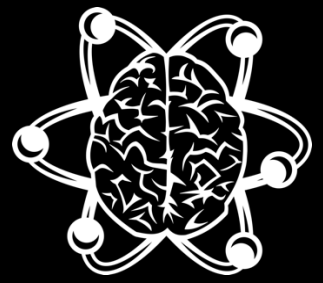
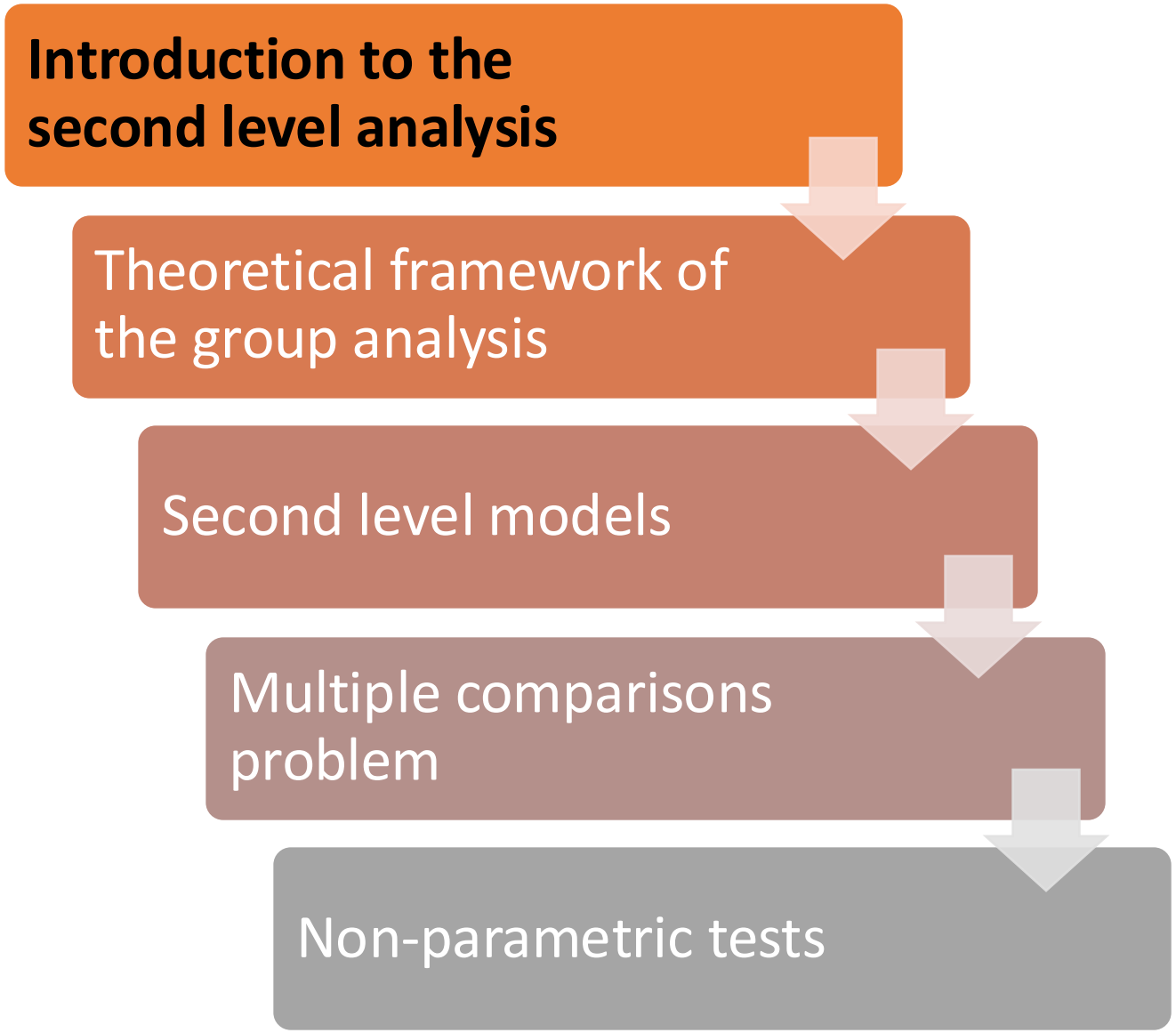


SECOND LEVEL ANALYSIS OF FMRI

Turku PET Centre Brain Imaging Course 2025

Severi Santavirta, Turku PET Centre

Topics


Introduction to the second level analysis
Theoretical framework of the group analysis
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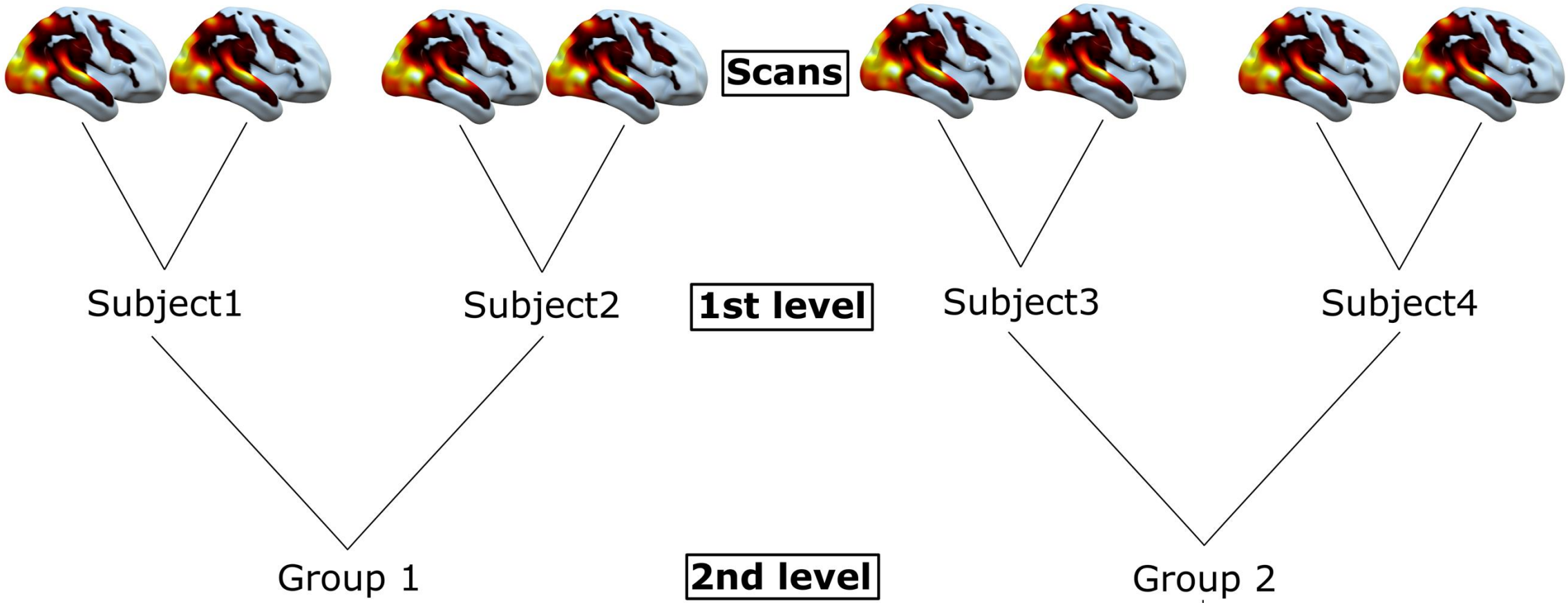
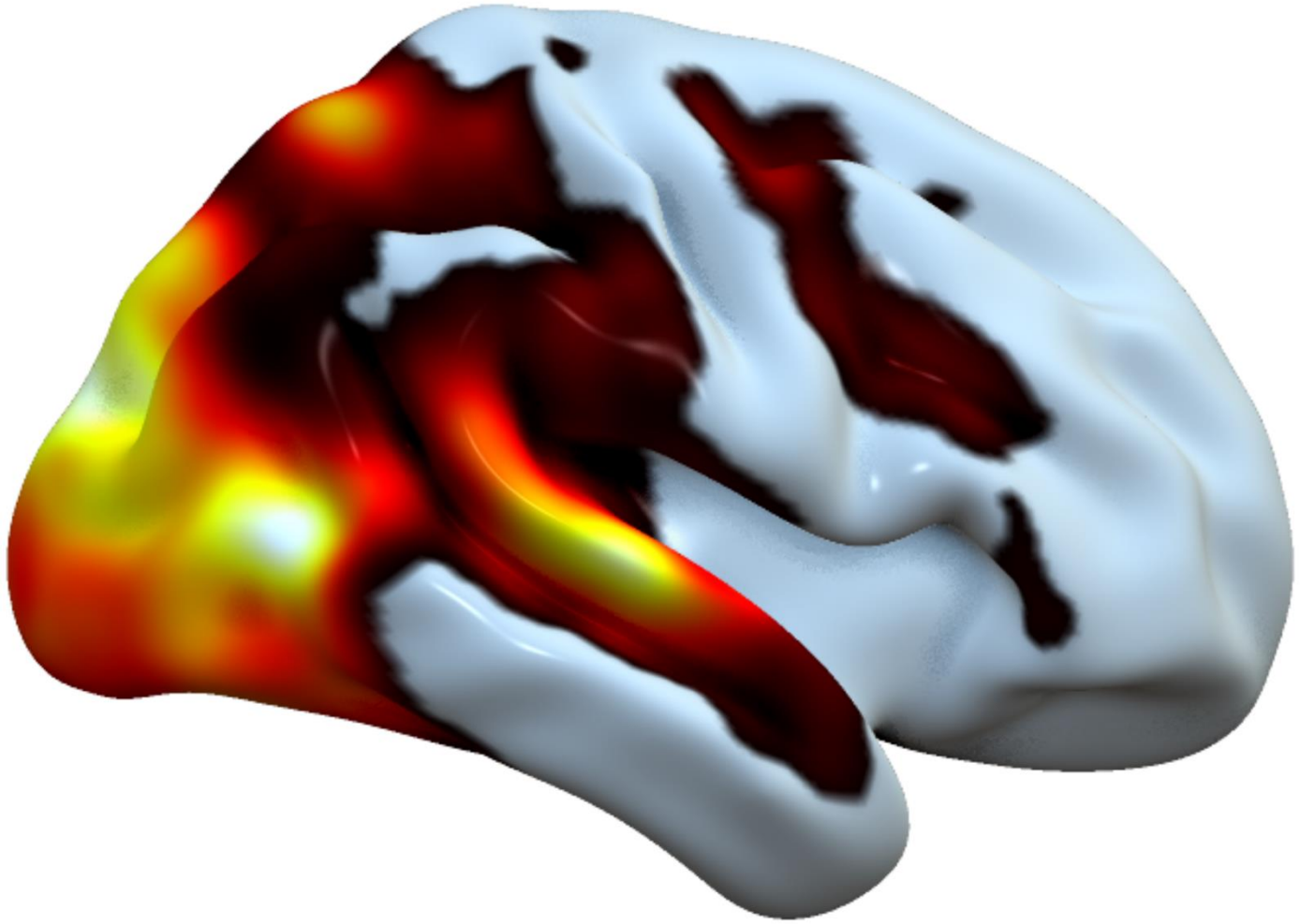
Hierarchical data



