

First level fMRI data analysis

Turku PET Centre Brain Imaging Course 2025

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Outline

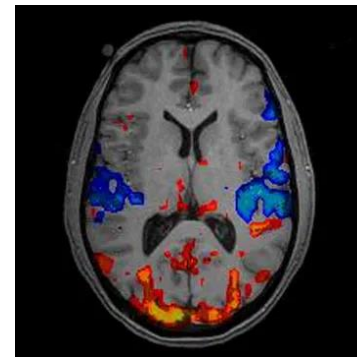
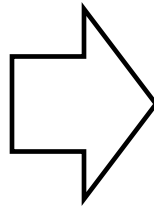
- What is 1st level analysis?
- Statistical models
- Contrasts
- Examples for illustration
 - Operation in SPM12
 - Reading the results, e.g., the contrasts

What is 1st level analysis?

- fMRI data analysis has two levels: 1st (within-subject) and 2nd (group-level).



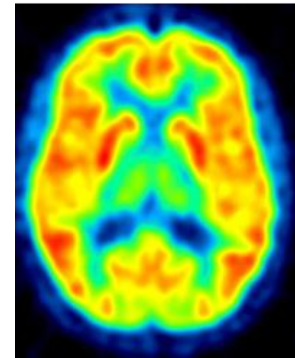
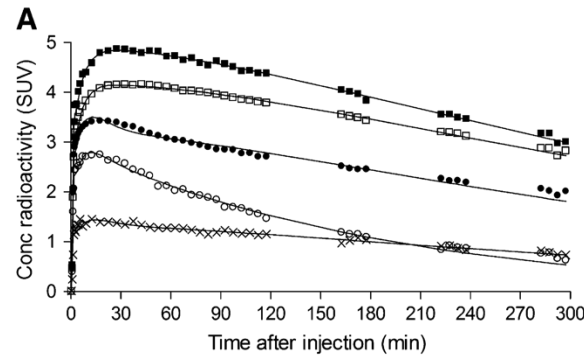
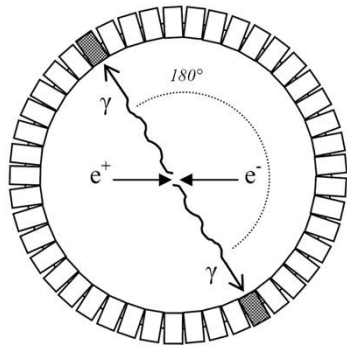
One subject's raw T2* data



The subject's statistical map

Within-subject: comparison between PET and fMRI data

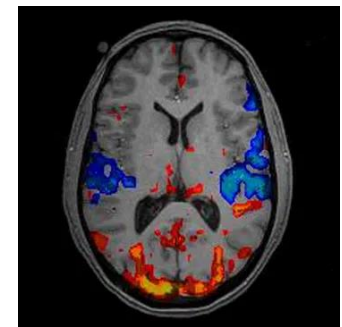
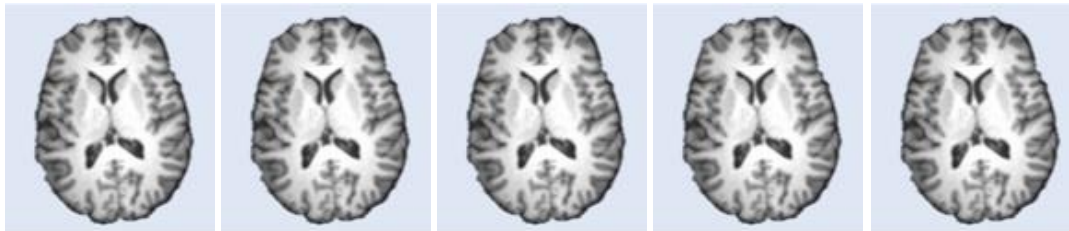
1. Traditional PET



Density
e.g., glucose uptake

2. fMRI

Time



Statistical map

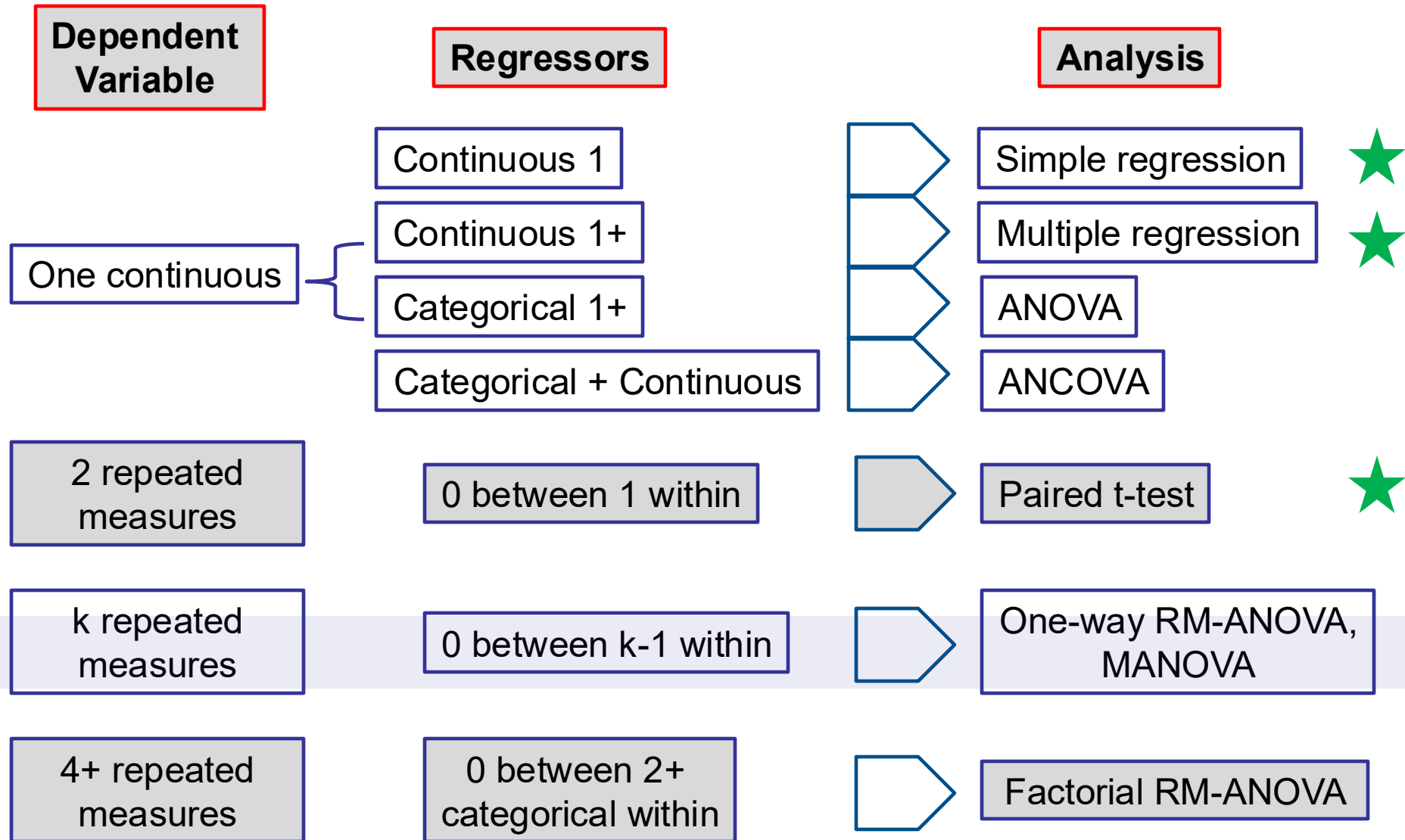
Task



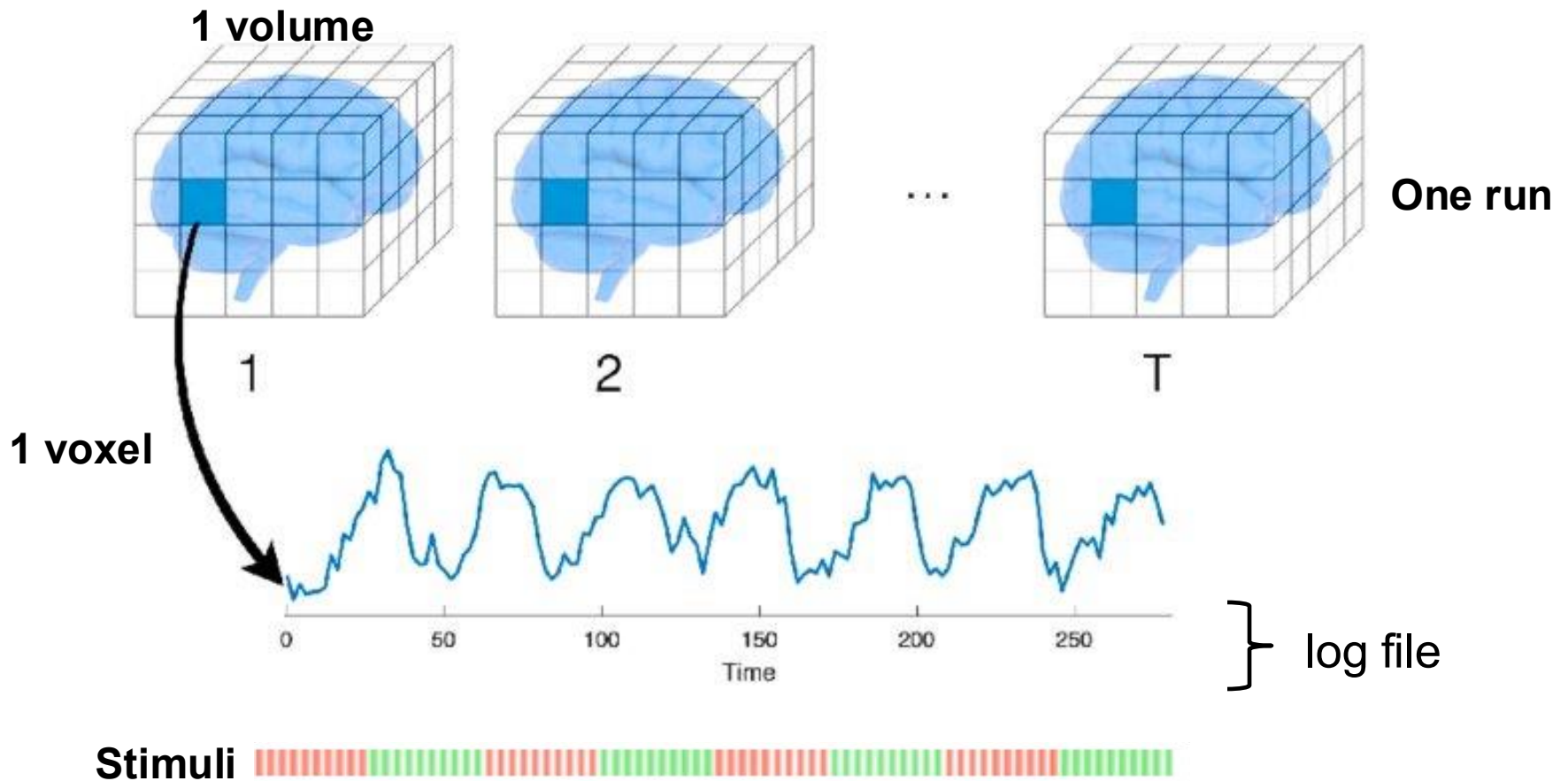
Why is 1st level analysis?

