# Single subject analysis using GLM

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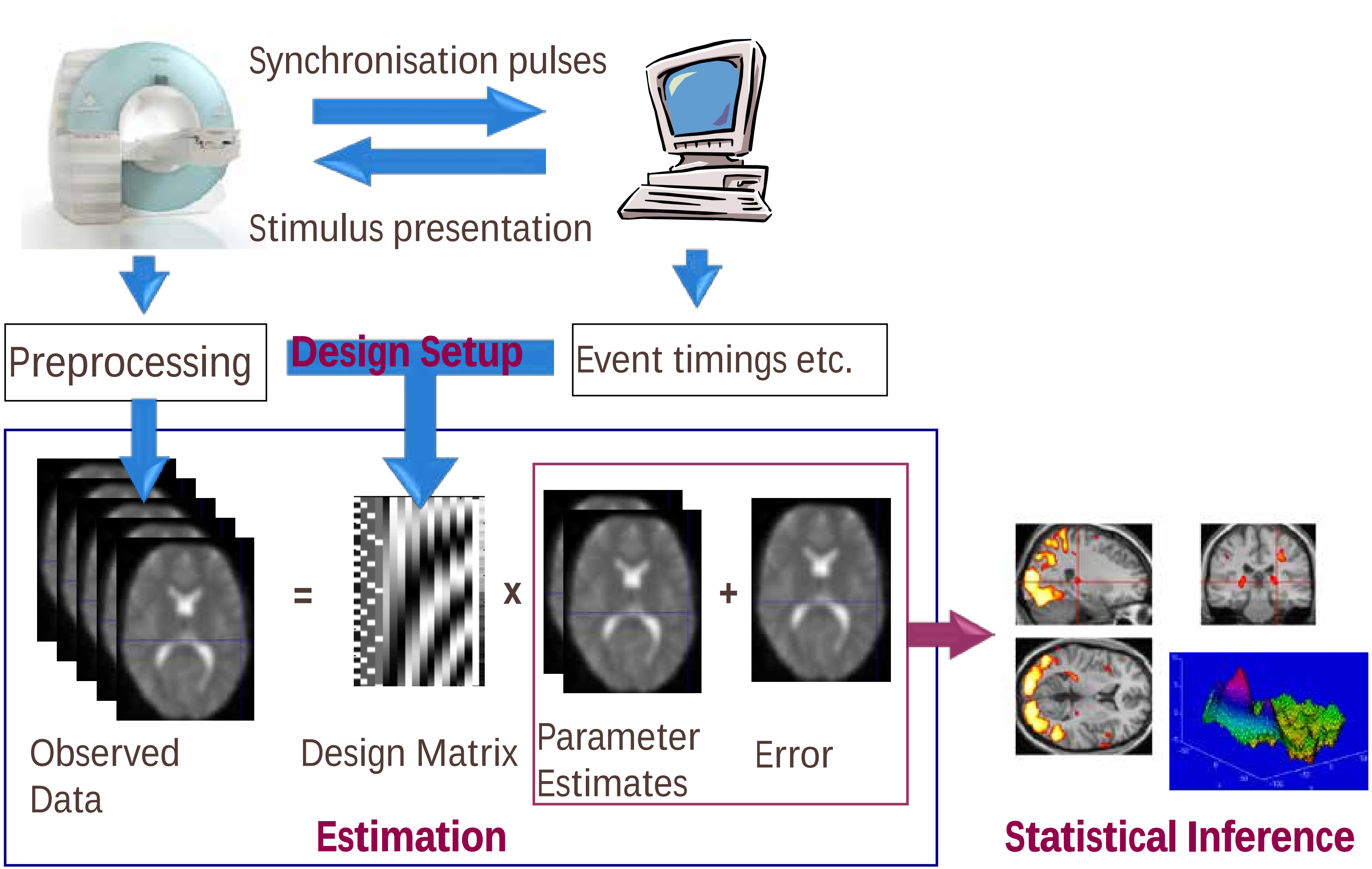
With thanks to Russell Thompson, Rik Henson, Matthew Brett and the authors of the HBF

# A functional experiment



# General Linear Model – Theory

- **GLM**
  - models observed data (dependent variable) – **Y**
  - as a linear combination (parameter estimate – **β**) of
  - regressors/predictor variables/explanatory variables (EV) (independent variables) – **X**

$$\mathbf{Y = X\beta+\varepsilon}$$

  - AN(C)OVA, t-test, (multiple) regression, LDA, CCA are also GLMs.
  - Relationship between a dependent variable and one or more independent variables

# General Linear Model – Theory

- **GLM**
  - For *i* observations modelled using *j* predictor variables:

← *j predictor variables* →

$$\begin{bmatrix} y_1 \\ y_2 \\ y_3 \\ \dots \\ y_i \end{bmatrix} = b_1 * \begin{array}{c} x_1 \\ \left\{\begin{bmatrix} x_{11} \\ x_{21} \\ x_{31} \\ \dots \\ x_{i1} \end{bmatrix}\right. \end{array} + b_2 * \begin{array}{c} x_2 \\ \left\{\begin{bmatrix} x_{12} \\ x_{22} \\ x_{32} \\ \dots \\ x_{i2} \end{bmatrix}\right. \end{array} + \dots + b_j * \begin{array}{c} x_j \\ \left\{\begin{bmatrix} x_{1j} \\ x_{2j} \\ x_{3j} \\ \dots \\ x_{ij} \end{bmatrix}\right. \end{array} + \begin{bmatrix} e_1 \\ e_2 \\ e_3 \\ \dots \\ e_i \end{bmatrix}$$

↑ *i observations* ↓

↑ *i noises* ↓

*j predictor weights*

# General Linear Model – Theory

- **GLM**
  - For *i* observations modelled using *j* predictor variables:

*j predictor variables*

*i observations*

*j predictor weights*

*i noises*

$$\begin{bmatrix} y_1 \\ y_2 \\ y_3 \\ \dots \\ y_i \end{bmatrix} = \begin{bmatrix} x_{11} & x_{12} & \dots & x_{1j} \\ x_{21} & x_{22} & \dots & x_{2j} \\ x_{31} & x_{32} & \dots & x_{3j} \\ \dots & \dots & \dots & \dots \\ x_{i1} & x_{i2} & \dots & x_{ij} \end{bmatrix} \times \begin{bmatrix} b_1 \\ b_2 \\ b_3 \\ \dots \\ b_j \end{bmatrix} + \begin{bmatrix} e_1 \\ e_2 \\ e_3 \\ \dots \\ e_i \end{bmatrix}$$

$$Y = X\beta + \varepsilon$$

# General Linear Model – Application

- **Mass univariate approach:**
  - Model $\mathbf{X}_{1\text{-}i,1\text{-}j}$ estimated for each voxel independently (multiple tests)
  - For each voxel:
    - $\mathbf{Y}_{1\text{-}i}$: timeseries of i observations at a single voxel
    - $\mathbf{B}_{1\text{-}j}$ for each of the j predictors

↓

  - Series of $\boldsymbol{\beta}_{1\text{-}j}$ images (beta0001-beta000j)

# General Linear Model – Application

- **Model:**
  - Contains all known source of variance:
    - Controlled factors (e.g. stimuli)[1]
    - All nuisance variables[1]
  - Assumption about noise (ε)
    - High-pass filter
    - Temporal autocorrelation
    - Spikes (aa)
    - Movement parameters (SPM)
    - PhysIO Toolbox
    - Imaging Wiki/PhysNoise
    - GLMDenoise (aa)
    - FIX (FSL)