Whole brain analysis
Voxel-level analysis
Massive univariate analysis

The same thing!

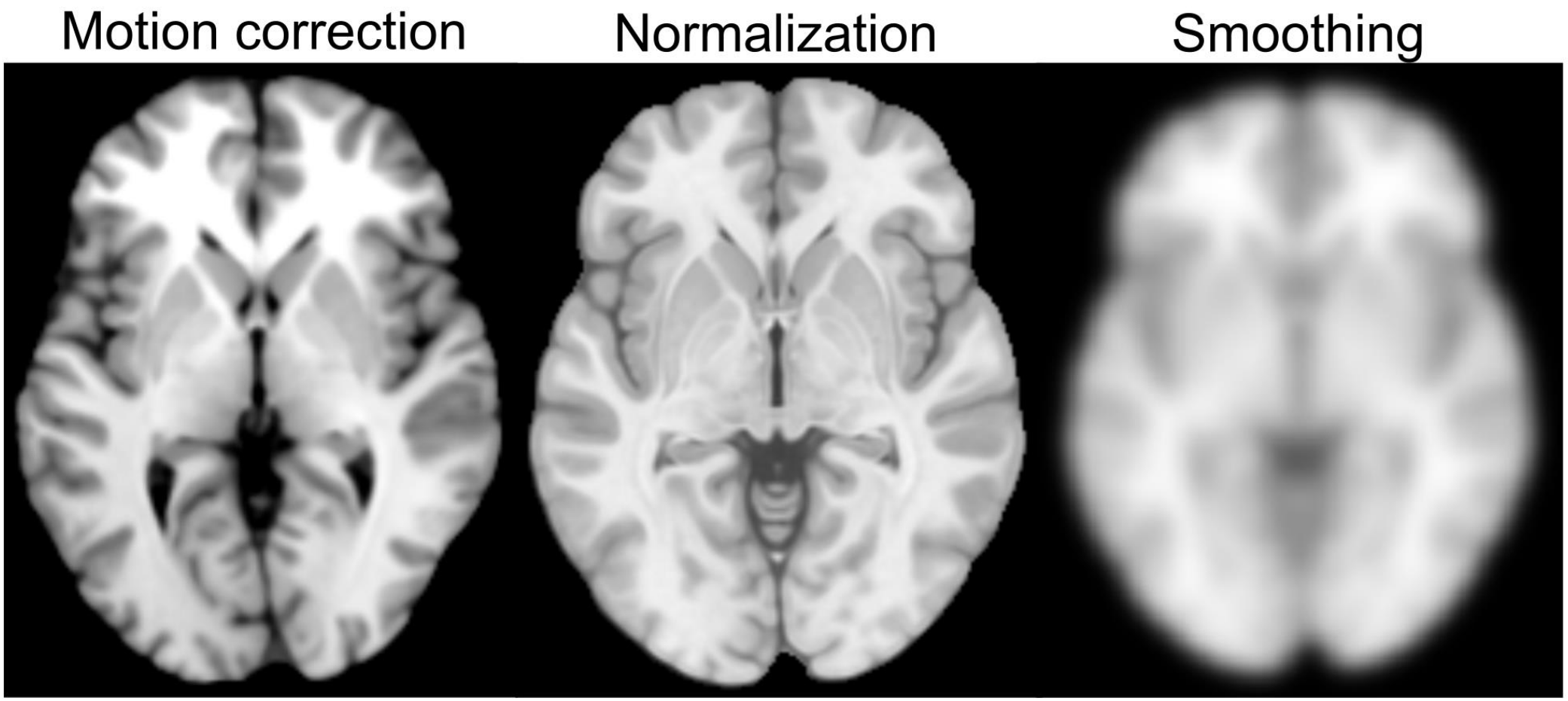
Region-of-interest (ROI) analysis



Temporal lobe	Temporal Pole Superior	
	Heschl	*
	Temporal Superior	
	Temporal Middle	*
Occipital lobe	Occipital Inferior	*
	Occipital Middle	*
	Occipital Superior	*
	Lingual	*
	Calcarine	*
	Cuneus	*
	Fusiform	*
Parietal lobe	Parietal Superior	*
	Parietal Inferior	
	Supramarginal	
	Rolandic Operculum	
	Precuneus	
	Angular	
	Paracentral Lobule	
	Postcentral	
Frontal lobe	Precentral	
	Supplementary Motor Area	
	Cingulate Posterior	*
	Cingulate Middle	*
	Cingulate Anterior	*
	Frontal Superior	
	Frontal Superior Medial	
	Frontal Middle	
	Frontal Inferior Triangular	
	Frontal Inferior Operculum	
	Frontal Inferior Orbital	
	Orbitofrontal Posterior	
	Orbitofrontal Lateral	
	Olfactory	
Subcortex	Parahippocampal	*
	Hippocampus	
	Insula	*
	Amygdala	
	Thalamus	
	Pallidum	
	Caudate	
	Putamen	

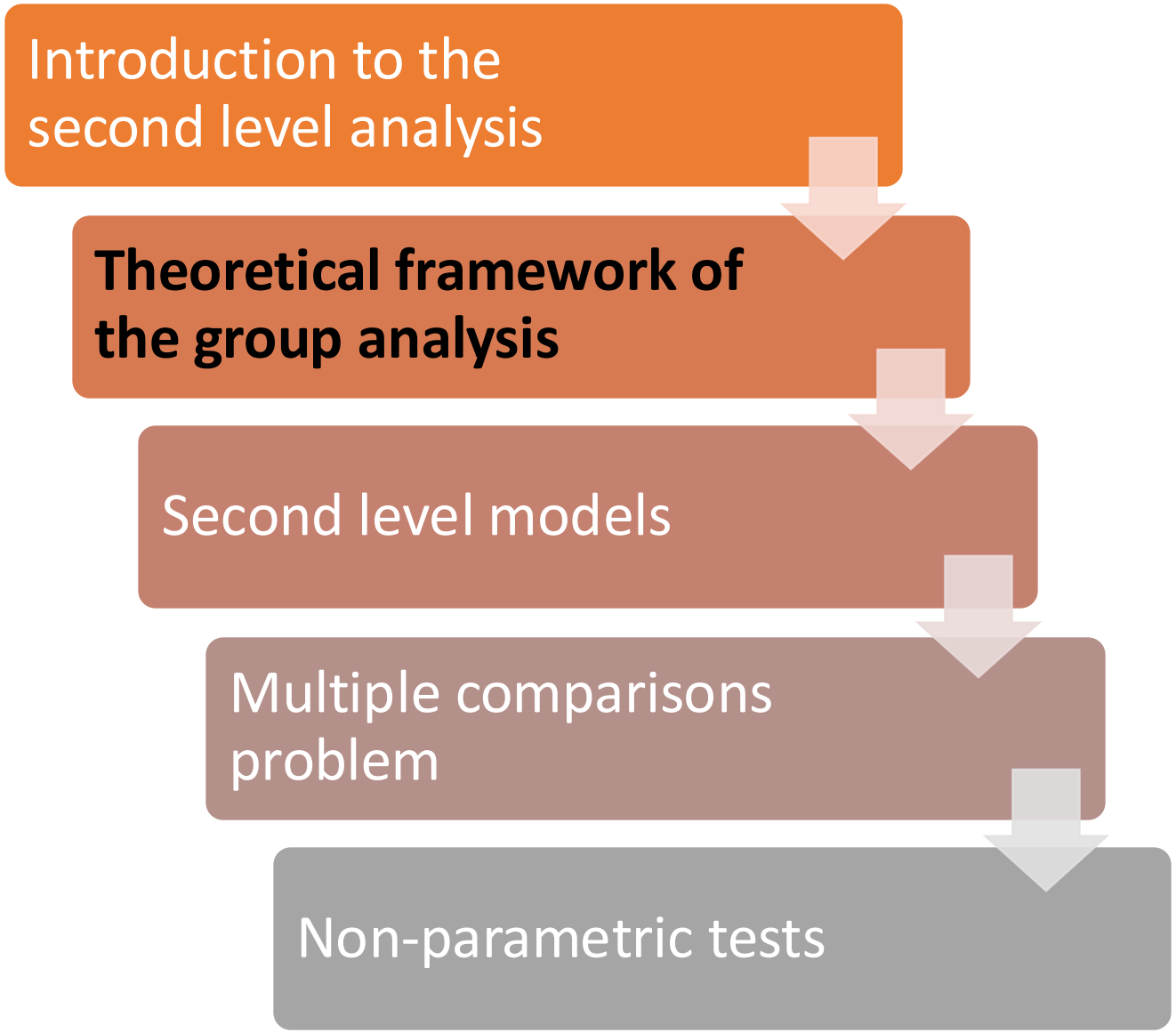
The data between subjects should be comparable

