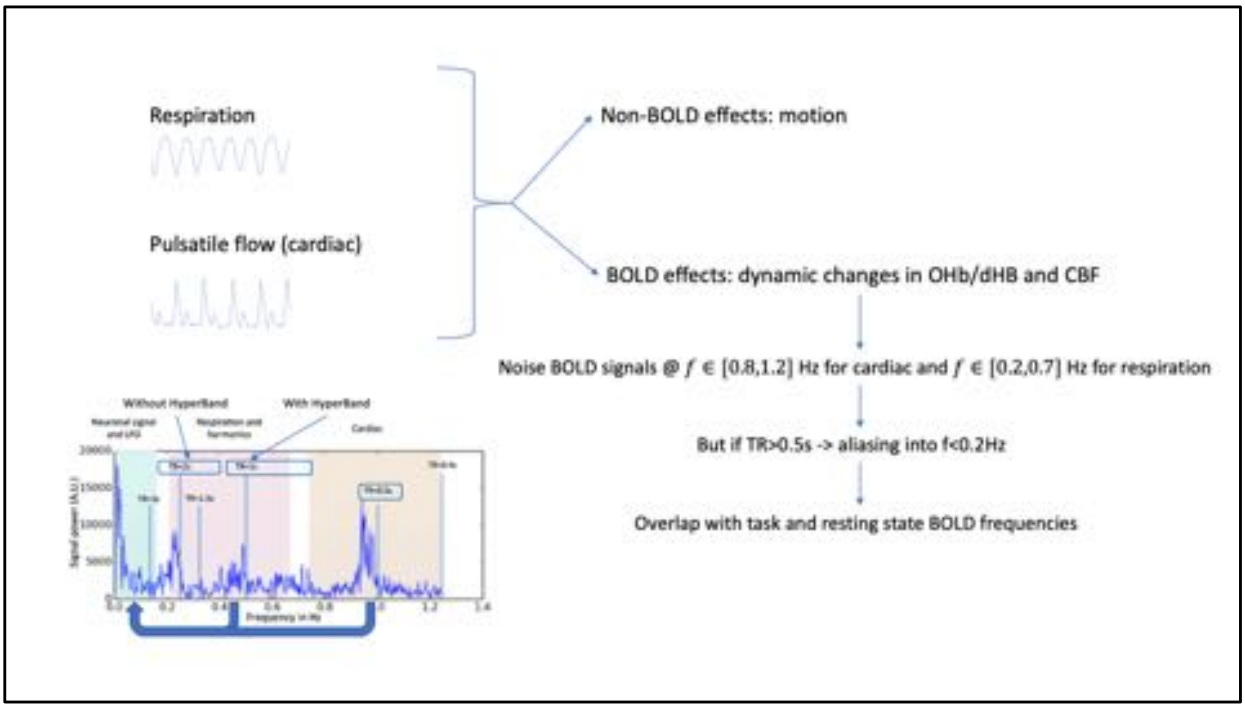


realignment



Motion effects on the signal:

- The signal in a voxel is coming from a collection of neighboring voxels (most visible at brain edges)
- Since the susceptibility artifact at the sinuses depends on the position and orientation of the head in the magnetic field B0, motion affect the susceptibility artifact
- Spins that move from one slice to the other will get excited at irregular intervals -> effects on their signal saturation

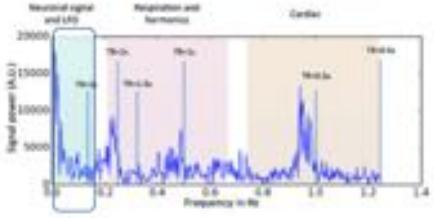


Power spectrum (left) and time domain data (right) presented in different spectral bands, from a voxel in a resting state data (TR = 0.4 s) of one participant. Three distinct spectral ranges corresponding to different physiological processes were marked. The spectral area captured by various TR values is also depicted on the power spectrum.

Tong et al. **Low Frequency Systemic Hemodynamic "Noise" in Resting State BOLD fMRI: Characteristics, Causes, Implications, Mitigation Strategies, and Applications**
2019

Denoising

Bandpass filtering



High-pass: f>0.01 (drift)

Low-pass: f<0.1 (respiratory and cardiac noise)

Noise regression

Prior to the analysis

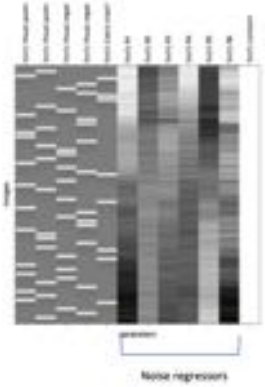
$Y_c = Y - \gamma.N$

Resting state analysis

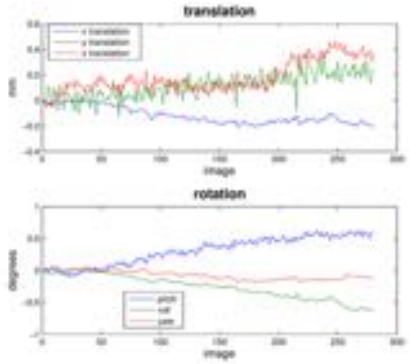
Which noise regressors?