# General Linear Model – Application

- **Model vs. Design:**
  - Model
    - Event-related (duration = 0)
    - Epoch-based (duration > 0)
      - Can be modelled with events
        - To model HRF along the epoch (e.g. FIR)
        - But: $\hat{\beta}pR_{event} \sim \hat{\beta}pR_{epoch}$ / nEvent[1]

  - Design
    - Blocked (fix SOA)
    - Randomized (variable SOA) – jittering
      - Lower predictability ( à  subject is more engaged)
      - Higher estimability ( ß  samples from various phases of the HRF)

# General Linear Model – Application

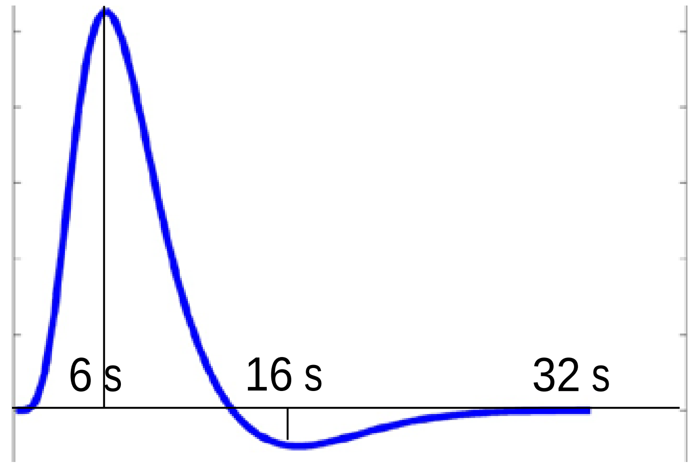
- **Model vs. Design:**

| Model / Design | Epoch-based | Event-related |
|---|---|---|
| **Blocked (fix SOA)** | "block-design" | "non-jittered event-related design" |
| **Randomized (variable SOA)** | "randomised block-design" | "jittered event-related design" |

# General Linear Model – Application

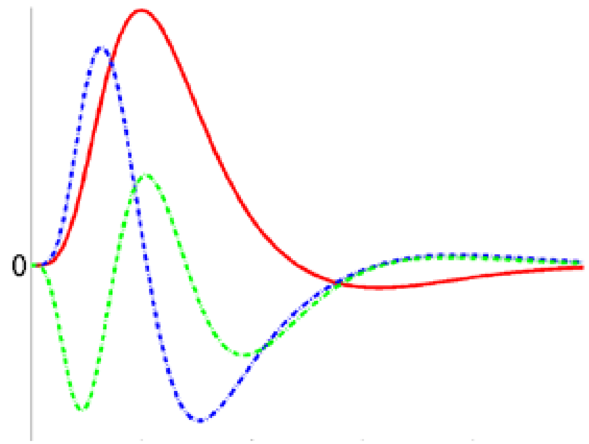
- **BOLD response – Kernel:**
  - stereotyped pattern of response
    (based on primary sensory areas)

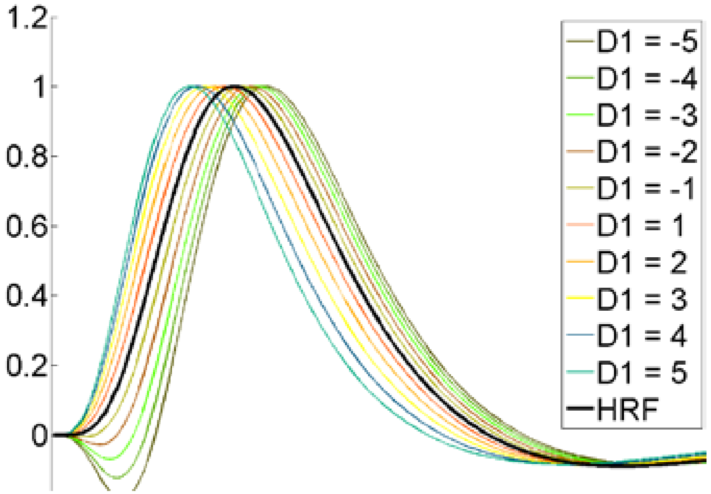
  - Canonical Haemodynamic Response Function
    (HRF) – *spm_hrf(TR,p)*
    - Double-gamma function
    - uses 7 parameters
      - p(1) - delay of response (relative to onset) 6
      - p(2) - delay of undershoot (relative to onset) 16
      - p(3) - dispersion of response 1
      - p(4) - dispersion of undershoot 1
      - p(5) - ratio of response to undershoot 6
      - p(6) - onset (seconds) 0
      - p(7) - length of kernel (seconds) 32

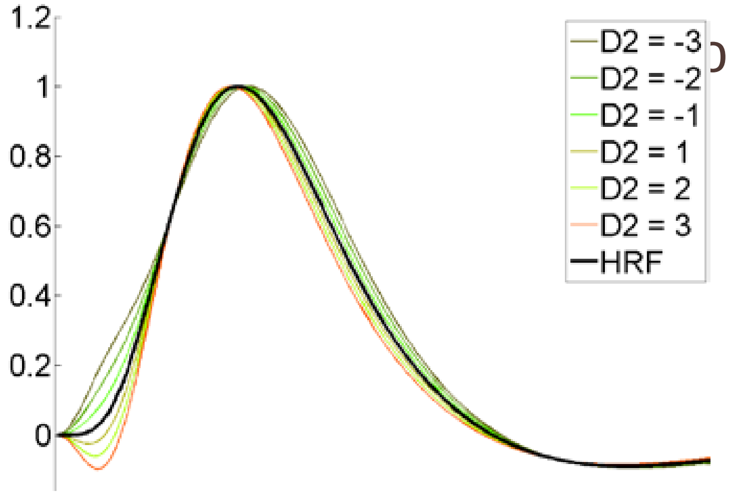


# General Linear Model – Application

- **HRF**
  - Derivatives (to “adjust” HRF[1]):