Preprocess data before analysis



What if I am only interested in doing ROI analysis?

Topics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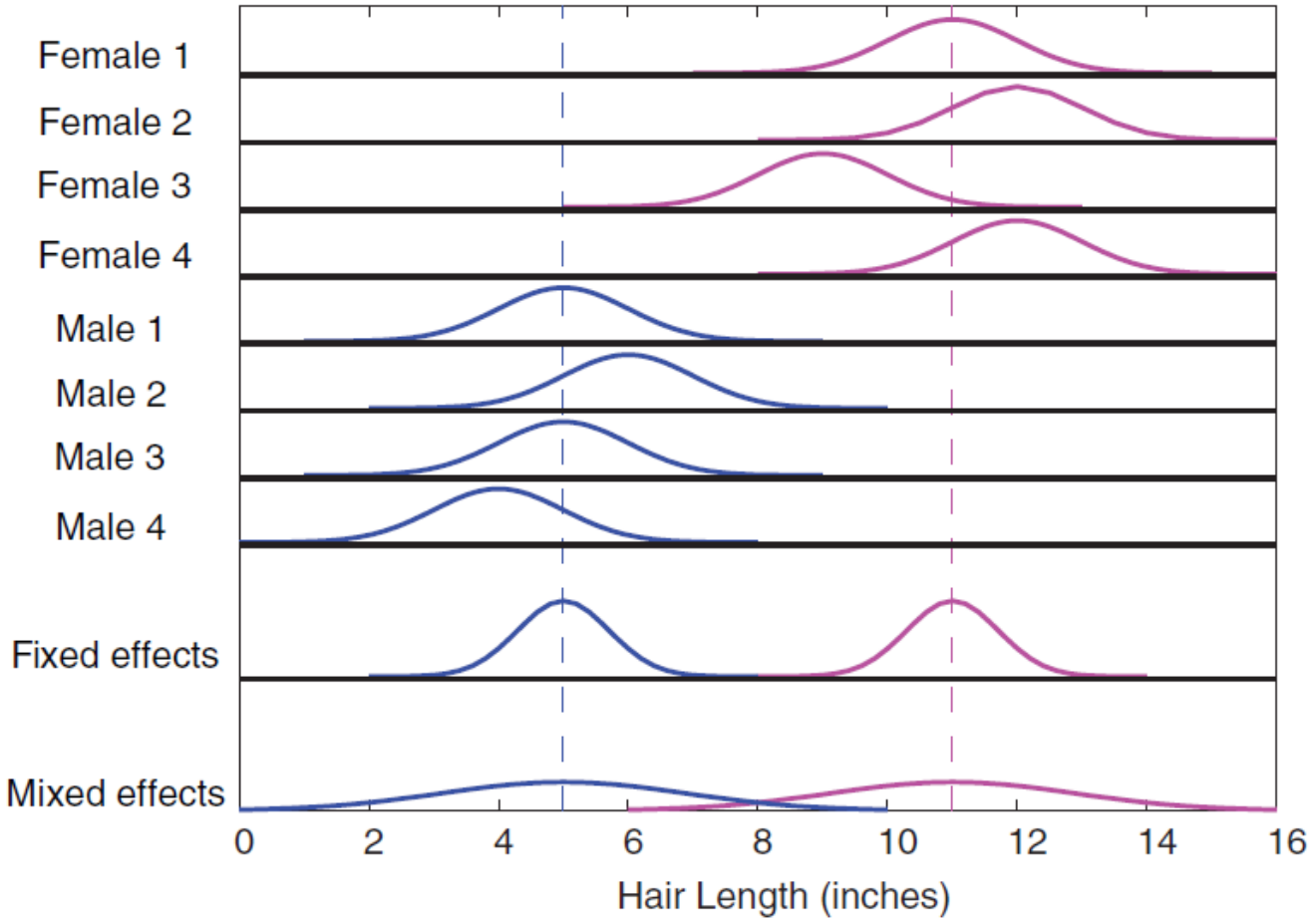
1st level analysis, 2nd level analysis, fixed effects, mixed effects… HELP!

Basic sources of variation in fMRI (or hair length)

Var^W = within-subject variance

Var^B = between-subject variance



(Poldrack, Nichols, & Mumford, 2011)

Fixed effects model		Mixed effects model
Var^W	Variance used in the analysis	$Var^W + Var^B$
Describe study sample only	Results	Generalize to population
Combine repeated measures within subjects	Application	Group analysis of fMRI

Mixed effects model in fMRI mathematically

- Within subject variance estimation (1st level model)

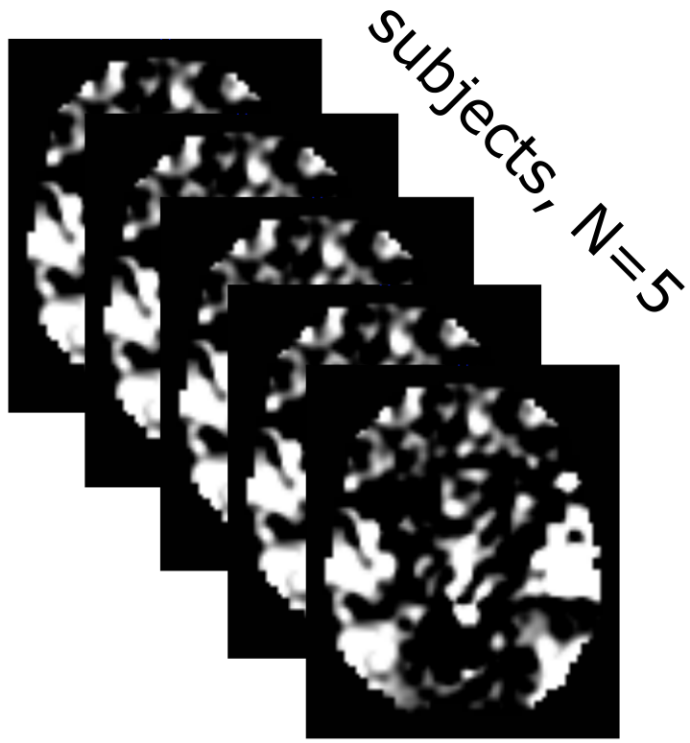
$$Y_s = \beta_s X + \varepsilon_s, \varepsilon_s \sim N(0, \sigma^2_{subject}) \quad (1)$$

- Between subject variance estimation (2nd level model)

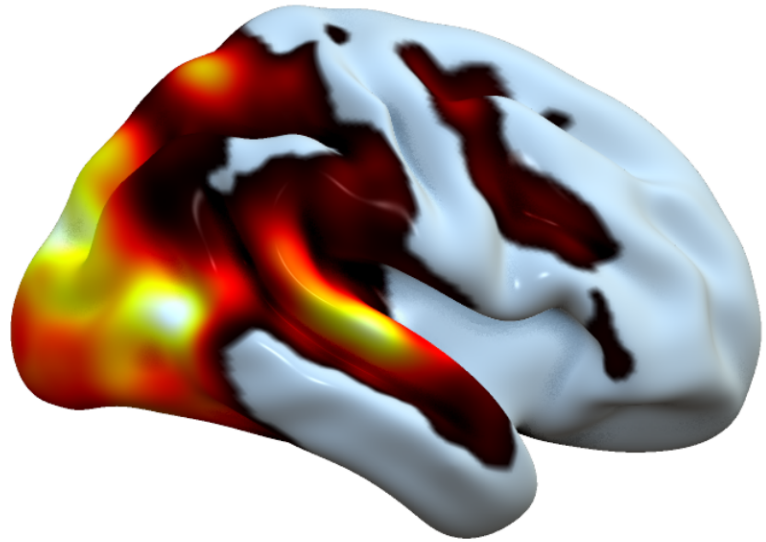
$$\beta_s = \beta_b X_b + \varepsilon_b, \varepsilon_b \sim N(0, \sigma^2_{between}) \quad (2)$$

- Full mixed effects model would estimate within & between subject variances simultaniously => Computationally demanding to estimate!

