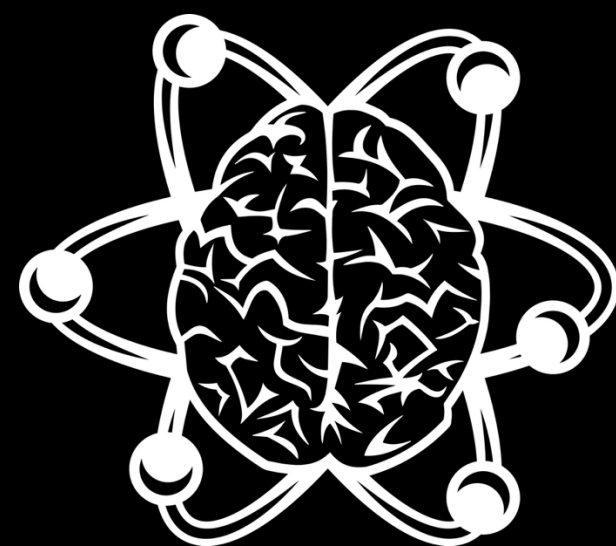
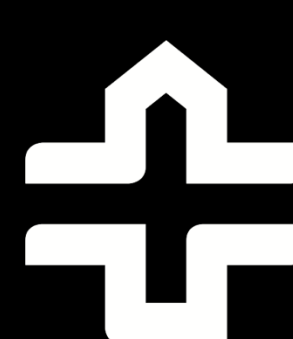




SECOND-LEVEL ANALYSIS OF PET AND MRI DATA

Turku PET Centre Brain Imaging Course 2025

Lauri Nummenmaa, Turku PET Centre



Basic problems associated with scientific measurement

ERRORS PRESENT AT ALL LEVELS; THEY ALSO ACCUMULATE FROM LEVEL TO LEVEL

TARGET
(e.g. specific
neuro-
receptor)

TRUE SCORE (T)
How target is
defined
(e.g. number of
receptors)

