# SECOND LEVEL ANALYSIS OF FMRI

Turku PET Centre Brain Imaging Course 2024

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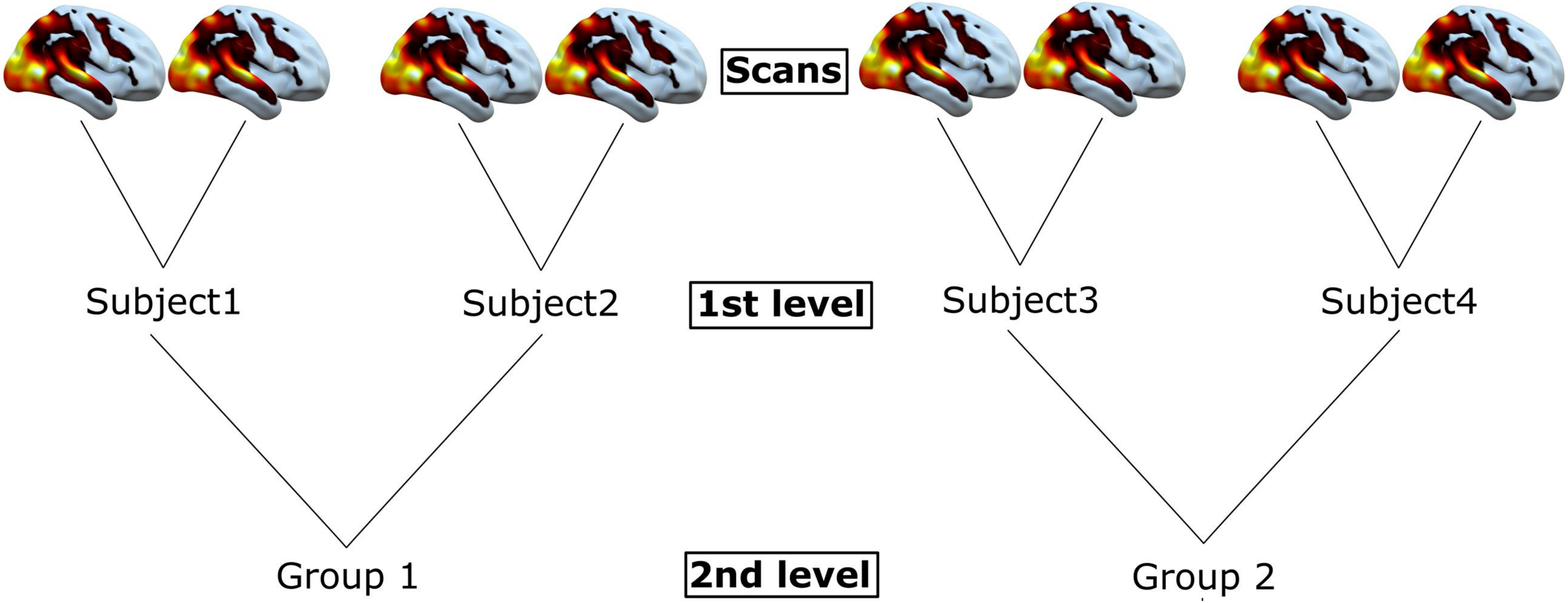
# Topics