Summary statistics approach (mixed effect model)



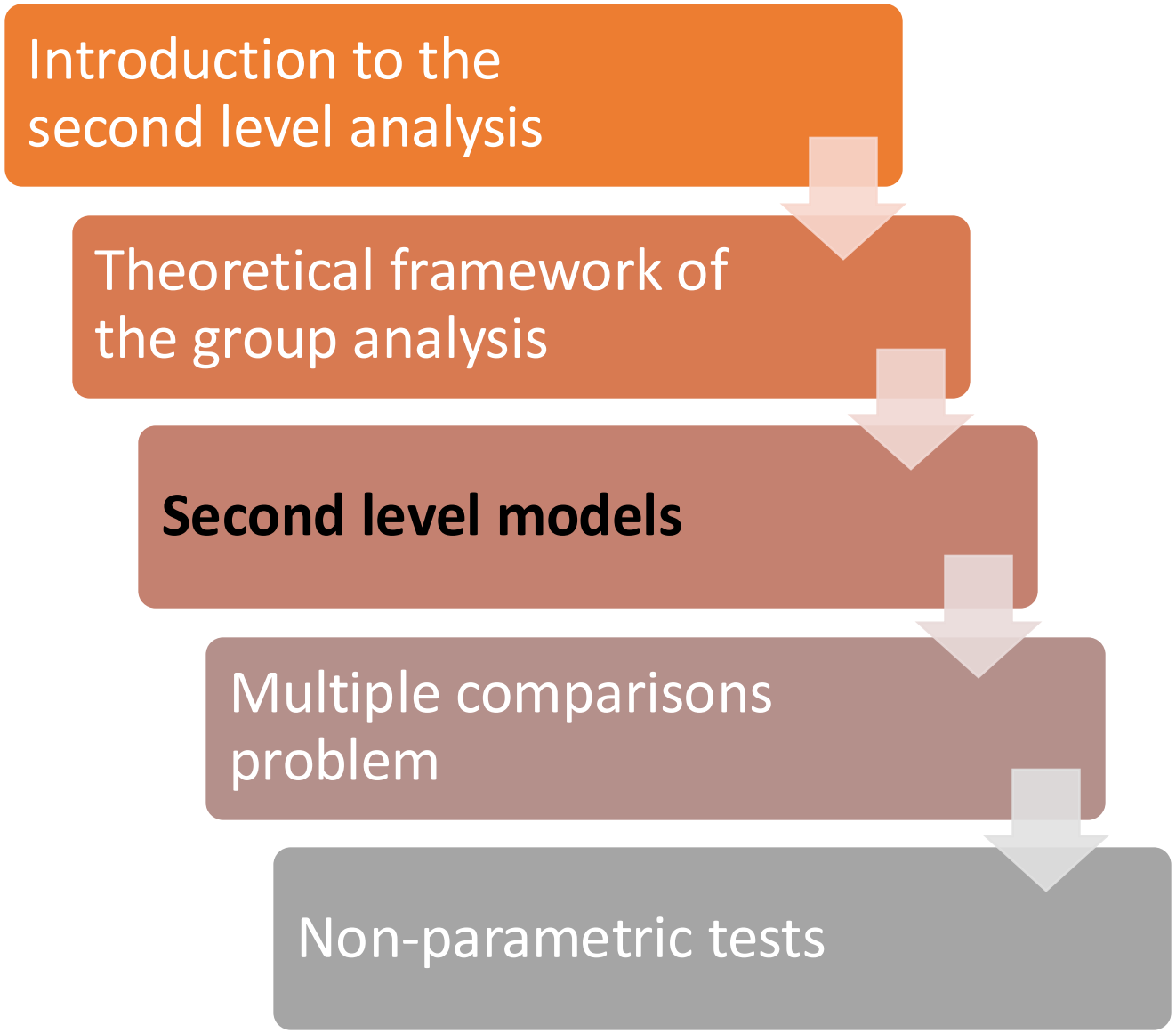
second level analysis



First-level statistical parametric maps

Population level results

Topics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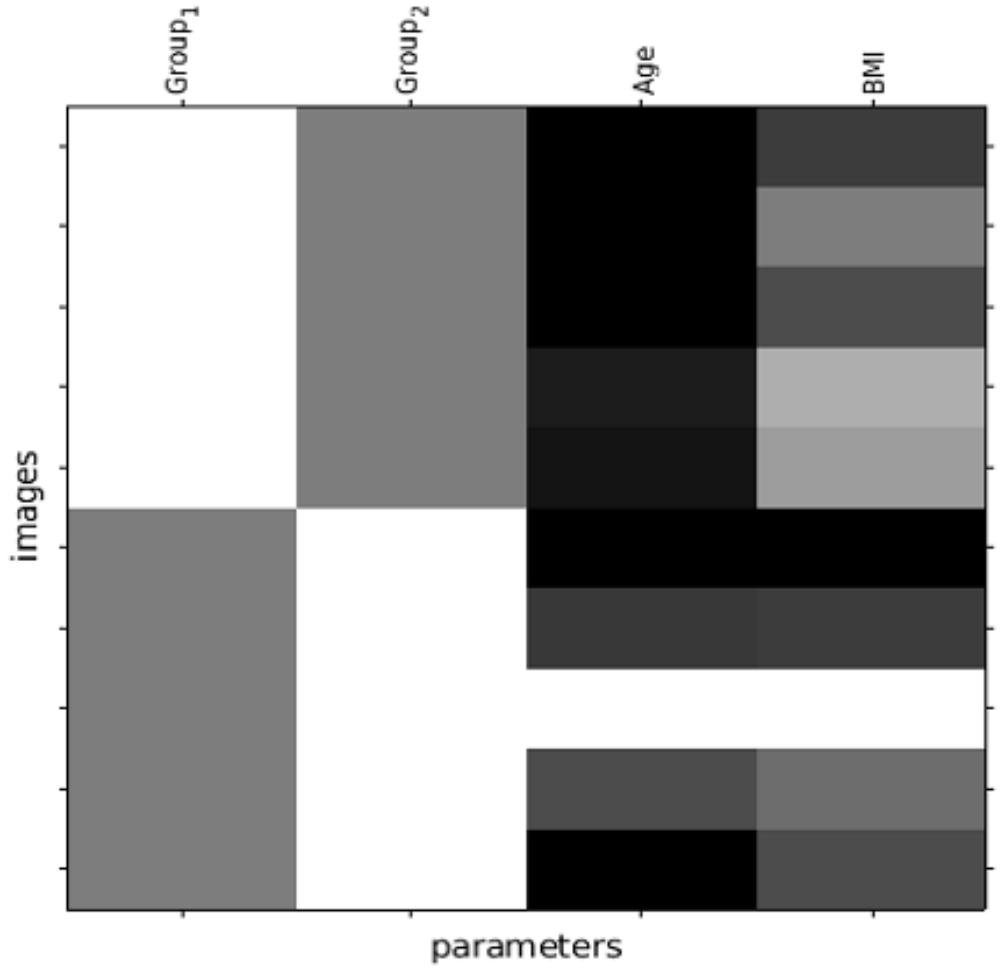
Second level design matrix in SPM

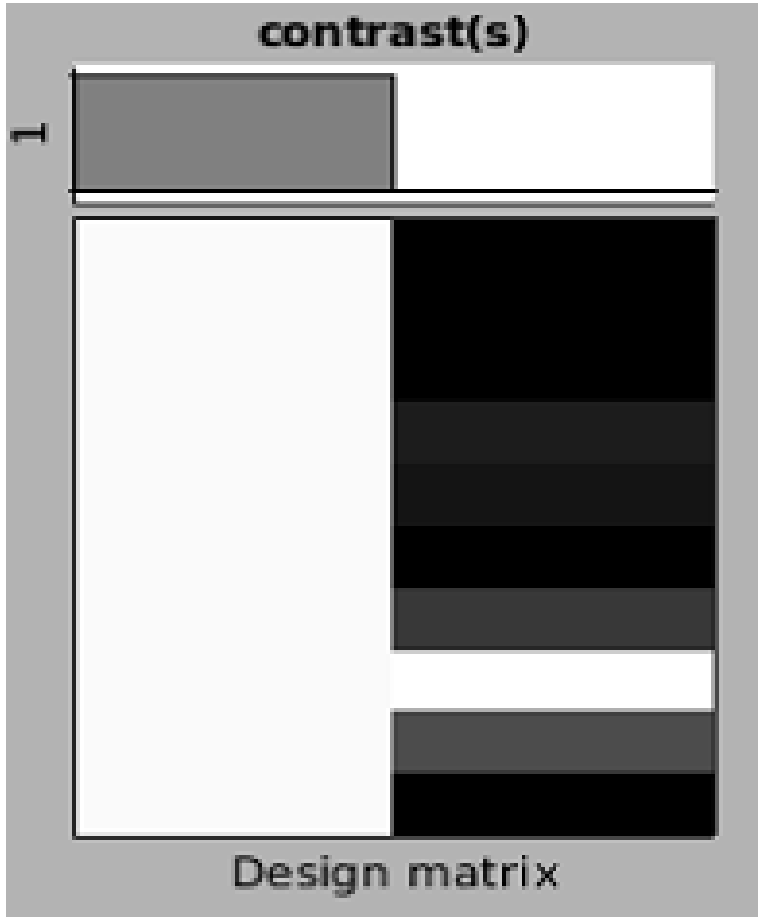
- Rows
  - Subjects
- Columns
  - Variables describing between subject variatio
  - Effect of each column will be estimated