GLM regressors

$Y = \beta.X$



Motion regressors



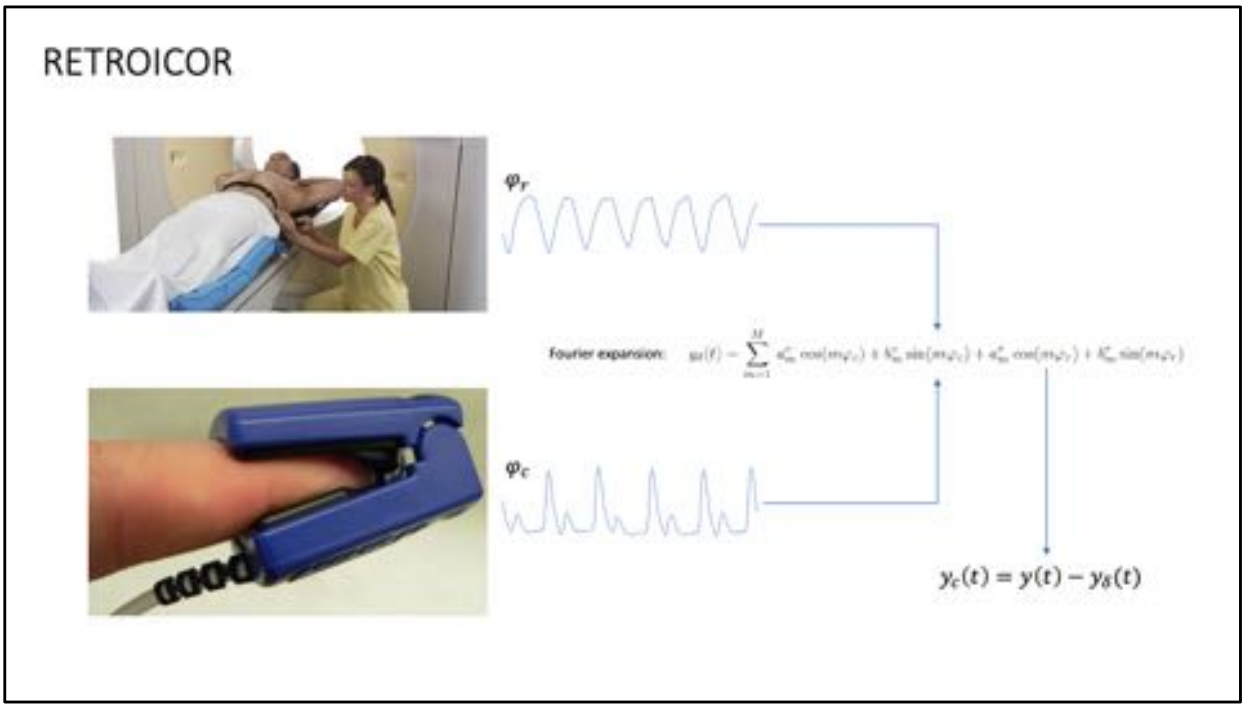
Realignment parameters → 6 regressors

+ temporal derivatives → 12 regressors

+ squared regressors → 24 regressors

To model higher order motion effects

Caution: the number of regressors affects the degrees of freedom of the statistical analysis!

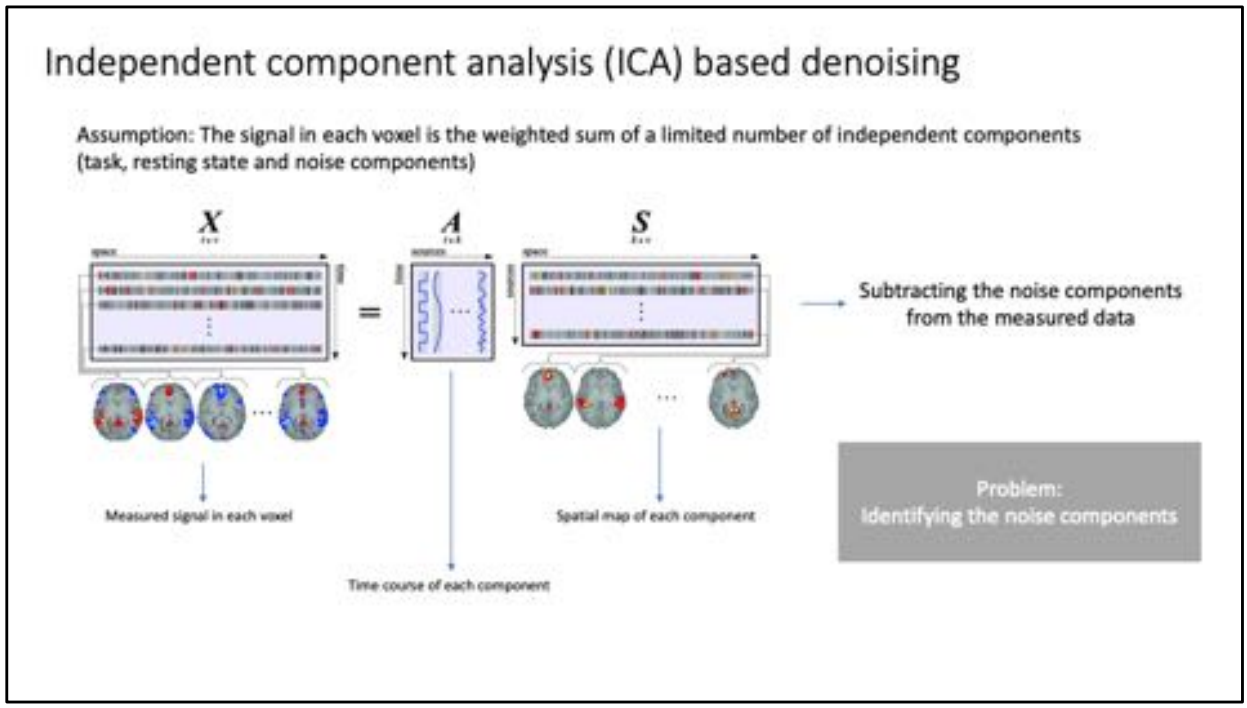


the physiological noise component $y_\delta(t)$ can be expressed as a low-order Fourier series expanded in terms of these phases:(1)
where the superscript on coefficients *a* and *b* refers to cardiac or respiratory function, and $\varphi_c(t)$ and $\varphi_r(t)$ are the phases in the respective cardiac and respiratory cycles at time *t*.

Glover et al. **Image-based method for retrospective correction of physiological motion effects in fMRI: RETROICOR. 44(1) 2000 (https://doi.org/10.1002/1522-2594(200007)44:1<162::AID-MRM23>3.0.CO;2-E)**

In our task fMRI experiment, we only added the 6 motion regressors as noise regressors because

1. We can not determine the physiologic noise signals in task fMRI
2. Physiologic noise does not correlate with the task
3. Adding more noise regressors to the design matrix can reduce the study outcome
4. The effect of adding more noise regressors to the design matrix is negligible



- The variation in the measured data Y can be explained by
    - The neural activity induced by the task conditions as modeled in X
    - The confounding signals as modeled in C

- 2 step approach
    - Determining the confounding signals
        - Based on principle component (PCA) or independent component analyses (ICA)
            - Determining the components
            - Arranging the components in good (neural activity) and bad (noise, artifacts, confounding signals) components
    - Subtracting the confounding signals from the measured data Y prior to the final analysis

- The measured signals in the brain can be separated in a limited number of spatially or temporally independent components
- Good components (BOLD signals) are discriminated from bad components (physiological noise, scanner noise, artifacts) based on their spatial location (eyes, CSF, macro-vasculature, non-brain areas) and frequency characteristics of the component signal
    - Manually -> time consuming and difficult to repeated (rater bias)
    - Machine learning -> depends on the learning dataset + for each change in the sequence parameters, a new learning dataset should be made

- Drawbacks:
    - Unknown number of independent number of independent components
        - Set by the operator
        - The components found depend on the number of components chosen
    - Often components with BOLD response and noise can be mixed -> throwing away

such mixed components = throwing away good activation results

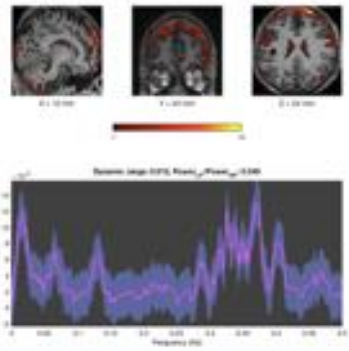
- ICA based denoising can be done in
    - GIFT (Matlab)
    - SPMdenoise (SPM toolbox)
    - MELODIC + FIX (FSL)