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

# Hierarchical data



**Scans**

Subject1

Subject2

**1st level**

Subject3

Subject4

Group 1

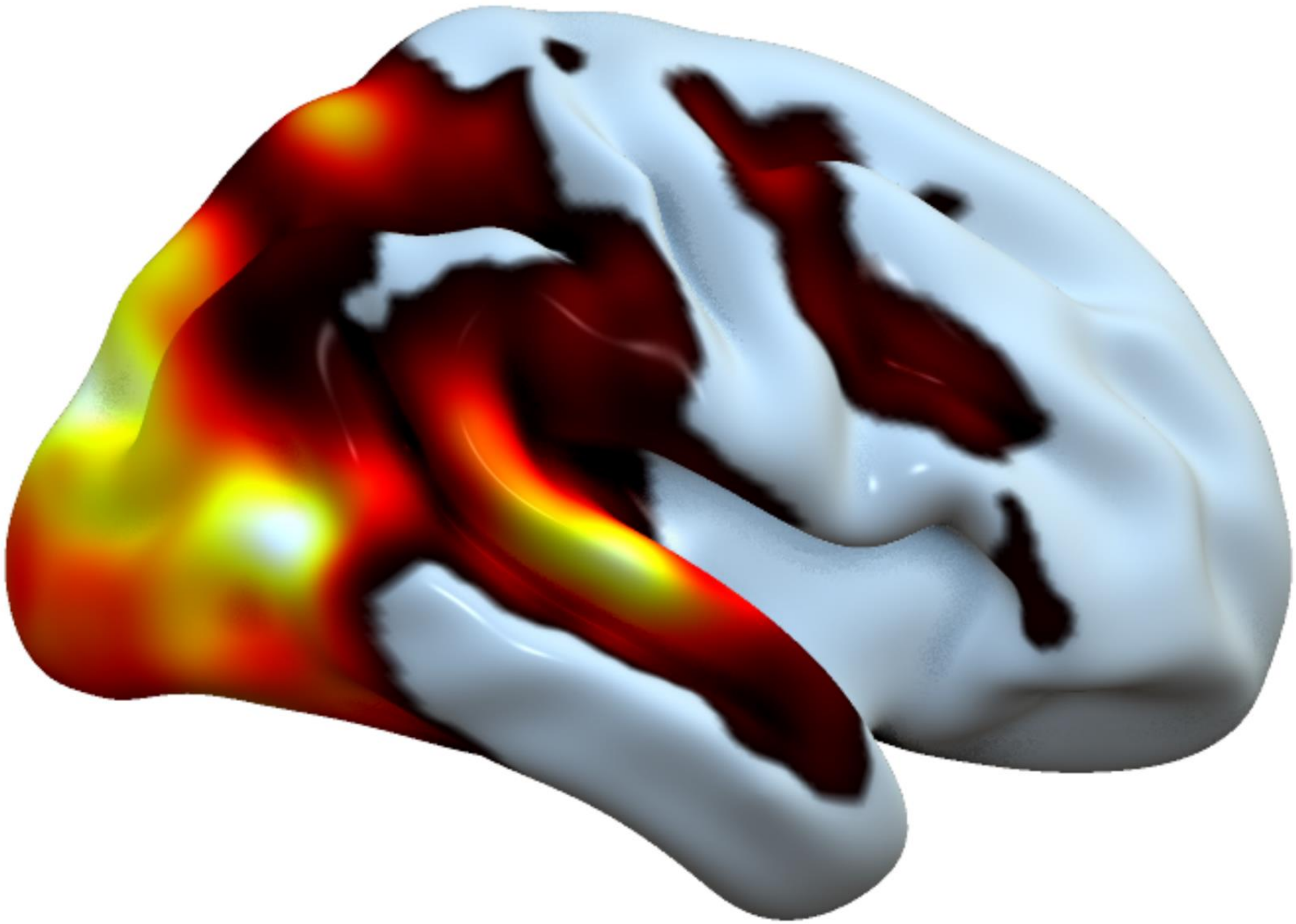
**2nd level**

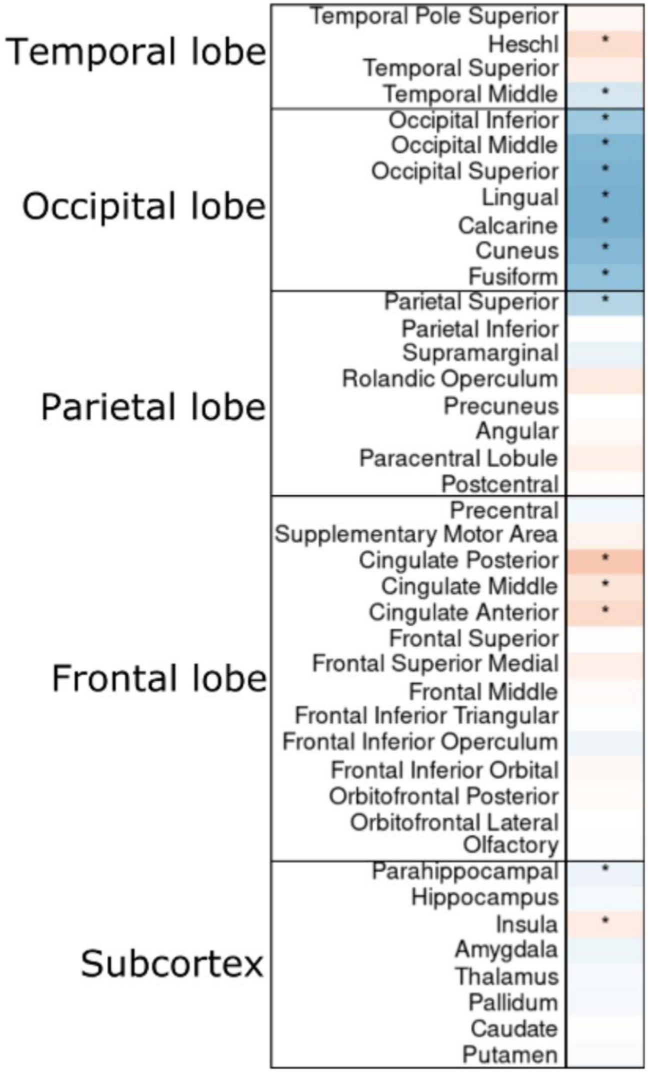
Group 2

Whole brain analysis
Voxel-level analysis
Massive univariate analysis

The same thing!

Region-of-interest (ROI) analysis

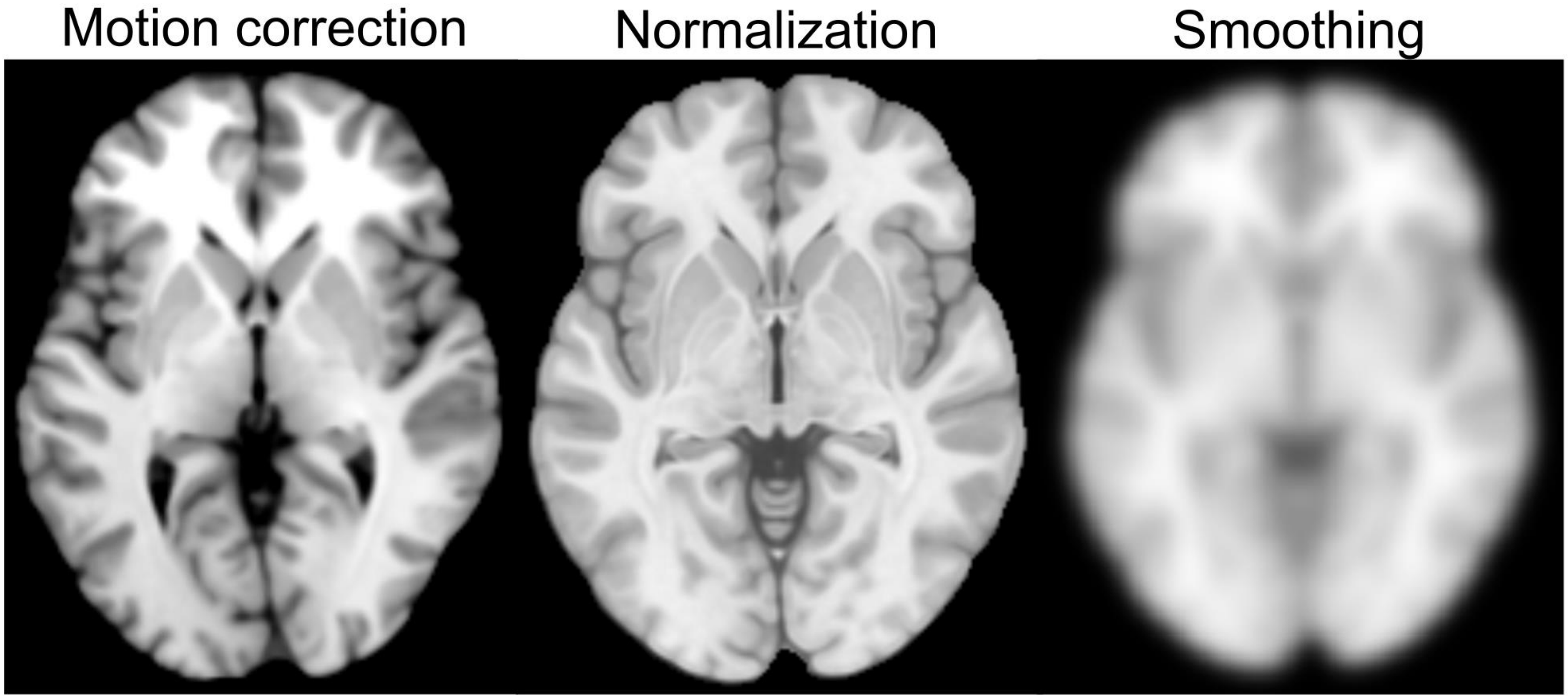




| | | |
|---|---|---|
| Temporal lobe | Temporal Pole Superior | |
| | Heschl | * |
| | Temporal Superior | |
| | Temporal Middle | * |
| Occipital lobe | Occipital Inferior | * |
| | Occipital Middle | * |
| | Occipital Superior | * |
| | Lingual | * |
| | Calcarine | * |
| | Cuneus | * |
| | Fusiform | * |
| 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?