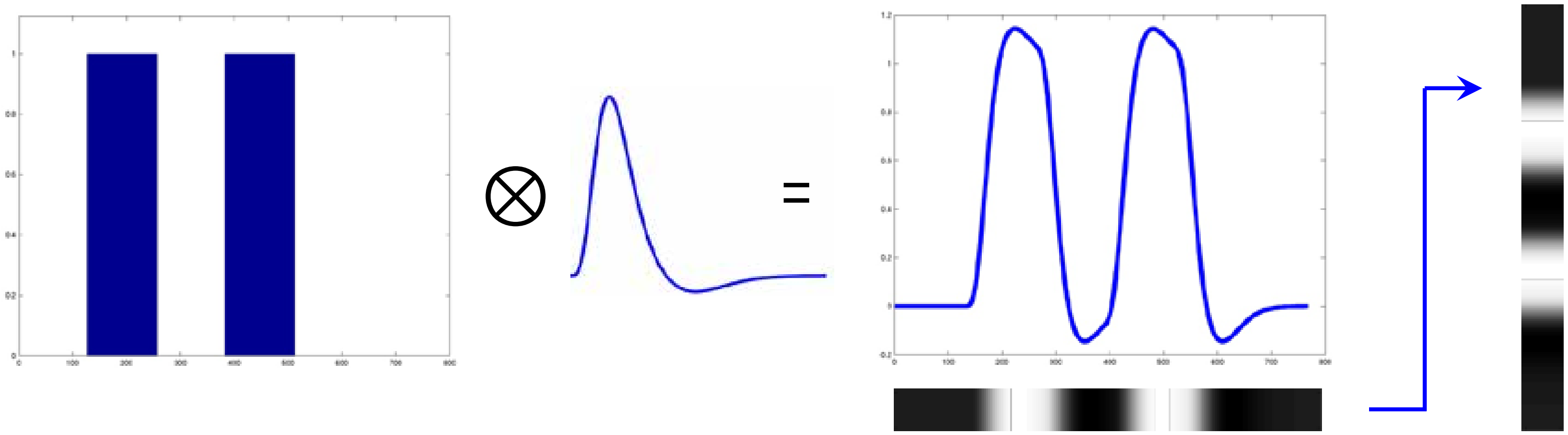


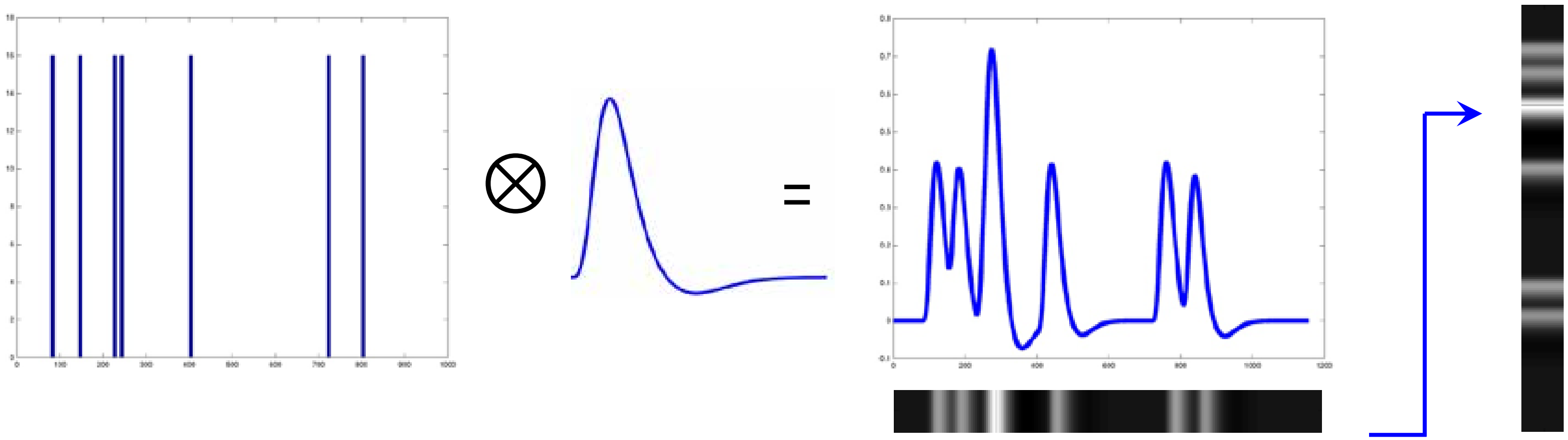



# General Linear Model – Application

- **BOLD response - Convolution:**
  - Epochs



  - Events



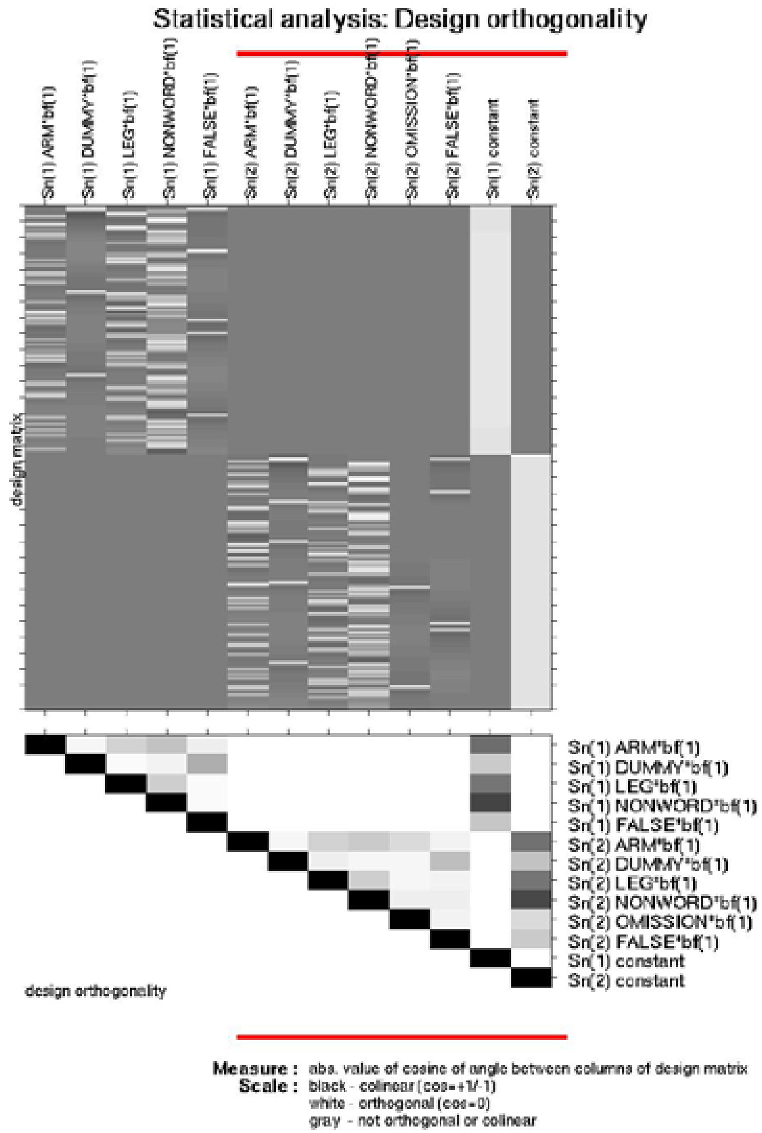
# General Linear Model – Application

- **Non-orthogonality**
  - Pairwise correlation (SPM)

  - Rank deficiency (more general)
    - If any column of **X** is a linear combination of any others

↓

  - Some parameters cannot be estimated uniquely (e.g. in case of EV1 and EV2)
    - ↓ efficiency for EV1, EV2 and EV1+EV2
    - Good efficiency for EV1-EV2