- BOLD signals are dominated by noise:
physiological factors (breathing, heart rate), head movement, scanner instabilities, magnetic susceptibility artifacts, and neuronal variability, leading to low signal-to-noise ratio
>> preprocessing NOT enough!
- Multiple repeated exposure to stimuli to increase signal-to-noise ratio.
- Statistical analysis to extract the signal-associated brain responses **>> statistical map**

The general linear model (GLM) family

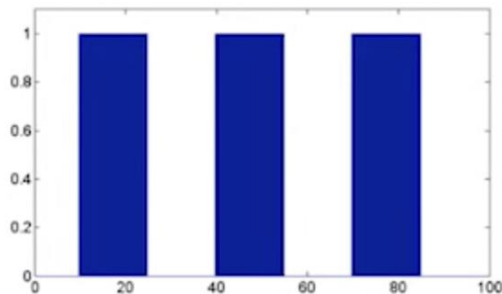


Voxel-level data modelling

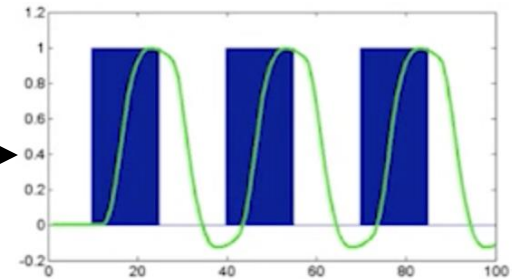
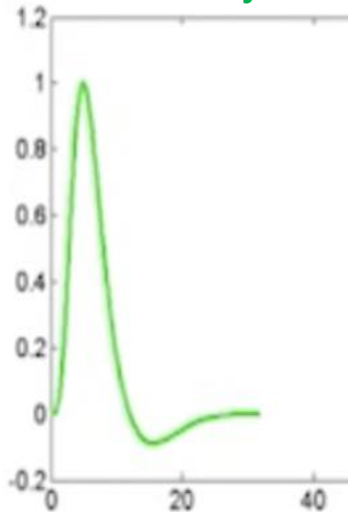


- BOLD responses are delayed: **peak at 4-6 s** and **baseline 20-30 s**.
- Convolved with the hemodynamic response function (**HRF**)
- The linear time-invariant (**LTI**) system

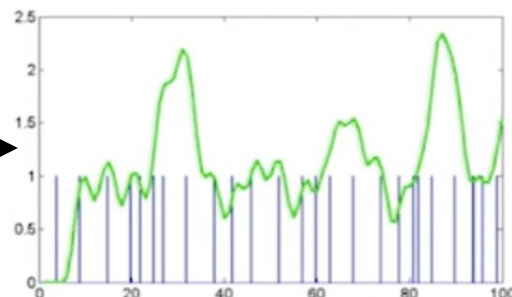
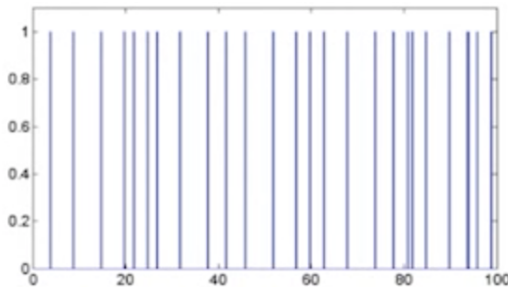
Block design



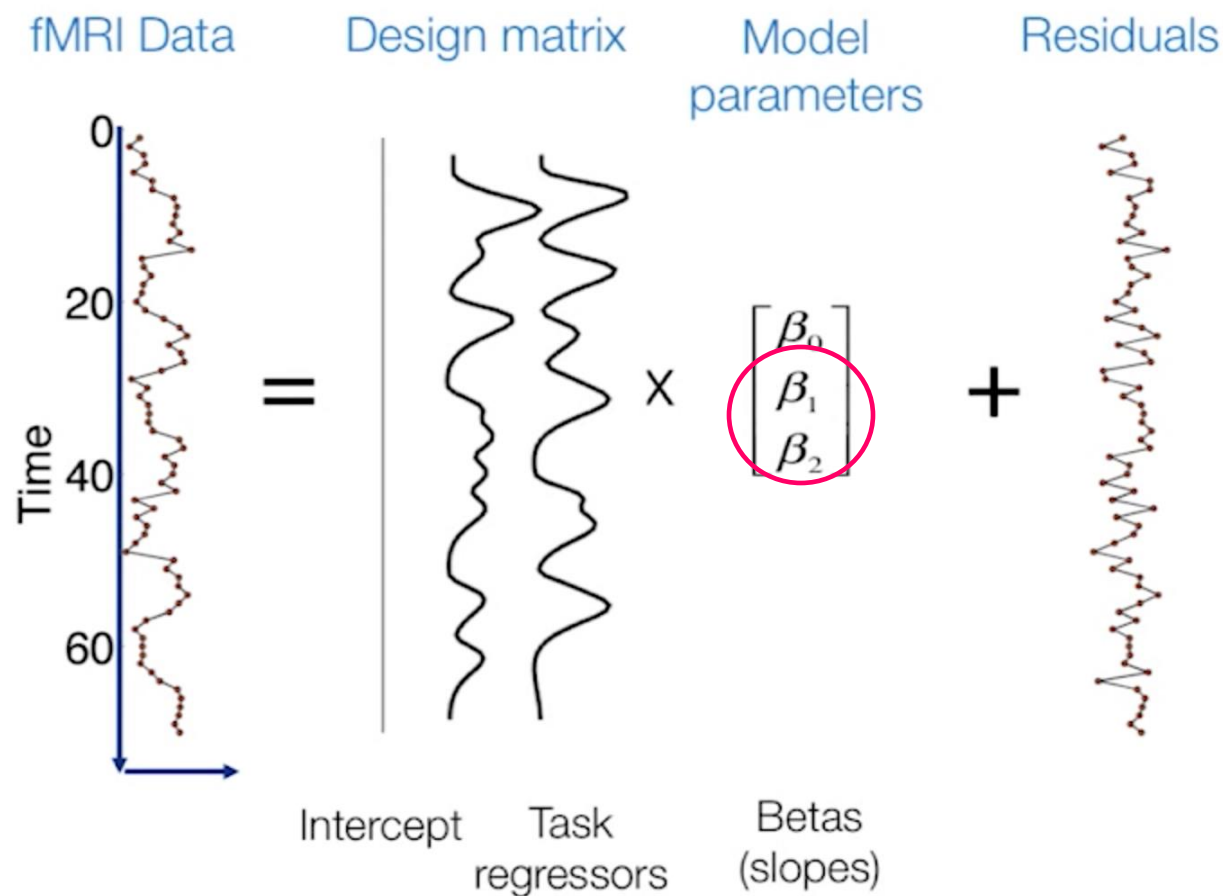
Hemodynamic
delay



Event-related design



Event-related design: two continuous regressors (laughter vs. scrambled laughter regressors)



Four example studies

1. Social laughter experiment
2. Naturlistic movie stimuli
3. Repeated measure food-reward responses
4. Resting states

The laughter experiment

Laughter is a contagious behavioural stimulus that is commonly used to study social brain functions. We have studied the social brain functions of participants with high psychopathy or autism traits.