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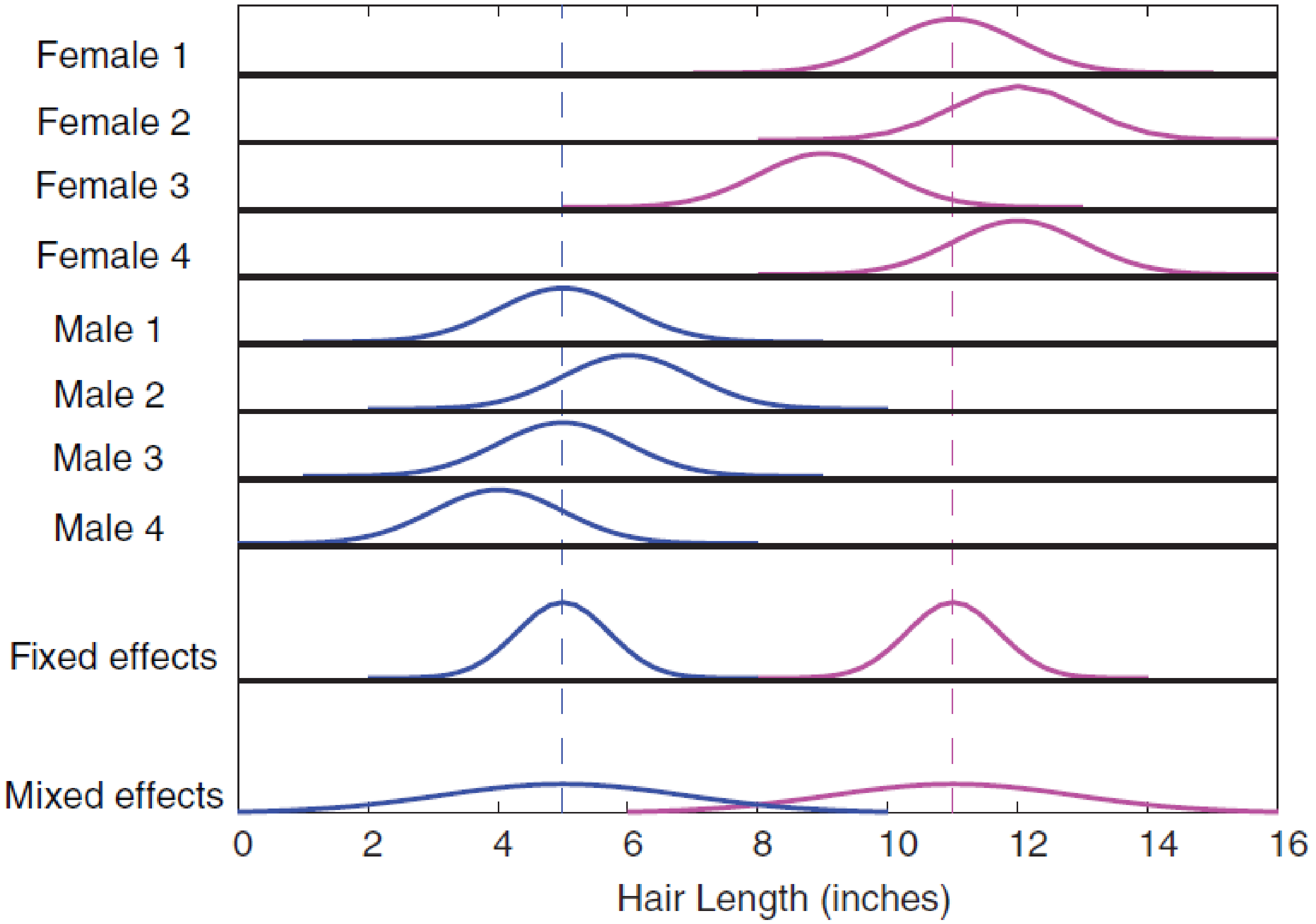
# 1st level analysis, 2nd level analysis, fixed effects, mixed effects… HELP!

**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** |
|---|---|---|
| $\text{Var}^{W}$ | Variance used in the analysis | $\text{Var}^{W} + \text{Var}^{B}$ |
| 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)

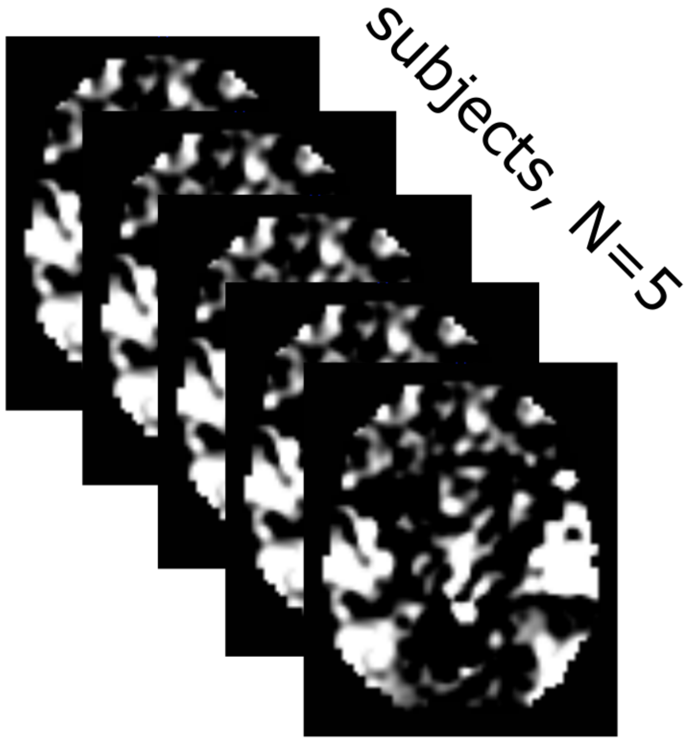
$$Y_s = \beta_s X + \varepsilon_s, \varepsilon_s \sim N(0, \sigma^2_{subject}) \qquad (1)$$

- Between subject variance estimation (2nd level model)

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

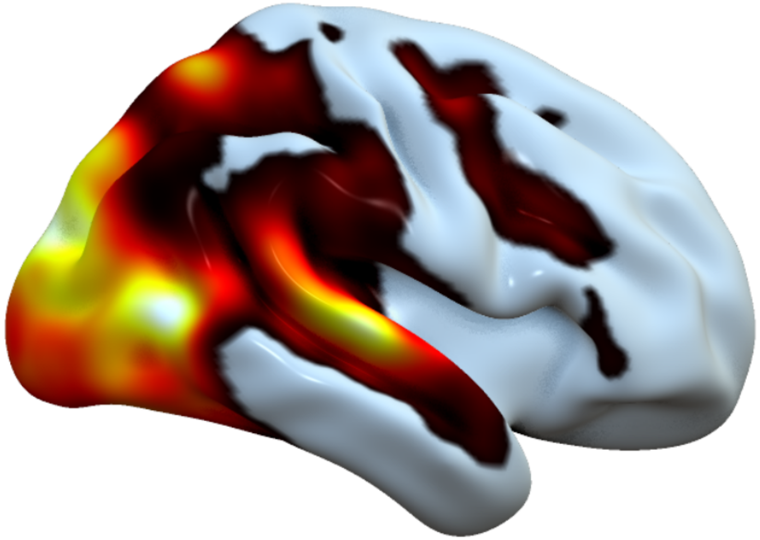
- Full mixed effects model would estimate within & between subject variances simultaniously => Computationally demanding to estimate!