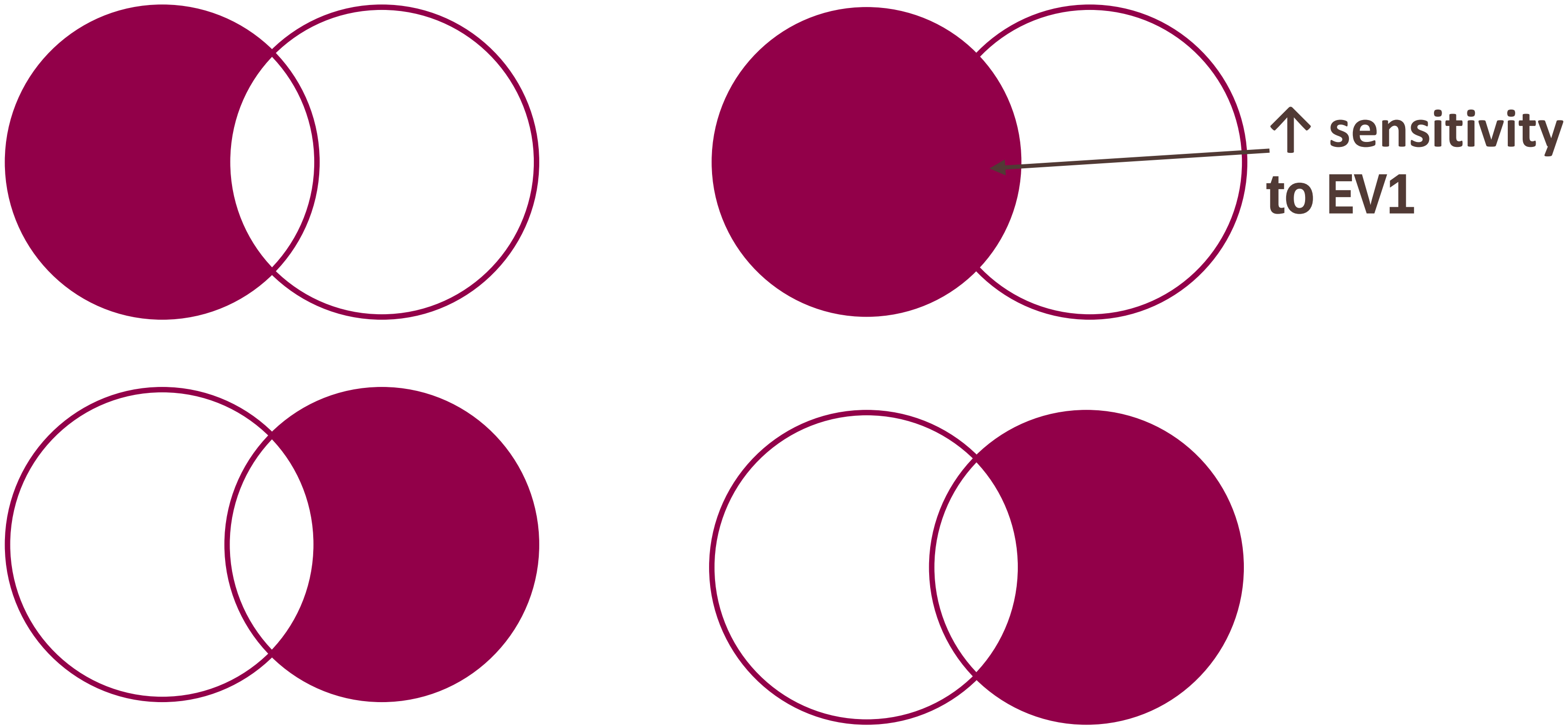
# General Linear Model – Application

- **Orthogonalisation**
  - Assigning shared explained variance

Without Orthogonalisation | Orthogonalise EV2 wrt. EV1

- Variance explained by EV1

- Variance explained by EV2



# General Linear Model – SPM input

- **Regressor = predictor variables/explanatory variables** – One ß for each regressor
  - Condition $\otimes$ HRF
    - Name
    - Onset
    - Duration
    - Modulation
  - Covariate: it will not be convolved with HRF

- **aa_user_fmri.m**
  - aas_addevent(aap, 'aamod_firstlevel_model', mriname, sessname, onsets, durations, modulation)

  - aas_addcovariate(aap, 'aamod_firstlevel_model', mriname, sessname, covarName, covarVector, HRF, interest)

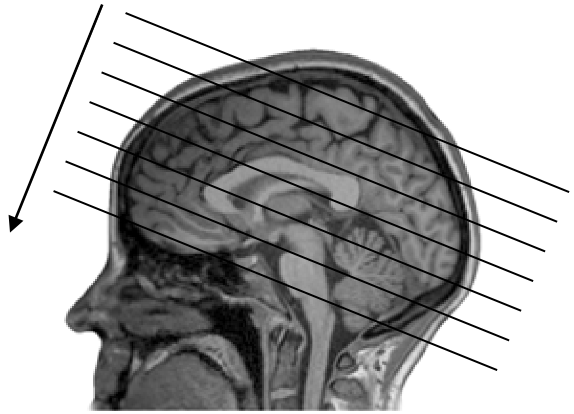
# General Linear Model – SPM input

- **Modulation: effect with many level (or continuous)**
    - Parametric modulation
        - Stimulus strength
        - Response accuracy
    - Temporal modulation = Parametric modulation using onset as parameter
        - Habituation
        - Learning
    - Polynomial Expansion: higher-order effects

    - Disadvantages
        - Correlated regressors à Orthogonalisation
            - < SPM12: always on (left to right)[1]
            - SPM12: switchable
        - Difficult to set up interactions between factors

# General Linear Model – SPM input

- **"Microtiming" ~ Slicetiming[1]**
  - Microtime resolution: time bins within one TR
    - Highest temporal precision = TR / MicRes
    - Divided equally for the whole TR
  - Microtime onset: first time bin



  - [2]Sparse EPI: e.g. TR = 3s, TA = 2s, 20 slices


    - Use middle slice (#10) as reference:

| **Slicetiming** | | **Microtiming[2]** | |
|---|---|---|---|
| **Slice order** | 20:-1:1 | **Microtime resolution** | 30 |
| **Reference slice** | 10 | **Micortime onset** | 10 |

# General Linear Model – SPM output

- beta_XXXX – $\hat{\boldsymbol{\beta}}$ (XXXX: for each EV)

- mask – brain mask (voxels included in the analysis)[1]

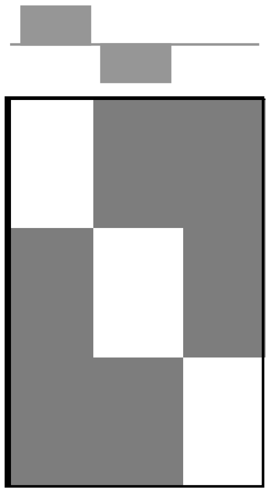
- ResMS – $\hat{\boldsymbol{\varepsilon}}$ (mean squared)
  - Heterogeneity may indicate unexplained variance in the data

- RPV – RESELs Per Voxel (local smoothness)
  - RPV = $\mathrm{FWHM}_{\text{in voxel}}^{-3}$
  - ≤ 0.037 (RESEL ≥ 3 voxel) to ensure the minimum smoothness for GRFT[2]

# General Linear Model – Contrasts

- **Specific hypotheses to be tested using t or F statistics**
  - How each predictor (column of the design matrix) plays a role
  - E.g. condition 1 > condition 2  à  contrast [1 -1 0]

$c = \boxed{1}\boxed{-1}\boxed{0}$



- **aa_user_fmri.m**
  - aas_addcontrast(aap, 'aamod_firstlevel_contrasts', mriname, format, vector, conname,contype)

# General Linear Model – Contrasts

- **F contrast:**
  - Model comparison: How much variance is explained by the model including the conditions of interest (e.g. movement parameters)?
  - Non-directional
  - Can contain more rows (OR)

