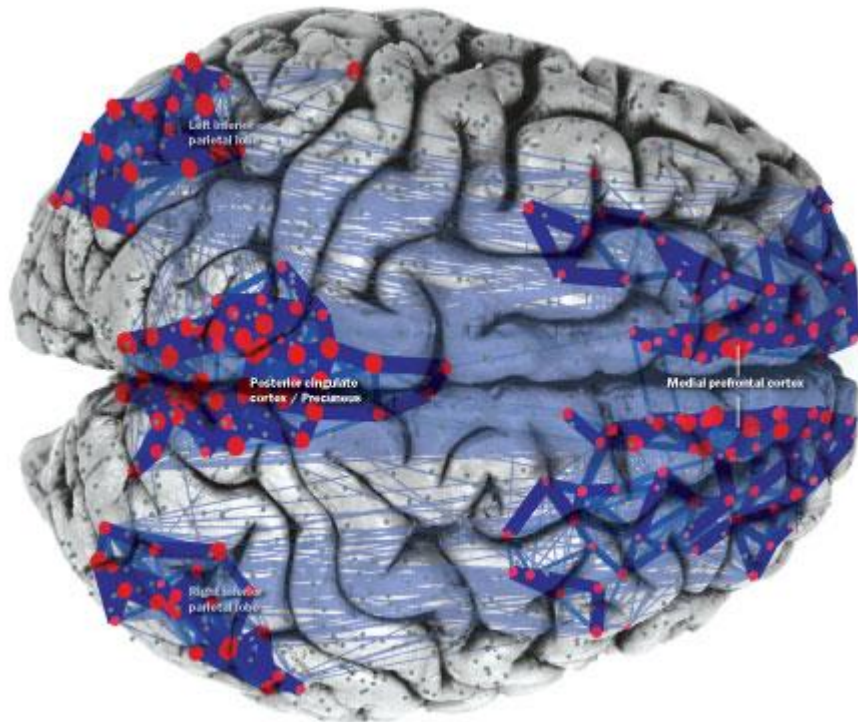# Resting state analysis with SPM

Hamburg SPM course 2015

Helmut Laufs

Enzo Tagliazucchi
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- Resting state activity is interesting *per se*

Aims

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- To know what SPM cannot easily do and apply self-made MATLAB scripts to do it

- Learn the basics of EEG-fMRI integration by finding the correlates of EEG rhythms during the resting state

First part: seed correlation

Methods I: Seed correlation

- Functional connectivity between a BOLD timeseries from a given location and the rest of the brain is investigated

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- Functional connectivity between a BOLD timeseries from a given location and the rest of the brain is investigated

Fox et al. 2007 Nat Rev Neurosci
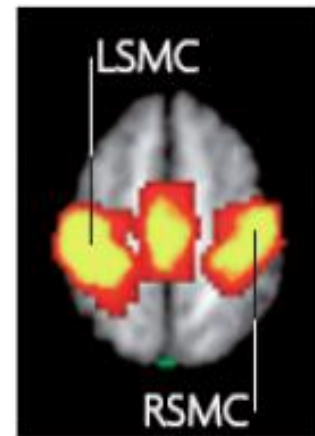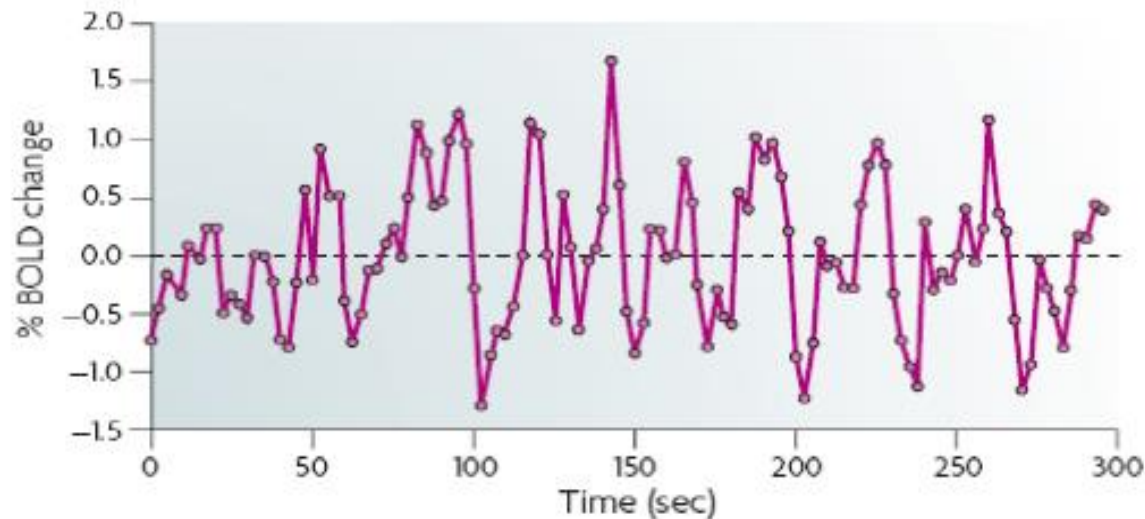
Methods I: Seed correlation

- Functional connectivity between a BOLD timeseries from a given location and the rest of the brain is investigated

- It is common to apply it individually for each subject (*first level analysis*) and the perform analysis at the group level or inter-group comparisons between different populations (*second level analysis*)
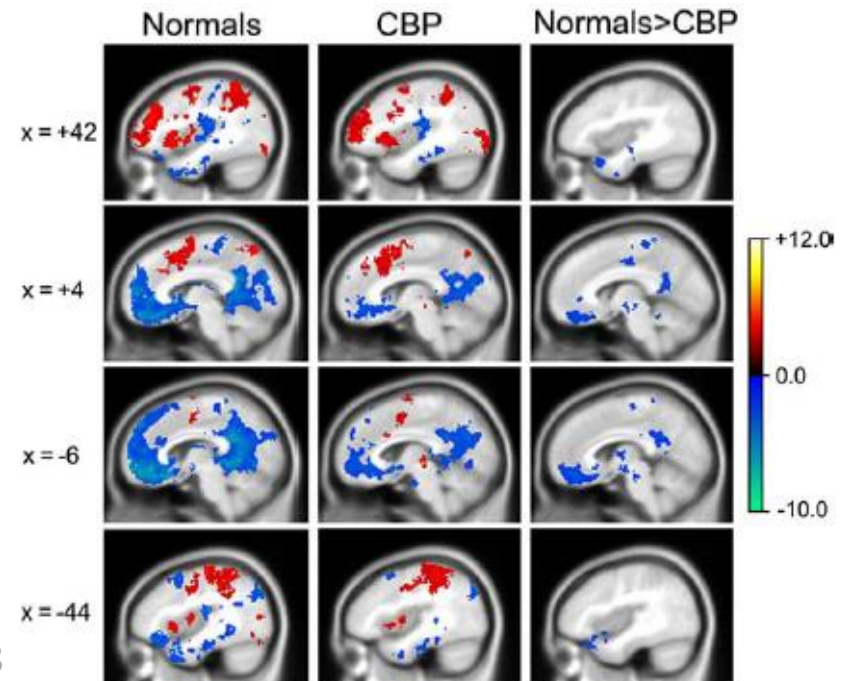
Baliki et al, J Neurosci 2008

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Y = spm_vol('aal_atlas.img'); aal = spm_read_vols(Y); ⬅ Code to open an fMRI volume using SPM
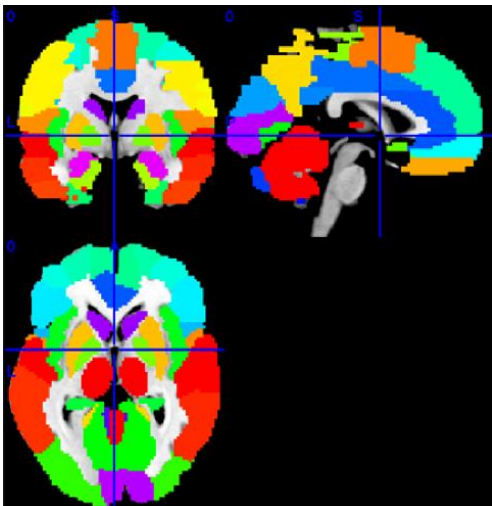
⬅ 90 cortical and sub-cortical regions + 26 cerebellar regions

How to do it

- First, extract a BOLD time course from a voxel or a ROI (*tools/extract_BOLD_regressor.m*)
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- Then, construct any other regressors you might want to include in the analysis: motion time series, derivatives of motion time series, cardiac and respiratory nosie.

RETROICOR method (Glover et al, 2000)

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- Put the BOLD regressor in the design matrix, together with the other regressors, and run a first level analysis for each subject you want in the analysis

SPM pipeline

Image time-series
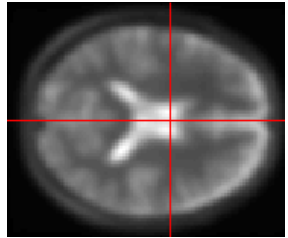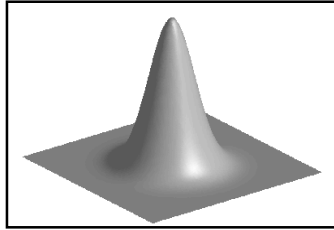
Kernel

Design matrix

Statistical parametric map (SPM)

Realignment → Smoothing → General linear model →

Normalisation

Statistical inference

Gaussian field theory

Template

Parameter estimates

p <0.05
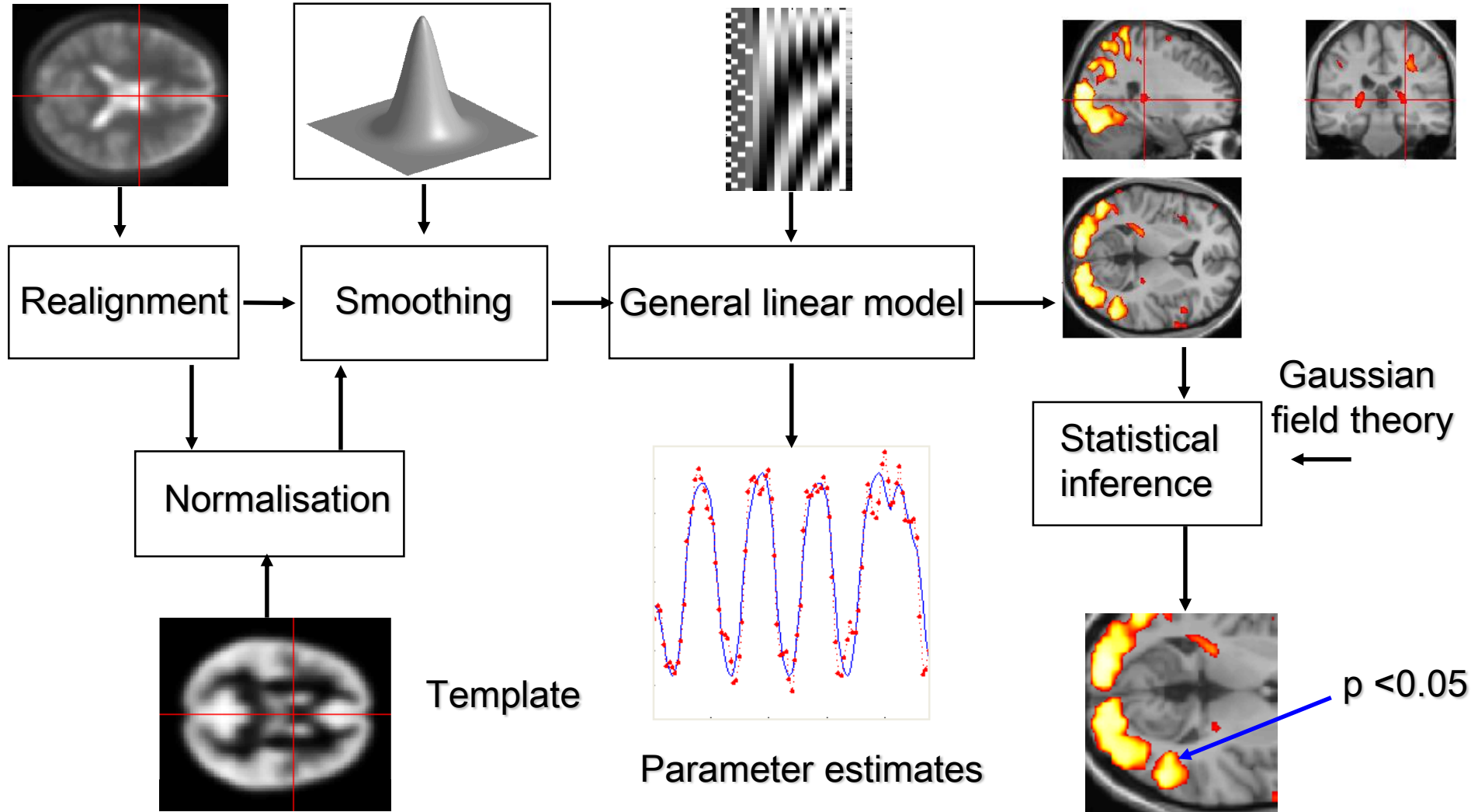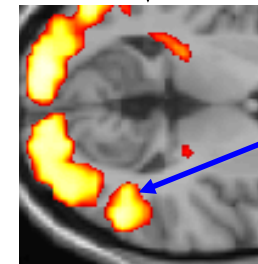
SPM pipeline