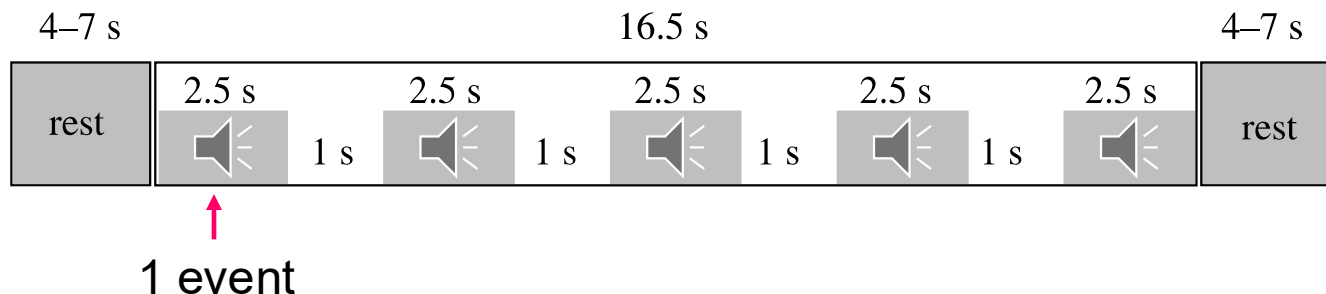
>> Sun L., et al., *Cerebral Cortex*, Volume 33, Issue 2, 15 January 2023

Four stimuli types:

Laughter / Crying vocalization /

Scrambled laughter / Scrambled Crying

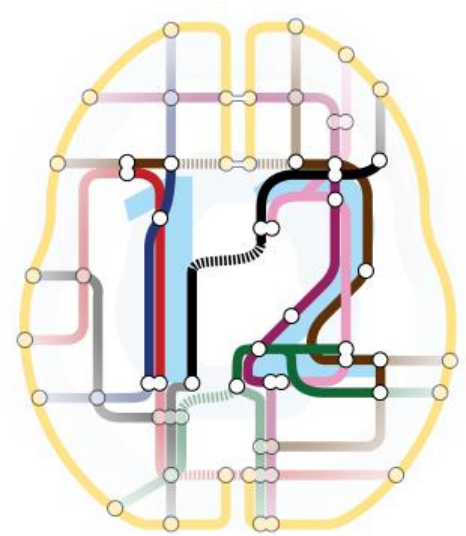
Block (also event-related) design



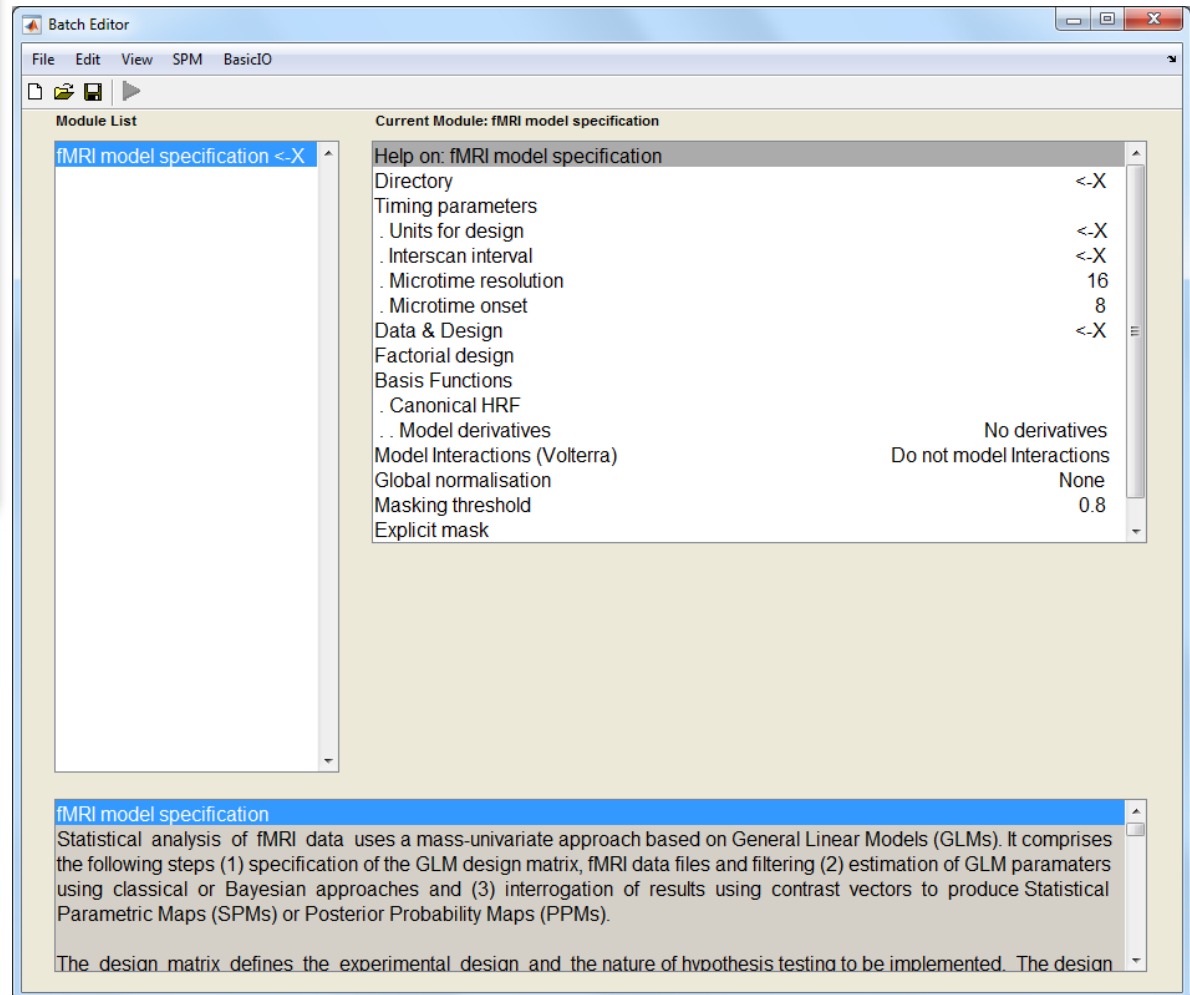
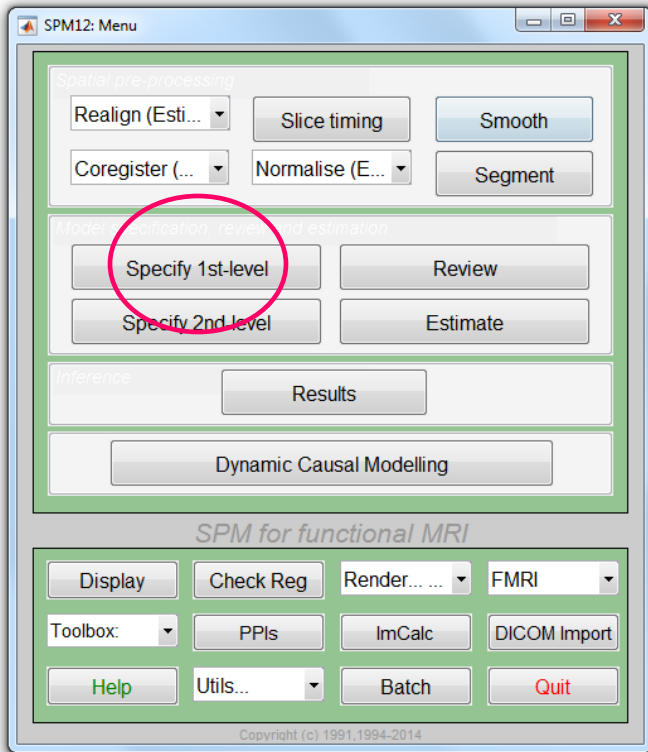
The 16.5 s block contains 5 Laughter, crying, or scrambled sound clips.

SPM12

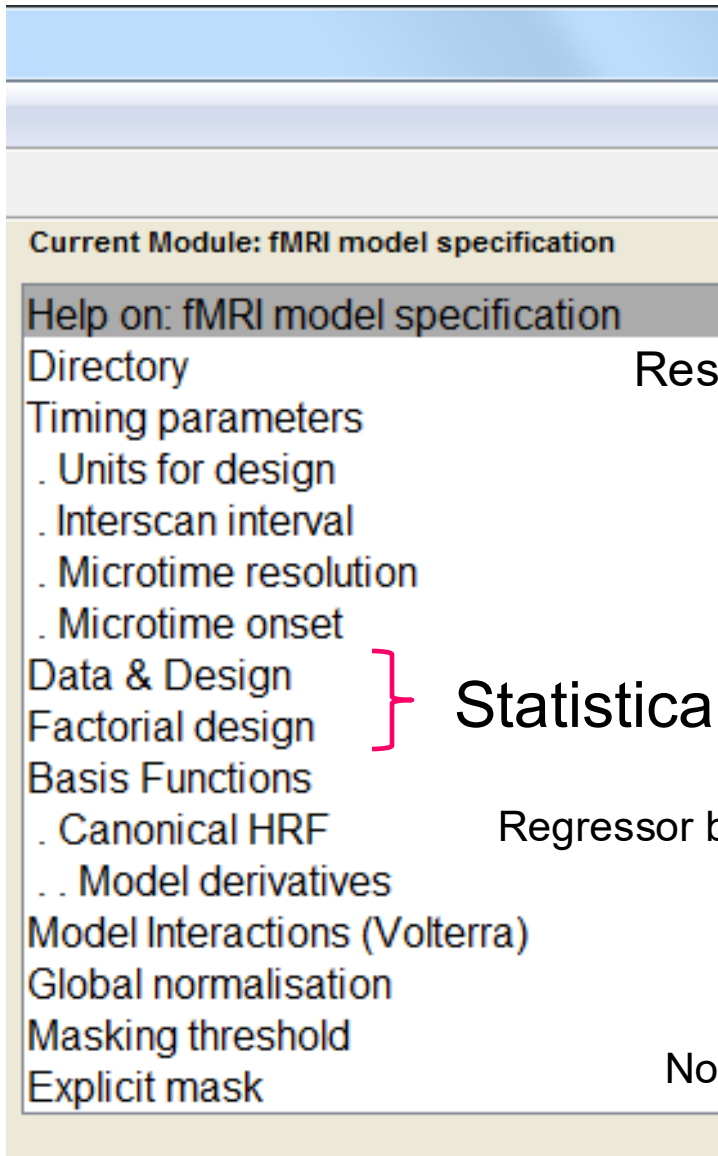
- SPM - theoretical concepts of Statistical Parametric Mapping in a complete analysis package.
- Run in matlab
- See more information:
<https://www.fil.ion.ucl.ac.uk/spm/software/spm12/>