    [1 0 0; % EV1 > baseline OR

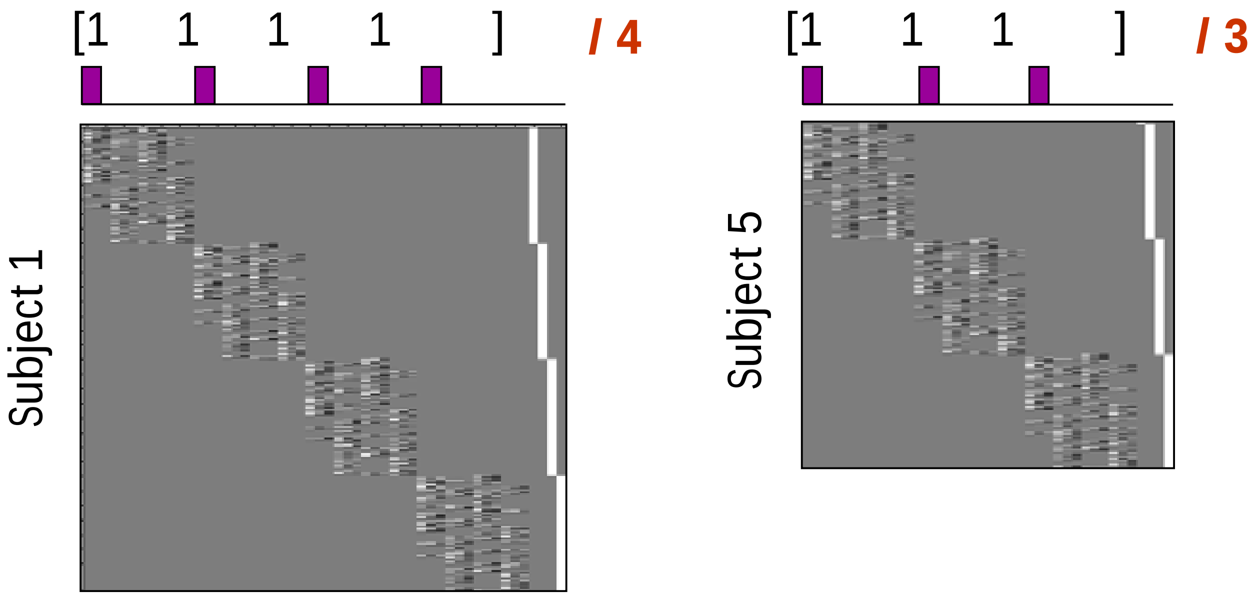
     0 1 0] % EV2 > baseline

    How much variance is explained by EV1 and EV2? Should we include them in the model?[1]
  - SPM output:
    - ess*.img (Extra Sum of Squares)
    - spmF*.img (F-map à p)

- **T contrast:**
  - Hypothesis test: How do the conditions of interest relate to each other and/or to the baseline?
  - Directional
  - Can contain only one row
  - SPM output:
    - con*.img (Contrast)
    - spmT*.img (T-map à p)

# General Linear Model – Contrasts

- **T contrast:**
  - The contrast vector sums to 0, 1 or -1
  - Positive weights sum to 1
  - Negative weights sum to -1
  - Scaling issue
    - T-stat does not depend on scaling (of both the regressors and the contrast).
    - Contrast[1] depend on scaling[2] à consistency across subjects!




courtesy to Ferath Kherif

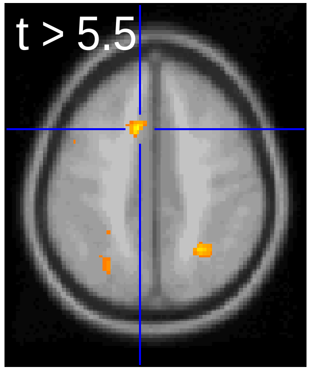
# General Linear Model – Contrasts

- **T contrast:**
  - Examples[1]:
    - [1 0 0] : condition 1 > unmodelled/implicit baseline
    - [2 -1 -1] : condition 1 > sum of conditions 2 and 3
    - [1 -0.5 -0.5] : condition 1 > average of conditions 2 and 3
    - [-3 -1 1 3] : linear increase over 4 conditions

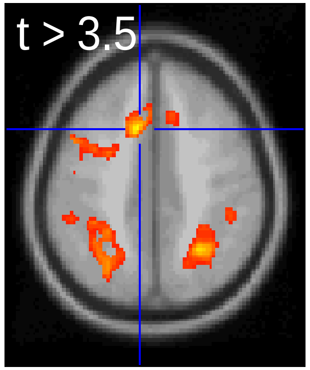
# Statistical Inference

- **How much is enough?**
  - Converting a continuous statistical value (p) to a binary decision à cut-off

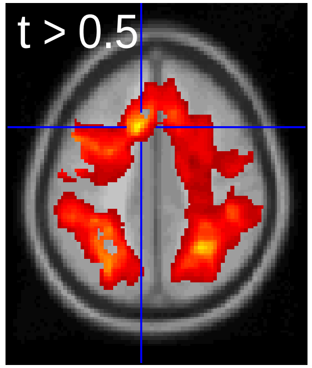
High Threshold




Medium Threshold




Low Threshold




Good Specificity

Poor Sensitivity
(risk of false negatives)

**Very Certain**[1]

Good Sensitivity

Poor Specificity
(risk of false positives)

**Quite Uncertain**[1]

# Statistical Inference

- **How to make certainty comparable?**

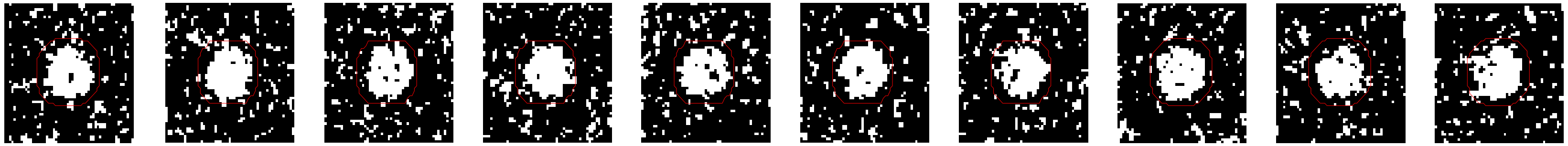
- **Multiple comparison**
  - Testing 100,000 random voxels at p = 0.05 à 5000 “significant” by chance (false positives, or “type I” errors)[1]

- **FamilyWise Error Rate (FWER):**
  - Chance of any false positive in the “family” (any similar measurement)

- **False Discovery Rate (FDR):**
  - Chance/Proportion of false positives in the rejected tests (i.e. suprathreshold results)
  - To enable in SPM:
    - *global defaults*
    - *defaults.stats.topoFDR = 0;*

# Statistical Inference

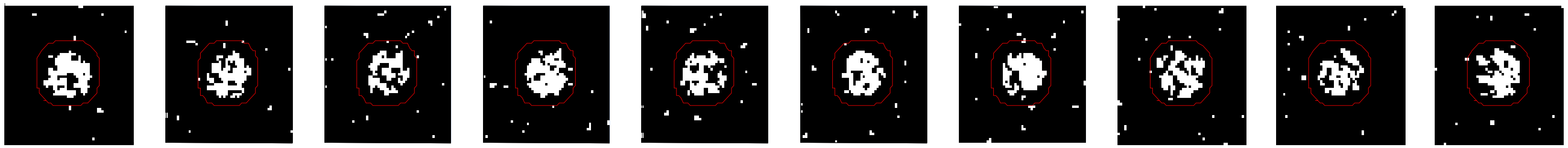
Control of Per Comparison Rate (i.e.none) at 10%



11.3% 11.3% 12.5% 10.8% 11.5% 10.0% 10.7% 11.2% 10.2% 9.5%

Percentage of Null Pixels that are False Positives

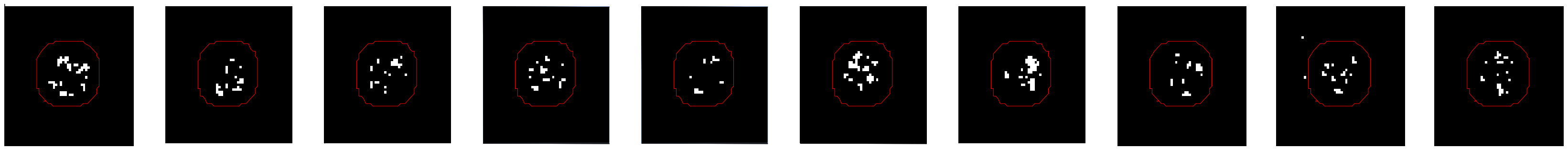
Control of False Discovery Rate at 10%



6.7% 10.4% 14.9% 9.3% 16.2% 13.8% 14.0% 10.5% 12.2% 8.7%

Percentage of Activated Pixels that are False Positives

Control of Familywise Error Rate at 10%



FWE

Occurrence of Familywise Error

# Statistical Inference

- **FWE**
  - Bonferroni correction: $p_{(corrected)} = p_{(uncorrected)} / n_{comparisons}$
    - Overly conservative in fMRI due to spatial dependence:
    - Nearby voxels are correlated: smoothness
      - Smoother data = More voxels in correlation à fewer indep. elements