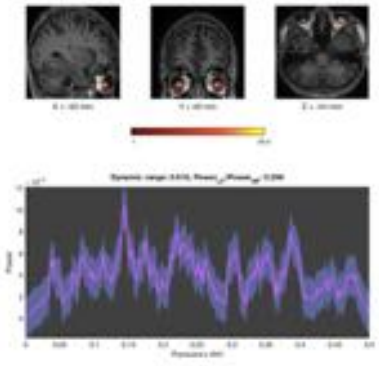
See also
Griffant et al. 2017: Hand classification of fMRI ICA noise components. NeuroImage 154:188-205

ICA based denoising: noise component identification

Based on the spatial location: brain edges, CSF, non-brain areas

Based on high (> 0.1 Hz) frequency content



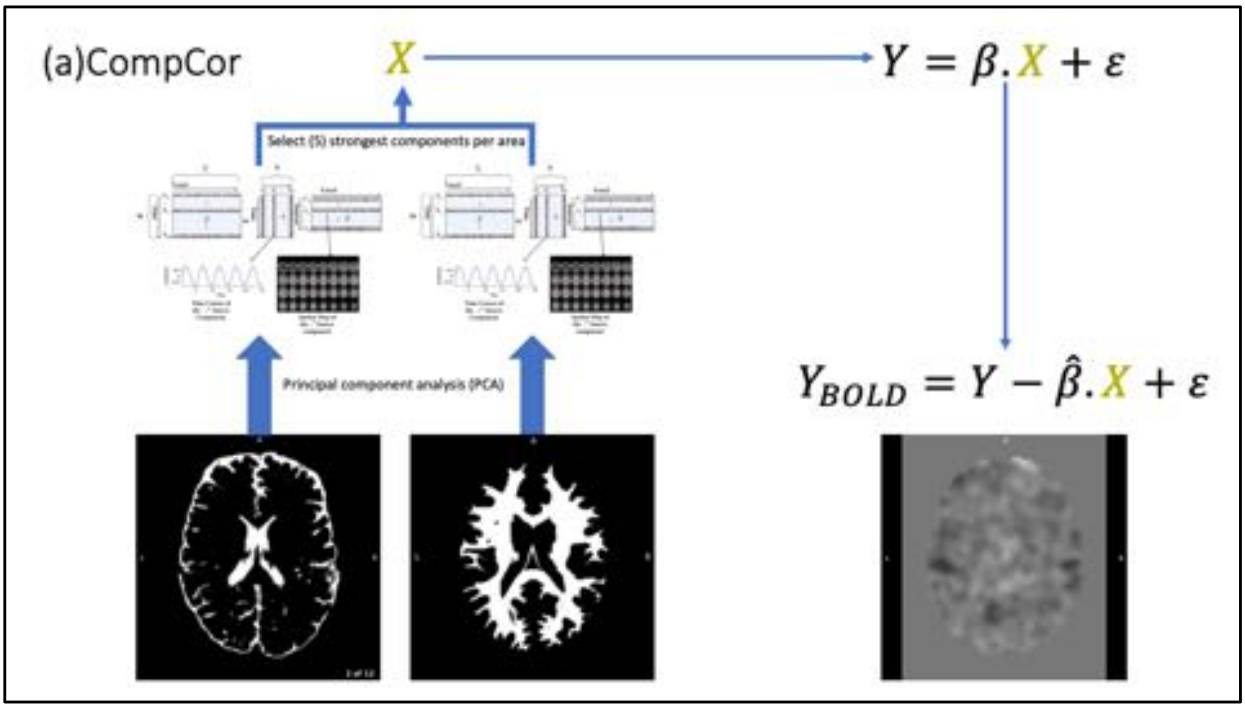


Manual -> time consuming and rater dependent

ICA-AROMA -> automatic motion artifact removal

Deep learning (DL) or AI based (e.g. Melodic) -> training of the algorithm

ICA-AROMA = ICA + Automatic Removal Of Motion artifacts



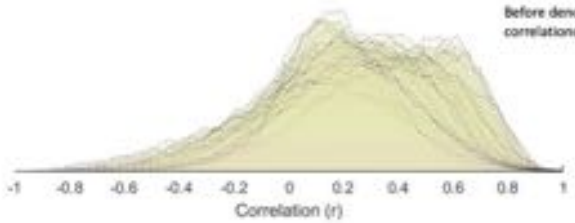
Alternatively,

- PCA components can be determined from areas supposed to have no neural activity (CSF, non-brain areas,…)
- Select the (5) strongest (explain most of the signal variability) components per area
- Fit the determined noise components and the realignment parameters (motion) to the signals in each voxel
- Subtract the fitted noise signals

- Standard, most packages use white matter and CSF as noise source areas. However, since more and more evidence is found that BOLD effects in the white matter linked to those in the gray matter exist, using solely CSF or CSF and non-brain areas as noises source, seems to be more appropriate.

Effect of denoising

Connectivity histogram before denoising

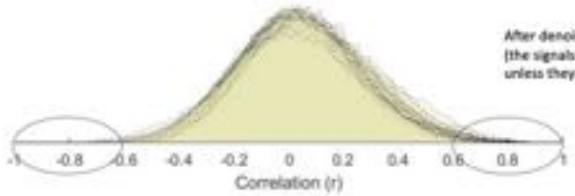


Before denoising: skewed distribution due to correlations induced by noise regressors

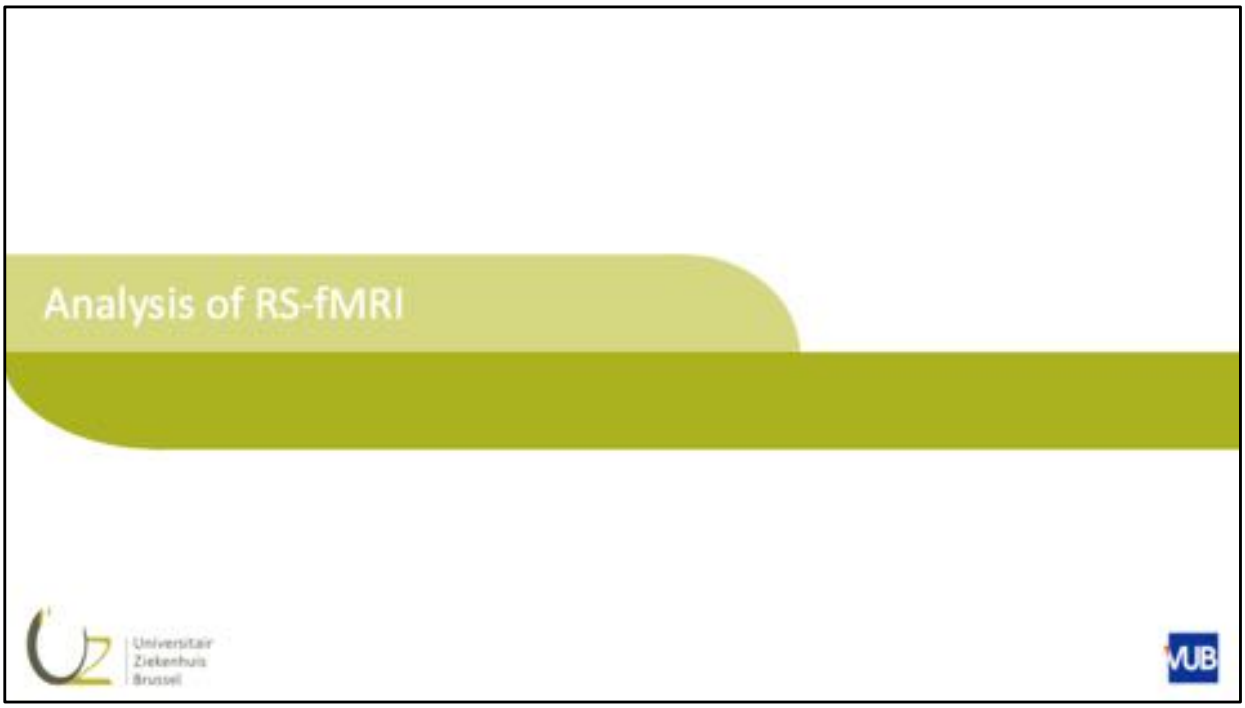
Evaluation of the denoising step:

Connectivity values between random selected voxel pairs

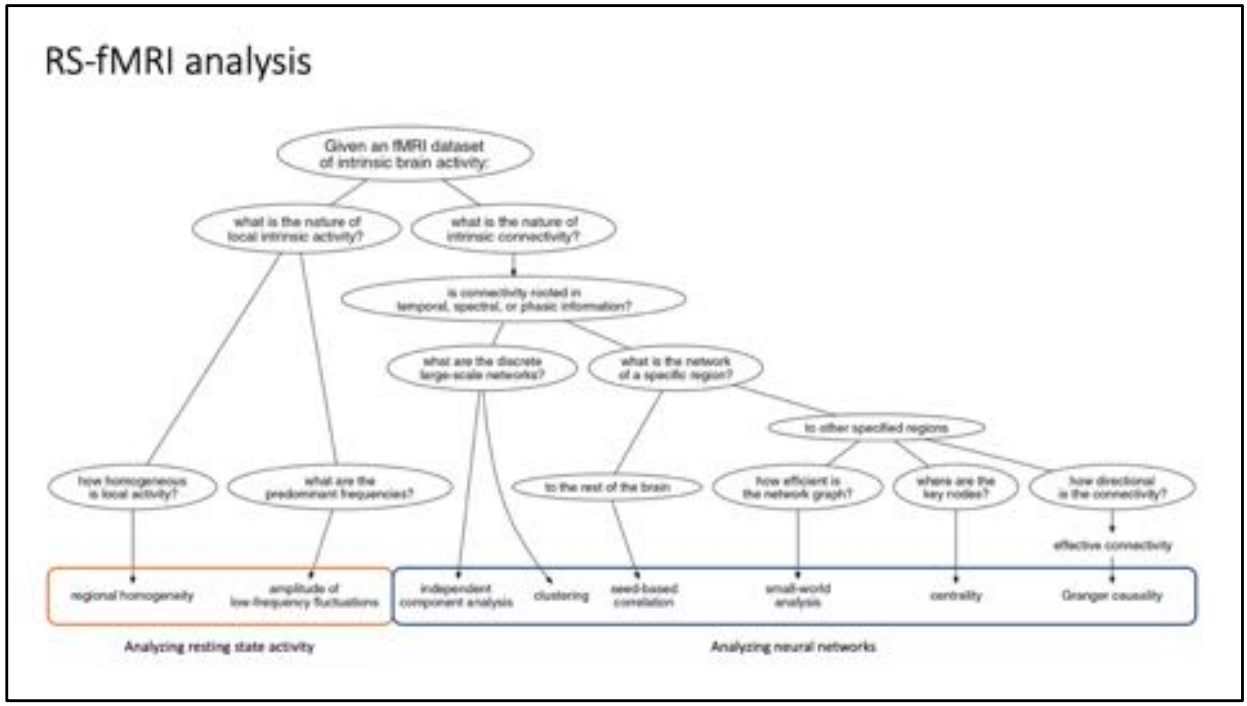
Connectivity histogram after denoising



After denoising: normally distributed correlations
(the signals of 2 randomly selected voxels normally do not correlate unless they are functionally connected)



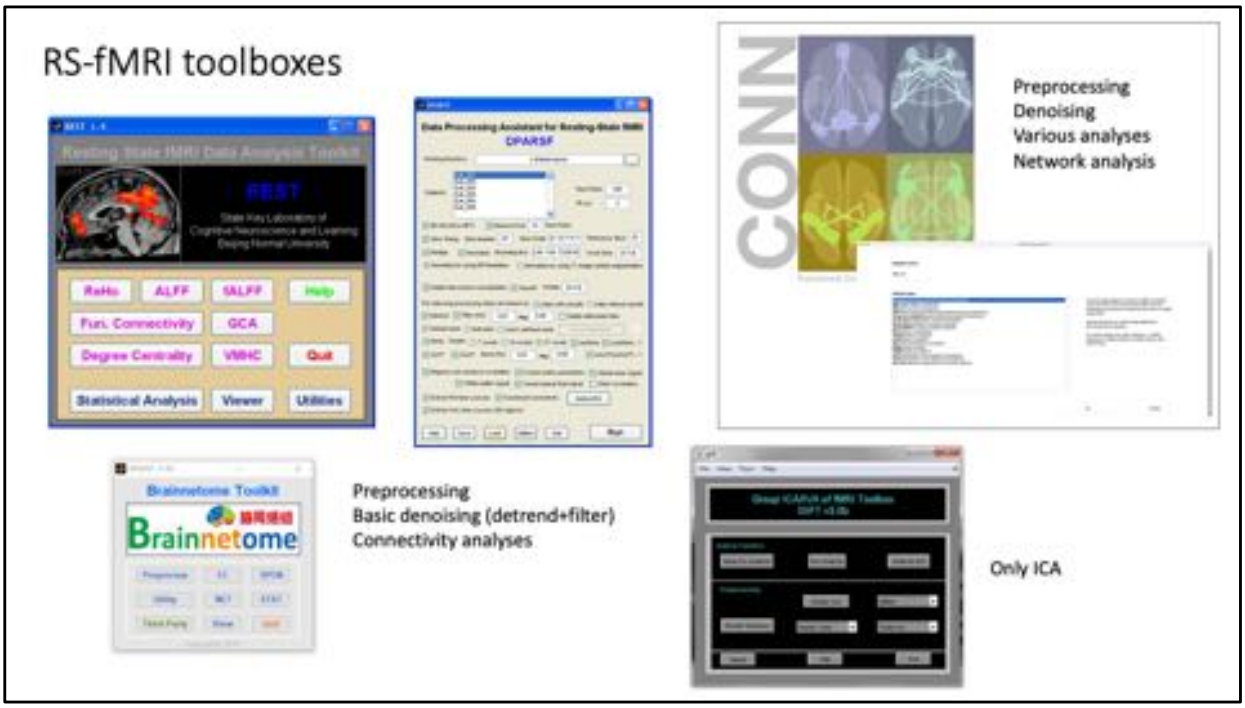
See also ‘Handbook of fcMRI methods in CONN’ on the website of CONN:
https://web.conn-toolbox.org/fmri-methods/connectivity-measures