Operation in SPM12



SPM12: parameter setting



Current Module: fmri model specification

Help on: fmri model specification

Directory Result to store

Timing parameters

. Units for design seconds

. Interscan interval 2.6

. Microtime resolution

. Microtime onset

Data & Design } Statistical model

Factorial design

Basis Functions

. Canonical HRF Regressor building

. . Model derivatives

Model Interactions (Volterra) }

Global normalisation

Masking threshold

Explicit mask No masks

Basic settings

Statistical model

Leave as default,
or specify if necessary

Current Module: fMRI model specification

Data & Design		
. Subject/Session		
.. Scans	→ All scan volumes	<-X
.. Conditions		
... Condition		
.... Name	→ Laughter	humans
.... Onsets		<-X
.... Durations		<-X
.... Time Modulation		No Time Modulation
.... Parametric Modulations		
.... Orthogonalise modulations		Yes
... Condition		
.... Name		Crying
.... Onsets		<-X



Data estimation

Module List

Model estimation	<-X
------------------	-----

Current Module: Model estimation

Help on: Model estimation

Select SPM.mat

Write residuals

Method

. Classical

SPM12: Menu

Realign (Esti...) Slice timing Smooth

Coregister (...) Normalise (E...) Segment

Specify 1st-level Review

Specify 2nd-level Estimate

Results

Dynamic Causal Modelling

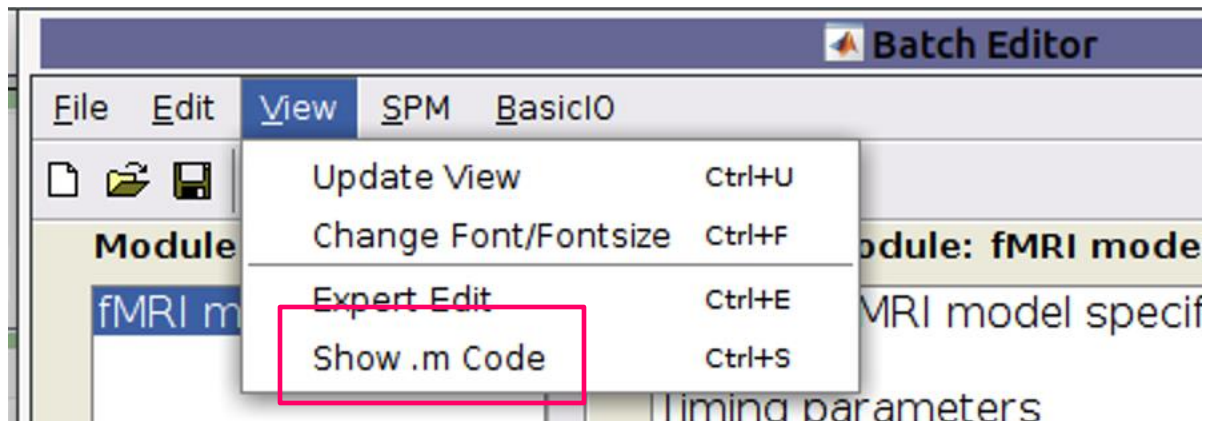
SPM for functional MRI

Display Check Reg Render... FMR

Toolbox: PPIs ImCalc DICOM Import

Help Utils... Batch Quit

Copyright (c) 1991, 1994, 2014

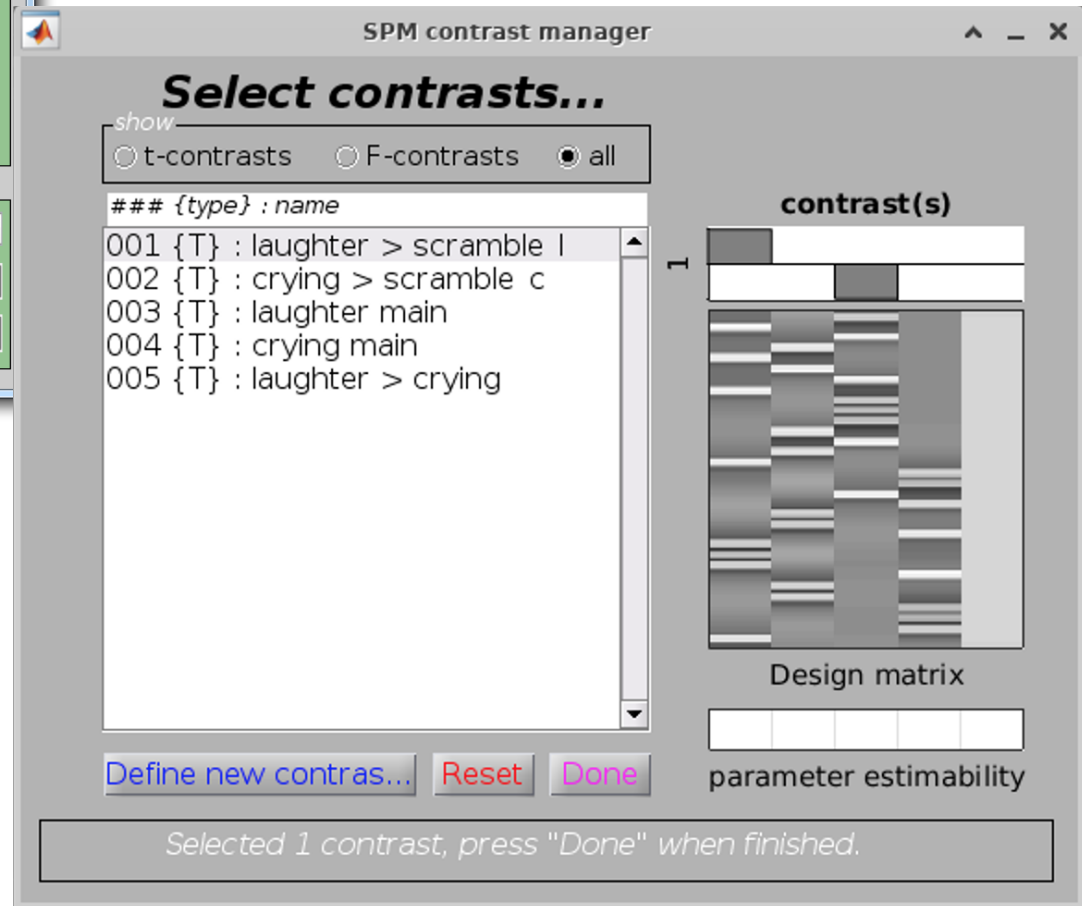
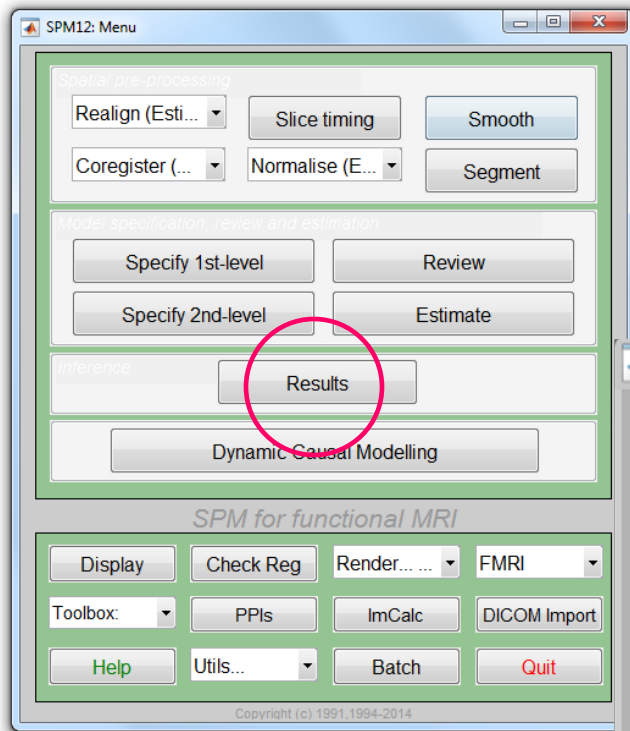


```
matlabbatch{1}.spm.stats.fmri_spec.dir = {output_directory};
matlabbatch{1}.spm.stats.fmri_spec.timing.units = 'secs';
matlabbatch{1}.spm.stats.fmri_spec.timing.RT = 2.6;
matlabbatch{1}.spm.stats.fmri_spec.timing.fmri_t = 45;
matlabbatch{1}.spm.stats.fmri_spec.timing.fmri_t0 = 23;
matlabbatch{1}.spm.stats.fmri_spec.ssess.scans = epi_files;
matlabbatch{1}.spm.stats.fmri_spec.ssess.cond(1).name = 'laughter';
matlabbatch{1}.spm.stats.fmri_spec.ssess.cond(1).onset = onset_laughter;
matlabbatch{1}.spm.stats.fmri_spec.ssess.cond(1).duration = duration_laughter;
matlabbatch{1}.spm.stats.fmri_spec.ssess.cond(1).tmod = 0;
matlabbatch{1}.spm.stats.fmri_spec.ssess.cond(1).pmod = struct('name', {}, 'param', {}, 'poly', {});
matlabbatch{1}.spm.stats.fmri_spec.ssess.cond(1).orth = 1;

matlabbatch{1}.spm.stats.fmri_spec.ssess.cond(2).name = 'crying';
matlabbatch{1}.spm.stats.fmri_spec.ssess.cond(2).onset = onset_crying;
matlabbatch{1}.spm.stats.fmri_spec.ssess.cond(2).duration = duration_crying;
matlabbatch{1}.spm.stats.fmri_spec.ssess.cond(2).tmod = 0;
matlabbatch{1}.spm.stats.fmri_spec.ssess.cond(2).pmod = struct('name', {}, 'param', {}, 'poly', {});
matlabbatch{1}.spm.stats.fmri_spec.ssess.cond(2).orth = 1;

matlabbatch{1}.spm.stats.fmri_spec.ssess.cond(3).name = 'scramble';
```

SPM12: setting contrasts

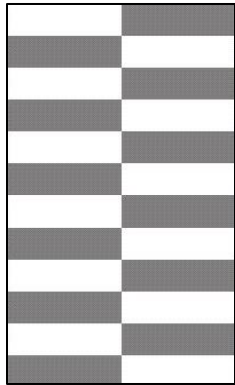


Contrasts

Experimental conditions: stimuli or interest vs. control stimuli

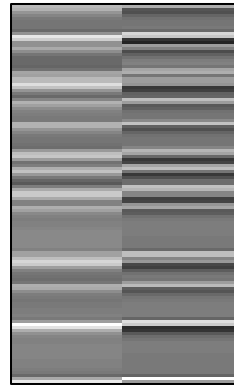
- Your interest is often the difference between the two conditions, which is “contrast”
 - You can calculate the difference, sum or separately each conditions, which are calculated by different linear contrasts.
-
- We only introduce T contrast in this lecture !!