↓

  - Gaussian Random Field Theory (GRFT) – default in SPM
    - Estimate the true number of independent/RESolution Elements (RESEL) using GRFT à RPV.img: RESEL Per Voxel (local smoothness)
    - Corrects for the number of RESELs à Less conservative than Bonferroni

# Statistical Inference

- **aa default**
  - aamod_firstlevel_threshold.xml

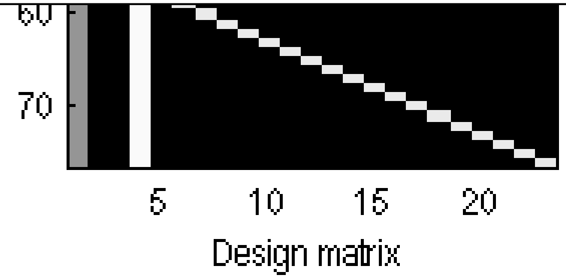
- **aa_user_fmri.m / aap_parameters.mat**
  - aap.tasksettings.aamod_firstlevel_threshold.threshold.correction = 'none';
  - aap.tasksettings.aamod_firstlevel_threshold.threshold.p = 0.001;
  - aap.tasksettings.aamod_firstlevel_threshold.threshold.extent = 0;

# Statistical Inference

## Levels of inference

- Peak
- Cluster
- Set

**SPMresults:** .\ANOVA
Height threshold T = 4.802783 {p<0.05 (FWE)}
Extent threshold k = 0 voxels



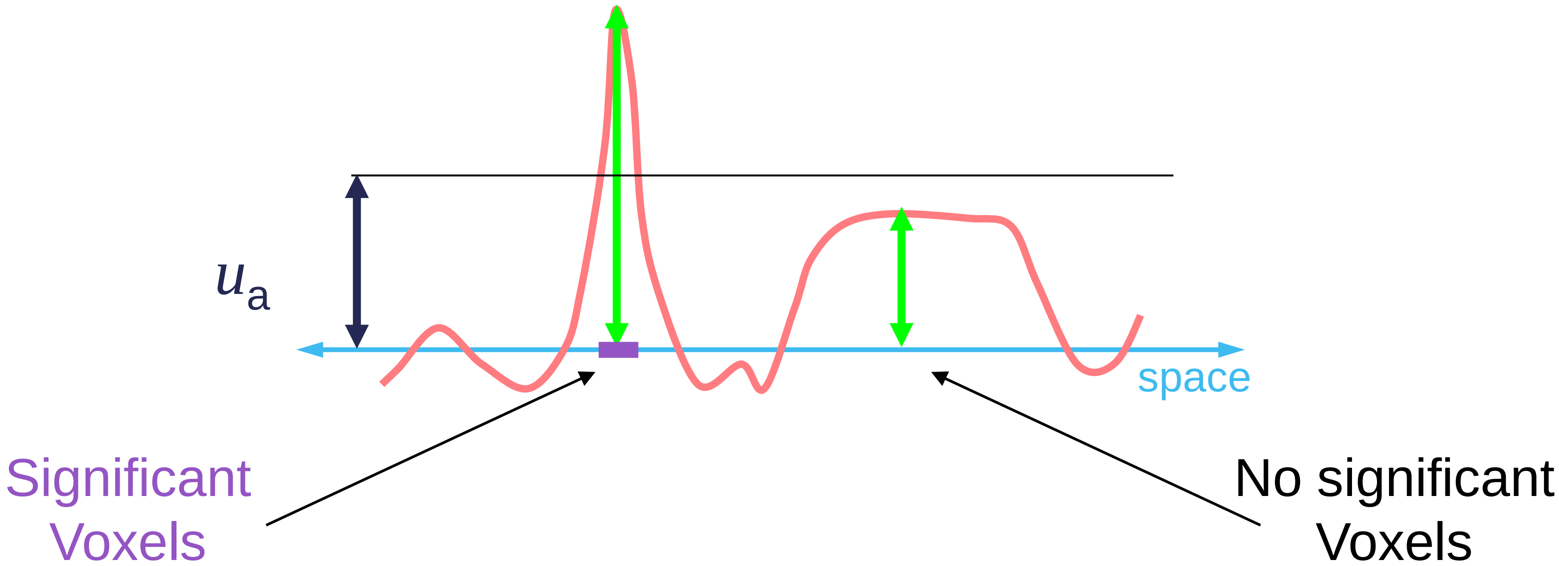

**Statistics:** *p-values adjusted for search volume*

| set-level | | cluster-level | | | | peak-level | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $p$ | $c$ | $p_{FWE-corr}$ | $q_{FDR-corr}$ | $k_E$ | $p_{uncorr}$ | $p_{FWE-corr}$ | $q_{FDR-corr}$ | $T$ | $(Z_{\equiv})$ | $p_{uncorr}$ | mm | mm | mm |
| 0.000 | 5 | 0.000 | 0.000 | 1292 | 0.000 | 0.000 | 0.000 | 8.99 | 7.00 | 0.000 | 36 | -22 | 62 |
| | | | | | | 0.000 | 0.000 | 8.90 | 6.95 | 0.000 | 40 | -18 | 56 |
| | | 0.000 | 0.004 | 321 | 0.001 | 0.000 | 0.000 | 7.63 | 6.26 | 0.000 | -18 | -50 | -26 |
| | | 0.003 | 0.085 | 84 | 0.068 | 0.002 | 0.062 | 5.84 | 5.12 | 0.000 | -58 | -18 | 18 |
| | | 0.003 | 0.085 | 97 | 0.052 | 0.007 | 0.185 | 5.45 | 4.84 | 0.000 | -56 | 6 | 10 |
| | | | | | | 0.012 | 0.271 | 5.28 | 4.72 | 0.000 | -44 | -4 | 8 |
| | | 0.035 | 0.703 | 4 | 0.703 | 0.041 | 0.810 | 4.87 | 4.42 | 0.000 | -60 | -22 | 40 |

# Statistical Inference

## Levels of inference

- **Peak-level (voxel-level) inference**
  - **Probability *p* of that or higher peak voxel intensity (i.e. T/F-value)**
  - Retains voxels above the threshold ( $p \leq u_a$ )
  - Best spatial specificity: interpretable for each voxel