# Summary statistics approach (mixed effect model)



First-level
statistical parametric maps

second level
analysis



Population level results

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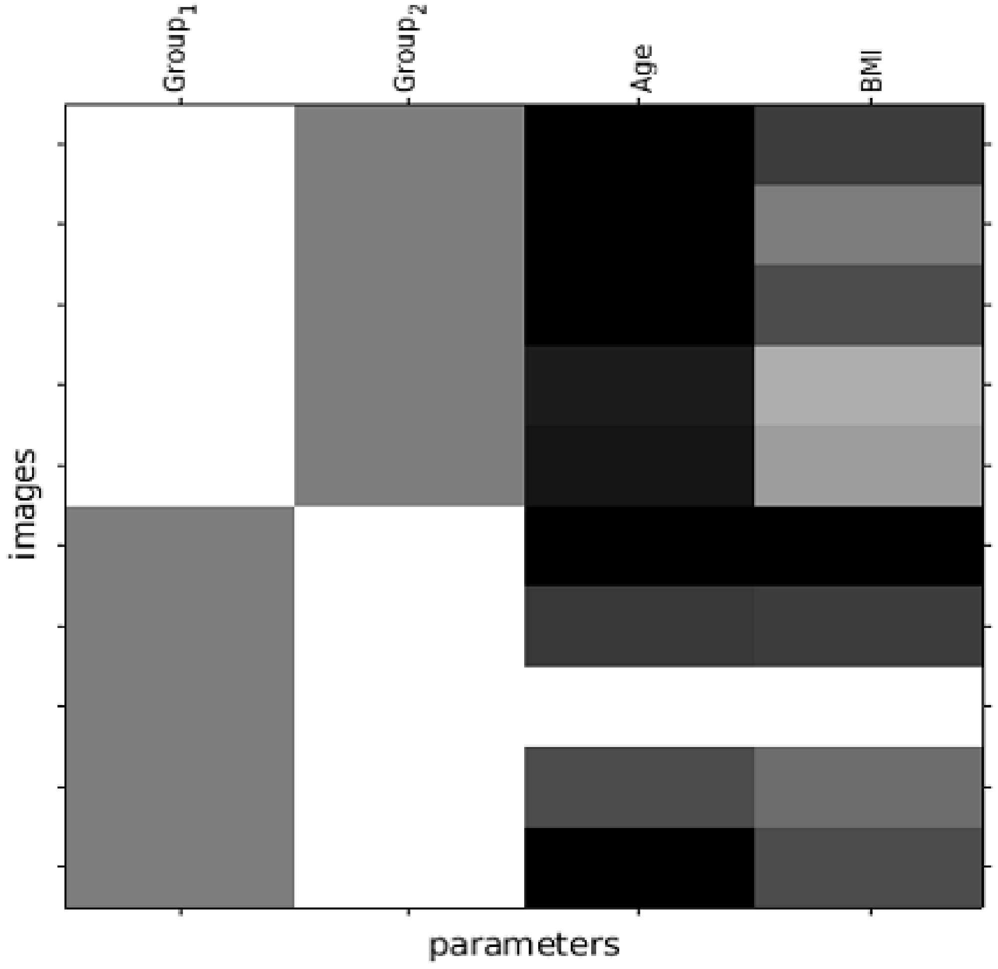
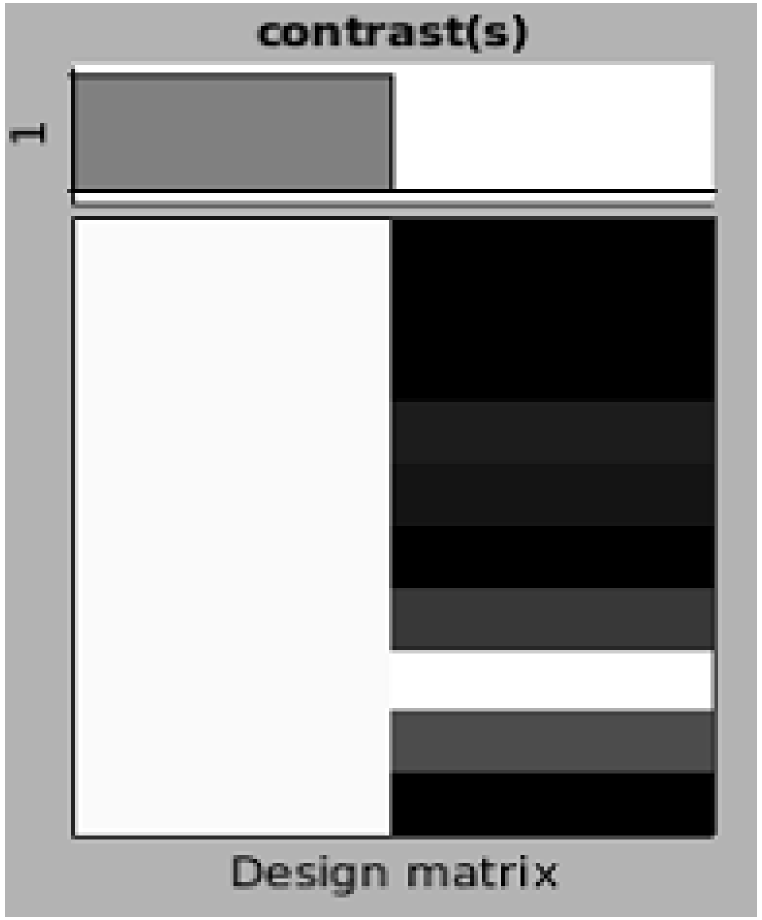
# Second level design matrix in SPM

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



# One sample T-test

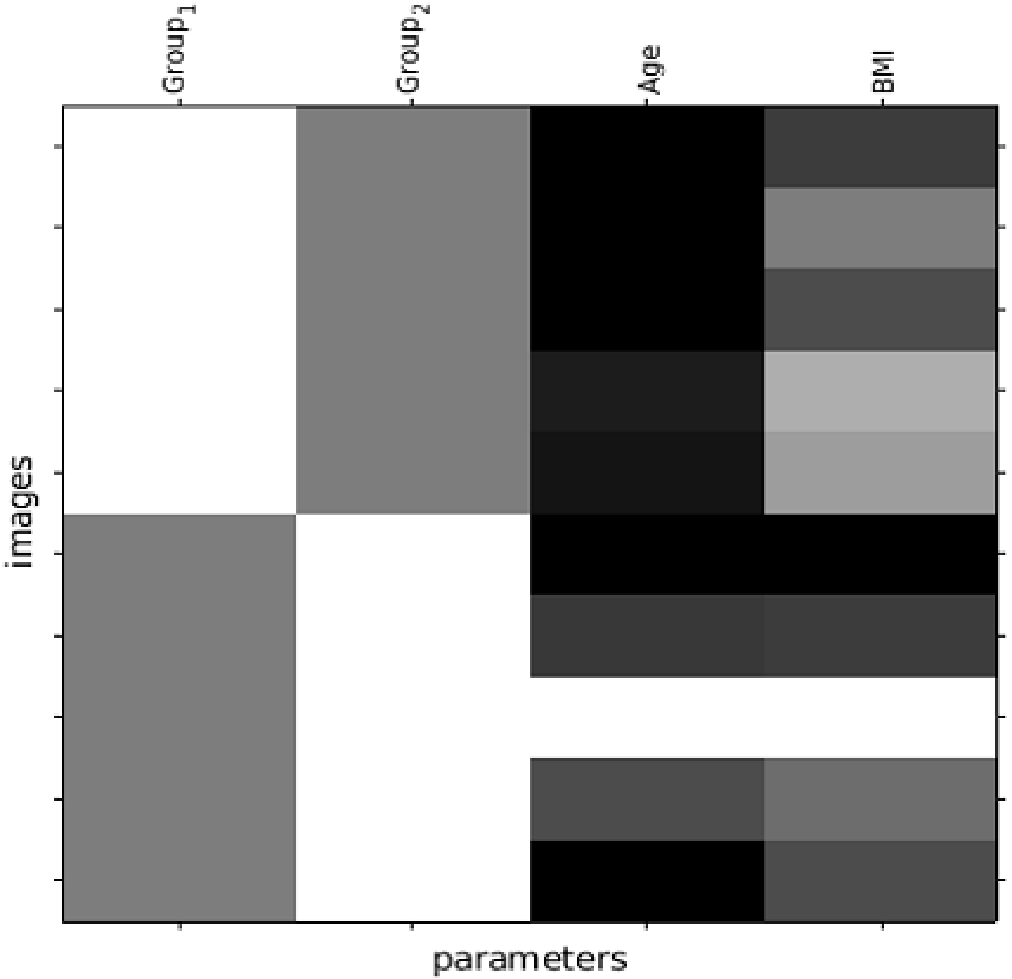
$\beta_{1st\ level} = \beta_{main} + \beta_{2nd\ level} X_{covariate}$



- **$\beta_{1st\ level}$**
  - Subjectwise association between the stimulus condition and BOLD signal
  - These are estimated in the 1st level analysis
- **$\beta_{main}$**
  - 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