Condition 1
Condition 2
Intercept



Block design

Condition 1
Condition 2
Intercept



Event-related design

Design matrix in
SPM 12

Difference between
conditions

$[1 \ -1]$ = “Con1 > Con2”

$[-1 \ 1]$ = “Con1 < Con2”

Separately

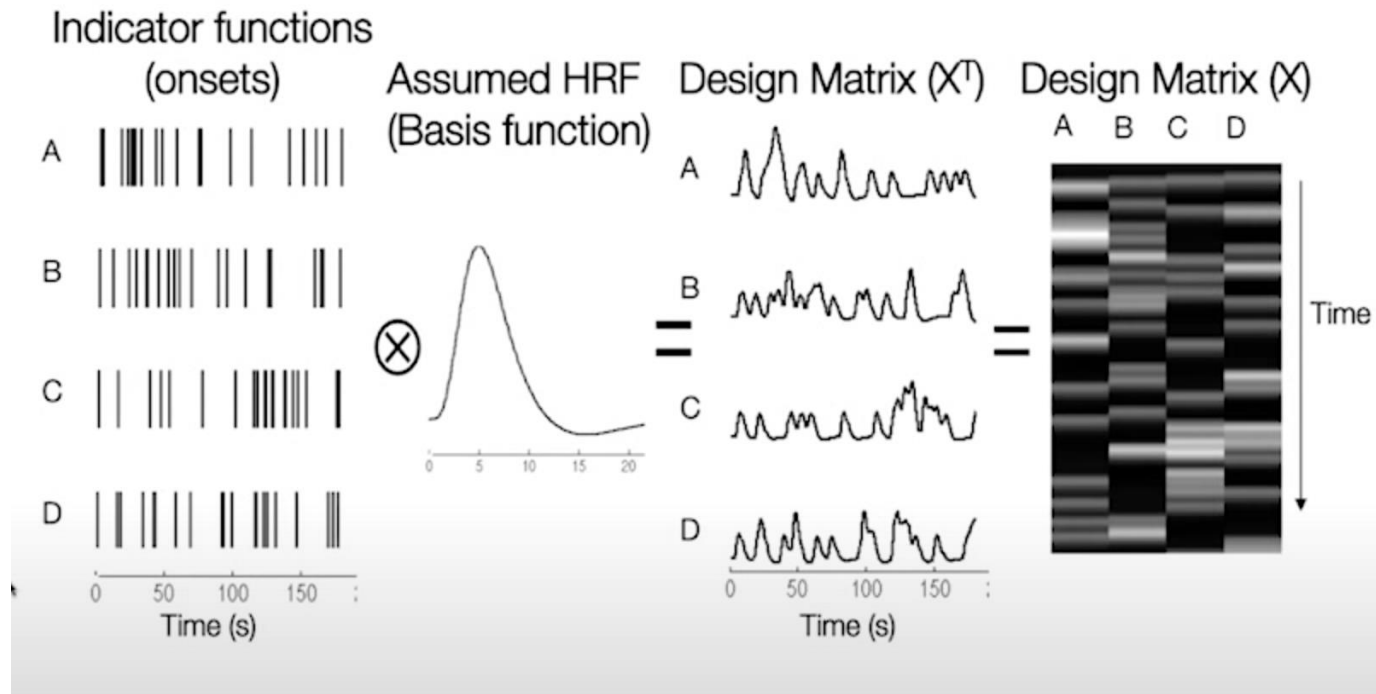
$[1 \ 0]$ or $[-1 \ 0]$ = “main effect Con1”

$[0 \ 1]$ or $[0 \ -1]$ = “main effect Con2”

Sum

$[1 \ 1]$ or $[-1 \ -1]$

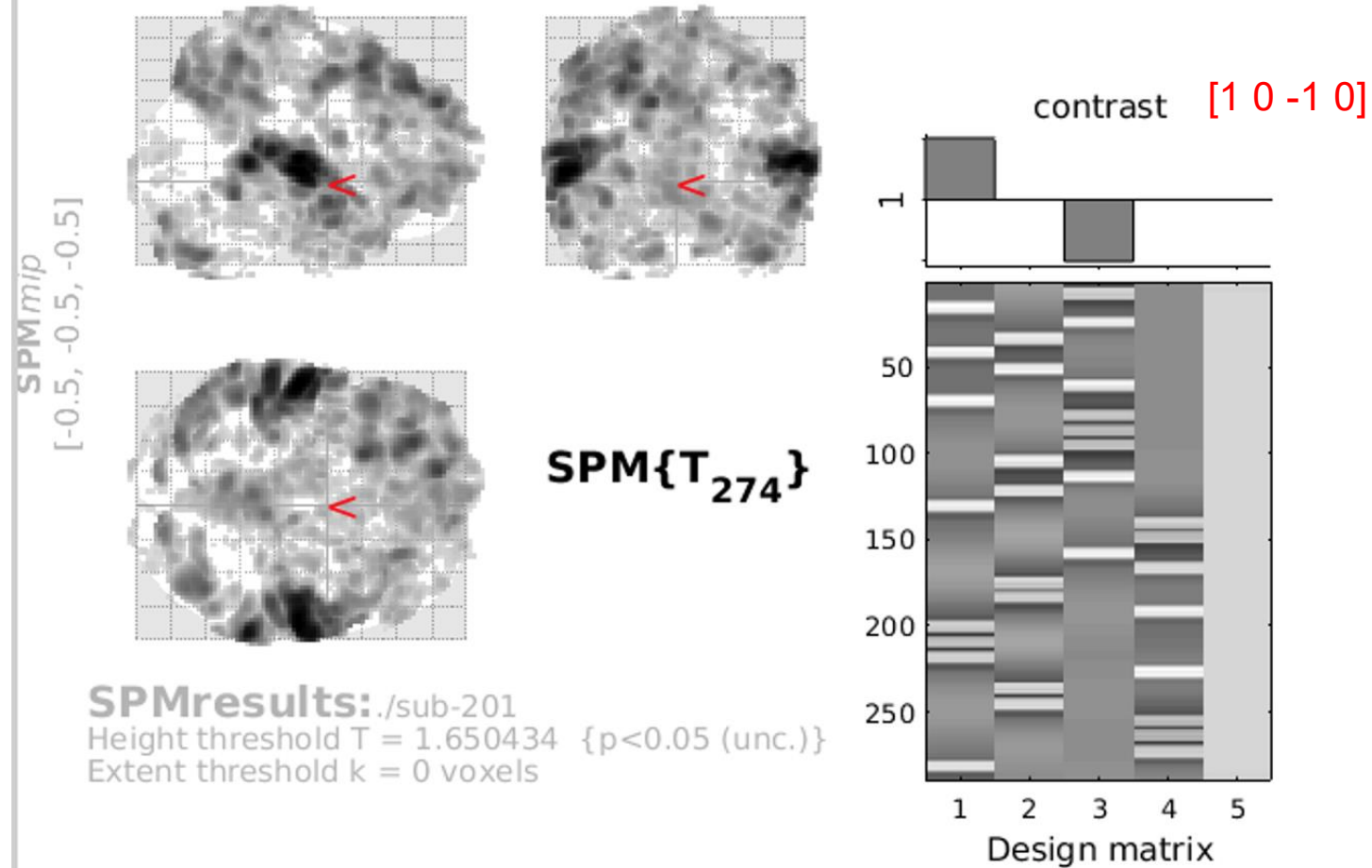
More regressors



- $[1 \ 1 \ -1 \ -1]$: $(A+B) > (C+D)$
- $[1 \ -1 \ 1 \ -1]$: $(A+C) > (B+D)$
- $[1 \ 0 \ 0 \ 0]$: main effect of A
- $[1 \ 1 \ 0 \ 0]$: Sum of $(A+B)$ vs the mean of the signal
-

SPM12: result

laughter > scramble_I



Example 2: Naturalistic stimuli

Naturalistic fMRI offers **ecological validity**, engages **complex brain functions**, **richer data**, **better participant engagement**, etc.

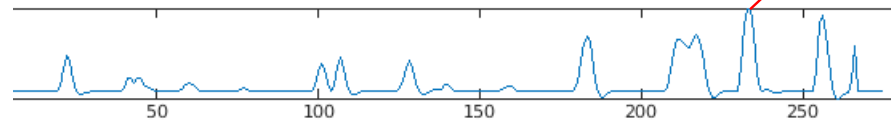
>> Santavirta S. et al., *NeuroImage*, Volume 272, 15 May 2023, 120025
>> Nummenmaa L., et al., *Cerebral Cortex*, Volume 31, Issue 9, September 2021, Pages 4104–4114

Movie-based fMRI

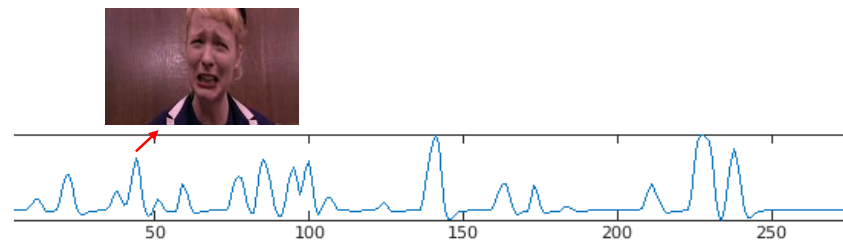
- ✓ Ratings of different dimensions (social, emotional, neutral, objective...)
- ✓ Each regressor should contain certain number of stimuli. CAN NOT be too small number!