One sample T-test

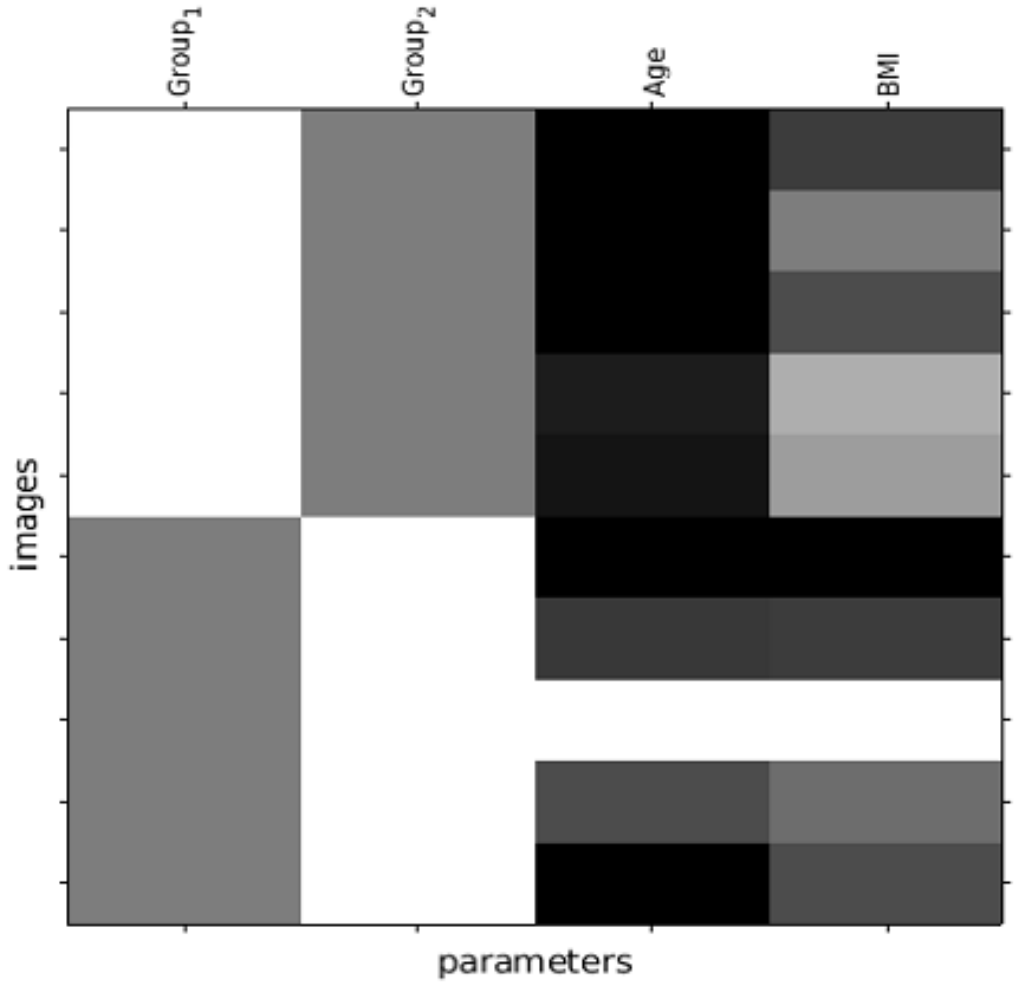
$$\beta_{1st\ level} = \beta_{main} + \beta_{2nd\ level} X_{covariate}$$

- **$\beta_{1st\ level}$**
  - Subjectwise association between the stimulus condition and BOLD signal
  - These are estimated in the 1st level analysis
- **β_{main}**
  - Is BOLD signal associated with the stimulus condition on the population level?
  - [1 0] in SPM contrast manager
- **$\beta_{2nd\ level}$**
  - Does a subjective factor (e.g age) explain the subjectwise differences in the association?
  - [0 1] in SPM contrast manager for positive effect
  - [0 -1] for negative effect



Two sample T-test

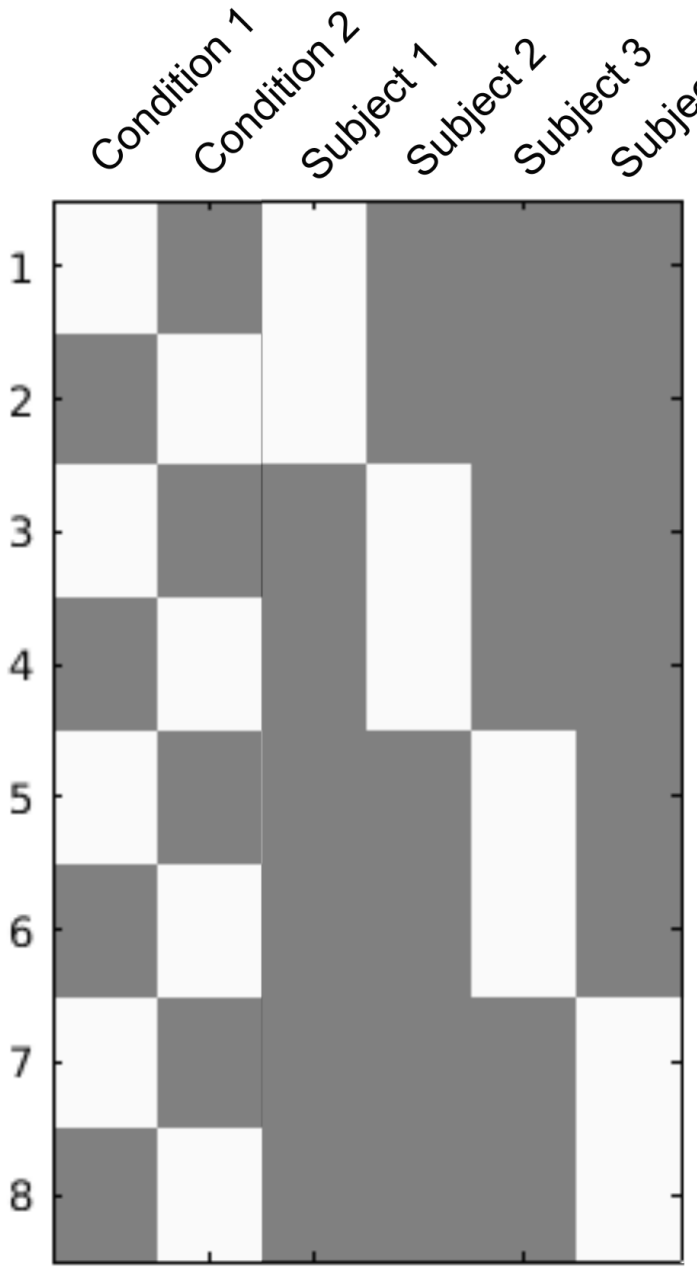
- H: Brain activity is different between two groups of subjects
- Group1 > Group2 with two additional covariates
  - SPM contrast [1 -1 0 0] = $\beta_{gr1} - \beta_{gr2}$
  - "Whether females have increased brain response for the 1st level condition than males when age and BMI are controlled for"



$$\beta_{contrast} = \beta_{gr1}X_{gr1} + \beta_{gr2}X_{gr2} + \beta_{age}X_{age} + \beta_{bmi}X_{bmi}$$

Paired T-test

- H: There is a difference between conditions in the brain response (two scans per subject)
- 4 subjects, 2 scans per subject
- Condition
  - Cross-sectional
    - Baseline vs. Stimulus
  - Longitudinal
    - Before treatment vs. After treatment
- Condition 2 > Condition 1
  - SPM contrast [-1 1 0 0 0 0] = $-\beta_{c1} + \beta_{c2}$
  - “Brain response associated with happy faces is higher after treatment"