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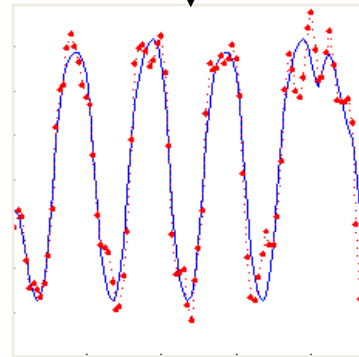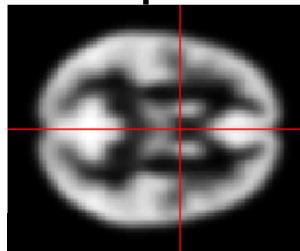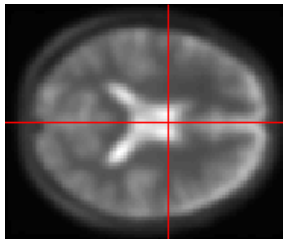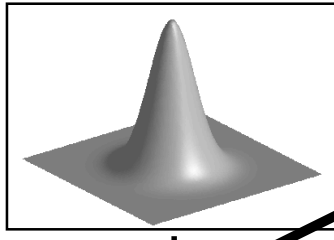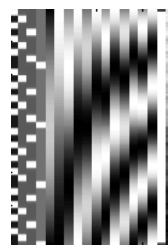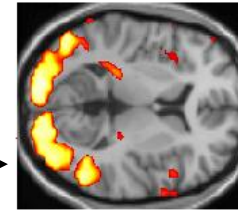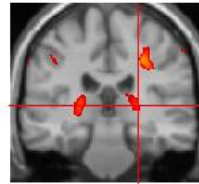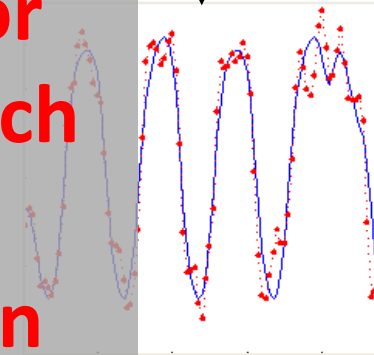Realignment → Smoothing → General linear model → Statistical inference ← Gaussian field theory

Normalisation

Parameter estimates
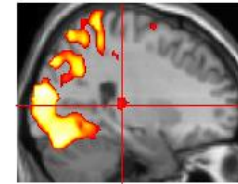
Instead of using a regressor based on the model, we use one (or more) regressors which are BOLD time series extracted from certain ROIs

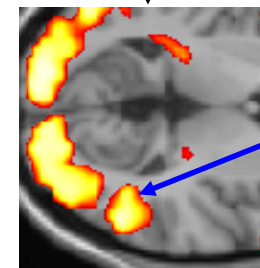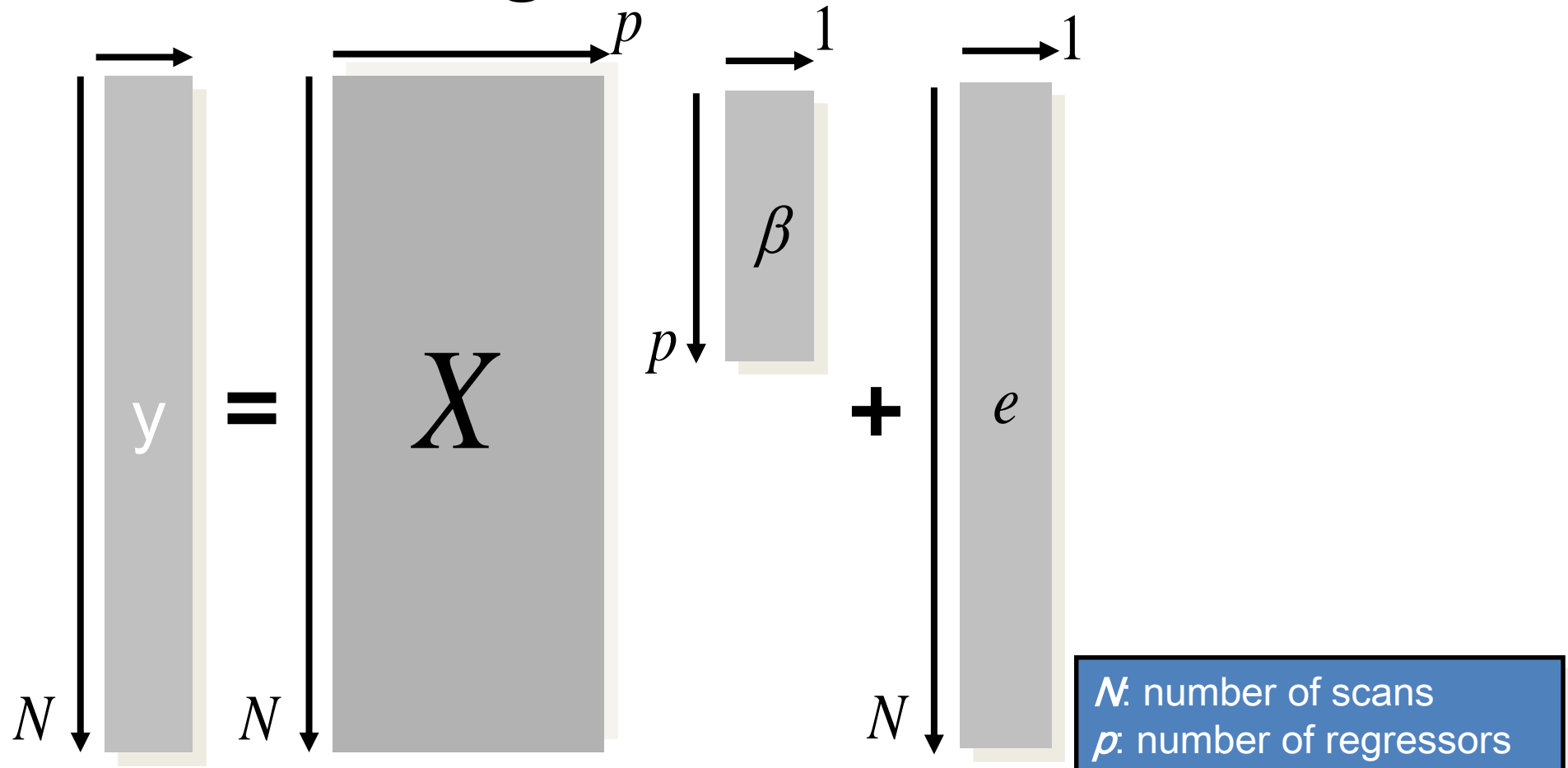p <0.05

Design matrix and GLM

$$y = X\beta + e \qquad e \sim N(0, \sigma^2 I)$$

N: number of scans
p: number of regressors

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$e \sim N(0, \sigma^2 I)$

Warning!!

BOLD regressors are NOT to be convolved with HRF. Other regressors might (like cardiac or respiratory) or might not (like motion)

N: number of scans
p: number of regressors
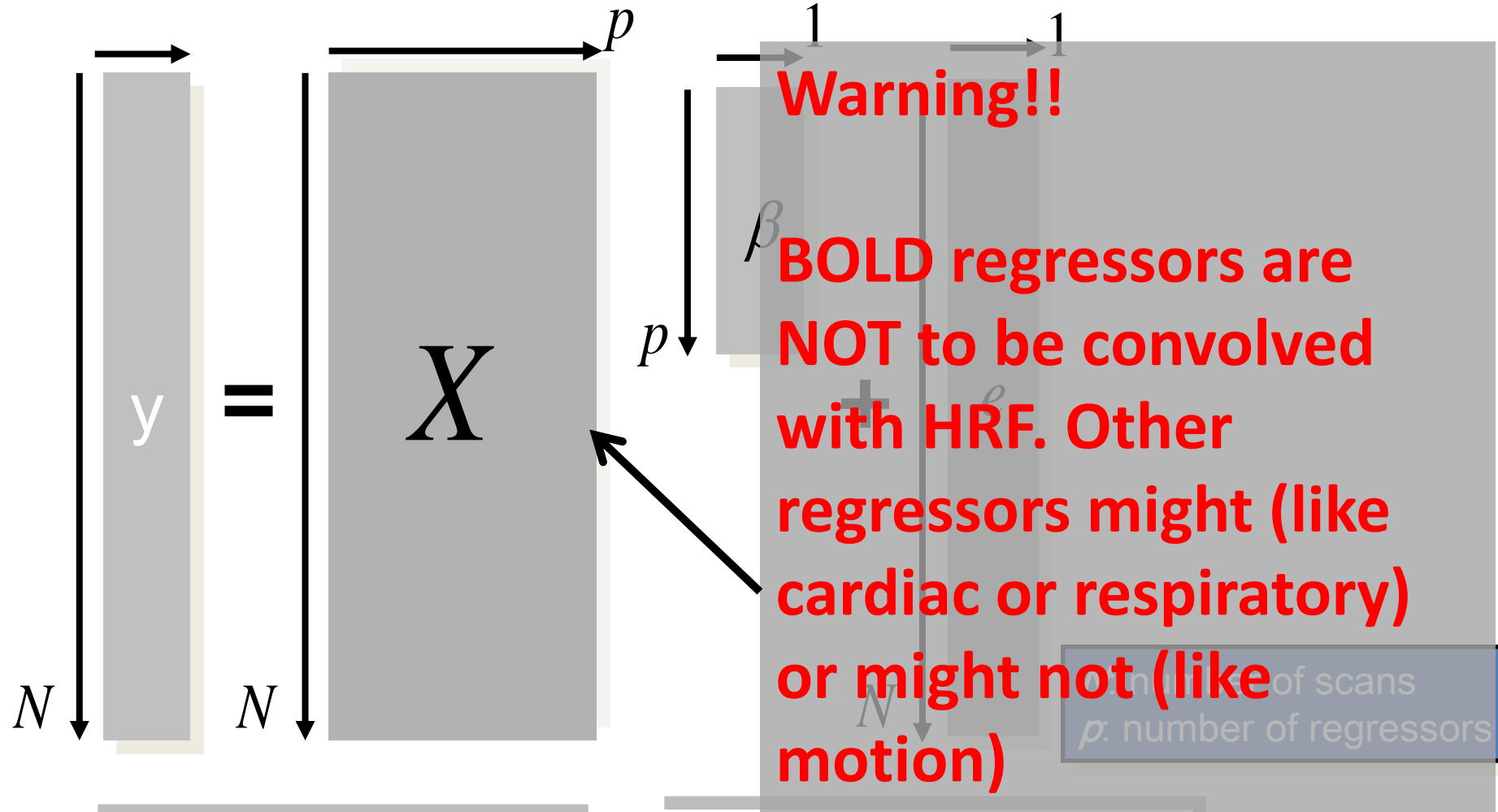
Dataset

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- First group has increased connectivity between left thalamus and both left and right pre-frontal cortex

Dataset

- Five subjects in two groups (50 volumes, 79 x 95 x 68 voxels)
- First group has increased connectivity between left thalamus and both left and right pre-frontal cortex
- Second group has increaased connectivity between left thalamus and left left pre-frontal cortex only

Steps (first level)

- Load the AAL atlas, which will be necessary to extract the thalamus BOLD timecourse

Y = spm_vol('aal_atlas.img'); aal = spm_read_vols(Y);

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Steps (first level)

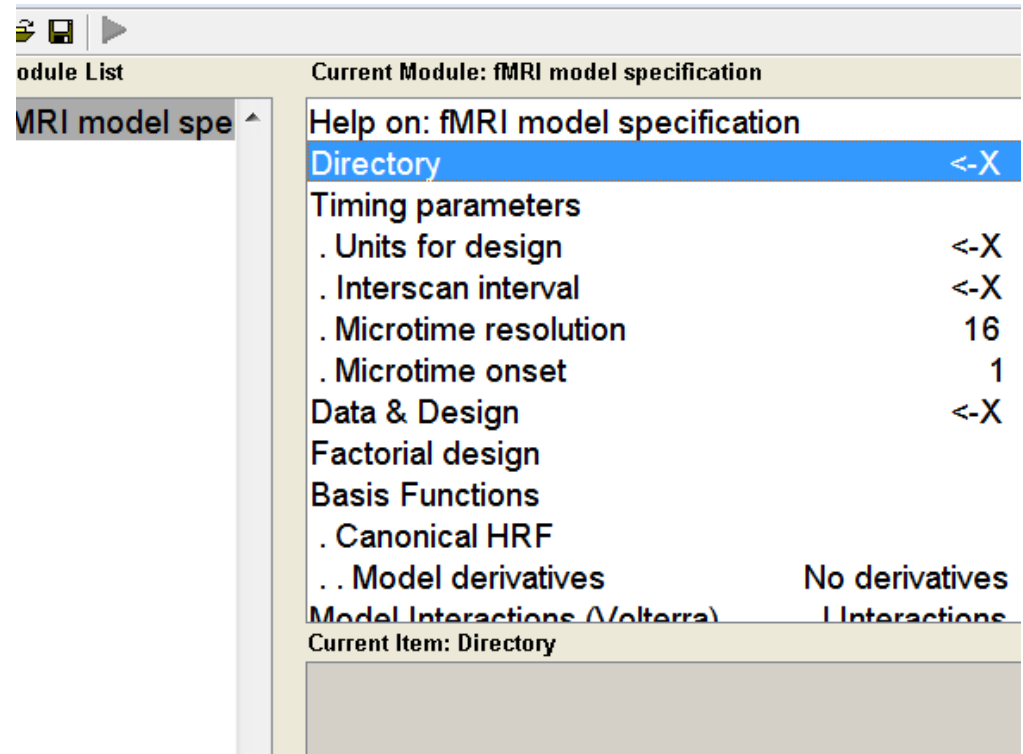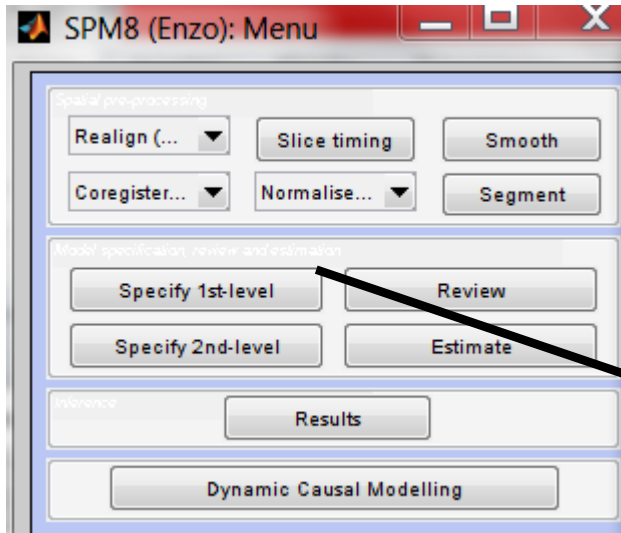- Load the AAL atlas, which will be necessary to extract the thalamus BOLD timecourse