- Kind of analyses done
    - Regional homogeneity of spontaneous activity -> local methods (ReHo)
    - Neural networks -> functional connectivity
    - Interactions between brain areas -> graph theory
    - Predominant variations in the RS-fMRI signal -> independent component analysis (ICA)
    - Delineate patterns of spontaneous activity -> pattern classification
    - Clusters of spontaneous activity -> cluster analysis

- The kind of analysis done, depends on your research question
    - Is your hypothesis about the degree of local activity
    - Is your hypothesis about functional connectivity/neural networks
    - …

See also
Van den Heuvel and Hulshoff Pol 2010. Exploring the brain network: A review on resting-state fMRI functional connectivity. Europ. Neuropsychopharm. 20:519-534
Margulies et al. 2010. Resting developments: a review of fMRI post-processing methodologies for spontaneous rain activity. Magn. Reson. Mater Phy. 23:289-307



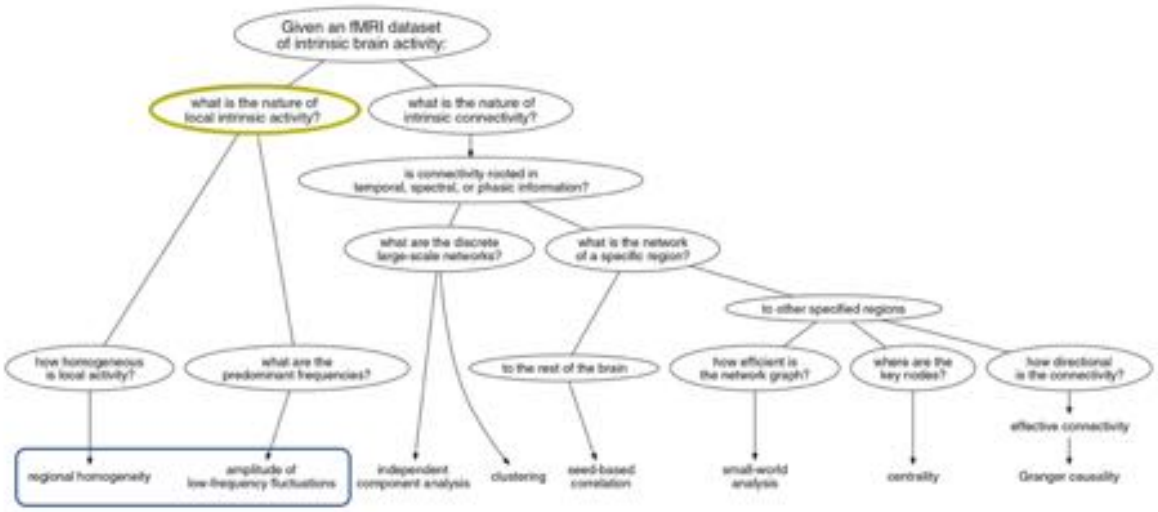
CONN toolbox: https://web.conn-toolbox.org

CONN is an SPM toolbox that runs in Matlab

Interesting documentation:
Whitfield-Gabrieli, S., & Nieto-Castanon, A. (2012). *Conn: A functional connectivity toolbox for correlated and anticorrelated brain networks*. Brain connectivity, 2(3), 125-141
Nieto-Castanon, A. (2020). *Handbook of functional connectivity Magnetic Resonance Imaging methods in CONN*. Boston, MA: Hilbert Press

Tip: transform the preprocessed fMRI scans from a set of 3D nifty files back into 1 4D niti files using ‘SPM -> utils -> 3D to 4D conversion‘ and delete the 3D files (makes data selection in CONN more easy)

RS-fMRI analysis

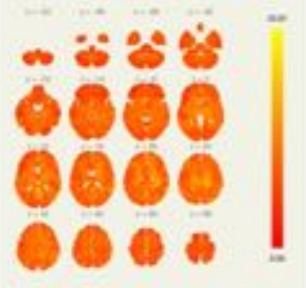


(Fractional) Amplitude of Low-Frequency Fluctuations ((f)ALFF)

(f)ALFF is an R-fMRI indicator that is used to detect **the regional intensity of spontaneous fluctuations in the BOLD signal, which pinpoints the spontaneous neural activity of specific regions and physiological states of the brain.**

ALFF maps represent a measure of BOLD signal power within the frequency band of interest (e.g. 0.01 - 0.10 Hz).
ALFF is defined as the root mean square of BOLD signal at each individual voxel after low- or band- pass filtering:

fALFF maps represent a relative measure of BOLD signal power within the frequency band of interest (e.g. 0.01 - 0.10 Hz) compared to that over the entire frequency spectrum.
fALFF is defined as the ratio of root mean square of BOLD signal at each individual voxel after vs. before low- or band-pass filtering:



$$ALFF(x) = \sqrt{\frac{1}{N}\cdot\sum_{t}(h(t) * S(x,t))^2}$$

$$fALFF(x) = \sqrt{\frac{\sum_{t}(h(t) * S(x,t))^2}{\sum_{t} S(x,t)^2}}$$

S is original BOLD timeseries before band- or low- pass filtering
h is a low- or band-pass filter
N is the number of timepoints

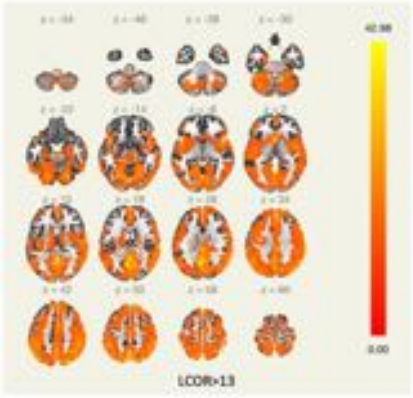
Different sensitivity and reliability for changes due to aging, disease states and treatment effects

↓

Best to look at both

Local correlation (LCOR) = Regional Homogeneity (ReHo)