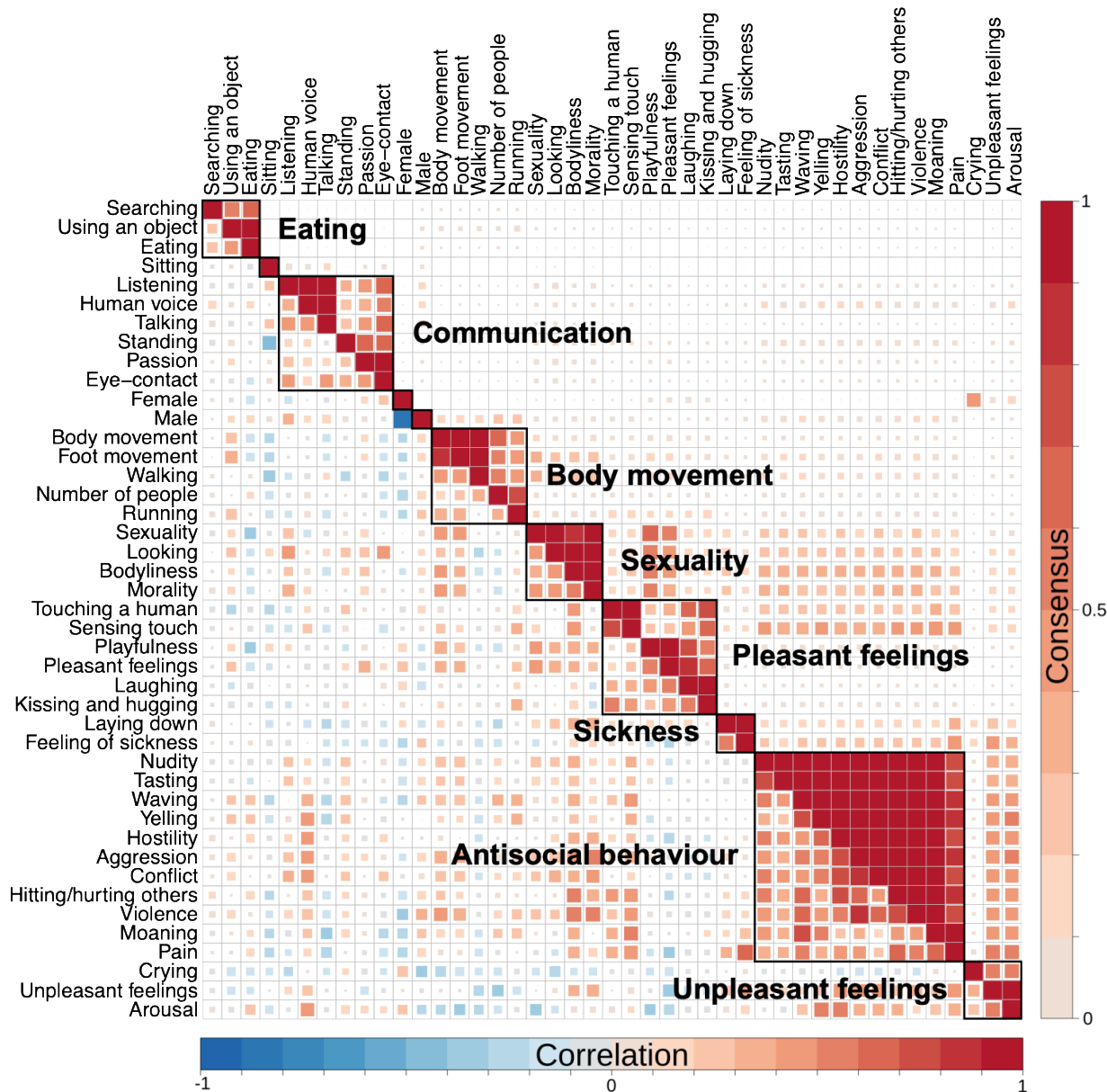


Self-control



Wanting





PCA
analysis

SPM12: parameter setting

Current Module: fMRI model specification

Help on: fMRI model specification

Directory

Timing parameters

- . Units for design
- . Interscan interval
- . Microtime resolution
- . Microtime onset

Data & Design

Factorial design

Basis Functions

- . Canonical HRF
- . . Model derivatives

Model Interactions ()

Global normalisation

Masking threshold

Explicit mask

Result to store

scan

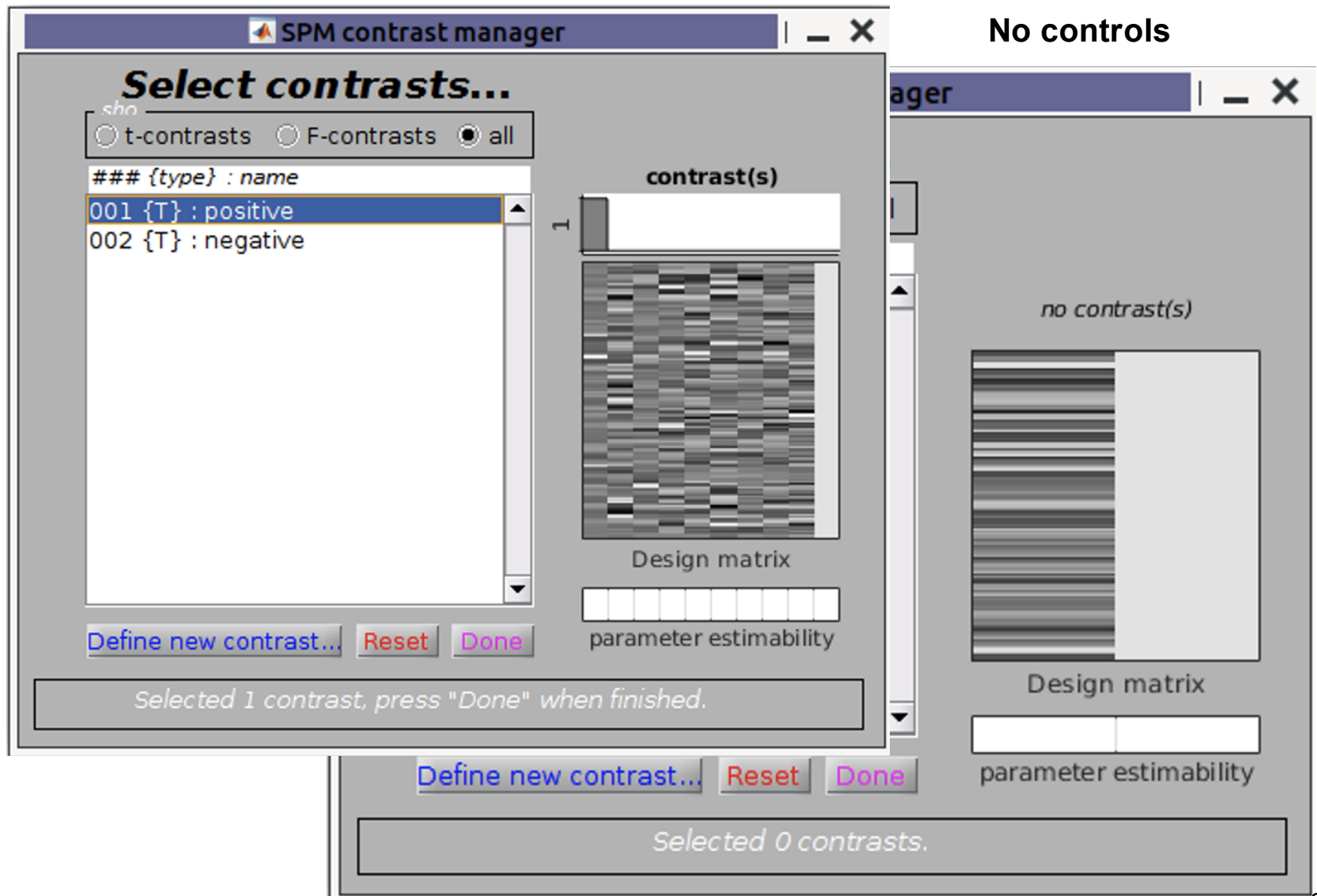
Dependent variables

Current Module: fMRI model specification

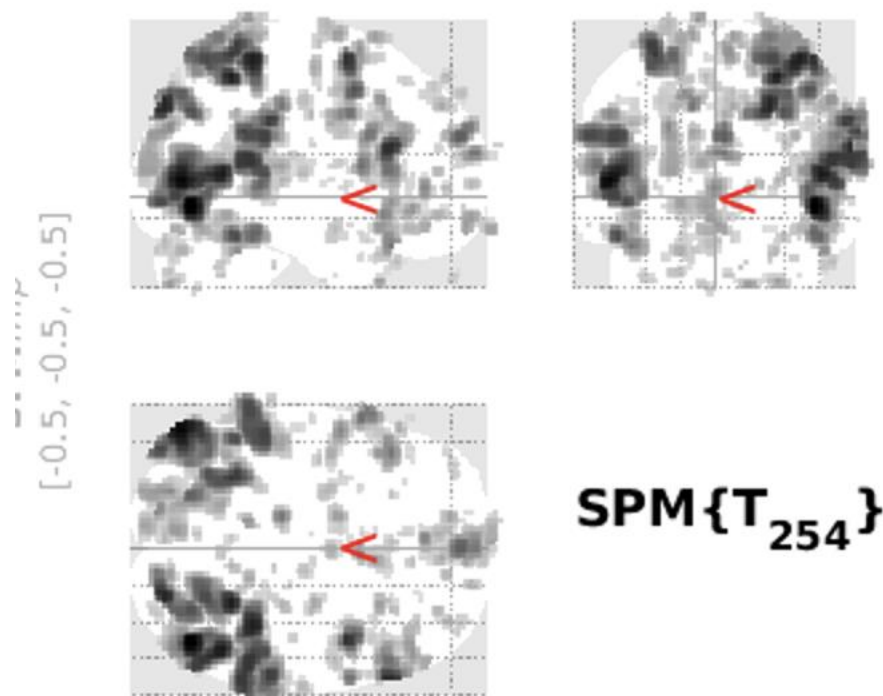
Data & Design

- . Subject/Session
- . . Scans → All scan volumes <-X
- . . Conditions
- . . . Condition
- Name → e.g. Self control humans
- Onsets <-X
- Durations → The same as volume No. <-X
- Time Modulation No Time Modulation
- Parametric Modulations
- Orthogonalise modulations Yes