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- In each subject file there is a file with noise regressors (noise_regressors.mat). These can be combined to form all the regressors to be included in the design matrix using the *create_all_regressors.m* script (**Check path**!).
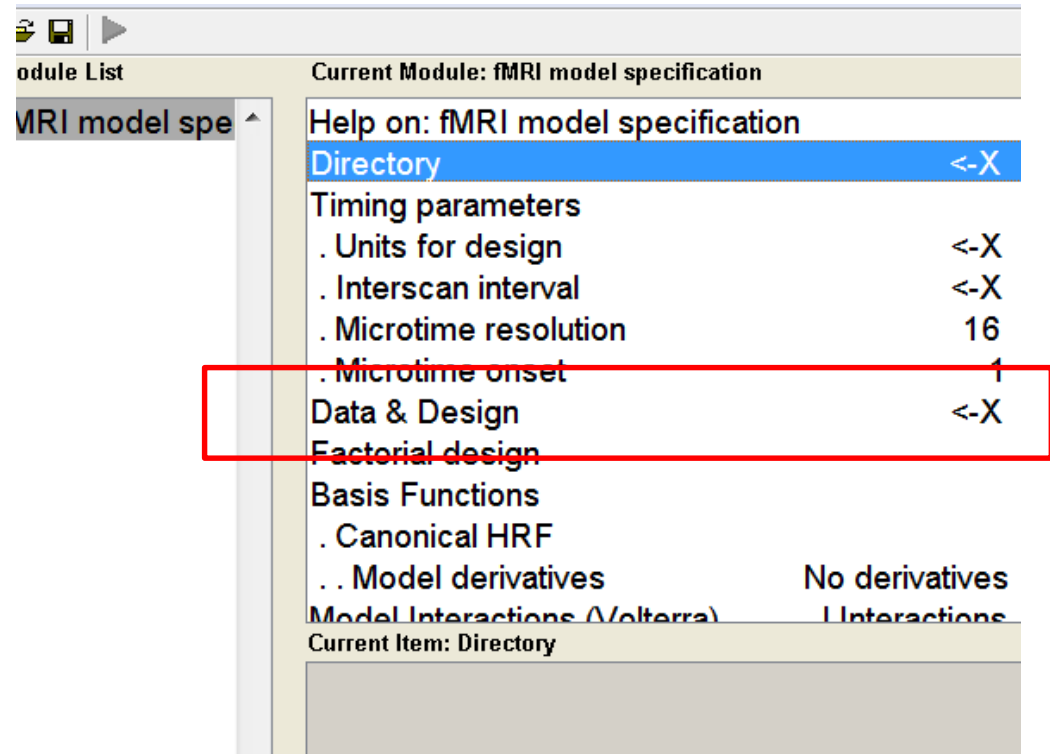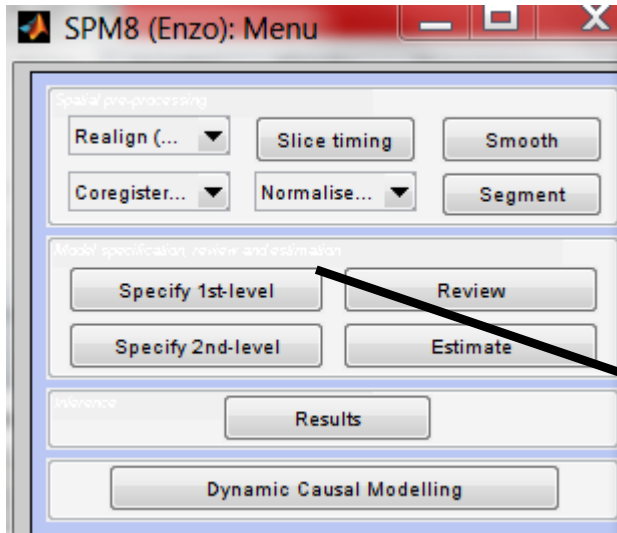
Set up model (first level)
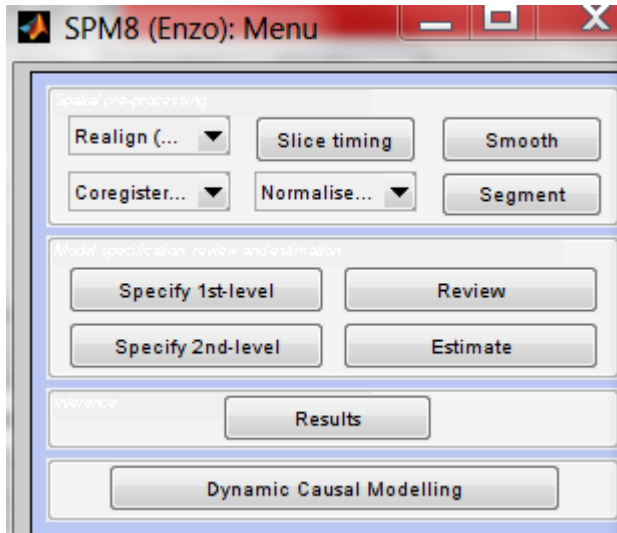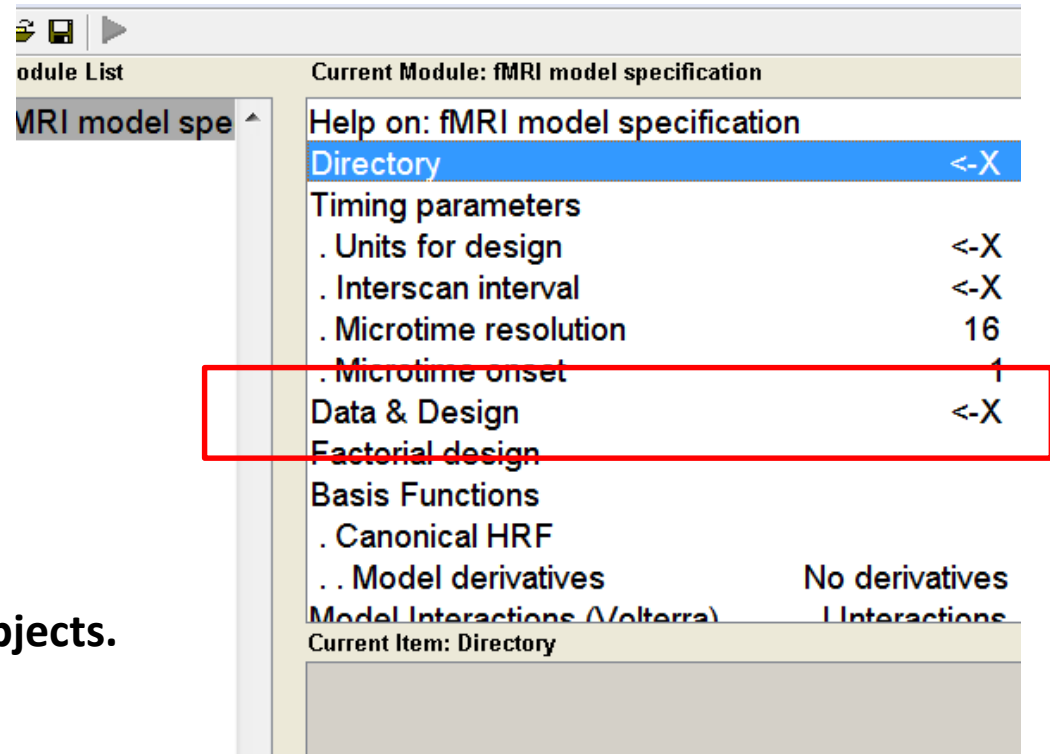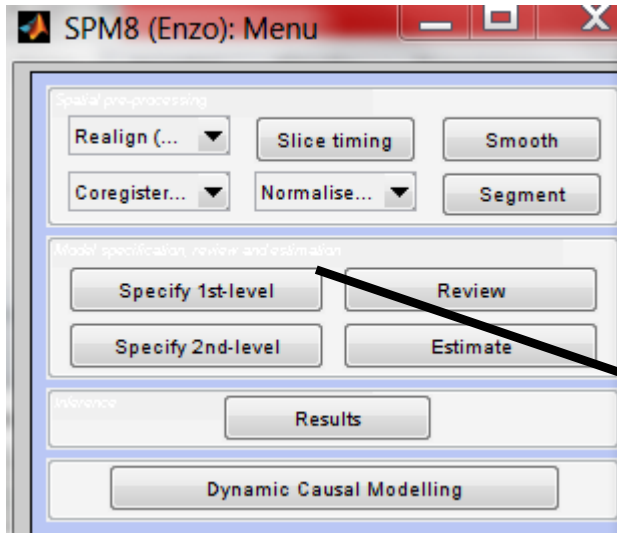
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Click Data & Design, add volumes (**careful! Check file order!!**) and add regressors using the Multiple Regressors option.

Select TR (2.08 s.) and select directory. **Model details do not concern us** (we defined our regressor)

Set up model (first level)

Use the batch script
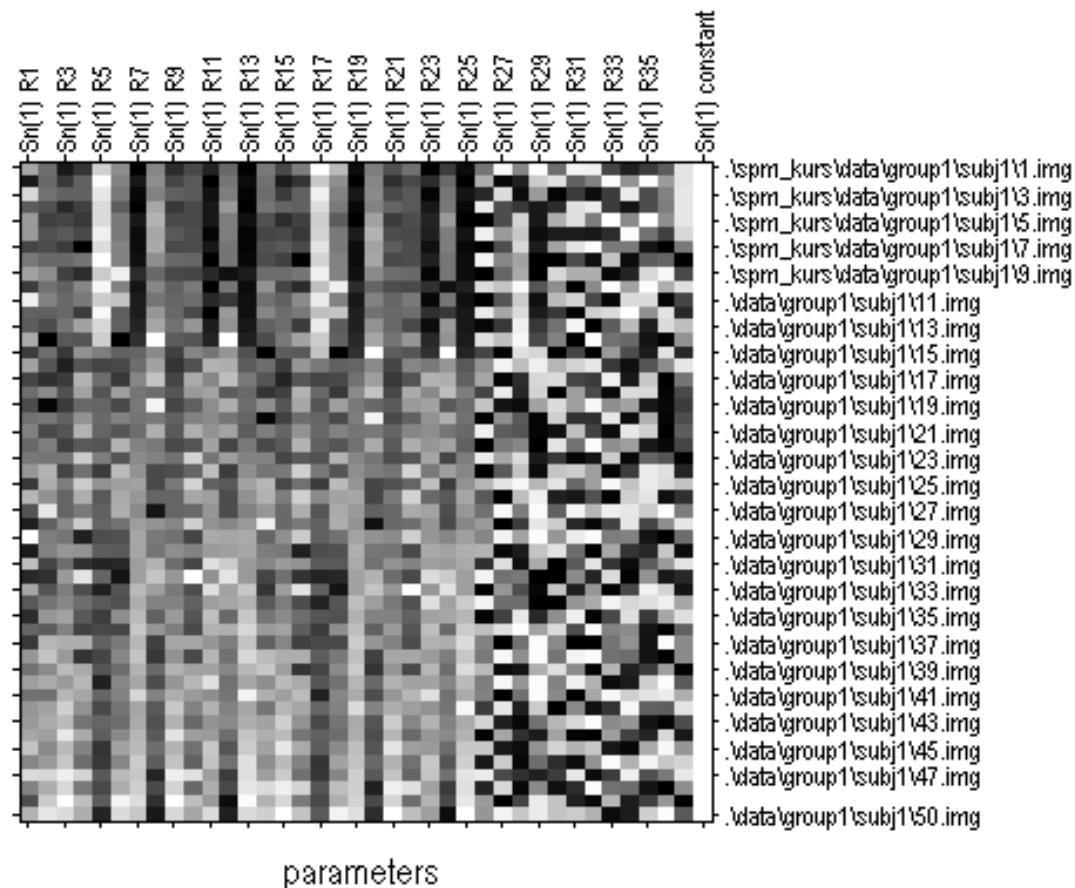*first_level_analysis_job.m* **for all subjects.**
Change directory paths in the file!!

Click Data & Design, add volumes (**careful! Check file order!!**) and add regressors using the Multiple Regressors option.