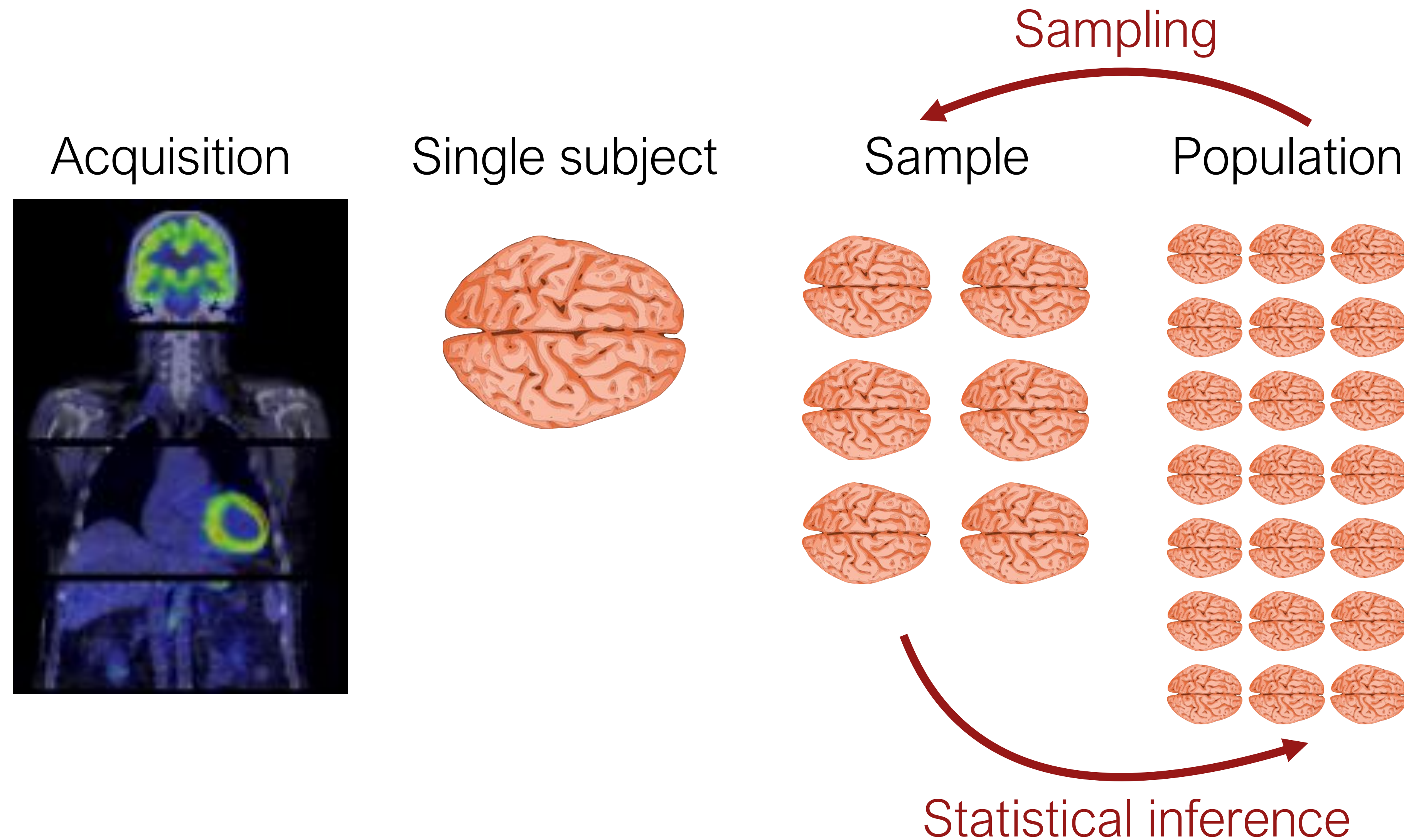


OBSERVED
SCORE
(Outcome
measure such
as BPND)

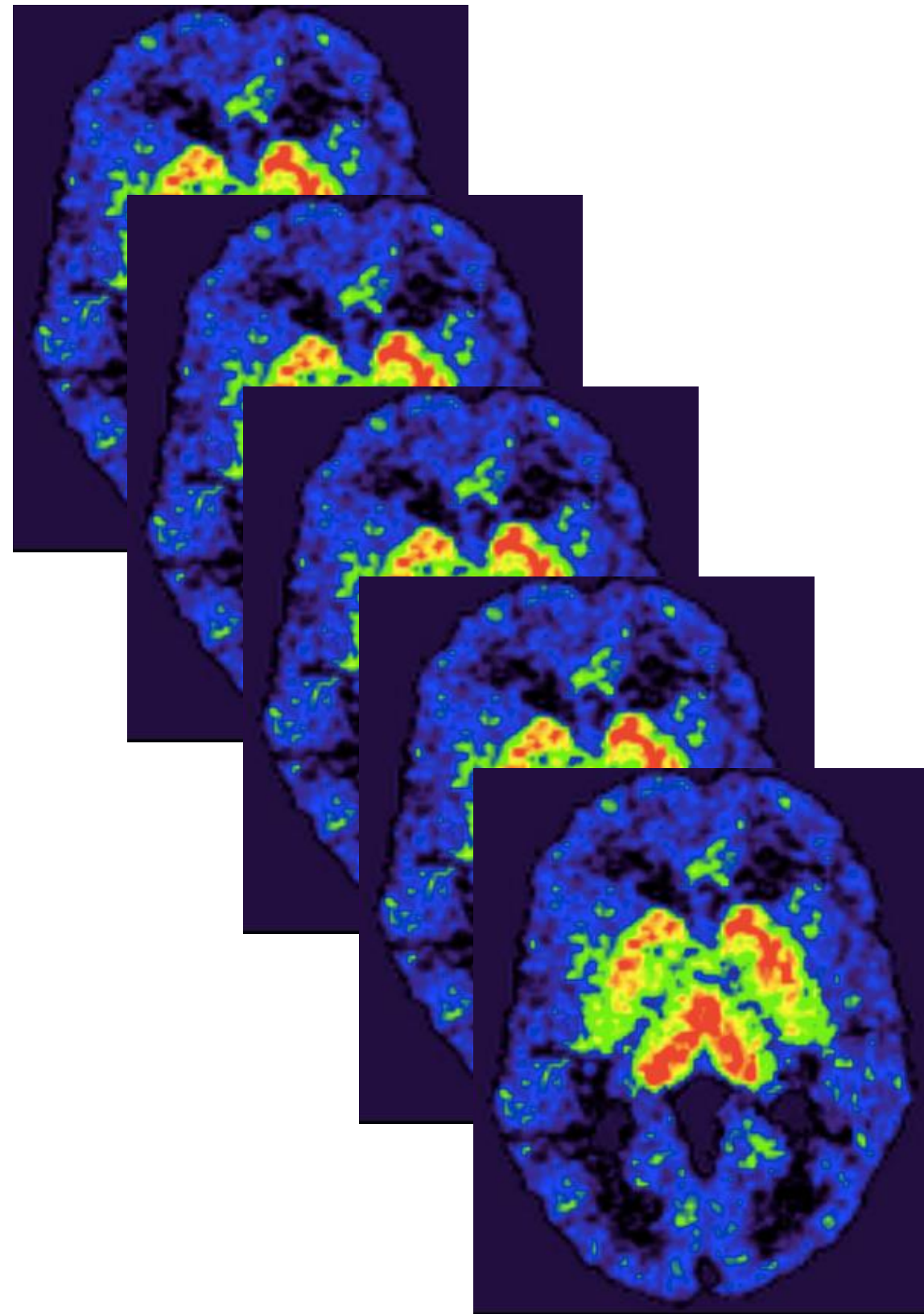
PREDICTION
OF BEHAVIOR
(e.g. anxiety-
like behaviour)