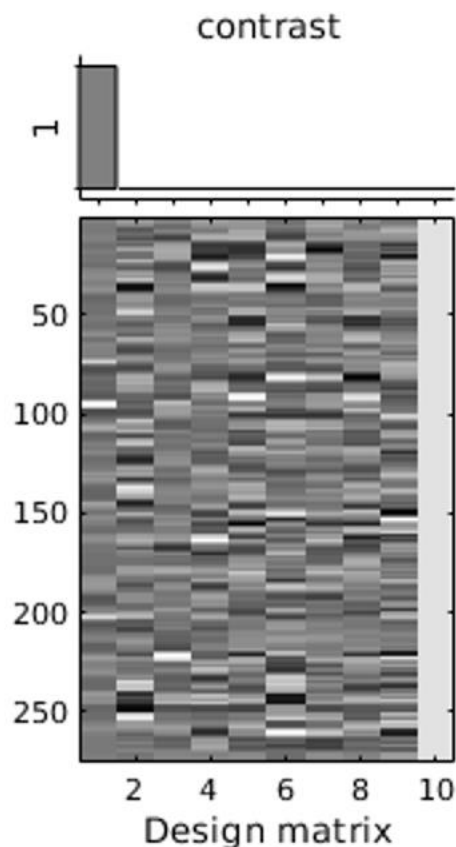
Controlling for low-level regressors



positive



SPMresults: ./Pleasant_feelings/sub-101
 Height threshold $T = 1.650875$ { $p < 0.05$ (unc.)}
 Extent threshold $k = 0$ voxels



Statistics: *p-values adjusted for search volume*

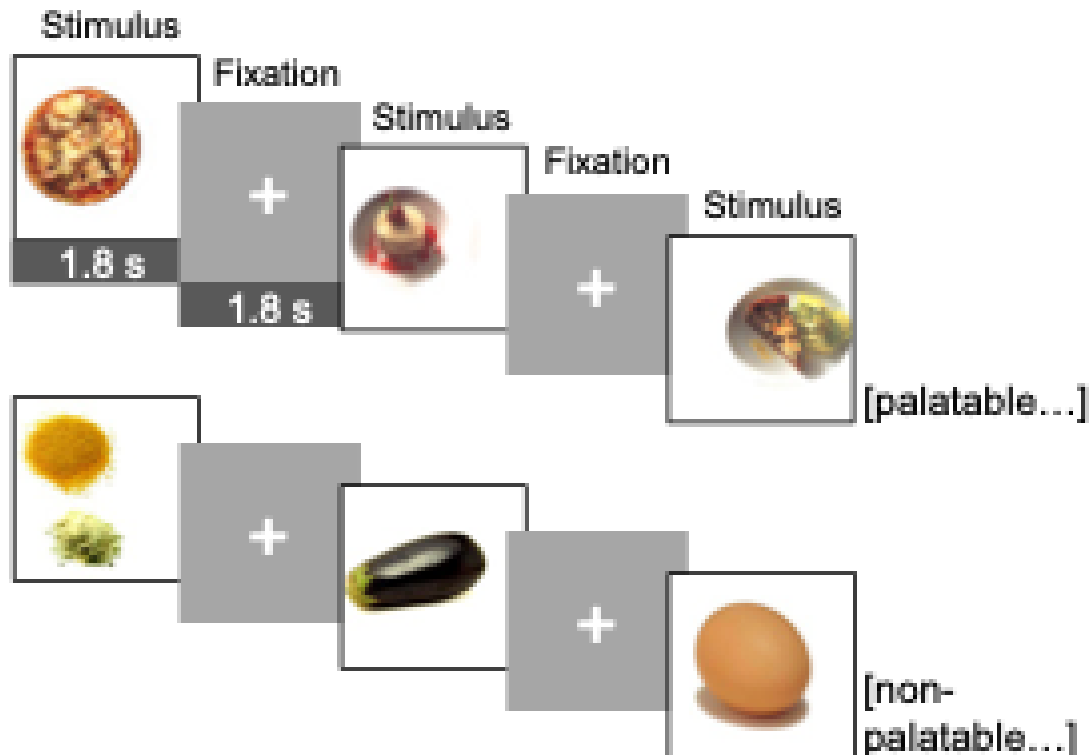
set-level		cluster-level				peak-level					mm mm mm		
p	c	$p_{FWE-corr}$	$q_{FDR-corr}$	k_E	p_{uncorr}	$p_{FWE-corr}$	$q_{FDR-corr}$	T	$(Z_{\equiv})$	p_{uncorr}			
1.000	100	0.009	0.002	1576	0.000	0.026	0.025	5.21	5.07	0.000	46	-72	-6
						0.211	0.055	4.67	4.57	0.000	48	-56	8
						0.701	0.100	4.23	4.15	0.000	54	-66	6
		0.034	0.005	1272	0.000	0.070	0.034	4.97	4.85	0.000	-52	-76	6
						0.700	0.100	4.15	4.00	0.000	50	-66	0

Example 3: Repeated measure food-reward

Food reward experiment has been used in decoding the brain conceptualization of satiation as induced by secretin hormone.

>> *Lauri S., Sun L., et al., Nature Metabolism, volume 3, pages 798–809 (2021)*

Twelve 16.2s blocks for each food category
Blocks in pseudo-randomized order
6 food stimuli intermixed with 3 fixations in one block



- Control condition vs. condition with infusion of secretin.
- Each subject **scanned twice** with fMRI: one in control condition, the other under intervention (secretin infusion).

Current Module: fMRI model specification

Data & Design

- . Subject/Session
- . . Scans
- . . Conditions
- . . . Condition
- Name
- Onsets
- Durations
- Time Modulation
- Parametric Modulations
- Orthogonalise modulations
- . . Multiple conditions
- . . Regressors
- . . Multiple regressors
- . . High-pass filter
- . Subject/Session
- . . Scans
- . . Conditions

Current Item: Data & Design

- New: Subject/Session
- Replicate: Subject/Session (1)
- Replicate: Subject/Session (2)
- Delete: Subject/Session (1)
- Delete: Subject/Session (2)

Current Module: fMRI model specification

- . . Scans
- . . Conditions
- . . . Condition
- Name
- Onsets
- Durations
- Time Modulation
- Parametric Modulations
- Orthogonalise modulations
- . . Condition
- Name
- Onsets
- Durations
- Time Modulation
- Parametric Modulations
- Orthogonalise modulations
- . . Multiple conditions
- . . Regressors
- . . Multiple regressors

Current Item: Conditions

- New: Condition
- Replicate: Condition (1)
- Replicate: Condition (2)
- Delete: Condition (1)
- Delete: Condition (2)