Select TR (2.08 s.) and select directory. **Model details do not concern us** (we defined our regressor)
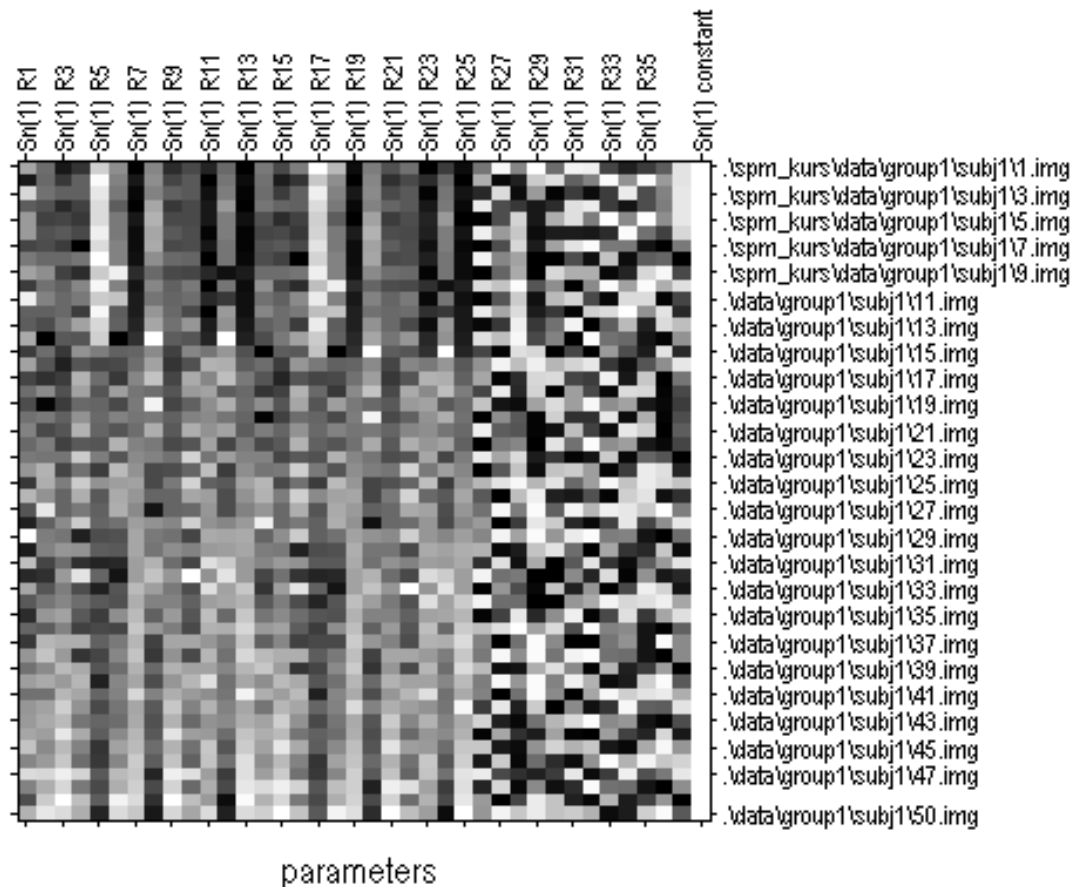
Design matrix (example)

Statistical analysis: Design
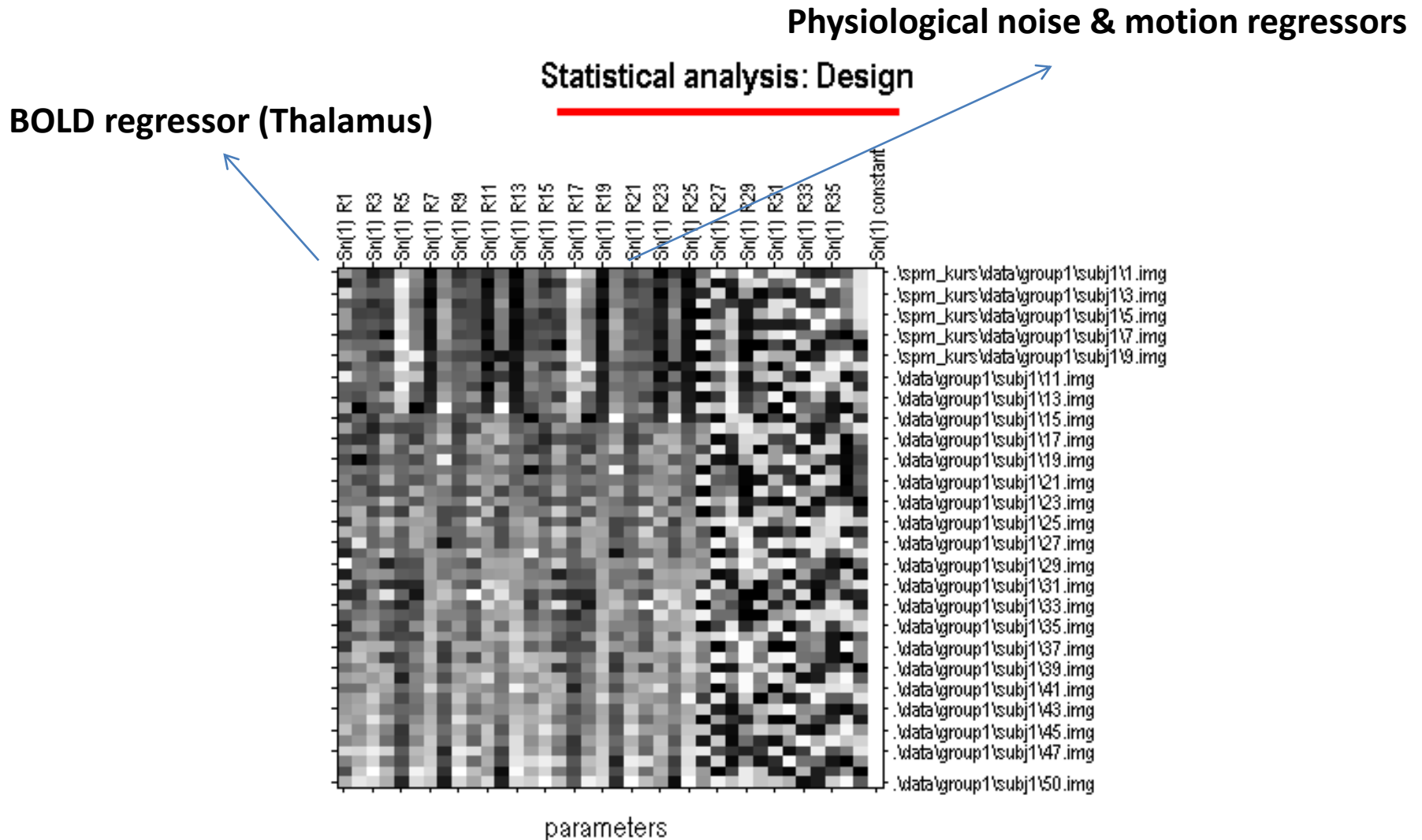
Design matrix (example)

BOLD regressor (Thalamus)
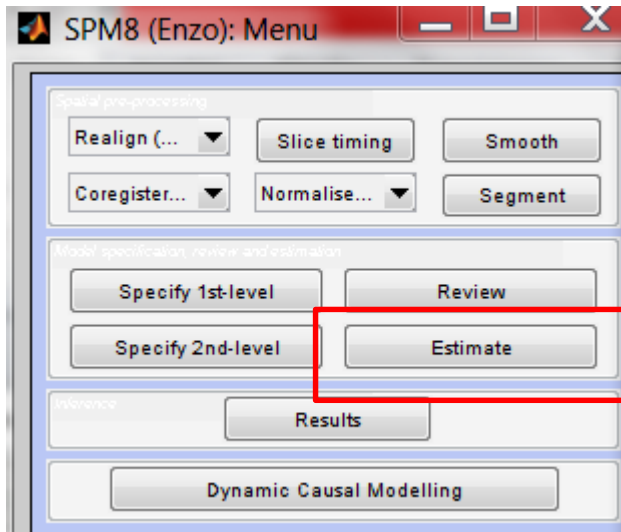

Statistical analysis: Design

Design matrix (example)

Physiological noise & motion regressors

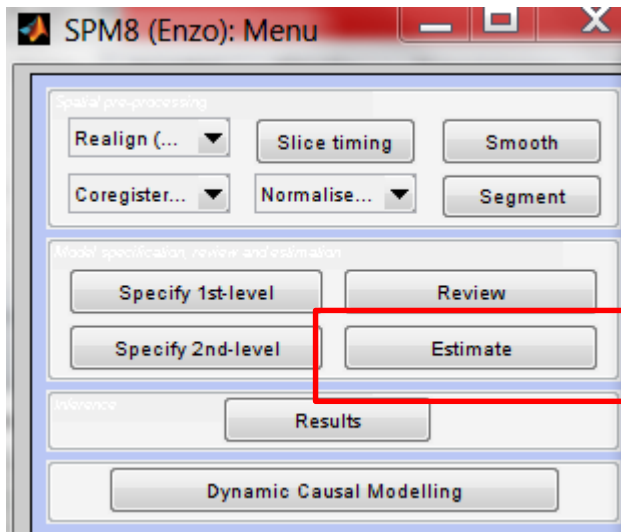**BOLD regressor (Thalamus)**

Statistical analysis: Design

parameters
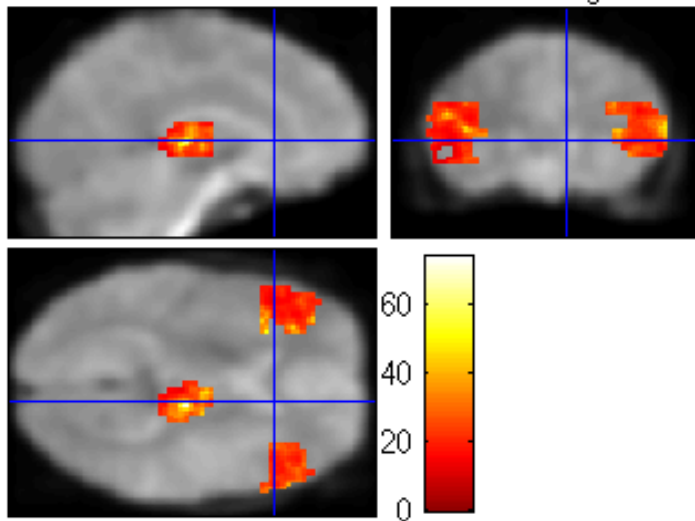
Estimate model (first level)

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Use the batch script *estimate_model_first_level_job.m* for all subjects. **Change directory paths in the file!!**

Results (first level)

Thalamic BOLD regressor
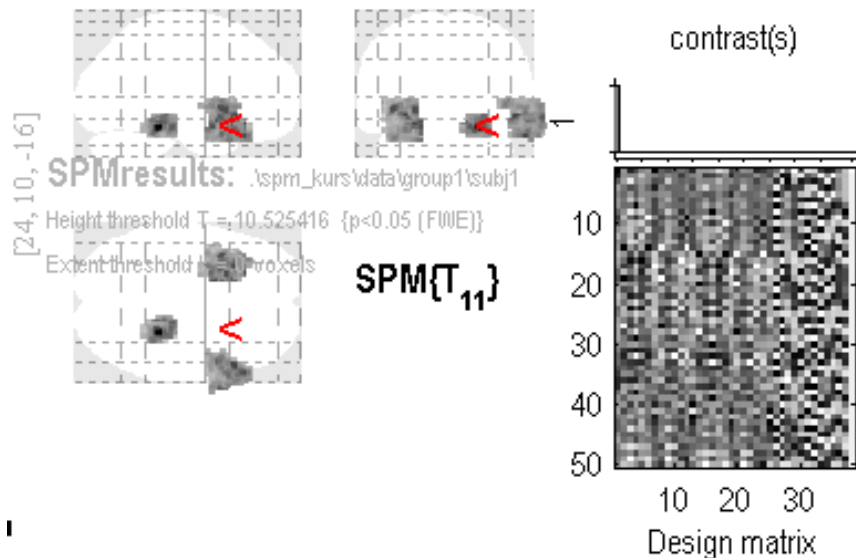
Results (first level)