- How well is target variable reflected in true score (construct validity)
- How well true score is reflected in observed score? (reliability)
- How well does observed score predict behaviour? (criterion-based validity)

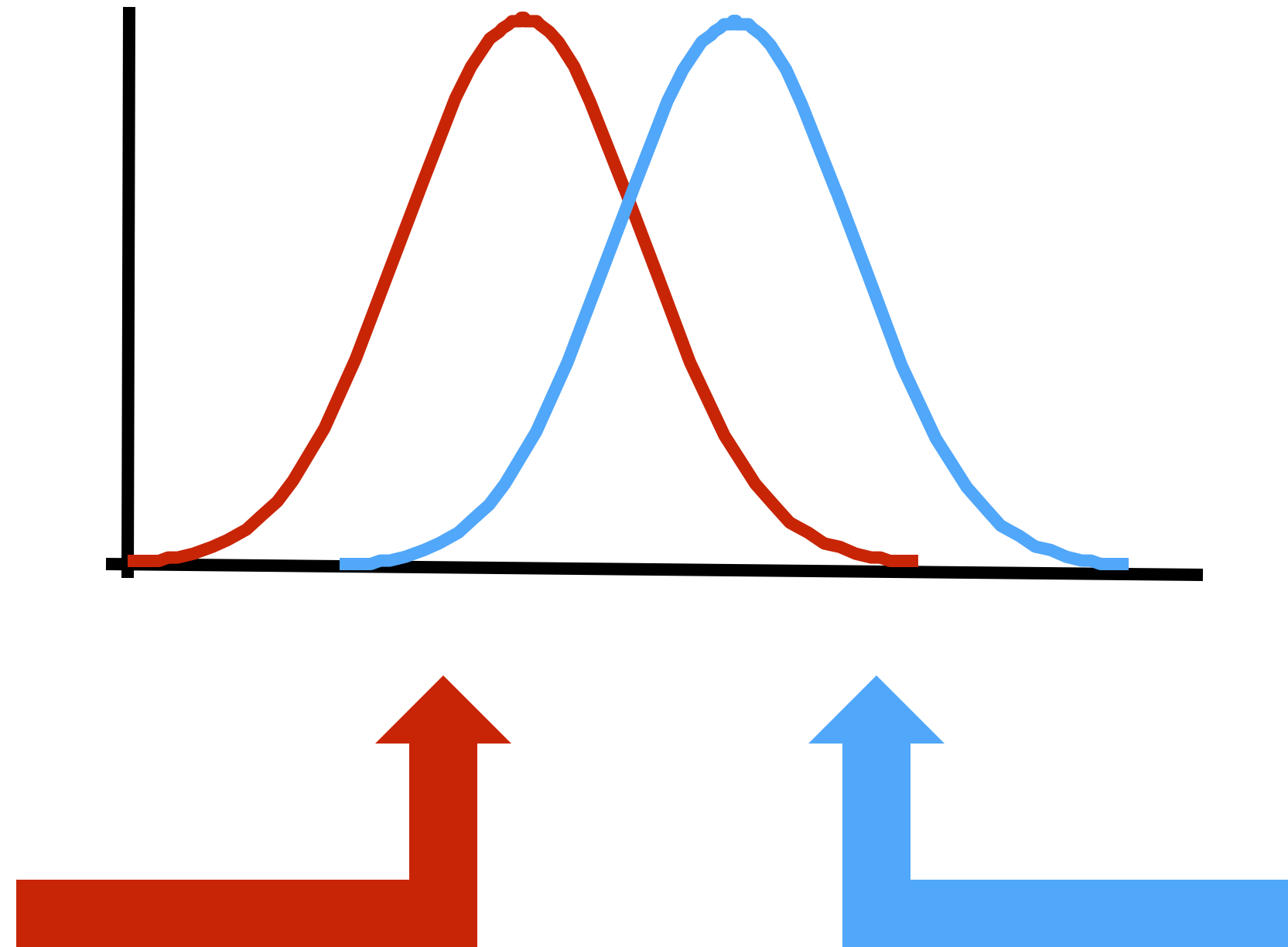
Making inferences about the population



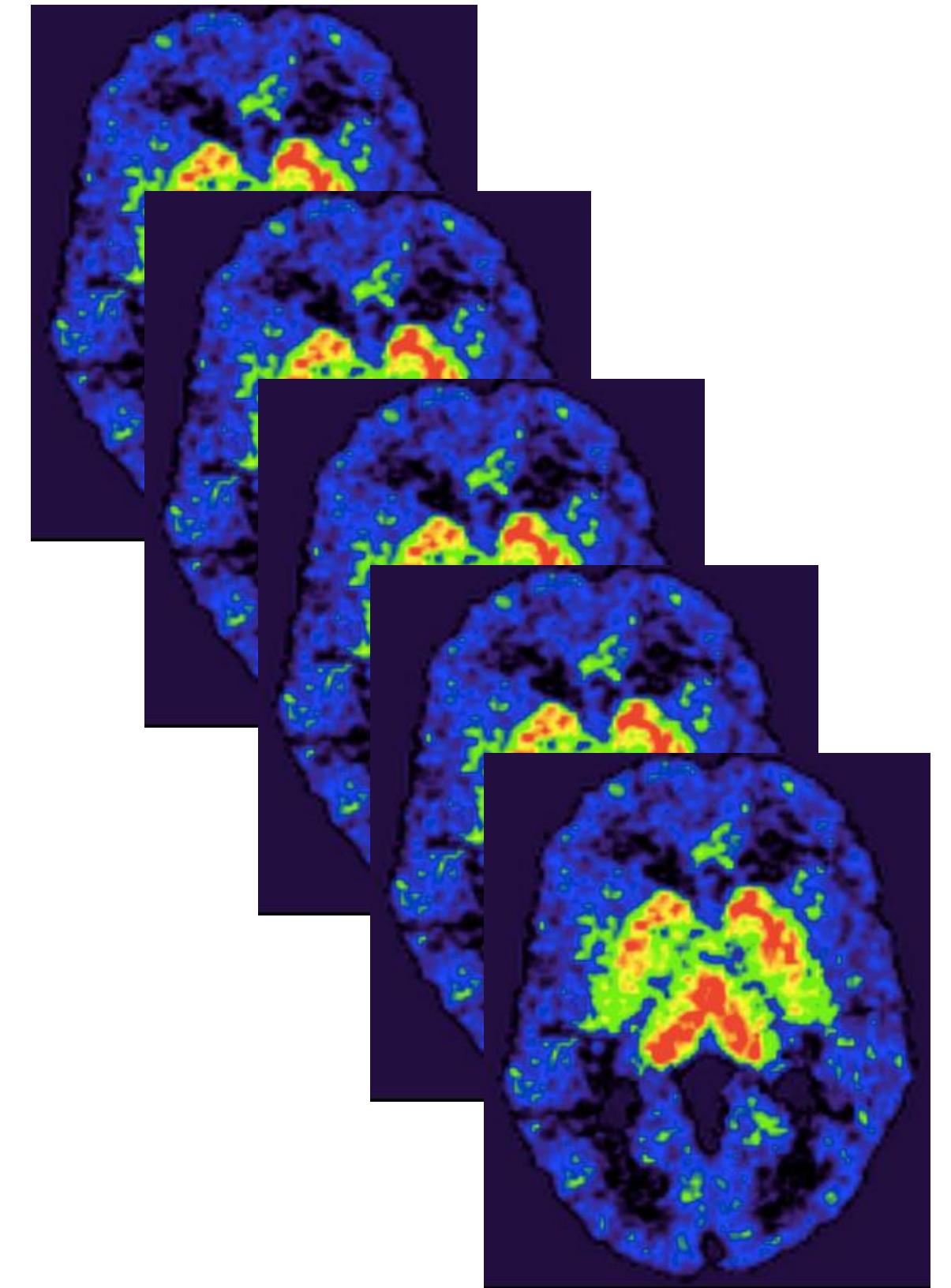