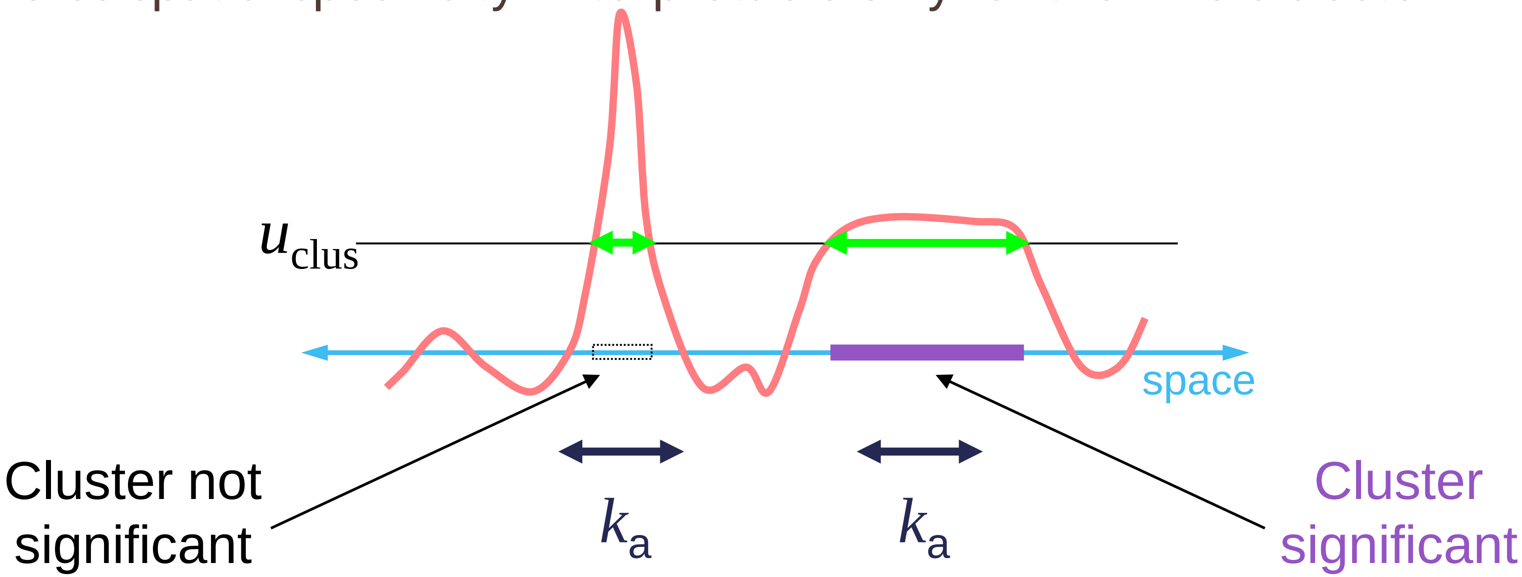

courtesy to Tom Nichols

# Statistical Inference

## Levels of inference

- **Cluster-level inference (default in FSL)**
  - **Probability *p* of that or larger number of neighbouring significant voxels**
  - Two thresholds
    - Define significant voxels by “cluster-forming” threshold ( $p \leq u_{clus}$ )
    - Retains only large clusters ( $p \leq P(k_a)$ )

  - Worse spatial specificity: interpretable only for the whole cluster



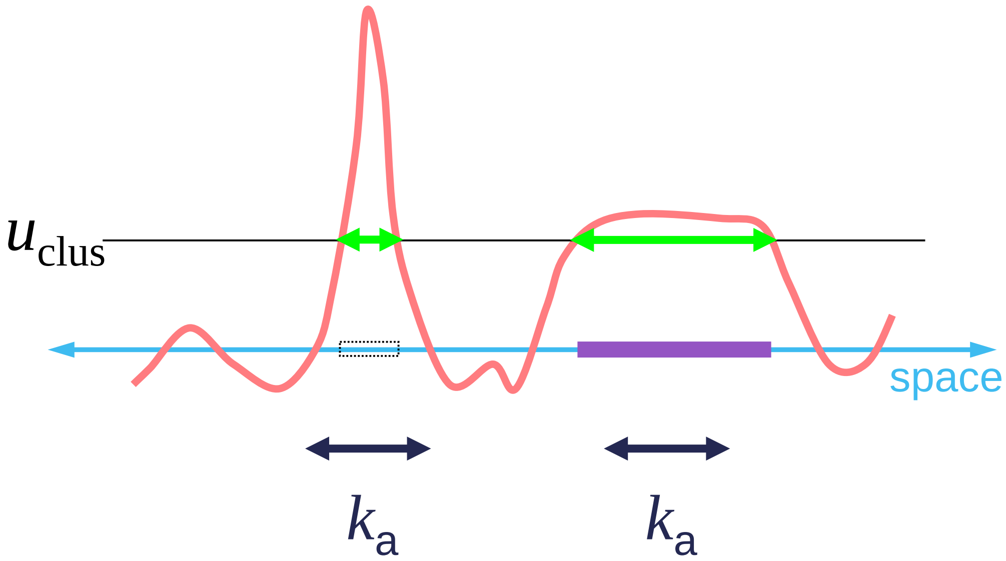

courtesy to Tom Nichols

# Statistical Inference

## Levels of inference

- **Set-level inference**
  - **Probability *p* of that or larger number of significant clusters**