Thalamic BOLD regressor
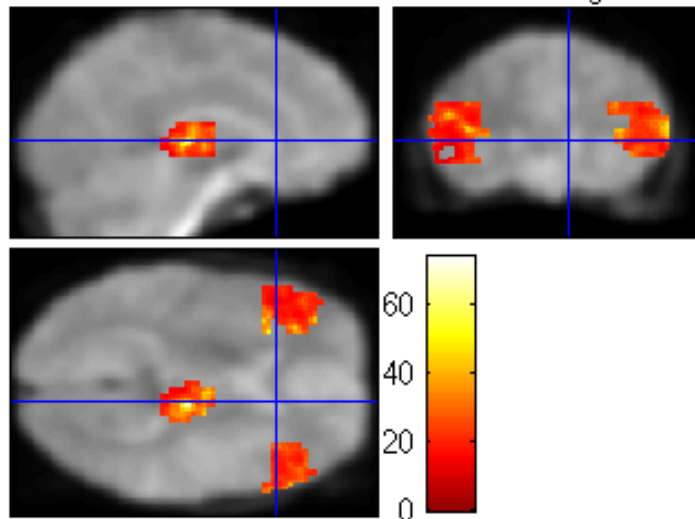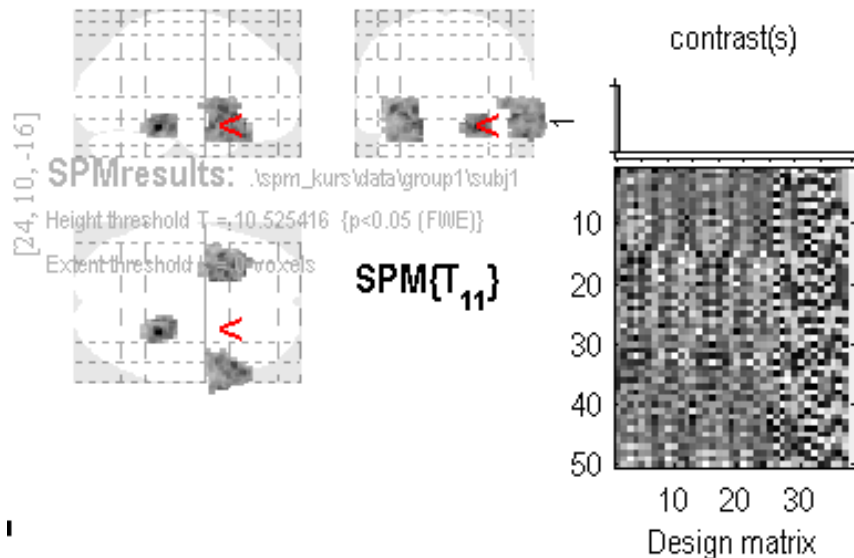
Cardiac regressor
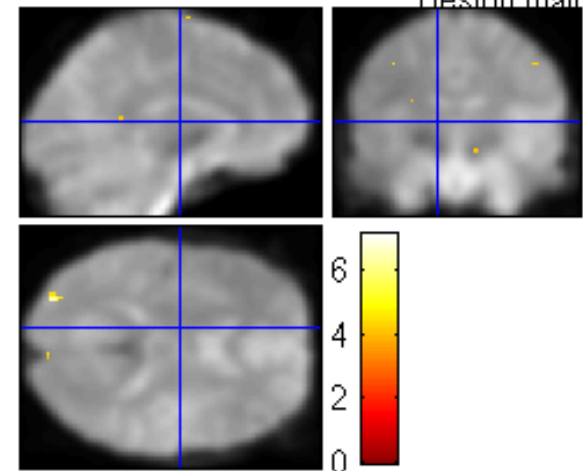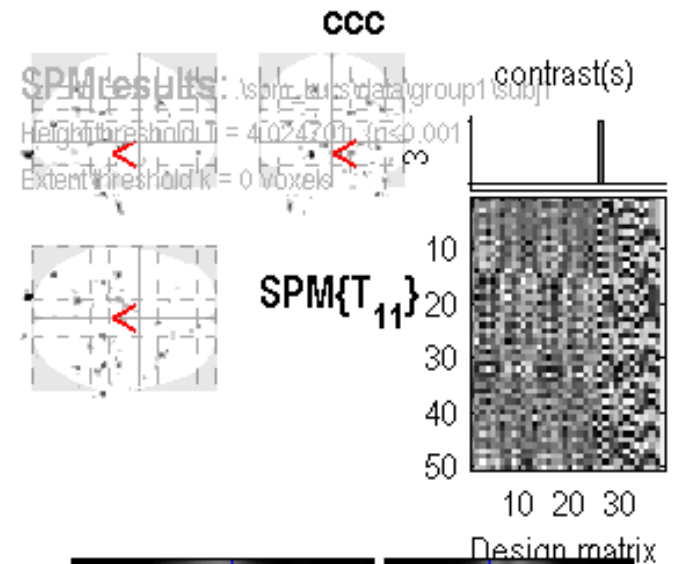
Second level analysis
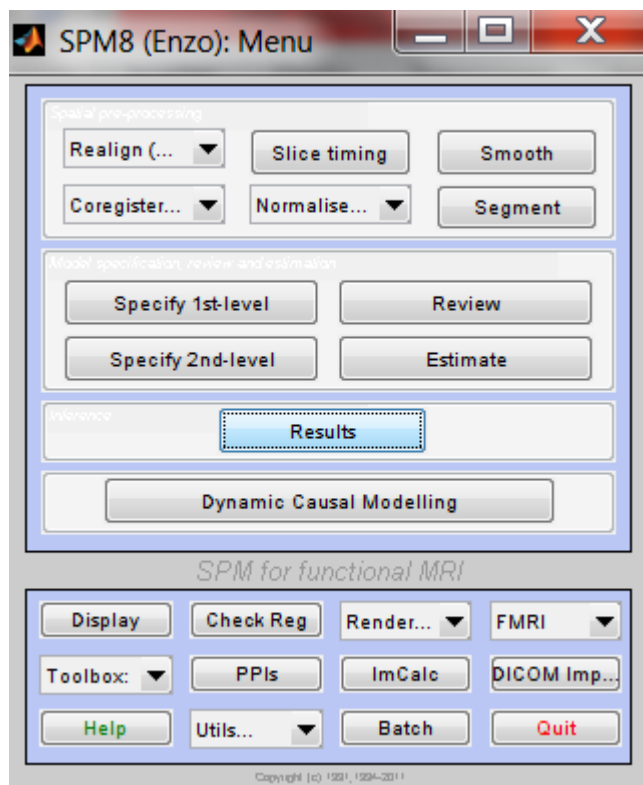
Second level analysis

Select two-sample t-test and the Beta_0001.img as „scans" for each subject in group1 and group 2
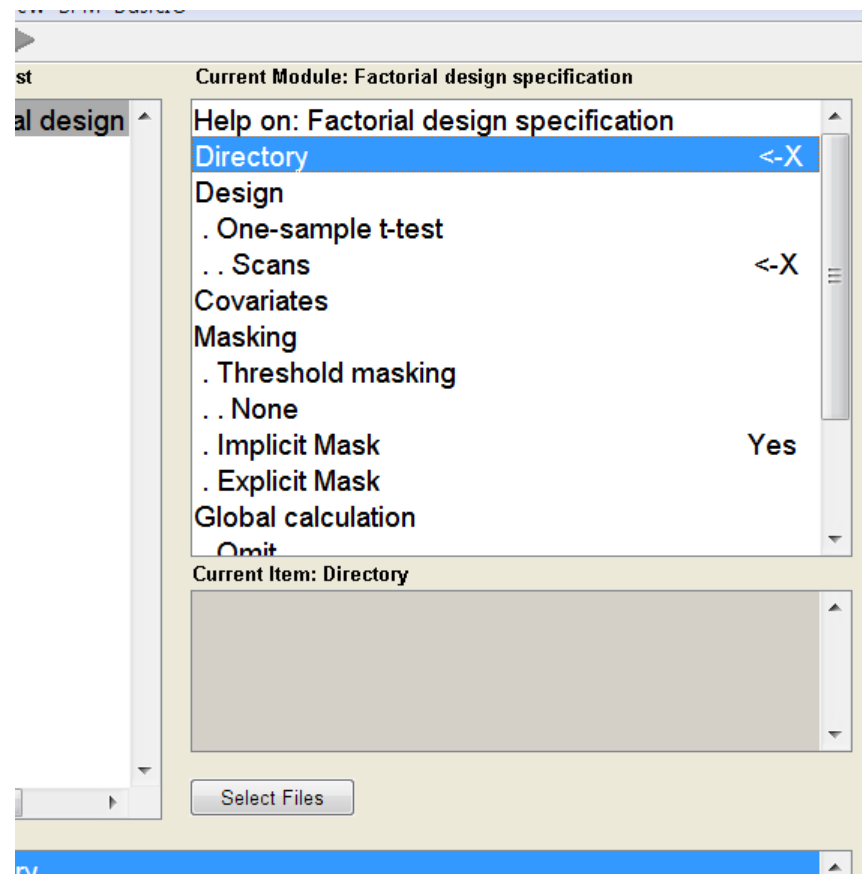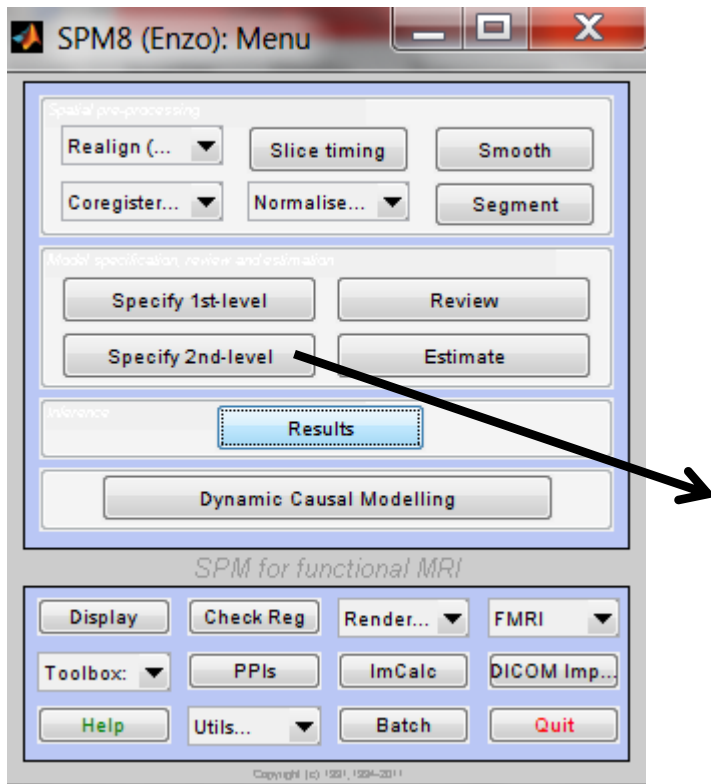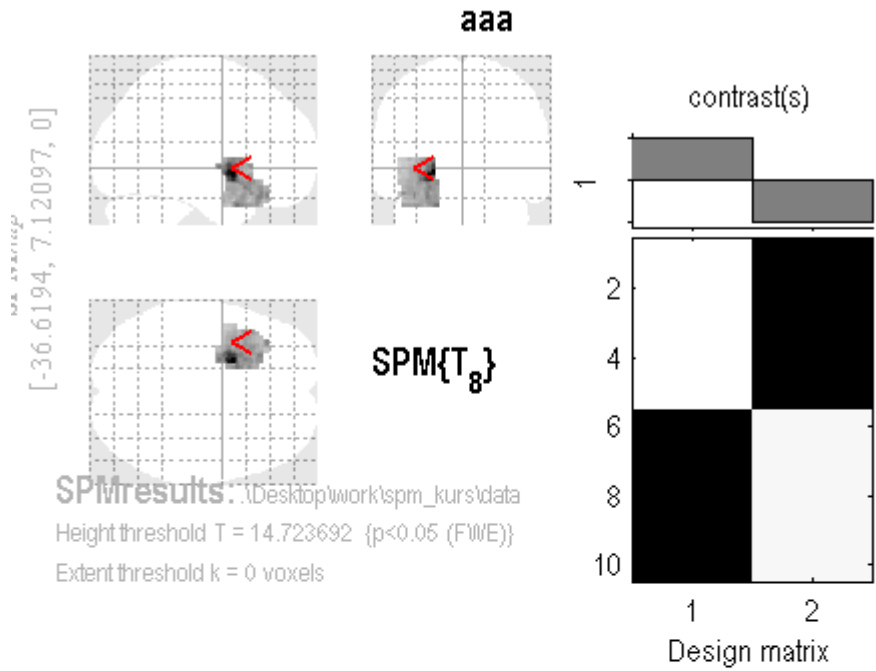
Results (second level)

group 1 > group 2
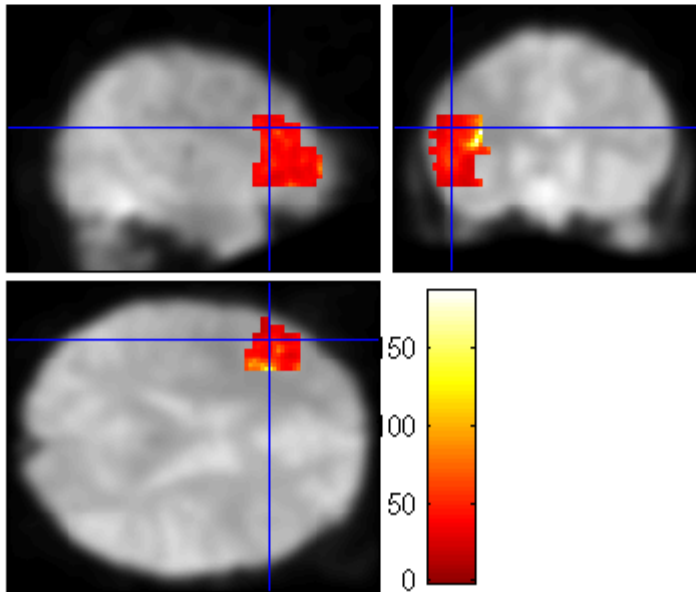
Interim summary

- Seed correlation can be performed in SPM by introducing a BOLD regressor in the Design Matrix, together with other physiological and motion confounds

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Interim summary

- Seed correlation can be performed in SPM by introducing a BOLD regressor in the Design Matrix, together with other physiological and motion confounds

- At the group level, seed correlation can be used to detect functional connectivity between populations (increasingly used as a candidate „biomarker" for disease)

- When used that way, it is very important to account for all possible confounds specific to each population (e.g., drugs? Depression? Motion? Vigilance?????)

What else to do…

- Play with seed correlation in this dataset (for example, using different seeds)

Second part: Dynamic RS-FC

Assumptions behind RS-FC

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- Empirically we know this is **NOT** the case.

- Correlations computed over sliding windows reveal changes in functional connectivity over time.