CONTROLS



ARE THESE BRAINS
STATISTICALLY
DIFFERENT?



PATIENTS



Starting point: Images where voxel intensities reflect the outcome measure

Sneak peek: Analysis of PET vs. fMRI data

- **PET data needs to be modelled** before population level inference
 - 4D image —> 3D image
 - Voxel intensities reflect outcome measure (receptor density, metabolism....)
- **Similarly, EPI data needs to be modelled** before population level inference
 - 4D image —> 3D image
 - Voxel intensities reflect the fit of the stimulation model to the BOLD time series

Univariate data
Regularly shaped

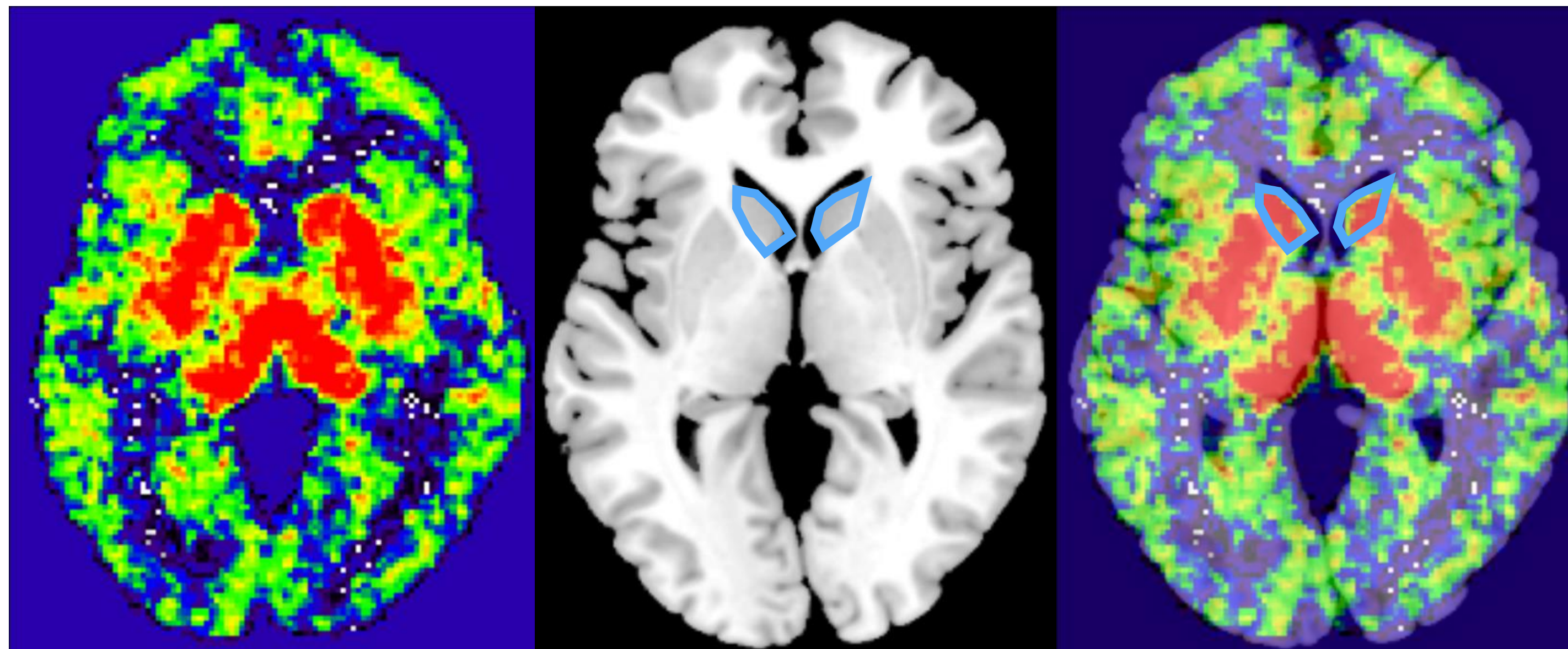
Controls		Patients
3		5
4	t-test	4
5		6
6		7
3		6
2		5
3		2
5		6
2		8