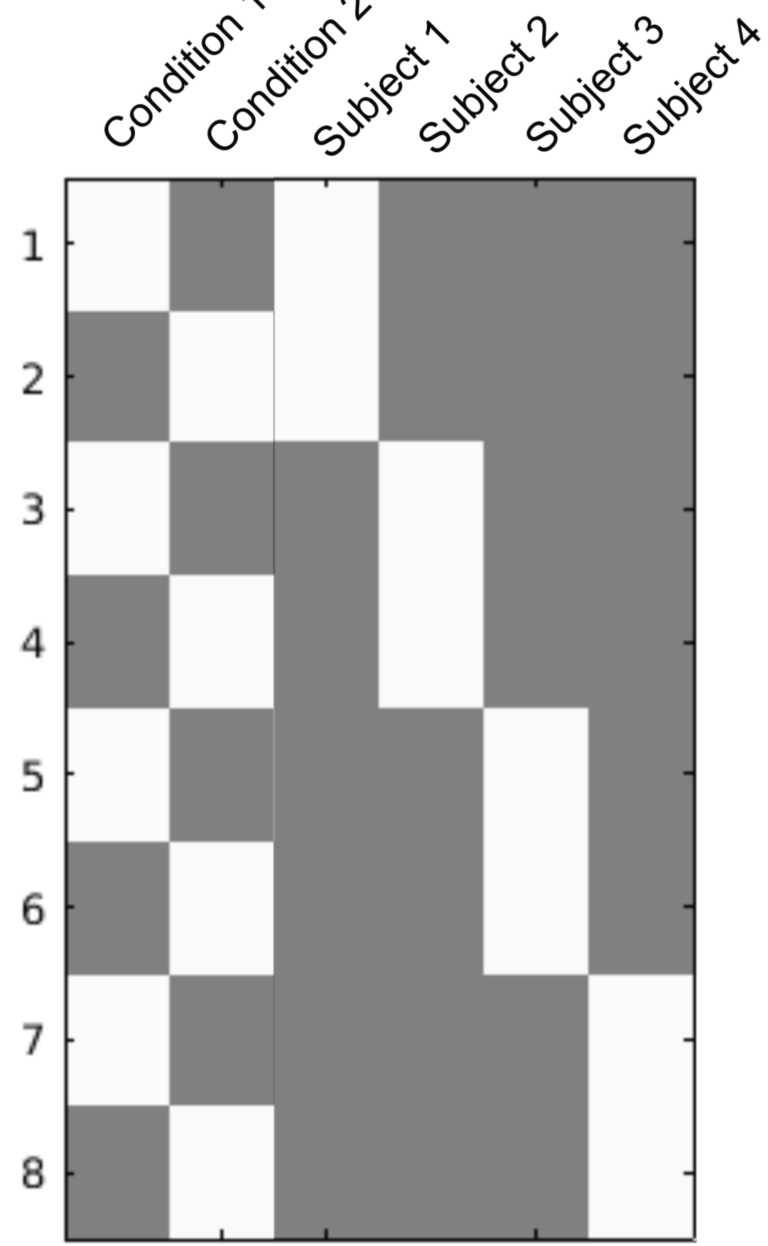
- H: Brain activity is different between two groups of subjects
- Group1 > Group2 with two additional covariates
  - SPM contrast [1 -1 0 0] = $\beta_{gr1} - \beta_{gr2}$
  - “Whether females have increased brain response for the 1st level condition than males when age and BMI are controlled for”



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

# 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"



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

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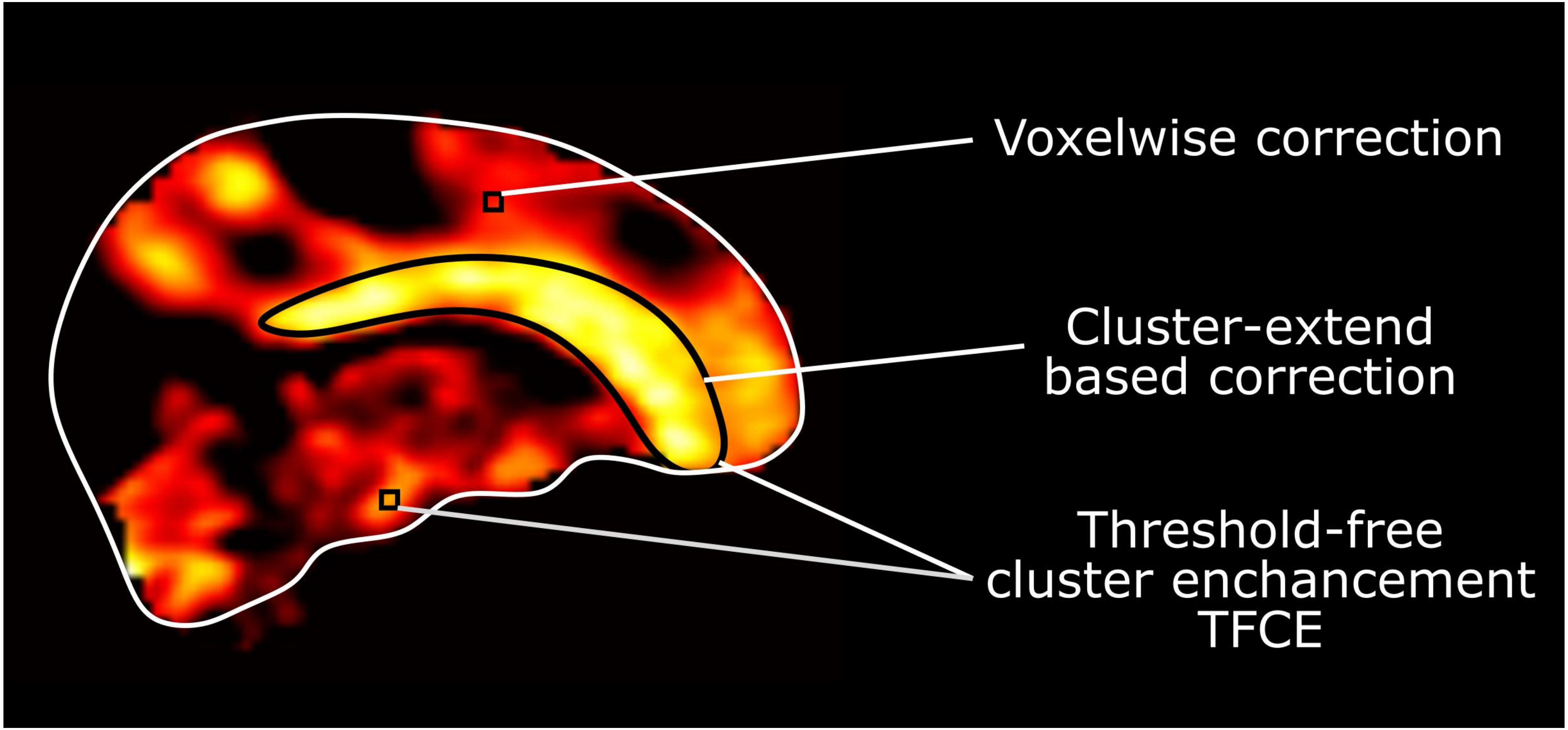
# Multiple comparisons correction methods



(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)