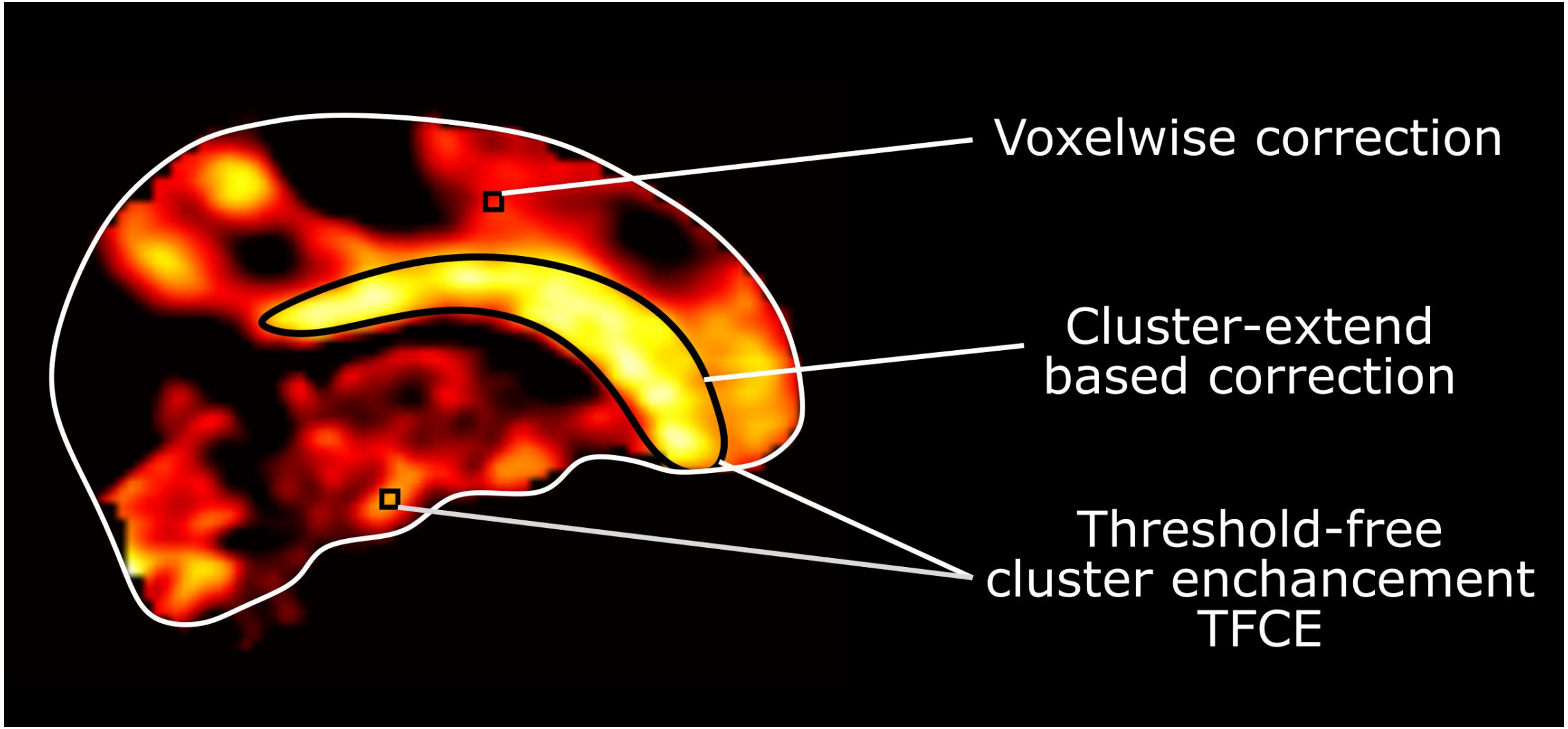


$\beta_{contrast} = \beta_{c1}X_{c1} + \beta_{c2}X_{c2} + \beta_{s1}X_{s1} + \beta_{s2}X_{s2} + \beta_{s2}X_{s2} + \beta_{s2}X_{s2}$

Topics

- Introduction to the second level analysis
- Theoretical framework of the group analysis
- Second level models
- **Multiple comparisons problem**
- Non-parametric tests

Multiple comparisons correction methods



(Review: Lindquist & Mejia, 2015)

Voxelwise multiple comparisons correction

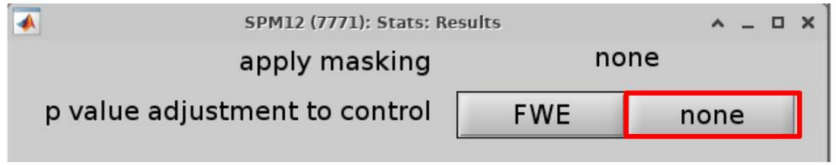
- Family-wise error rate (FWER) (Lindquist & Mejia, 2015)
  - Probability of making one or more false positives
  - Bonferroni correction
    - “5% probability that there is **at least one** false positive finding”
    - 0.05 / number of tests = corrected p-value threshold
- False discovery rate (Benjamini & Hochberg, 1995)
  - “On average **no more than 5%** of our findings are false positives”

Cluster-extend based correction (Lindquist & Mejia, 2015)

- Accounts for the spatial dependency between voxels
- “What is the probability to observe an activating cluster of this size under the null hypothesis of no activation”
- Twp-step procedure
  1. Choose primary voxel-level threshold
     1. e.g. $p < 0.001$
  2. Choose minimum size of a cluster
     1. As number of voxels, e.g. 50
     2. Usually selected based on the desired cluster level FWER level
- Be cautious, may yield more false positives than expected

1

2



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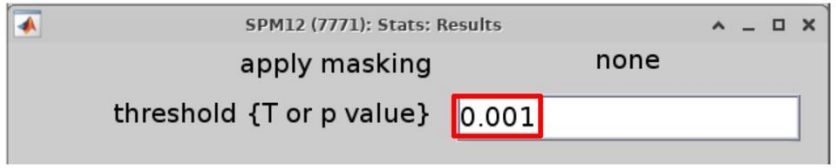

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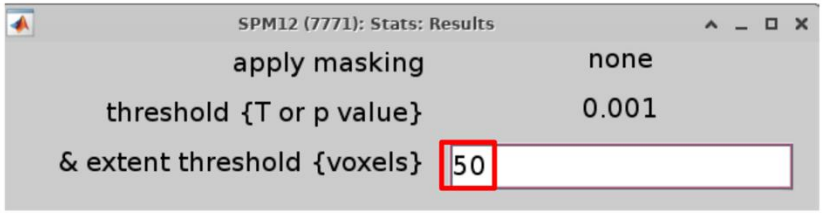
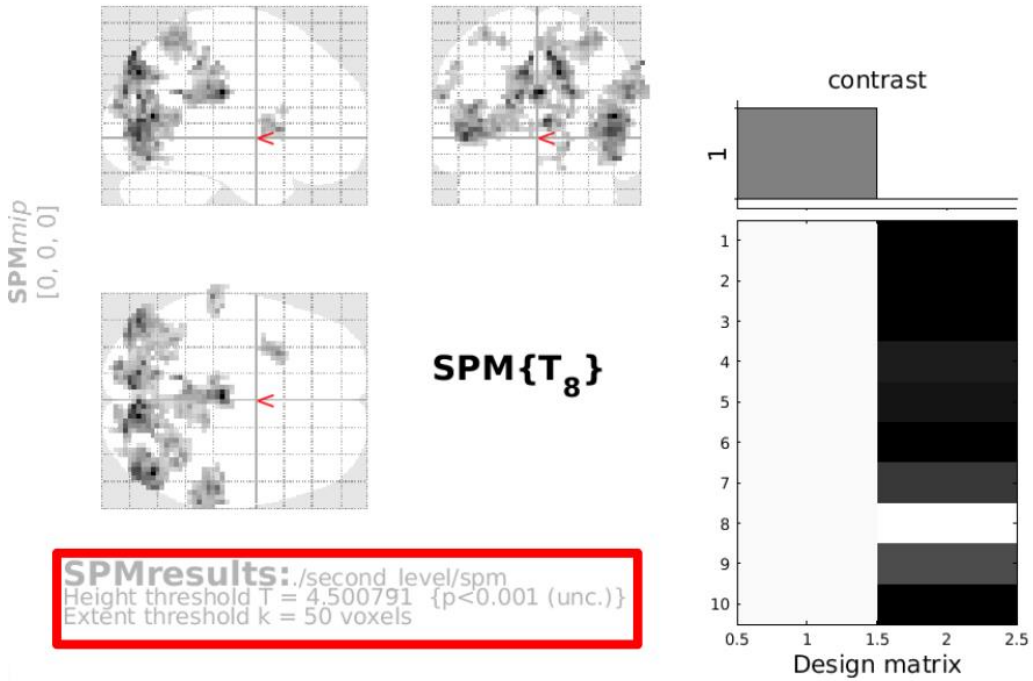


Main effect

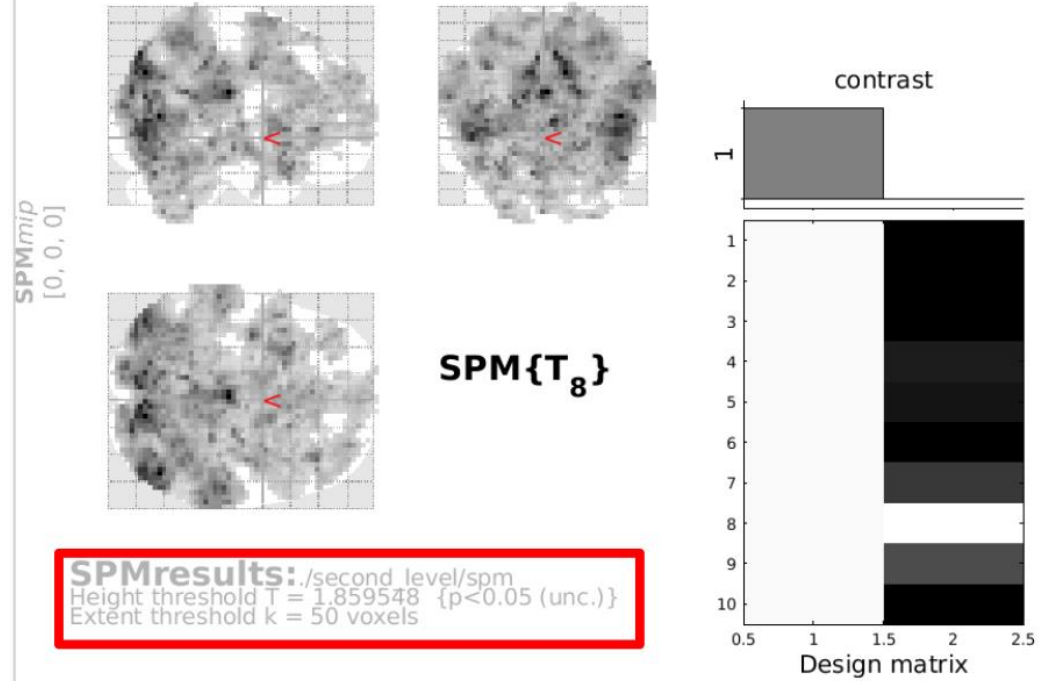


SPMresults: ./second_level/spm
Height threshold T = 4.500791 {p<0.001 (unc.)}
Extent threshold k = 50 voxels