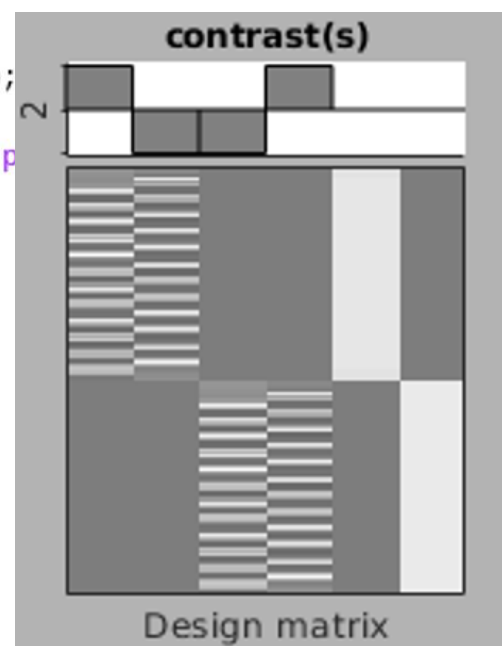
Step 2

```

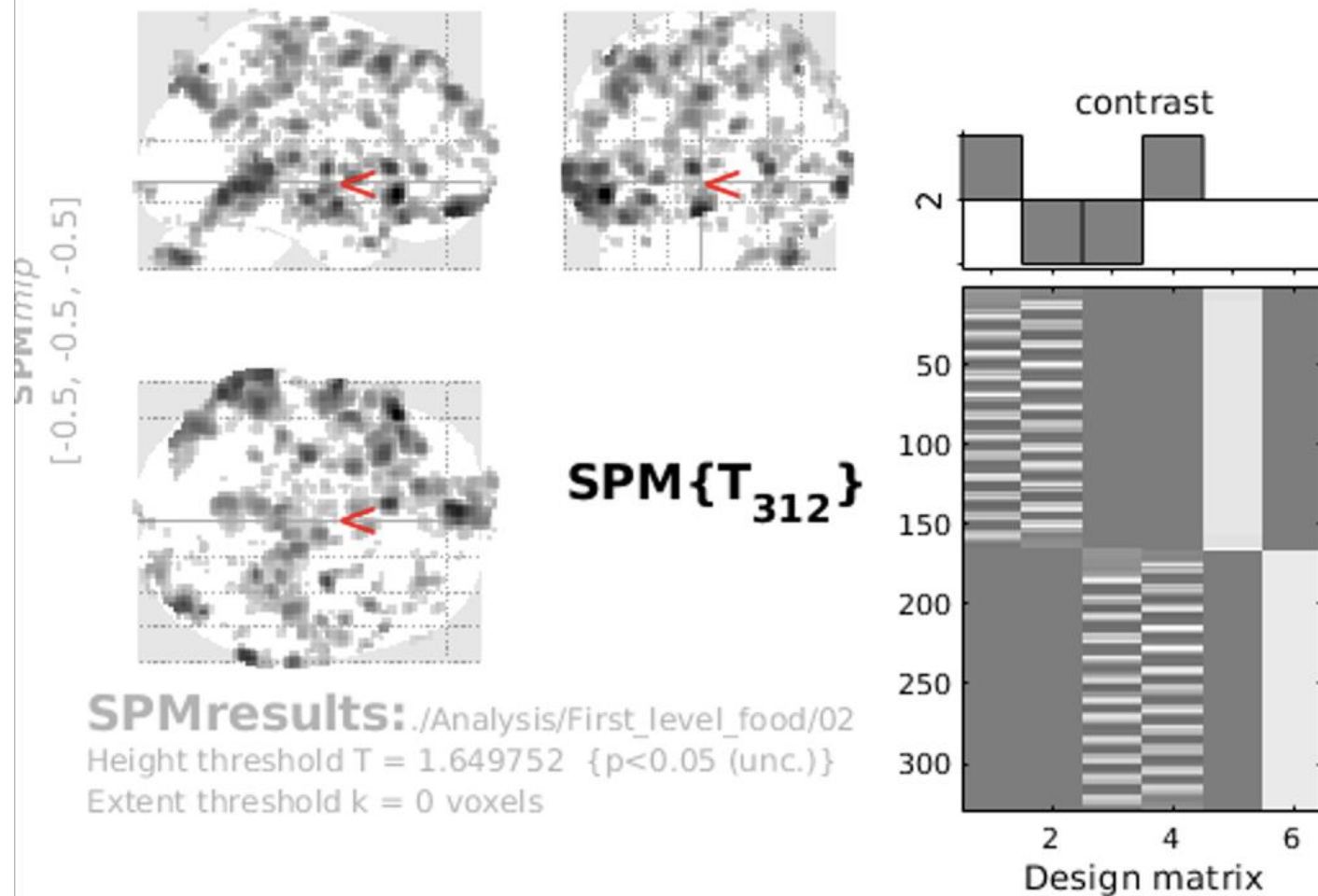
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matlabbatch{1}.spm.stats.fmri_spec.ses(1).cond(1).duration = duration_apep;
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matlabbatch{1}.spm.stats.fmri_spec.ses(1).cond(2).duration = duration_bland;
matlabbatch{1}.spm.stats.fmri_spec.ses(1).cond(2).tmod = 0;
matlabbatch{1}.spm.stats.fmri_spec.ses(1).cond(2).pmod = struct('name', {}, 'param', {}, 'poly', {});
matlabbatch{1}.spm.stats.fmri_spec.ses(1).cond(2).orth = 1;

matlabbatch{1}.spm.stats.fmri_spec.ses(2).scans = epi_files2;
matlabbatch{1}.spm.stats.fmri_spec.ses(2).cond(1).name = 'appetite';
matlabbatch{1}.spm.stats.fmri_spec.ses(2).cond(1).onset = s2_onset_apep;
matlabbatch{1}.spm.stats.fmri_spec.ses(2).cond(1).duration = s2_duration_apep;
matlabbatch{1}.spm.stats.fmri_spec.ses(2).cond(1).tmod = 0;
matlabbatch{1}.spm.stats.fmri_spec.ses(2).cond(1).pmod = struct('name', {}, 'p

```



interaction



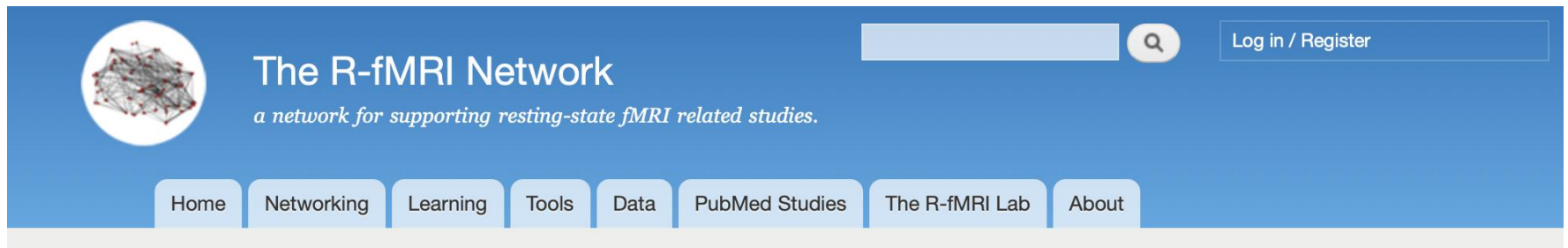
Example 4: resting state fMRI

- ReHo = regional homogeneity: larger value indicates a higher regional synchronization.
- ALFF = amplitude of low-frequency fluctuation: indicate the magnitude of neural activity
- **FC = functional connectivity (between ROIs): inter-regional correlations**

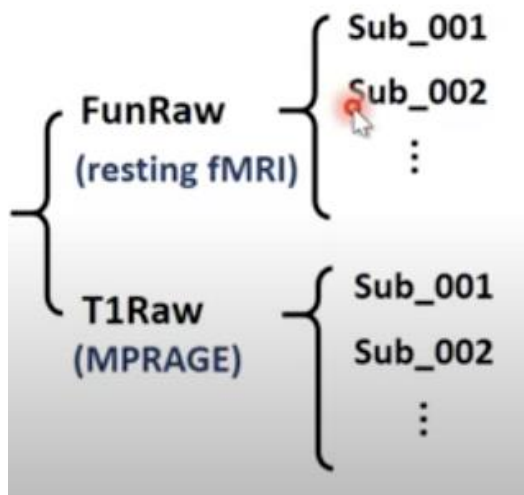


Toolbox for rs-fMRI analysis

- Matlab based
- Download DPARSF from: <http://rfmri.org/DPARSF>



Data folder



Data Processing Assistant for Resting-State fMRI

Advanced Edition **DPARSF A**

Working Directory: ...

Participants:

Time Points:
TR (s):

Template P... ☒ EPI DICOM to NIFTI ☒ T1 DICOM to NIFTI ☐ BIDS to DPARSF

☐ Apply Mats ☒ Remove First Time Points ☒ Slice Timing Slice Number: Slice Order:

Reference Slice: FieldMap Correction ☒ Realign ☐ Voxel-Specific Head Motion

☒ Reorient Fun* ☒ AutoMask ☐ Crop T1 ☒ Reorient T1* ☒ Bet ☒ T1 Coreg to Fun

☐ Segment ☒ New Segment + DARTEL Affine Regularisation in Segmentation: ☐ East Asian ☒ European

☒ Nuisance Covariates Regression Polynomial trend: Head Motion model: ☐ Rigid-body 6 ☐ Derivative 12

☒ Friston 24 ☐ Voxel-specific 12 ☐ Head motion scrubbing regressors

Nuisance regressors (WM, CSF, Global) ☐ Other covariates ☐ Add mean back ☐ Filter (Hz): ~

☒ Normalize Bounding Box: Voxel Size:

☐ Normalize by using EPI templates ☐ Normalize by using T1 image unified segmentation ☒ Normalize by DARTEL

☐ Smooth ☐ Smooth by DARTEL FWHM:

☒ Default mask ☐ No mask ☐ User-defined mask Use Default Mask ... ☐ Warp Masks into Individual Space

☐ Detrend ☐ Nuisance Covariates Regression ☒ ALFF+fALFF Band (Hz): ~ ☒ Filter

☐ Scrubbing ☒ ReHo Cluster: ☐ 7 ☐ 19 ☒ 27 voxels ☐ Smooth ReHo ☒ Degree Centrality

☐ Functional Connectivity ☒ Extract ROI time courses Define ROI ☐ Define ROI Interactively* ☐ CWAS

☒ Normalize to Symmetric Template ☒ Smooth ☒ VMHC ☐ Normalize Derivatives ☒ Smooth Derivatives

Parallel Workers #: Functional Sessions #: Starting Directory Name:

Help Save Load Utilities Quit **Run**

Summary

- The first level analysis is a **within-subject analysis, necessary due to the low signal-noise ratios.**
- **Experimental design decides the statistical model.**
- We have showed 4 example studies on how to conduct the first level analysis.
- Using **Contrasts** to view the results
- Contrast images are ready for second level analysis.

References

- Sun, Lihua, et al. "Aberrant motor contagion of emotions in psychopathy and high-functioning autism." *Cerebral Cortex* 33.2 (2023): 374-384.
- Santavirta, Severi, et al. "Functional organization of social perception networks in the human brain." *NeuroImage* 272 (2023): 120025.
- Nummenmaa, Lauri, et al. "Brain basis of psychopathy in criminal offenders and general population." *Cerebral cortex* 31.9 (2021): 4104-4114.
- Laurila, Sanna, et al. "Secretin activates brown fat and induces satiation." *Nature metabolism* 3.6 (2021): 798-809.
- Yan, Chaogan, and Yufeng Zang. "DPARSF: a MATLAB toolbox for" pipeline" data analysis of resting-state fMRI." *Frontiers in systems neuroscience* 4 (2010): 1377.



Thanks!

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