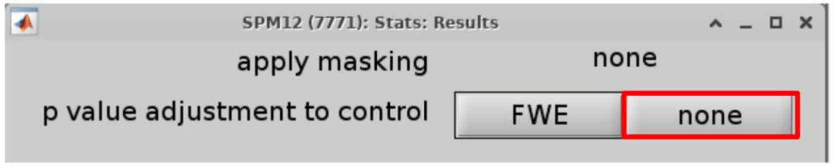
- Accounts for the spatial dependency between voxels
- “What is the probability to observe an activating cluster of this size under the null hypothesis of no activation”
- Twp-step procedure
    1. Choose primary voxel-level threshold
        1. e.g. $p < 0.001$
    2. Choose minimum size of a cluster
        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


SPM12 (7771): Stats: Results

apply masking

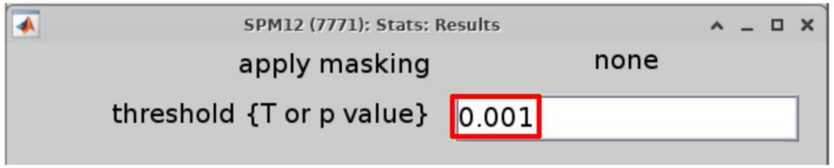
2


SPM12 (7771): Stats: Results

apply masking none

p value adjustment to control

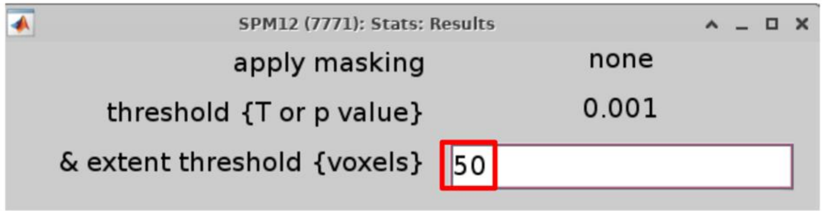
3


SPM12 (7771): Stats: Results

apply masking none

threshold {T or p value} 0.001