Statistics: ***p-values adjusted for search volume***

set-level		cluster-level				peak-level					mm	mm	mm
p	c	$p_{FWE\text{-}corr}$	$q_{FDR\text{-}corr}$	k_E	p_{uncorr}	$p_{FWE\text{-}corr}$	$q_{FDR\text{-}corr}$	T	(Z_E)	p_{uncorr}			
0.000	**10**	**0.000**	**0.000**	**453**	**0.000**	**0.014**	**0.507**	**14.69**	**5.04**	**0.000**	**48**	**-69**	**12**
						0.099	0.507	11.33	4.65	0.000	45	-78	3
						0.188	0.581	10.40	4.52	0.000	48	-72	-15
		0.000	**0.000**	**166**	**0.000**	**0.016**	**0.507**	**14.39**	**5.01**	**0.000**	**0**	**-24**	**27**
						0.242	0.581	10.06	4.46	0.000	-12	-51	27
						0.593	0.581	8.91	4.27	0.000	0	-33	24
		0.000	**0.000**	**1011**	**0.000**	**0.019**	**0.507**	**14.10**	**4.98**	**0.000**	**12**	**-78**	**39**
						0.040	0.507	12.77	4.83	0.000	6	-81	51
						0.061	0.507	12.06	4.75	0.000	3	-78	9
		0.000	**0.000**	**202**	**0.000**	**0.296**	**0.581**	**9.79**	**4.42**	**0.000**	**66**	**-39**	**45**
						0.296	0.581	9.79	4.42	0.000	57	-27	24
						0.425	0.581	9.32	4.34	0.000	57	-33	30
		0.000	**0.000**	**57**	**0.000**	**0.536**	**0.581**	**9.03**	**4.29**	**0.000**	**-63**	**-30**	**24**
						0.999	0.606	7.34	3.94	0.000	-54	-27	45
						1.000	0.824	5.45	3.43	0.000	-57	-33	33
		0.000	**0.000**	**68**	**0.000**	**0.764**	**0.581**	**8.61**	**4.21**	**0.000**	**-27**	**12**	**3**
						1.000	0.641	6.70	3.79	0.000	-30	3	3
						1.000	0.719	6.05	3.61	0.000	-21	15	15
		0.000	**0.000**	**55**	**0.000**	**0.831**	**0.591**	**8.51**	**4.19**	**0.000**	**18**	**-66**	**-6**
						1.000	0.694	6.24	3.66	0.000	18	-60	0
						1.000	0.705	6.20	3.65	0.000	12	-72	-9
		0.000	**0.000**	**130**	**0.000**	**0.991**	**0.606**	**8.17**	**4.12**	**0.000**	**30**	**-60**	**57**

Main effect

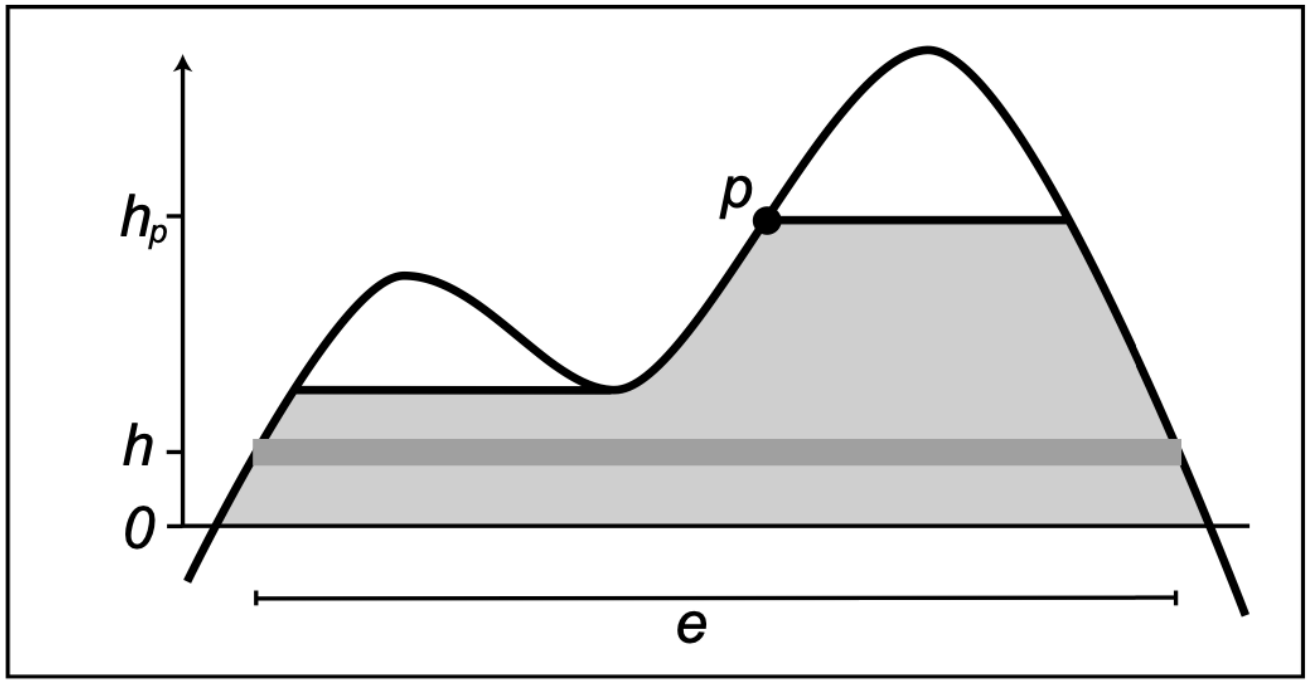


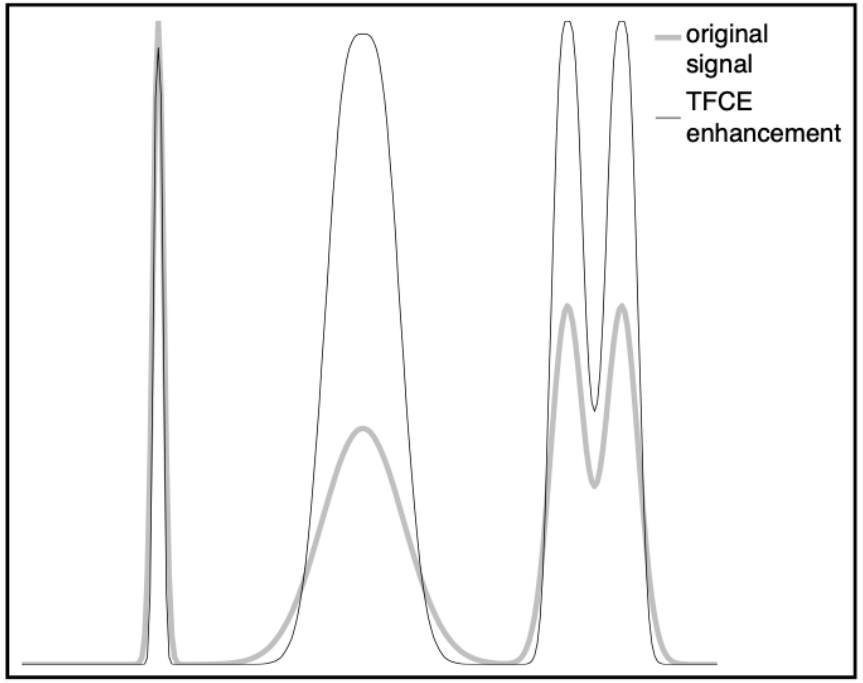
SPMresults: ./second_level/spm
Height threshold T = 1.859548 {p<0.05 (unc.)}
Extent threshold k = 50 voxels

Statistics: ***p-values adjusted for search volume***

set-level		cluster-level				peak-level					mm	mm	mm
p	c	$p_{FWE\text{-}corr}$	$q_{FDR\text{-}corr}$	k_E	p_{uncorr}	$p_{FWE\text{-}corr}$	$q_{FDR\text{-}corr}$	T	(Z_E)	p_{uncorr}			
1.000	**3**	**0.000**	**0.000**	**18190**	**0.000**	**0.014**	**0.309**	**14.69**	**5.04**	**0.000**	**48**	**-69**	**12**
						0.016	0.309	14.39	5.01	0.000	0	-24	27
						0.019	0.309	14.10	4.98	0.000	12	-78	39
		0.003	**0.000**	**668**	**0.000**	**0.536**	**0.355**	**9.03**	**4.29**	**0.000**	**-63**	**-30**	**24**
						0.999	0.370	7.34	3.94	0.000	-54	-27	45
						1.000	0.503	5.45	3.43	0.000	-57	-33	33
		0.893	**0.253**	**163**	**0.013**	**1.000**	**0.490**	**5.60**	**3.48**	**0.000**	**-36**	**39**	**24**
						1.000	0.503	5.34	3.39	0.000	-39	45	33
						1.000	0.658	4.22	2.98	0.001	-24	42	33