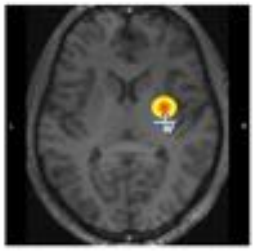
LCOR maps represent **a measure of local coherence at each voxel**, characterized by **the strength and sign of connectivity between a given voxel and its neighboring areas**.
LCOR is defined as the average of correlation coefficients between each individual voxel and a region of neighboring voxels



$$LCOR(x) = \frac{\int w(x-y)r(x,y)dy}{\int w(x-y)dy}$$

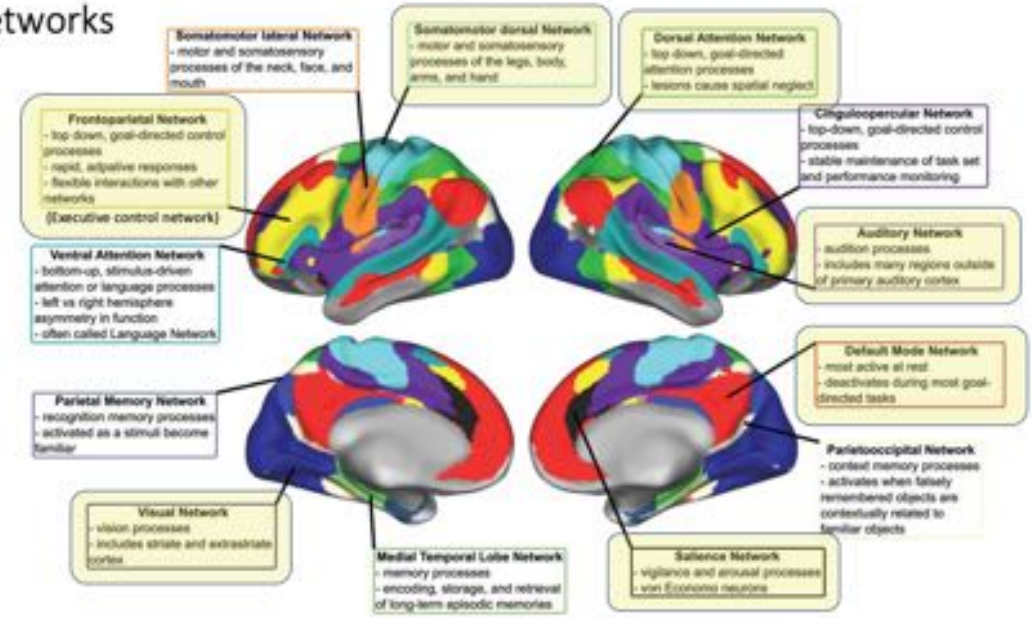
r is the map of voxel-to-voxel correlations between every pair of voxels

w is an isotropic Gaussian weighting function with size sigma characterizing the size of the local neighborhood ($w(x) = e^{\frac{|x|^2}{2\sigma^2}}$)