Sliding window advances one volume at a time

fMRI BOLD extracted from brain parcellation (AAL - 90 regions)

Sliding windowed BOLD correlation

Tagliazucchi et al., Front. Neurosci 2012
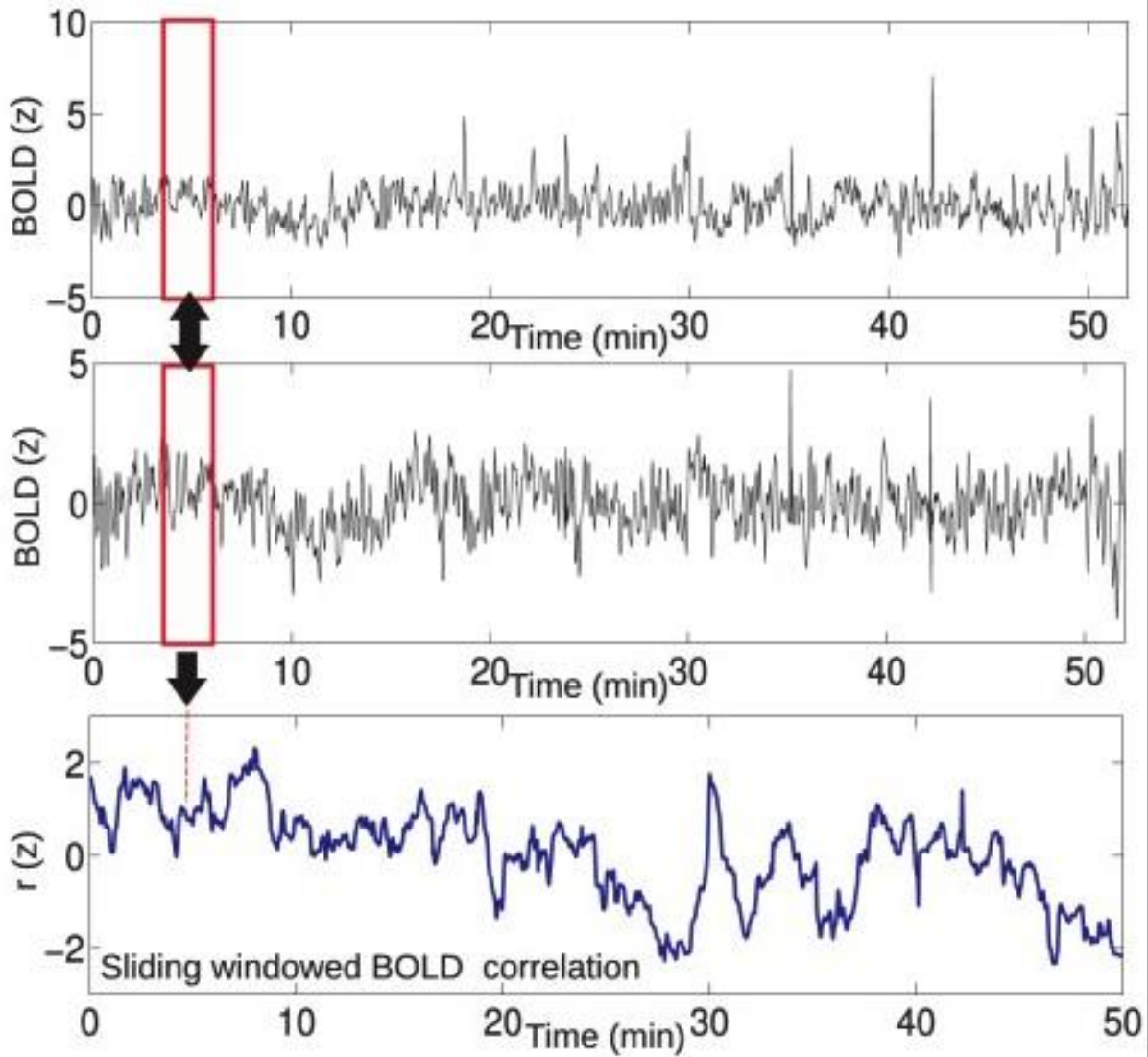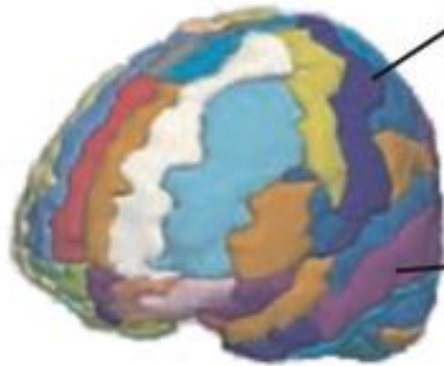
This computation is repeated for each pair of the 90 regions, giving a correlation matrix for each point in time. The correlation matrix is always symmetric and has in its *i-th* and *j-th* entry the correlation between the *i-th* and *j-th* regions.

Region i

Region j
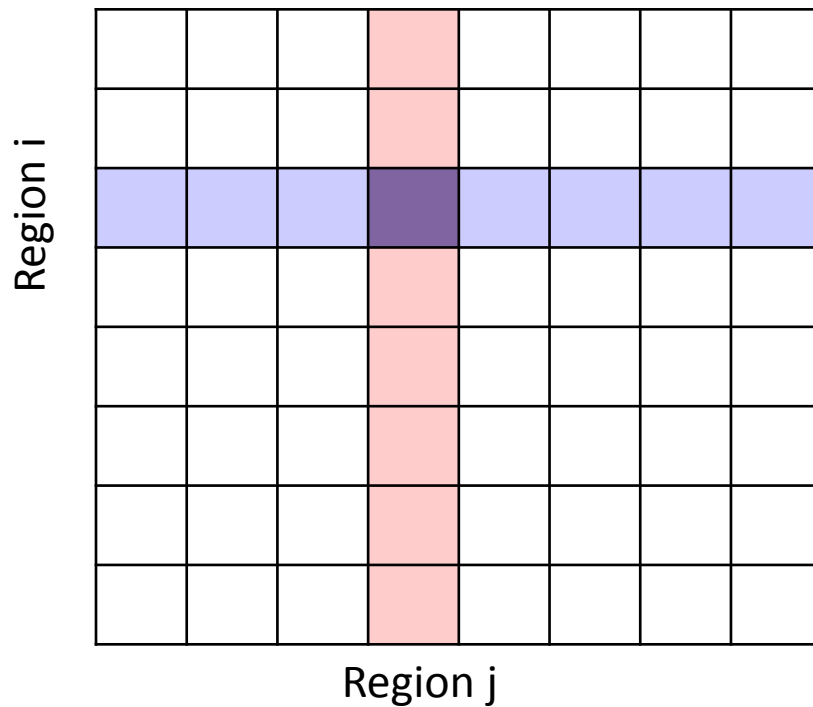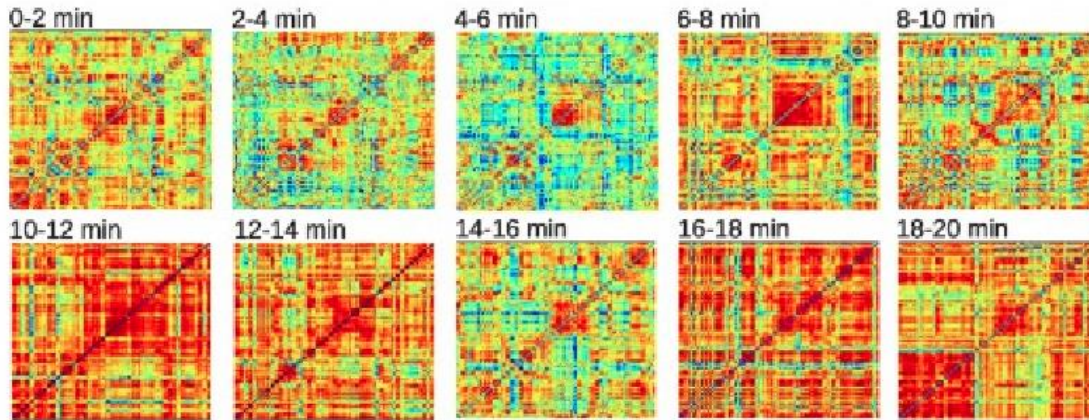
Correlation matrices are very different when computed over different moments in time
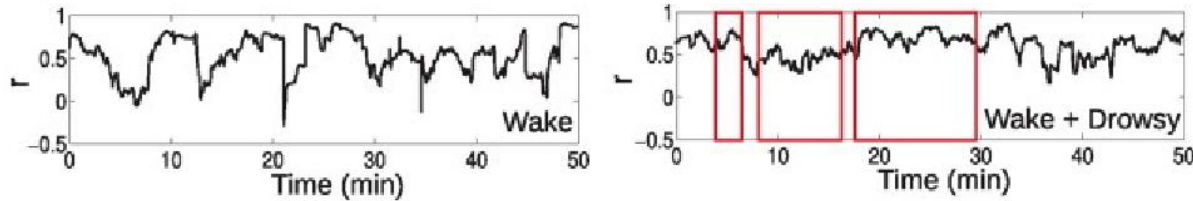
This variability in FC may reflect processes that become active at different times during rest, the results of drowsiness and vigilance loss, mind wandering and self-generated thought, etc.
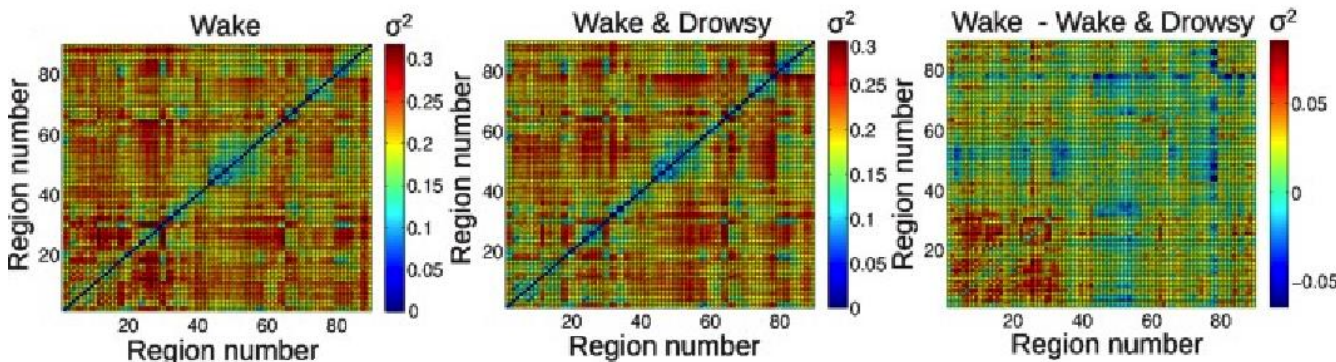
How to disentangle dynamic RS-FC?

- We compute FC (correlation matrices) over time using a sliding window of certain length

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- We investigate the variability in connectivity time courses over time (FC variance)

- We look for differences between groups of subjects.

Simulated data
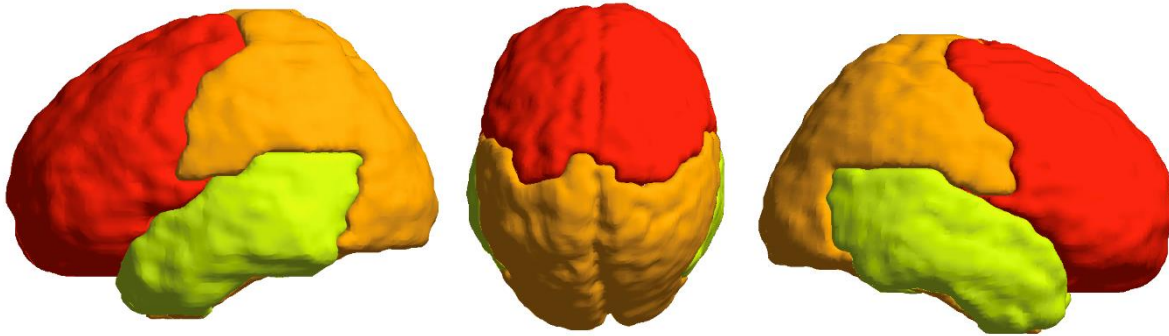
The 90 AAL regions are divided into three groups of regions

Simulated data
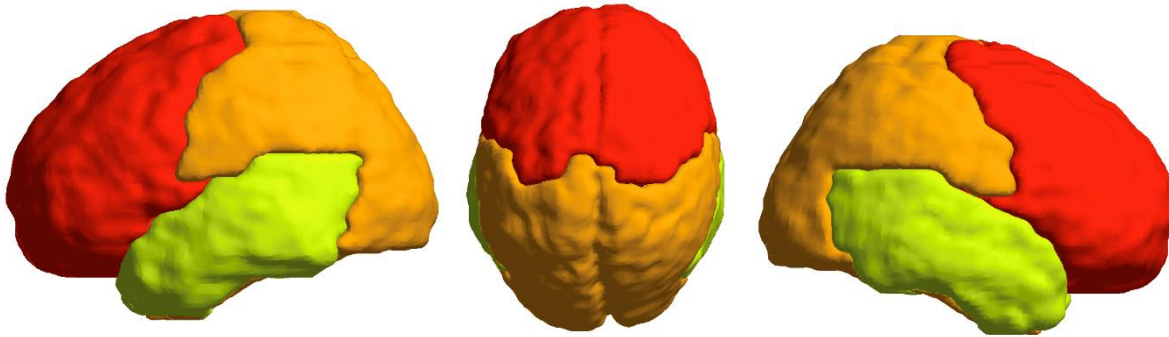
The 90 AAL regions are divided into three groups of regions

Two FC states are alternated randomly. Each region is connected to itself (R=1), anticorrelated with another region (R=-1) and not correlated with the remaining two (R=0)