Threshold-free cluster enchancement (TFCE)



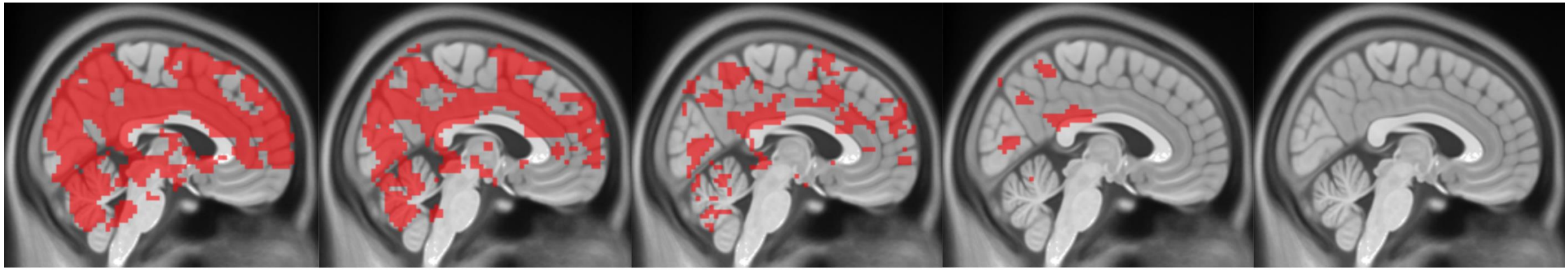


TFCE = h_p (voxelwise t-value) * e (amount of supporting voxels)

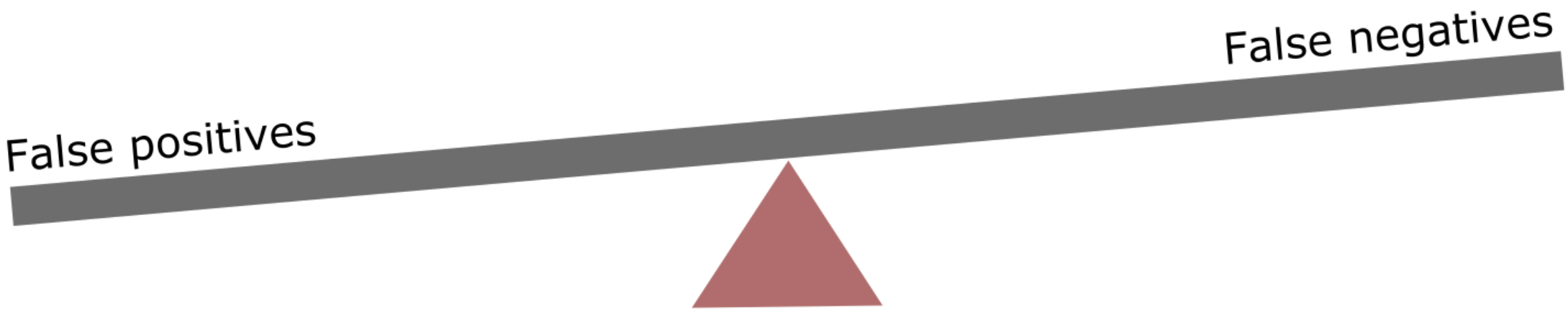
→ The voxelwise significance is adjusted by the amount supporting voxels

→ Significance of each voxel is assessed with permutations and then corrected for multiple comparisons

(Smith & Nichols, 2009)



TFCE (randomise)	Cluster FWE p<0.05,k=50	FDR a=0.05 (randomise)	Cluster FWE p<0.001,k=50	Bonferroni/FWE p<0.05



Conservative correction inflates effect size estimates

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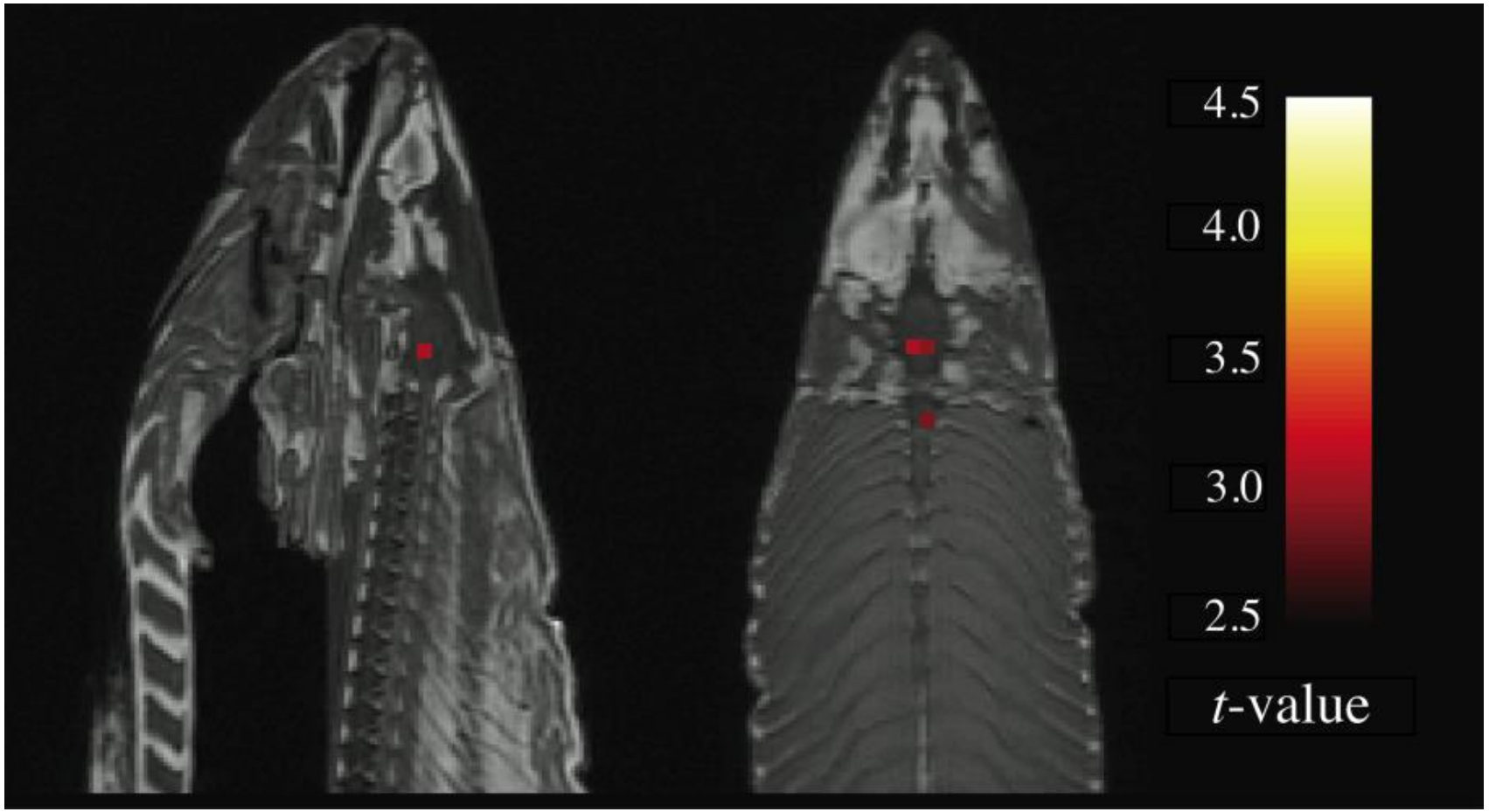
- **Non-parametric tests**

Non-parametric tests

- Motivation for non-parametric tests in group analyses (Eklund, Nichols, & Knutsson, 2016)
  1. Voxelwise multiple comparisons methods may produce too conservative findings and cluster-based methods more false positives than expected
  2. Non-parametric tests have been shown to correct better for multiple comparisons.
  3. Non-parametric tests do not make assumptions of the distribution of the statistic.
- Tools for non-parametric tests
  - SnPM (Doc: https://warwick.ac.uk/fac/sci/statistics/staff/academic-research/nichols/software/snpm)
  - FSL Randomise (Winkler, Ridgway, Webster, Smith & Nichols, 2014)
    - One and two sample (unpaired/paired) T-tests, repeated measures anova
    - Easy to output statistical result maps with various different multiple comparisons methods
      - Including TFCE method
    - Doc: https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Randomise

Do not end up finding activations in a dead salmon's brain.

Correct for multiple comparisons!



(Bennet, 2011)

Computer demonstrations

- horizon.utu.fi
  - Virtual desktop: **Ubuntu Mate 22.04 PSYK8965**
  - Only registered students with **utu accounts** can access.
  - Make sure that you have **unix permissions** set up well before the classes!
    - idm.utu.fi -> My information -> Add UNIX access

References

Benjamini, Y., & Hochberg, Y. (1995). Controlling the False Discovery Rate - a Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society Series B-Statistical Methodology, 57*(1), 289-300. doi:DOI 10.1111/j.2517-6161.1995.tb02031.x

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