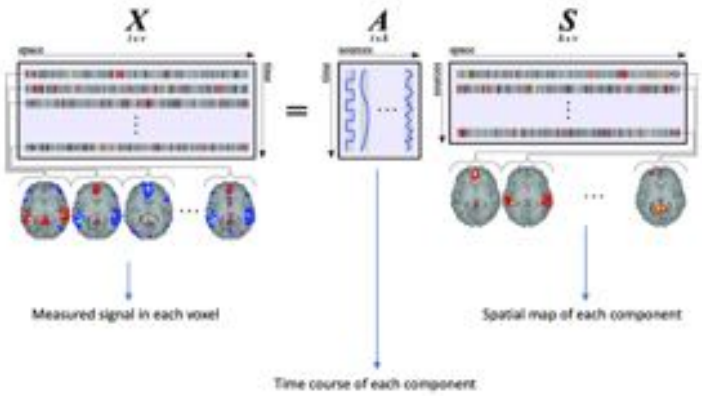


RS-fMRI analysis

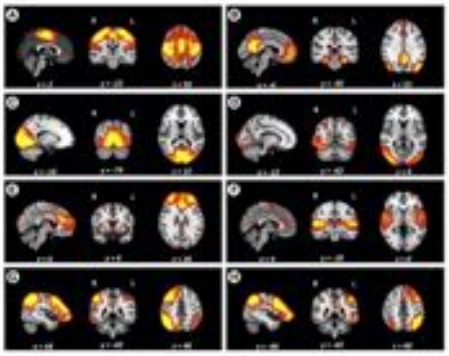
Brain networks



Splitting the fMRI signals in independent networks = ICA

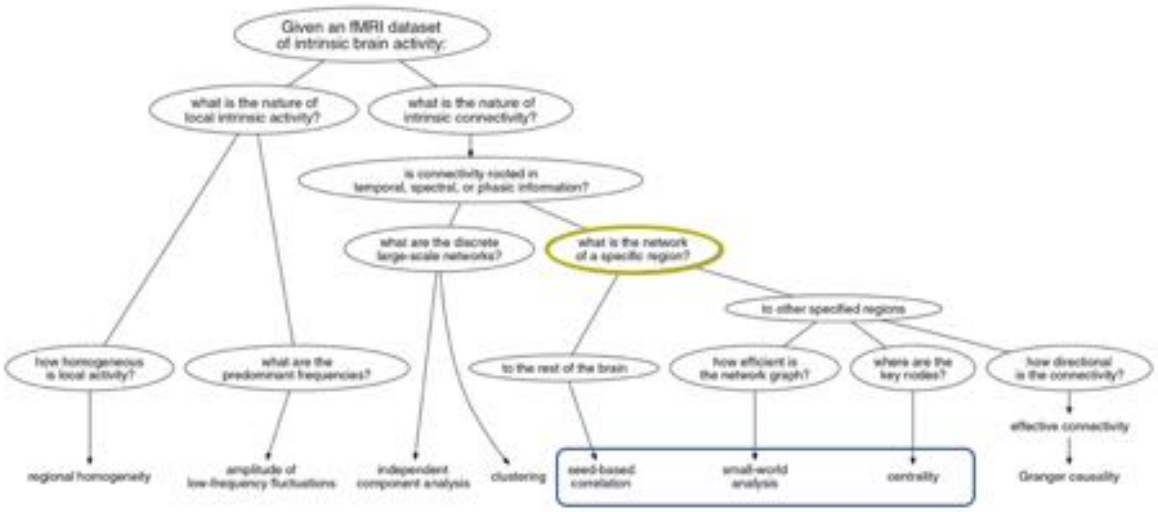


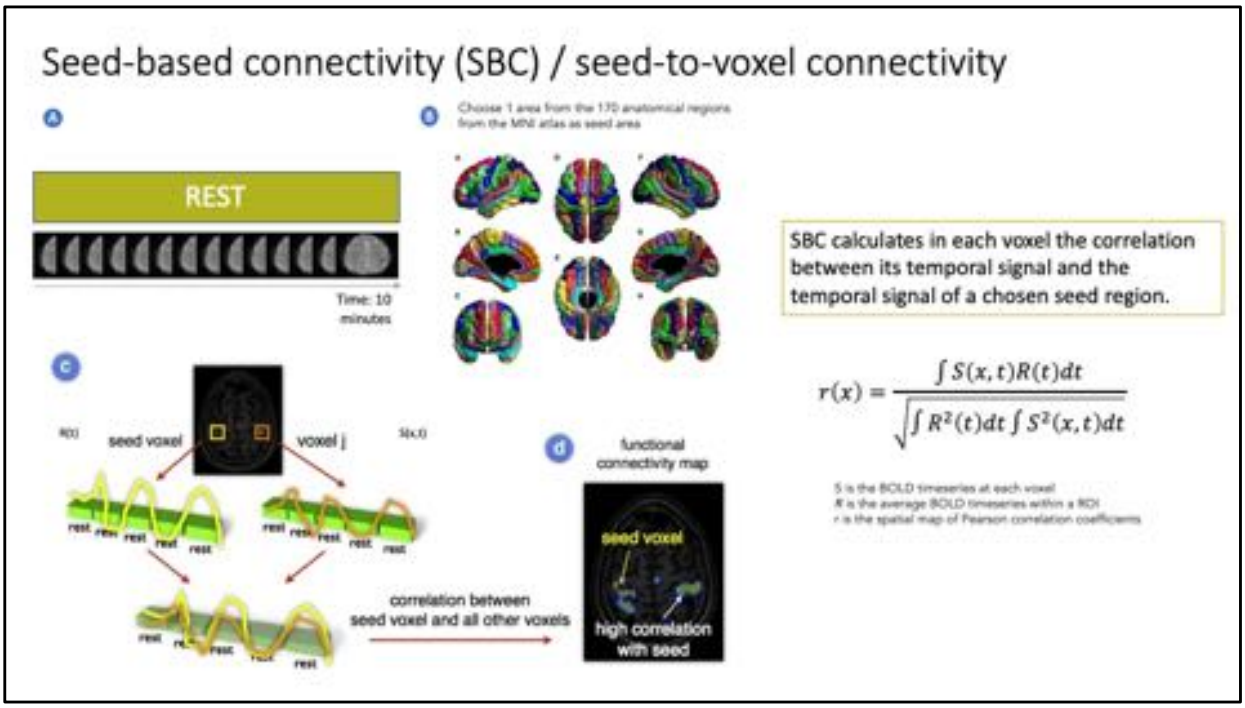
Each component = a functional network



The resting state networks. Group mean (A) motor, (B) default mode, (C) medial visual, (D) lateral visual, (E) executive, (F) auditory, (G) right dorsal visual stream, and (H) left dorsal visual stream networks.

RS-fMRI analysis





- The most widely used analysis method is the seed-based analysis
    - Seed = source region-of-interest (ROI)
    - The time series of the seed area is correlated to the time series in
        - All voxels -> seed-to-voxel analysis
        - Other seed areas -> seed-to-seed analysis

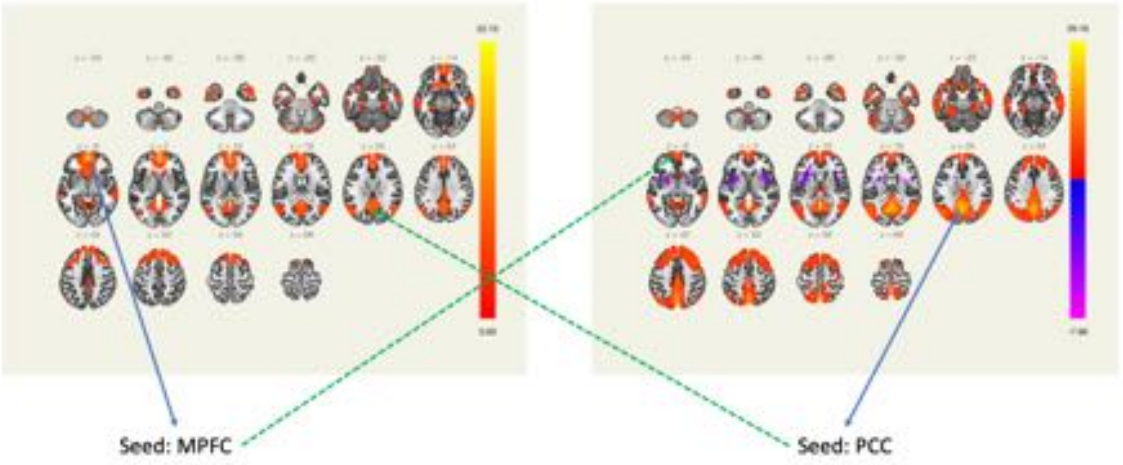
- Interpretation: if areas are connected to each other (part of the same functional network), there is some degree of synchronization of their signal fluctuations -> correlated signals

- The correlations does not show causal interactions, nor the direction of the interaction

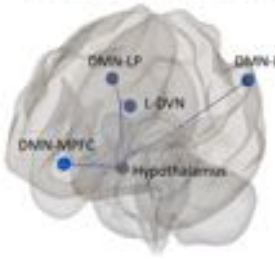
- Drawback: study results dependent on the definition of the seed
    - As can be seen in the images: the found network differs dependent on the chosen seed region

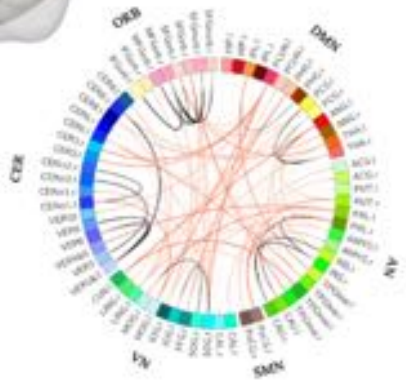
See also
Van den Heuvel and Hulshoff Pol 2010. Exploring the brain network: A review on resting-state fMRI functional connectivity. Europ. Neuropsychopharm. 20:519-534
Margulies et al. 2010. Resting developments: a review of fMRI post-processing methodologies for spontaneous rain activity. Magn. Reson. Mater Phy. 23:289-307

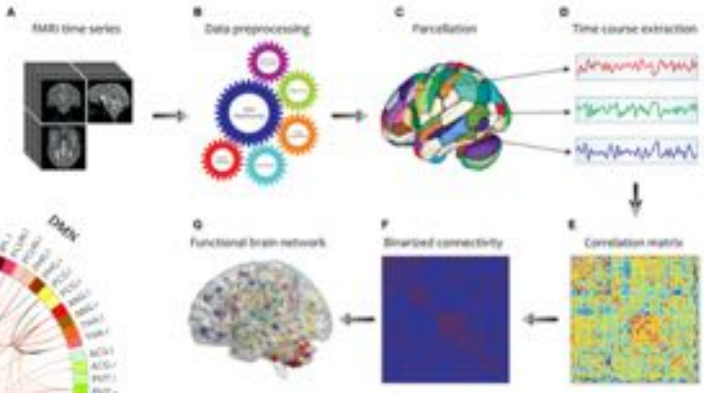
Seed-based connectivity (SBC): Default mode network (DMN)



ROI-to-ROI connectivity (RRC)