State 1　　　　　　**State 2**

R=0

R=-1

Simulated data

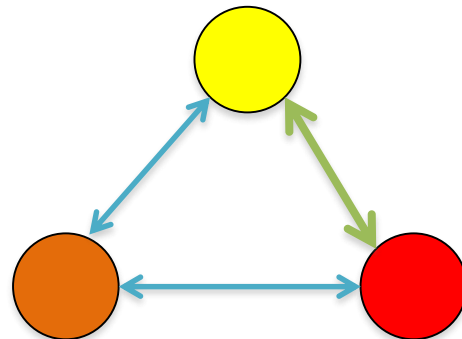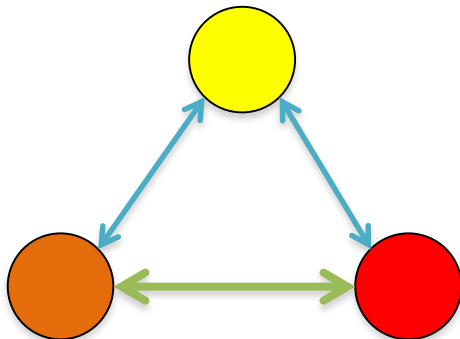
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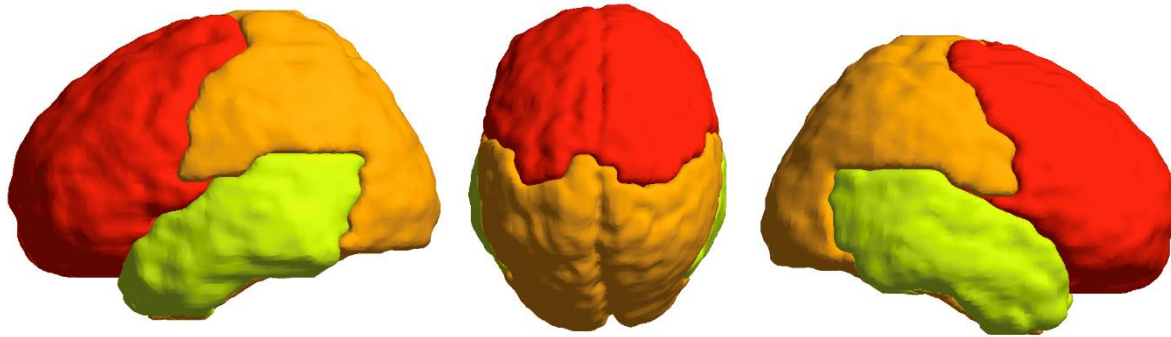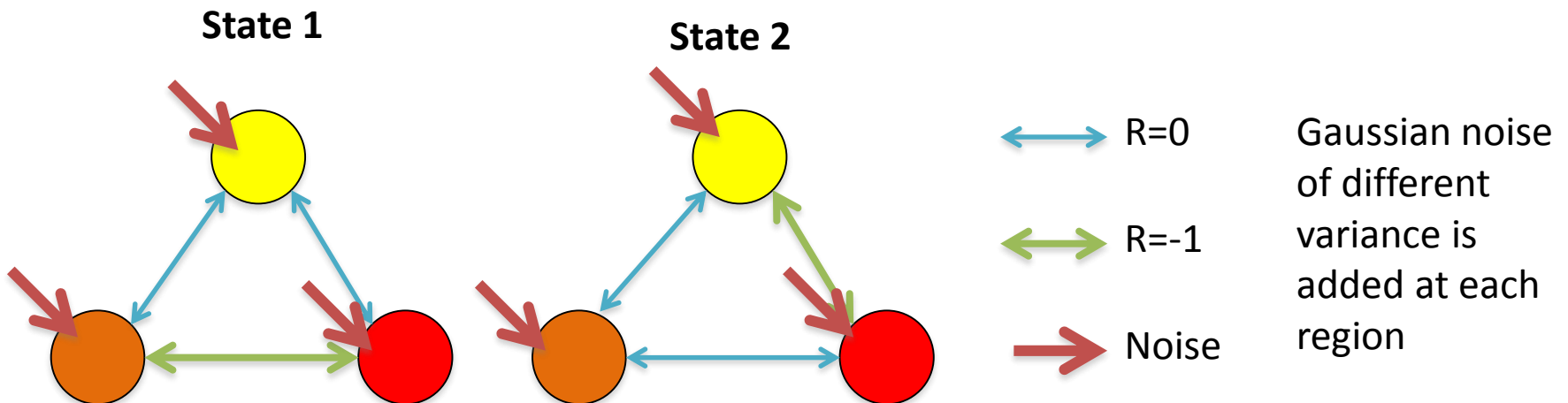

State 1

State 2

R=0

R=-1

Noise

Gaussian noise of different variance is added at each region

Parameters

How many subjects per group

How long do states last in group 1

How many times state 1 appears in both groups

How many times state 2 appears in both groups

```
num_1 = 5; % number of subjects in group 1
num_2 = 5; % number of subjects in group 2

duration_1 = 50; % duration of transient connectivity states in group 1
duration_2 = 50; % duration of transient connectivity states in group 2

num_state_1_1 = 4; % number of "state 1" connectivity state in group 1
num_state_1_2 = 4; % number of "state 1" connectivity state in group 2

num_state_2_1 = 4; % number of "state 2" connectivity state in group 1
num_state_2_2 = 4; % number of "state 2" connectivity state in group 2

noise_state_1_1 = [0.1, 0.1, 0.1]; % noise vector for state 1 in group 1
noise_state_1_2 = [0.1, 0.1, 0.1]; % noise vecotr for state 1 in group 2

noise_state_2_1 = [0.1, 0.1, 0.1]; % noise vector for state 2 in group 1
noise_state_2_2 = [0.1, 0.1, 0.1]; % noise vecotr for state 2 in group 2

window_length = 20; % window length for the sliding correlation analysis
num_clus = 2; % number of clusters for the separation into connectivity states
```
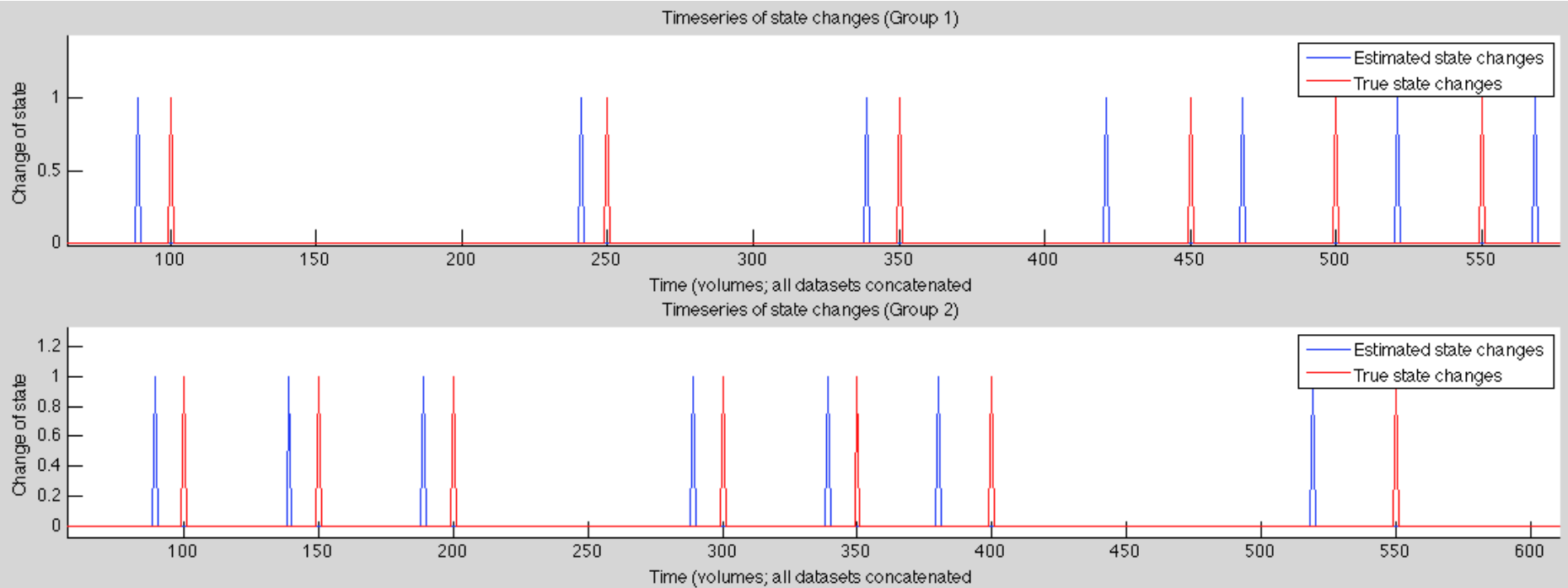
Noise definition

Analysis parameters

Output

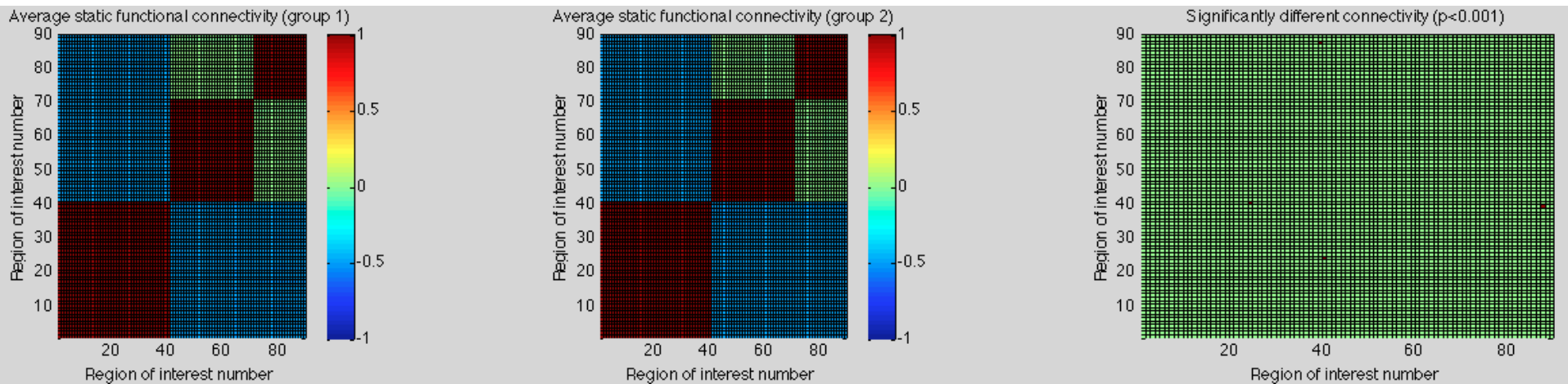Time series of state changes: the „spikes" represent that a change of connectivity state occured at the time.

In this example the estimated state changes (blue) are close to true state changes (red), but generally earlier (why?)

Output

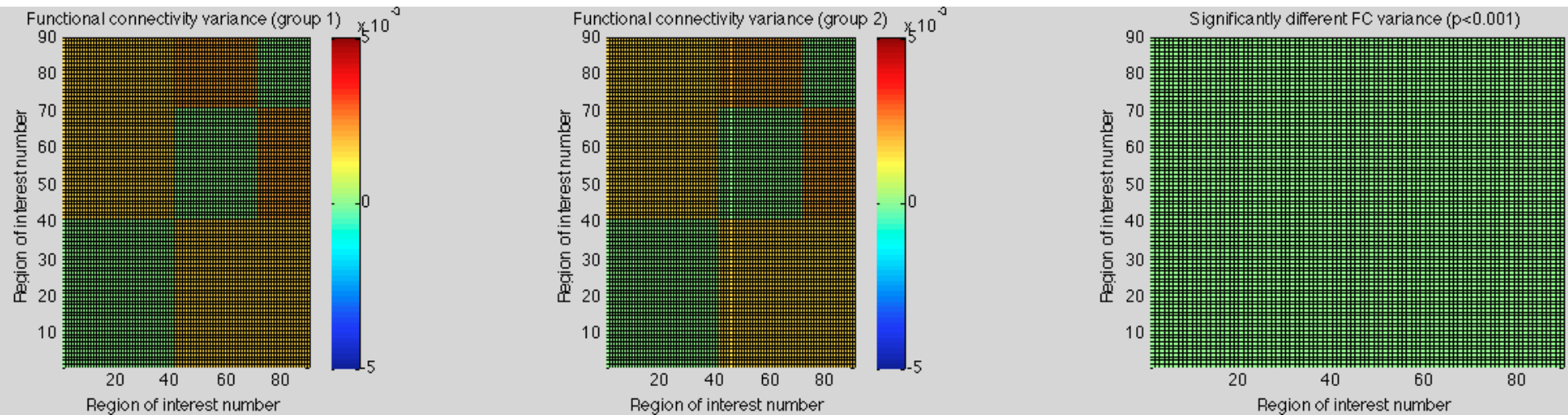Correlation matrices computed over the whole session for both groups.