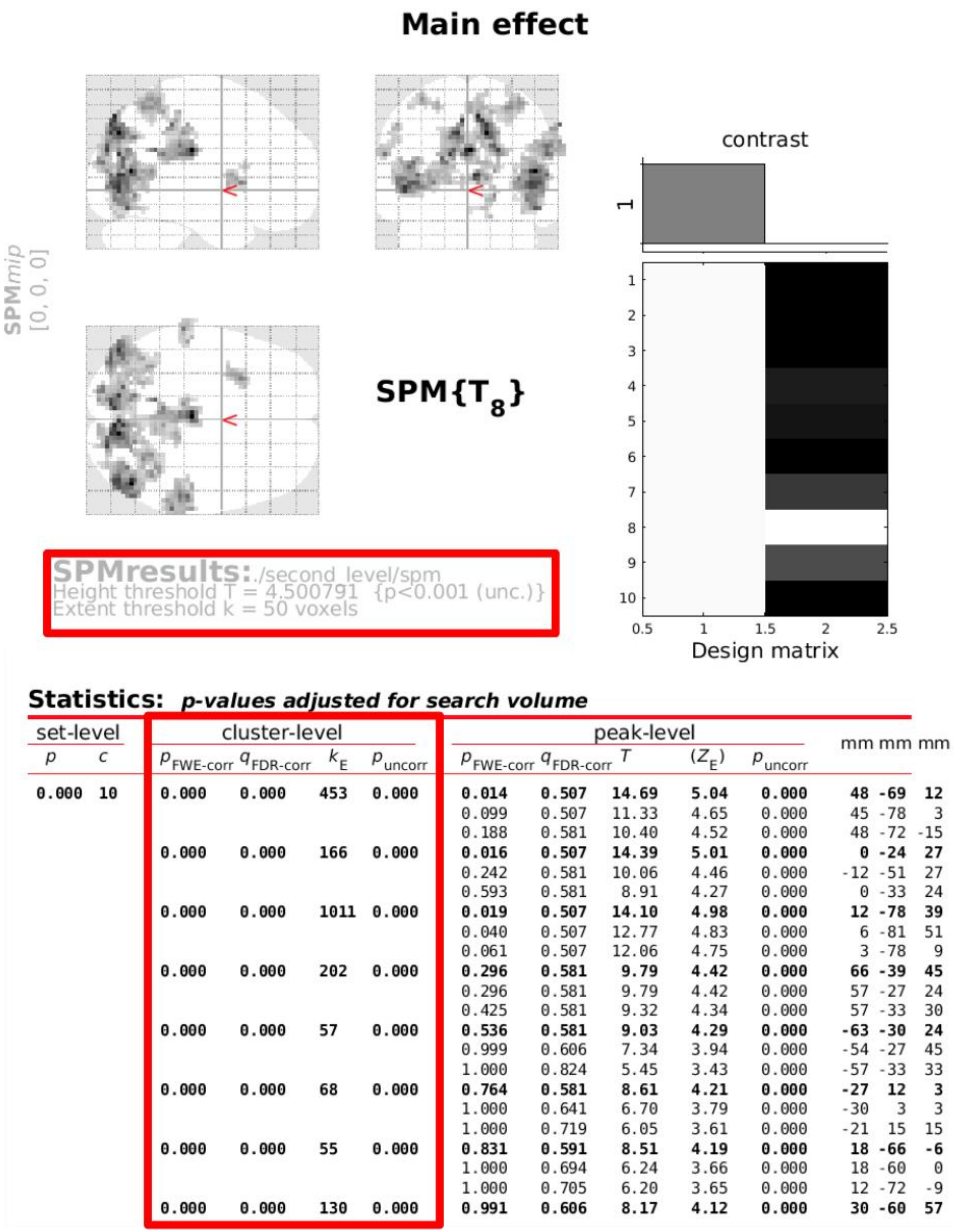
4


SPM12 (7771): Stats: Results

apply masking none

threshold {T or p value} 0.001

& extent threshold {voxels} 50

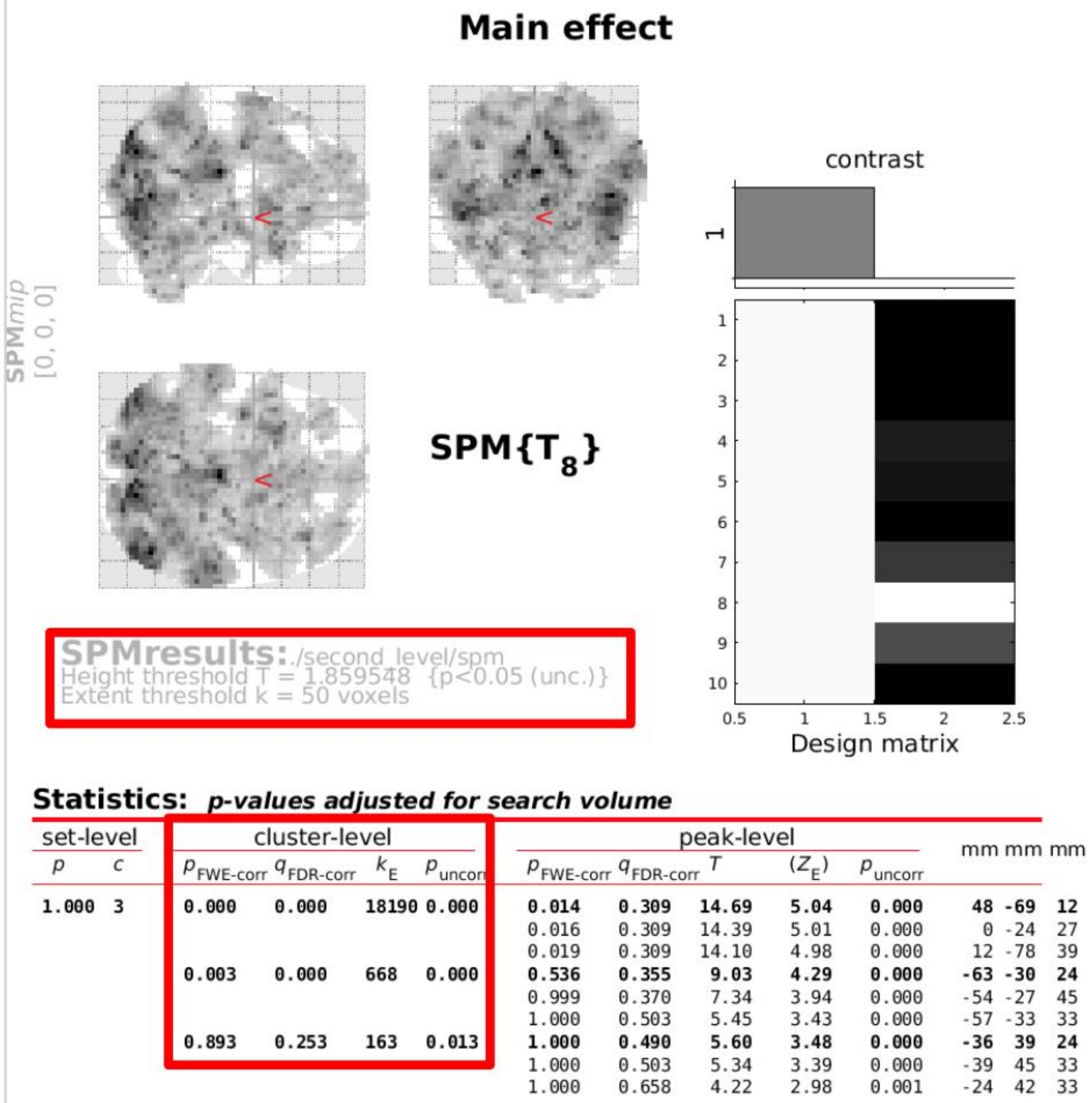


## Main effect

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 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $p$ | $c$ | $p_{FWE-corr}$ | $q_{FDR-corr}$ | $k_E$ | $p_{uncorr}$ | $p_{FWE-corr}$ | $q_{FDR-corr}$ | $T$ | $(Z_E)$ | $p_{uncorr}$ | | | |
| 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 |



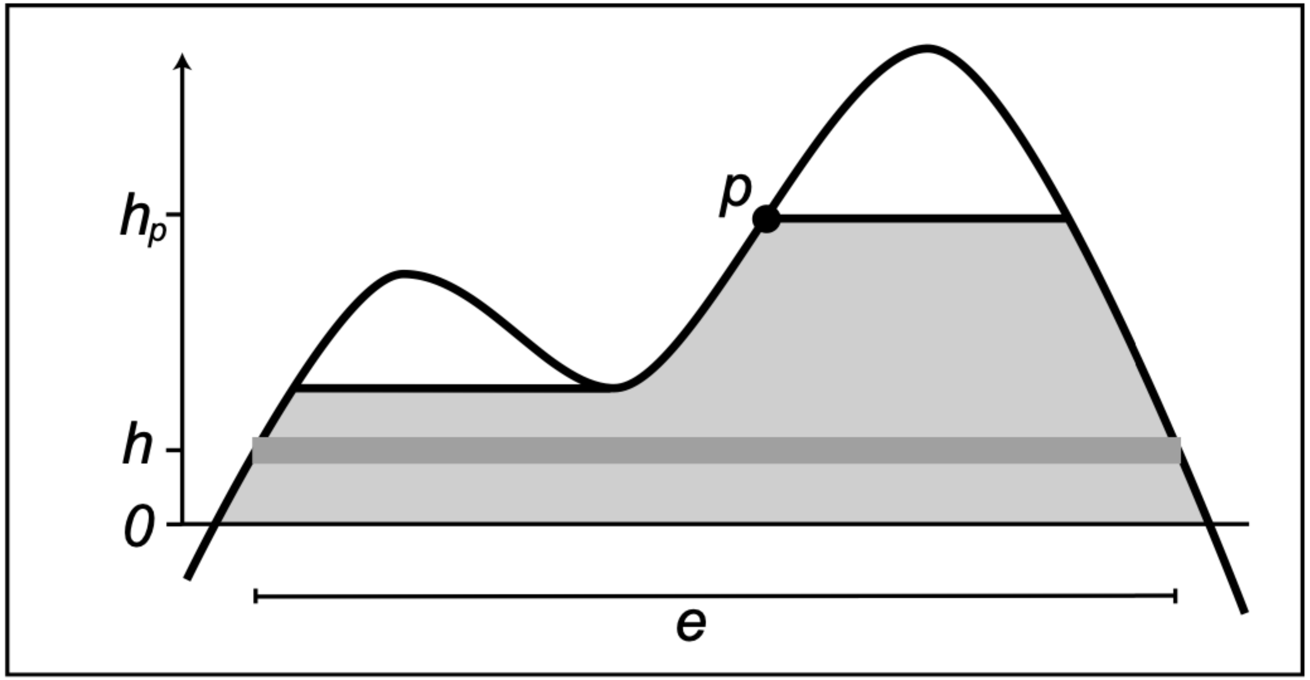
## Main effect

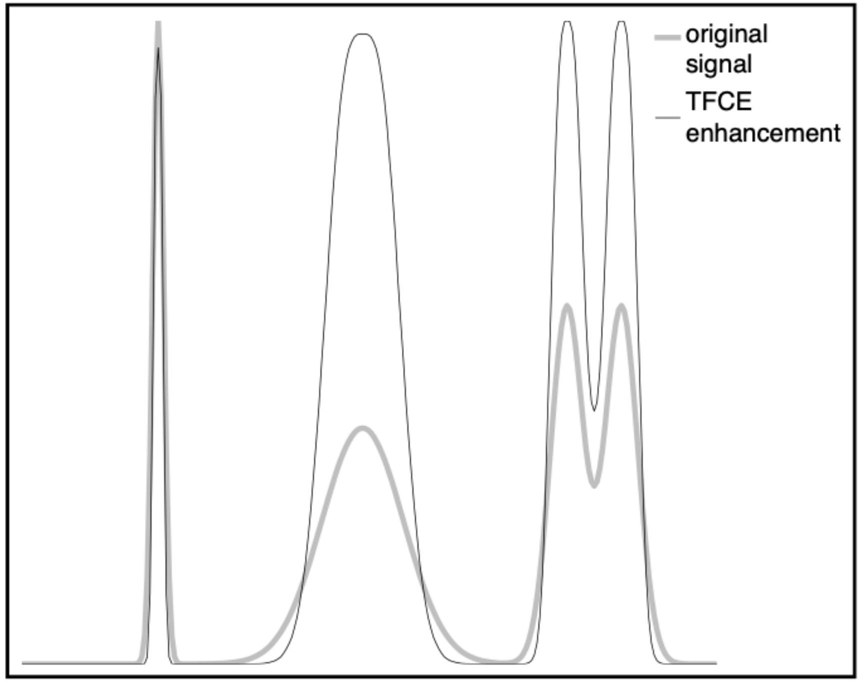
SPMresults: ./second_level/spm
Height threshold T = 1.859548 {p<0.05 (unc.)}
Extent threshold k = 50 voxels