3D neuroimaging data
Irregularly shaped



ROI-based analyses

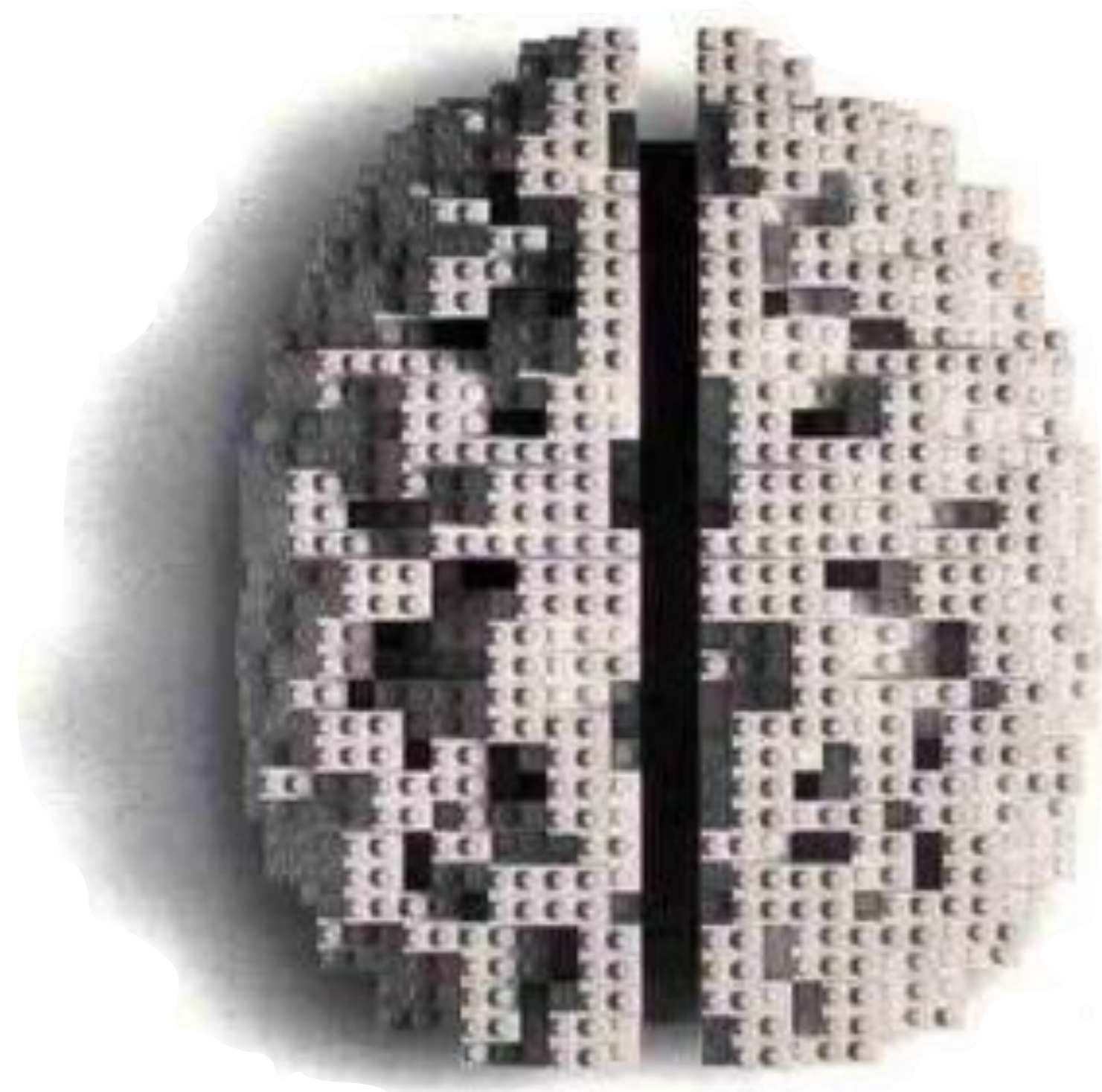
Univariate data
regularly shaped
can use univariate stats