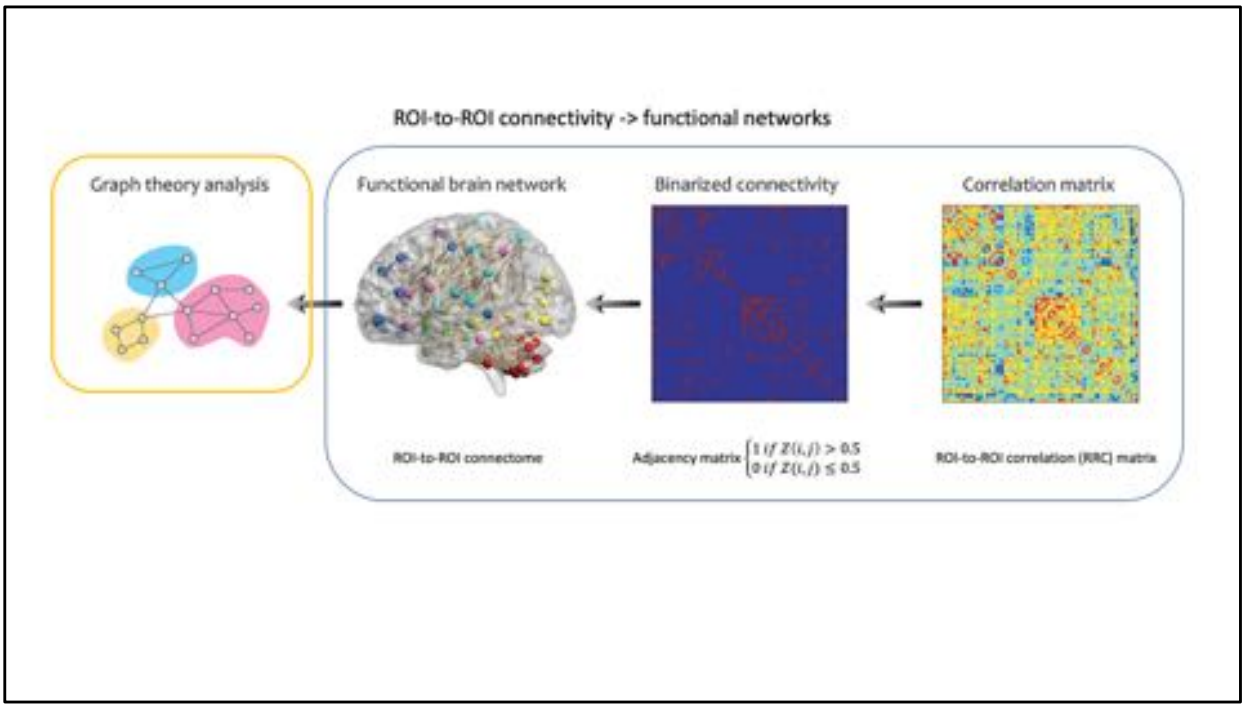






$$r(i,j) = \frac{\int R_i(t)R_j(t)dt}{\sqrt{\int R_i^2(t)dt \int R_j^2(t)dt}}$$

R is the BOLD timeseries within each ROI
r is a matrix of correlation coefficients

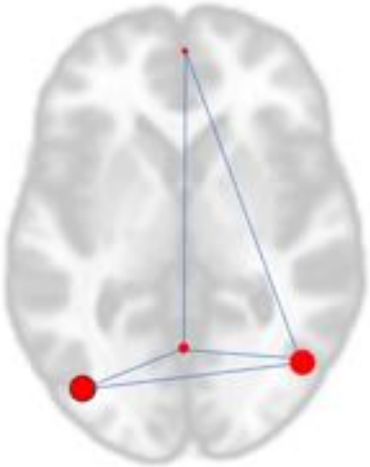


Farahani et al. **Application of Graph Theory for Identifying Connectivity Patterns in Human Brain Networks: A Systematic Review.** Front. Neurosci., 06 June 2019 | https://doi.org/10.3389/fnins.2019.00585

Graph theory based network measures

Nodes = ROIs in the graph

Edges = connections between the nodes



Degree and Cost at each node/ROI represent **measures of network centrality**, characterizing **the degree of local connectedness** of each ROI within a graph

Degree = number of edges from/to each node

At ROI level: $d_i = \sum_j A_{i,j}$

At network level: $d = \frac{\sum_i d_i}{N}$

A is the adjacency matrix

N is the total number of nodes

Cost = proportion of edges from/to each node

At ROI level: $c_i = \frac{\sum_j A_{i,j}}{N-1}$

At network level: $c = \frac{\sum_i c_i}{N}$

Average path distance and Global efficiency at a node **represents a measure of this node centrality within the network**, characterizing **the degree of global connectedness** of each ROI.

Average path distance = average number of edges traversed in an optimal path between one node and an other node

At ROI level: $L_i = \frac{\sum_{j \neq i} D_{i,j}}{N-1}$

D is the shortest-path distance matrix

N is the total number of nodes

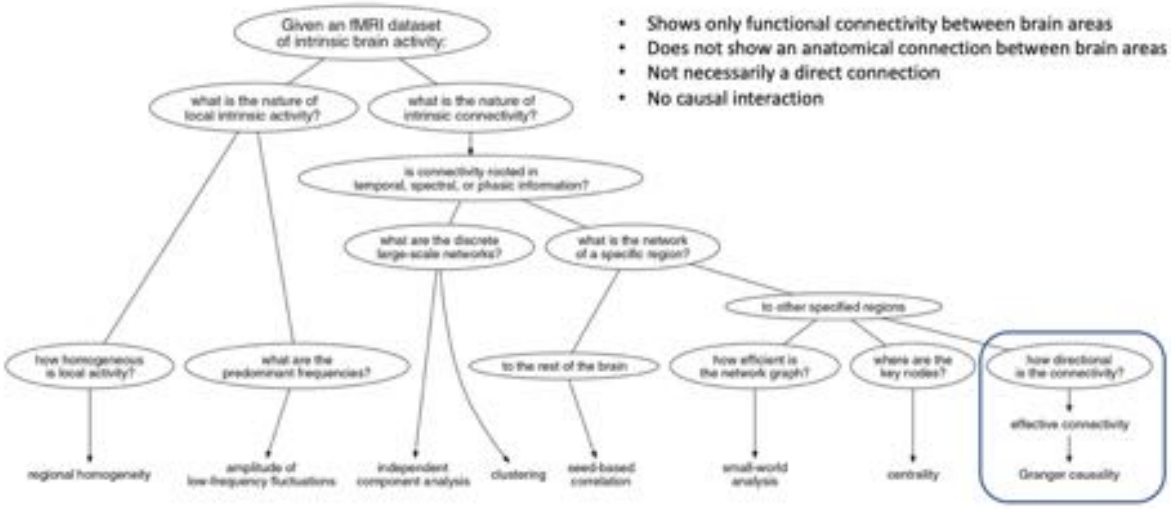
At network level: $L = \frac{\sum_i L_i}{N}$

Global efficiency = average inverse distance between a node and all other nodes

At ROI level: $GE_i = \frac{\sum_{j \neq i} 1/D_{i,j}}{N-1}$

At network level: $GE = \frac{\sum_i GE_i}{N}$

Seed-to voxel and ROI-to-ROI connectivity



- Shows only functional connectivity between brain areas
- Does not show an anatomical connection between brain areas
- Not necessarily a direct connection
- No causal interaction

DANK U - THANK YOU

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