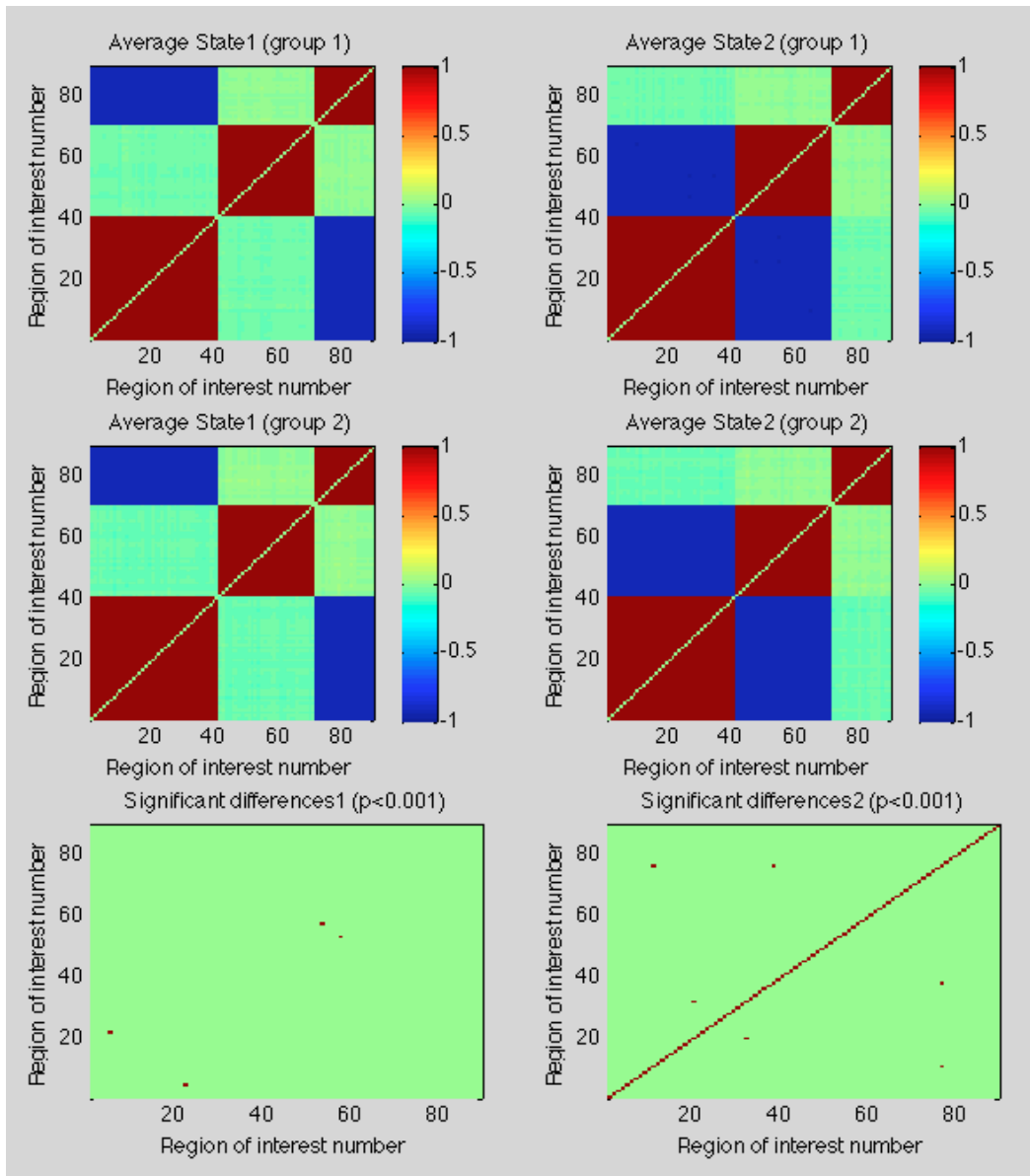**If the noise variance at each region is equal between groups, there are no differences.**

Output

Variance of the dynamical connectivity between each pair of regions

If the number of states and their duration is the same between groups, there are no differences in the variance (i.e. no group „switches" state more often than the other)

Output

Connectivity states estimated by the clustering algorithm with k = 2

If the noise is equal between groups, no significant differences are found.

Note that the ordering of the states identified by the algorithm do not need to match the ordering of what we called „state 1" and „state 2" (the ordering of the output is arbitrary).

What happens if the noise is different between groups?
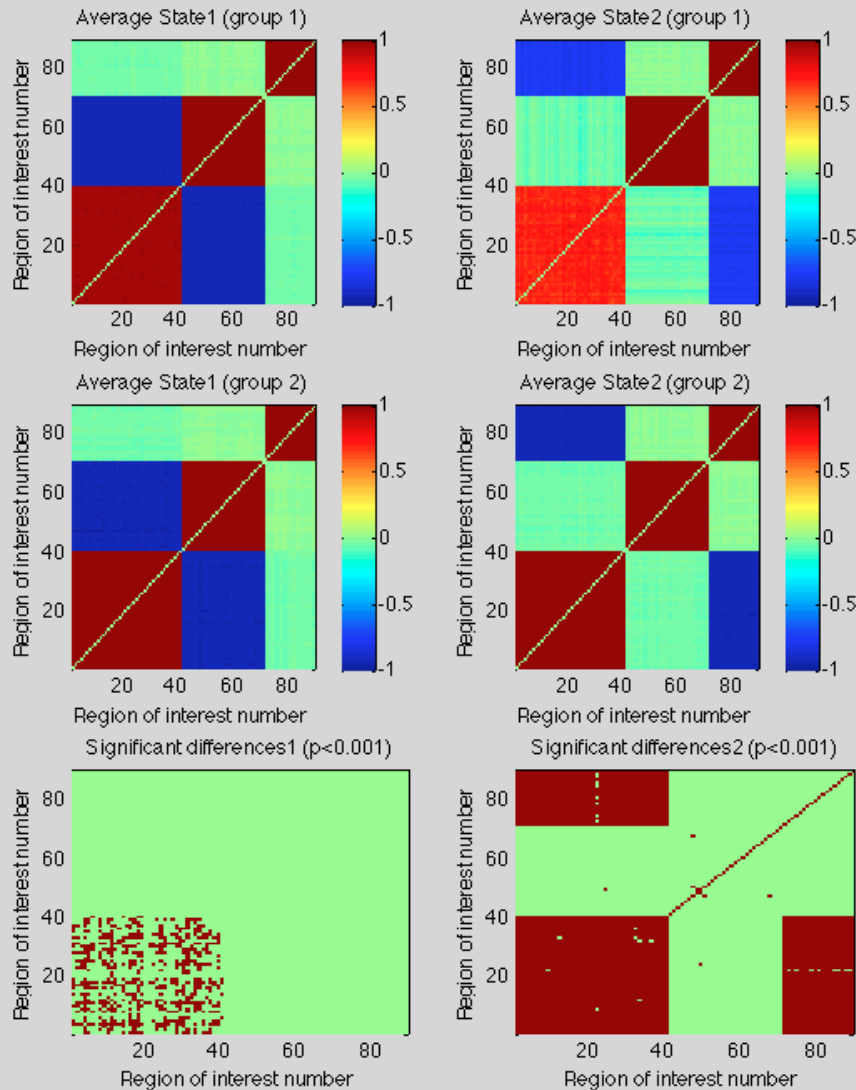

```
noise_state_1_1 = [0.5, 0.1, 0.1];
noise_state_1_2 = [0.1, 0.1, 0.1];

noise_state_2_1 = [0.1, 0.1, 0.1];
noise_state_2_2 = [0.1, 0.1, 0.1];
```

More noise in region 1 of state 1 in group 1 reduced the connectivity of region 1 with itself, and with its anticorrelated region.
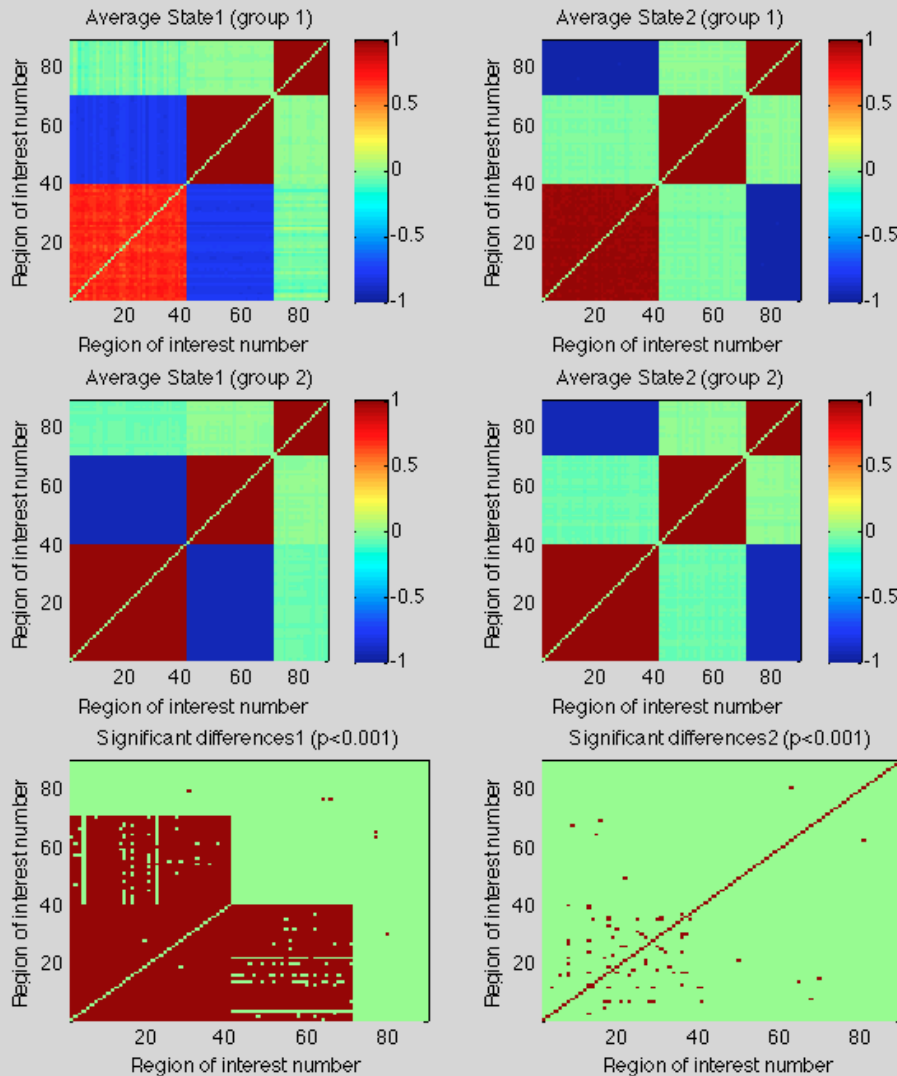
What happens if the noise is different between groups?


```
noise_state_1_1 = [0.1, 0.1, 0.1];
noise_state_1_2 = [0.1, 0.1, 0.1];

noise_state_2_1 = [0.5, 0.1, 0.1];
noise_state_2_2 = [0.1, 0.1, 0.1];
```

More noise in region 1 of state 2 in group 1 reduced the connectivity of region 1 with itself, and with its anticorrelated region.

What happens if the switching between states is more frequent in one group?

```
duration_1 = 100; % duration of transient connectivity states in group 1
duration_2 = 40;  % duration of transient connectivity states in group 2

num_state_1_1 = 1;  % number of "state 1" connectivity state in group 1
num_state_1_2 = 10; % number of "state 1" connectivity state in group 2

num_state_2_1 = 3;  % number of "state 2" connectivity state in group 1
num_state_2_2 = 10; % number of "state 2" connectivity state in group 2
```
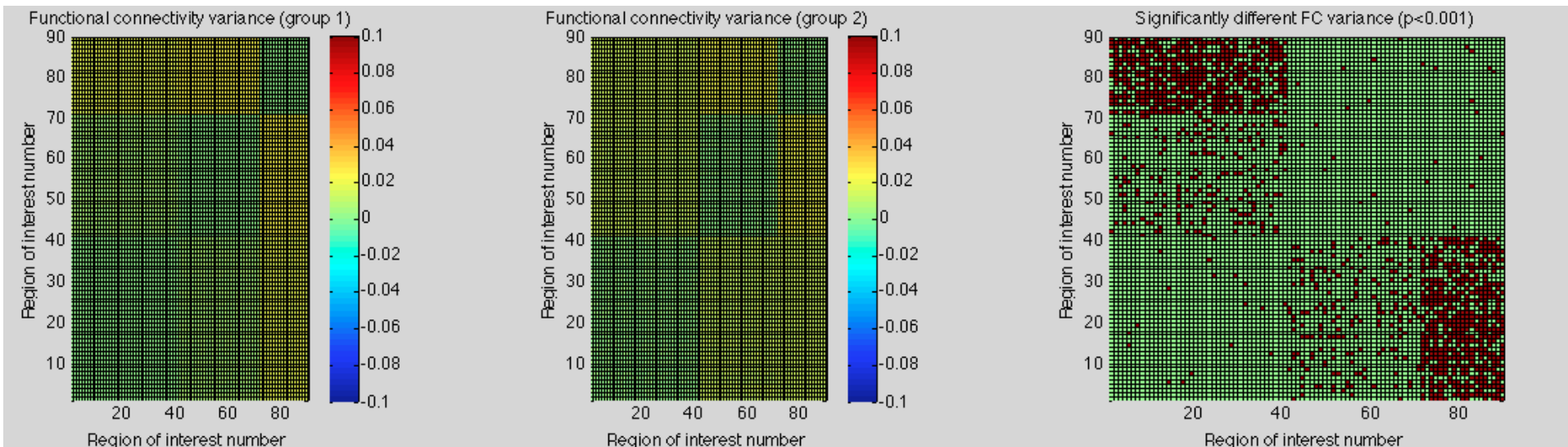
List of possible things to try out:

- Use different levels of noise in different regions/states/groups and see what happens.

- Are the differences in FC connectivity of the states always followed by changes in static FC?

- What happens if you use more than two clusters? E.g. 3 or 4 clusters.

- What happens if one group switches states and the other doesn't?

- Try this out with your own data! You need to extract the timeseries from the AAL template and use them as inputs to dynamical_FC_analysis_main.m

Limitations

- When we simulate the data, we know the number of clusters *a priori* but if this is not known in real data, one should try different values or use a criterion (e.g. GAP statistic, "Elbow" method, etc.)

- *If the groups have different number of states, the pairwise comparison between states is not possible.*

- *Even if FC doesn't really have different states, the clustering algorithm will find clusters nevertheless.*