  - Worst spatial specificity: interpretable only globally




Only 1 significant cluster

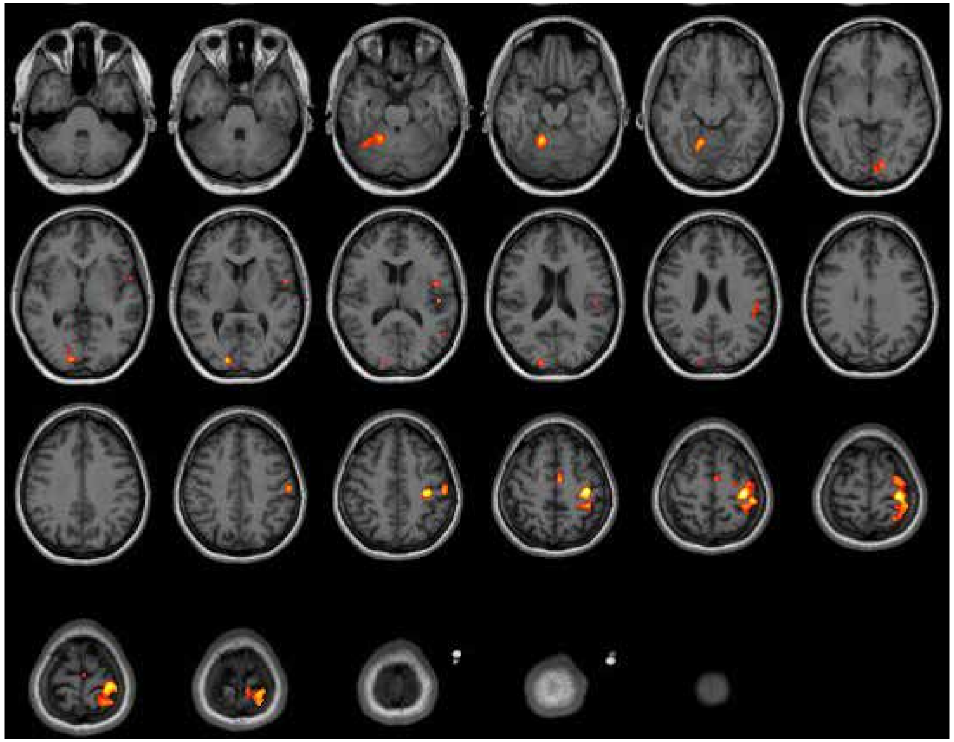


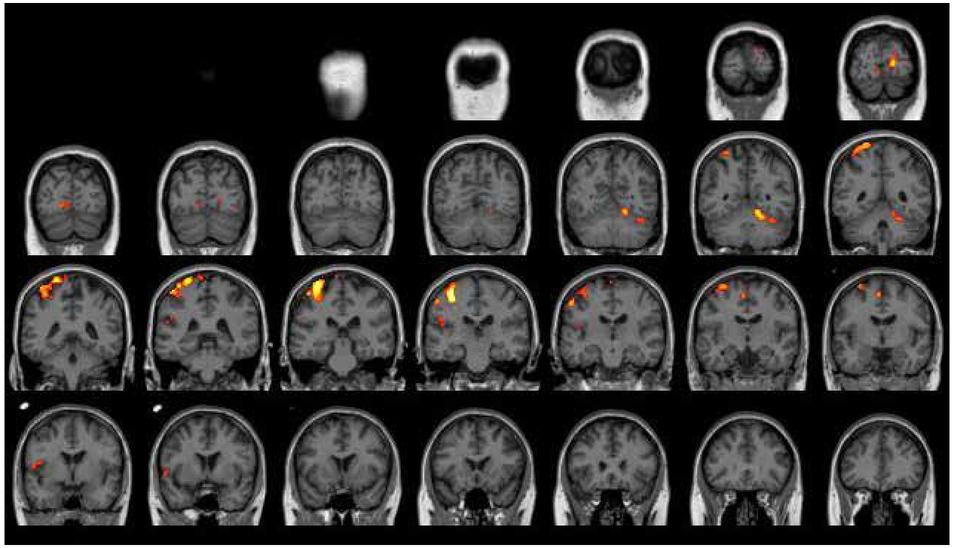
courtesy to Tom Nichols

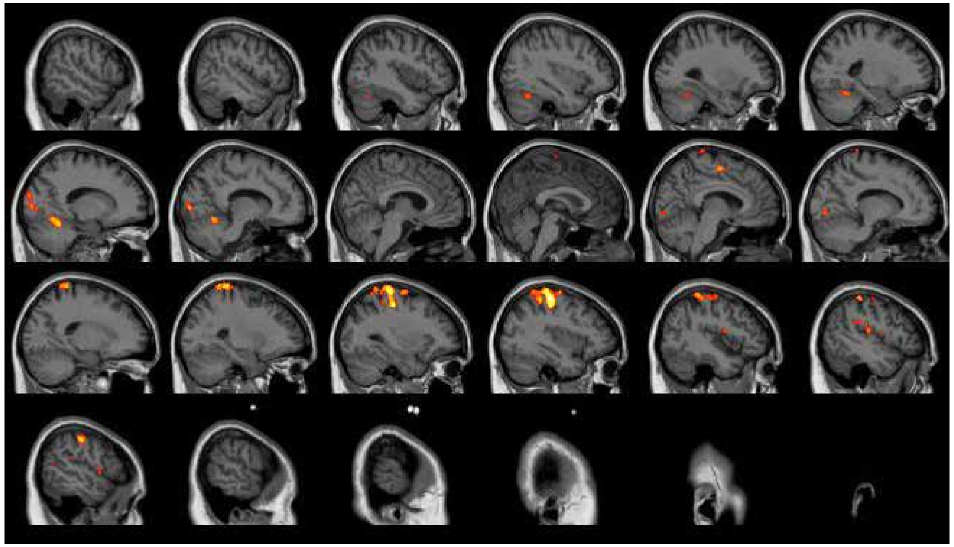
# Statistical Inference

## Visualisation

- **aamod_firstlevel_threshold**



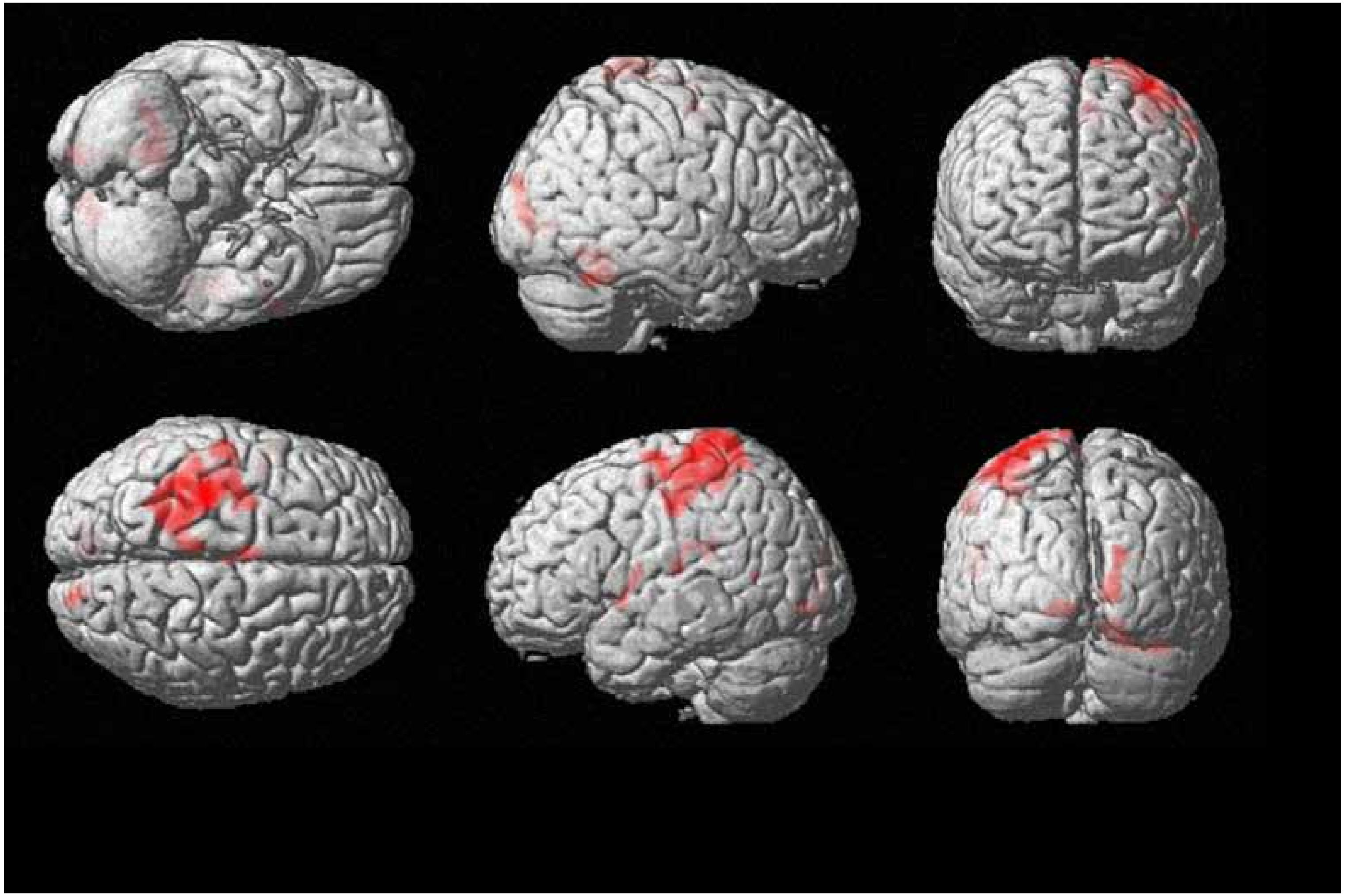




# Statistical Inference

## Visualisation

- **aamod_firstlevel_threshold**



# Statistical Inference

## Visualisation

- **aamod_firstlevel_threshold_register2FS**
  - Launch a VNC session on a **login-gpu01-03** machine
    - *ssh login-gpu0X* à remember the machine name (e.g. login-gpu01)
    - *vncstart* à remember the desktop number (50+account number; e.g. 51)
    - TurboVNC (e.g. login-gpu01:51)

  - FreeView
    - *cdw*
    - *cd Material/4_aa/AA_fMRI/aamod_firstlevel_threshold_register2FS_00001/S1*
    - *vglrun freeview*

    - View à Viewport Layout à 1 & 3 Horizontal
    - View à Viewport Layout à 3D
    - View à Show Slices (3D View): off

# Statistical Inference

## Visualisation

- **aamod_firstlevel_threshold_register2FS**
  - Load volume (brain.mgz) and overlay (rthr*.nii)
    - File à Load Volume à brain.mgz
    - File à Load Volume à ../stats/rthrT_0001.nii à Color map: Heat

  - Load Surface (lh.*) and overlay (rthr*2FS_lh.mgh)
    - File à Load Surface à lh.pial
    - Curvature: binary
    - Overlay à Load generic... à ../stats/rthr**T_0001**2FS_lh.mgh
    - Configure Overlay à Min = 3