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

| set-level | | cluster-level | | | | peak-level | | | | | mm | mm | mm |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $p$ | $c$ | $p_{FWE-corr}$ | $q_{FDR-corr}$ | $k_E$ | $p_{uncorr}$ | $p_{FWE-corr}$ | $q_{FDR-corr}$ | $T$ | $(Z_E)$ | $p_{uncorr}$ | | | |
| 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)



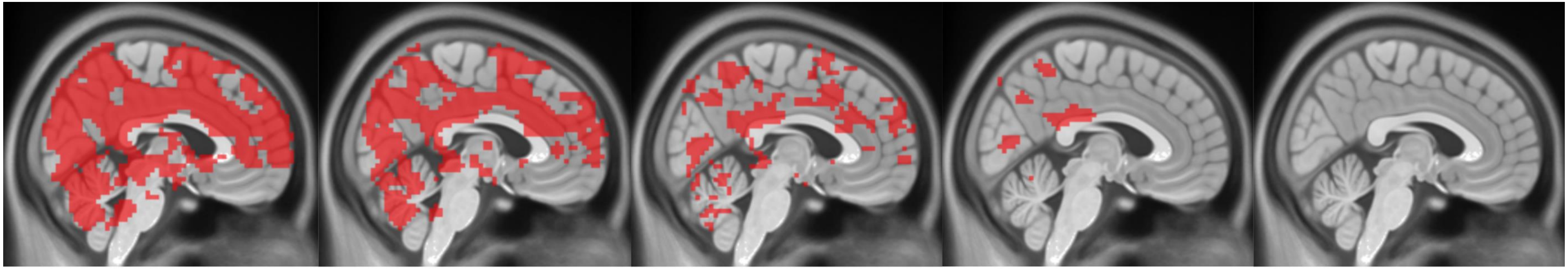


TFCE = $h_p$ (voxelwise t-value) * e (amount of supporting voxels)

→ 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)



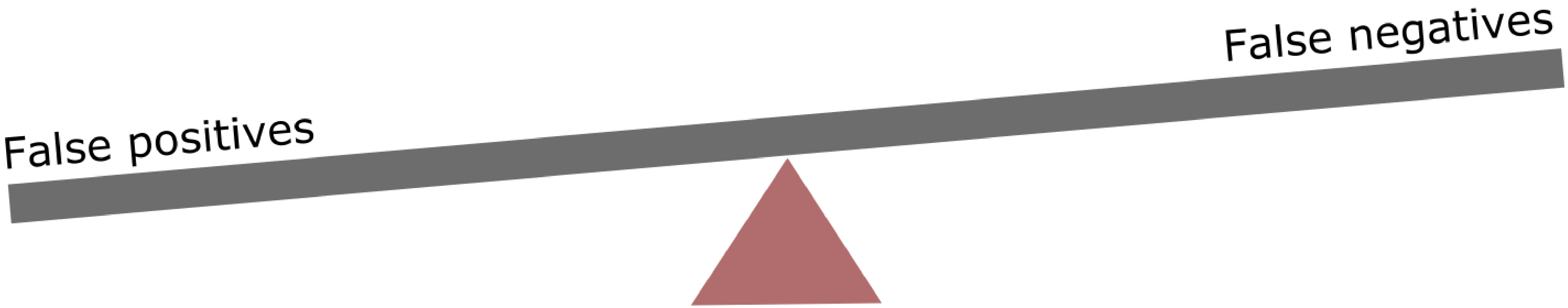
TFCE
(randomise)

Cluster FWE
p<0.05,k=50

FDR a=0.05
(randomise)

Cluster FWE
p<0.001,k=50

Bonferroni/FWE
p<0.05



False negatives

False positives

Conservative correction inflates effect size estimates

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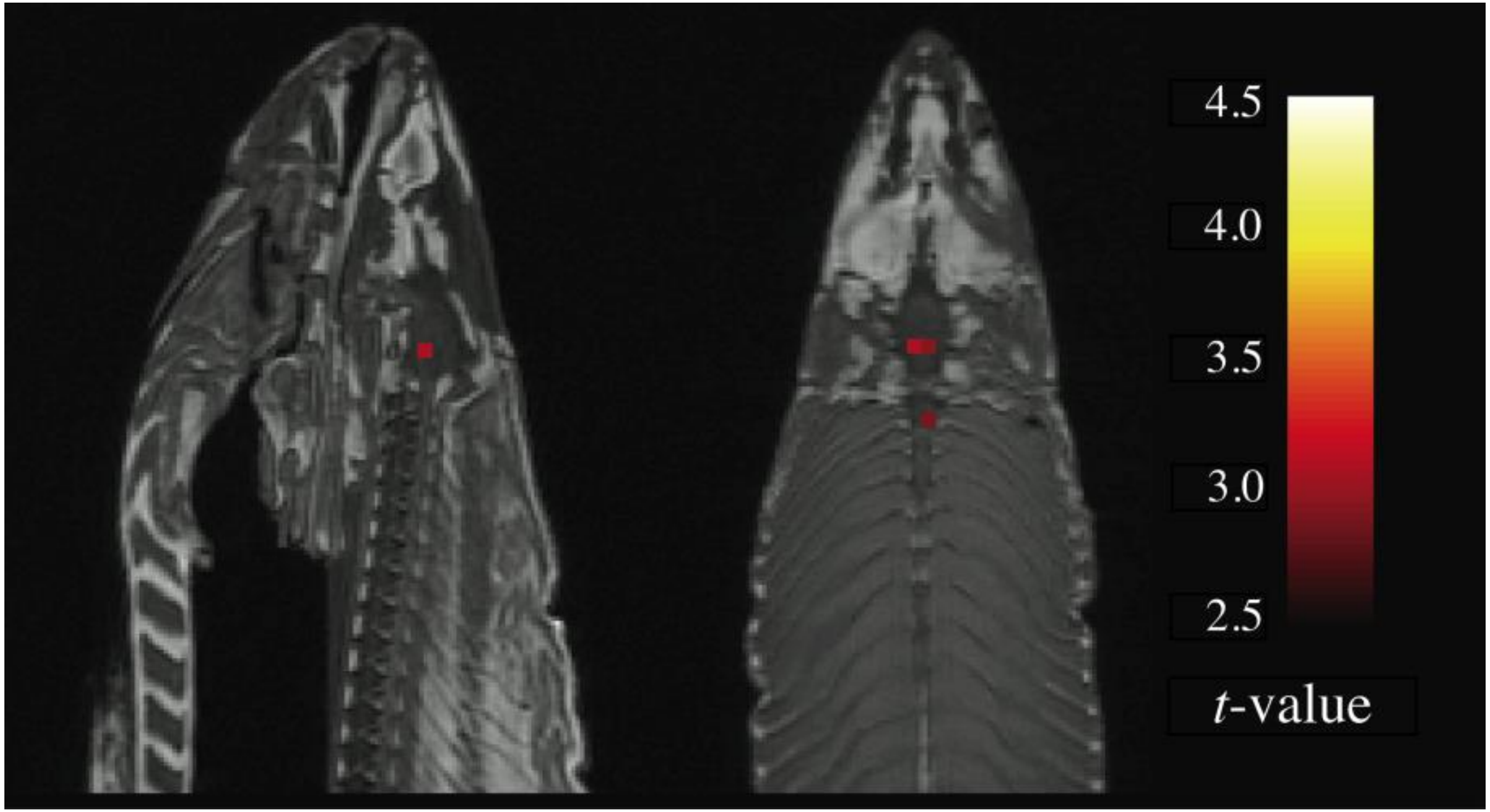
# Non-parametric tests

- 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)

# References

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