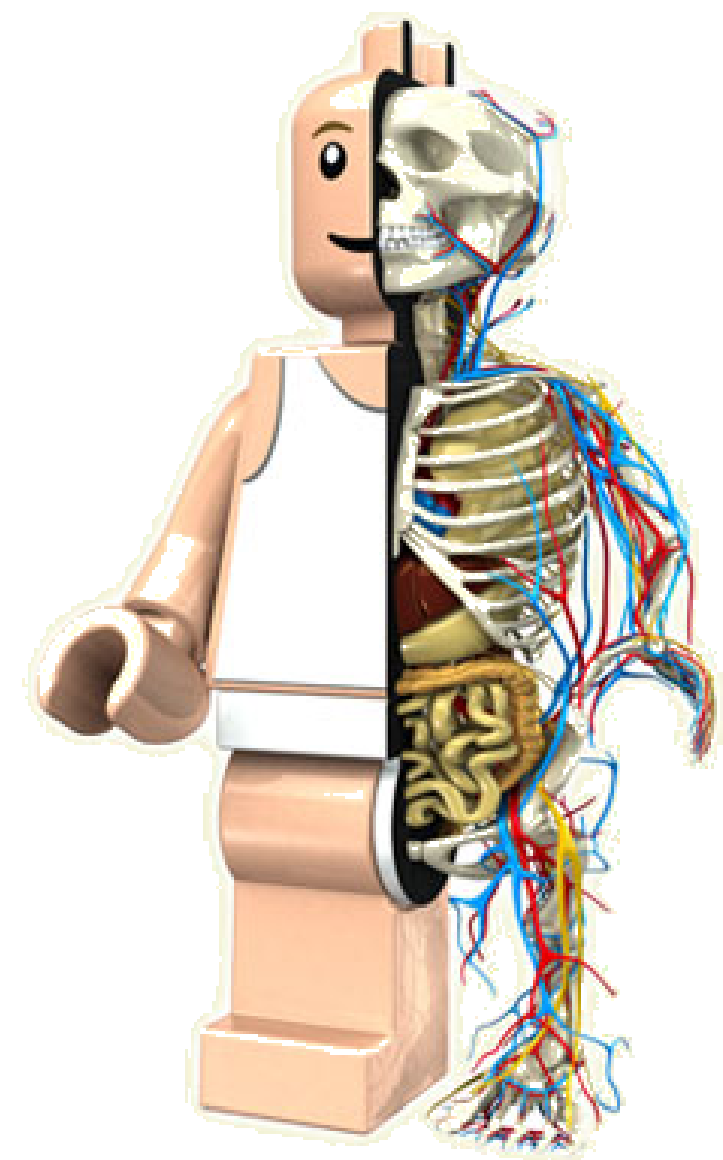


Extract
outcome
measure
in ROI

Controls	Patients
3	5
4	4
5	6
6	7
3	6
2	5
3	2
5	6
2	8

- **Pros:** Anatomically accurate if ROIs well defined, data can be analyzed with simple univariate statistical tests
- **Cons:** Laborious, using many ROIs not feasible, averaging within ROI not always appropriate





Controls

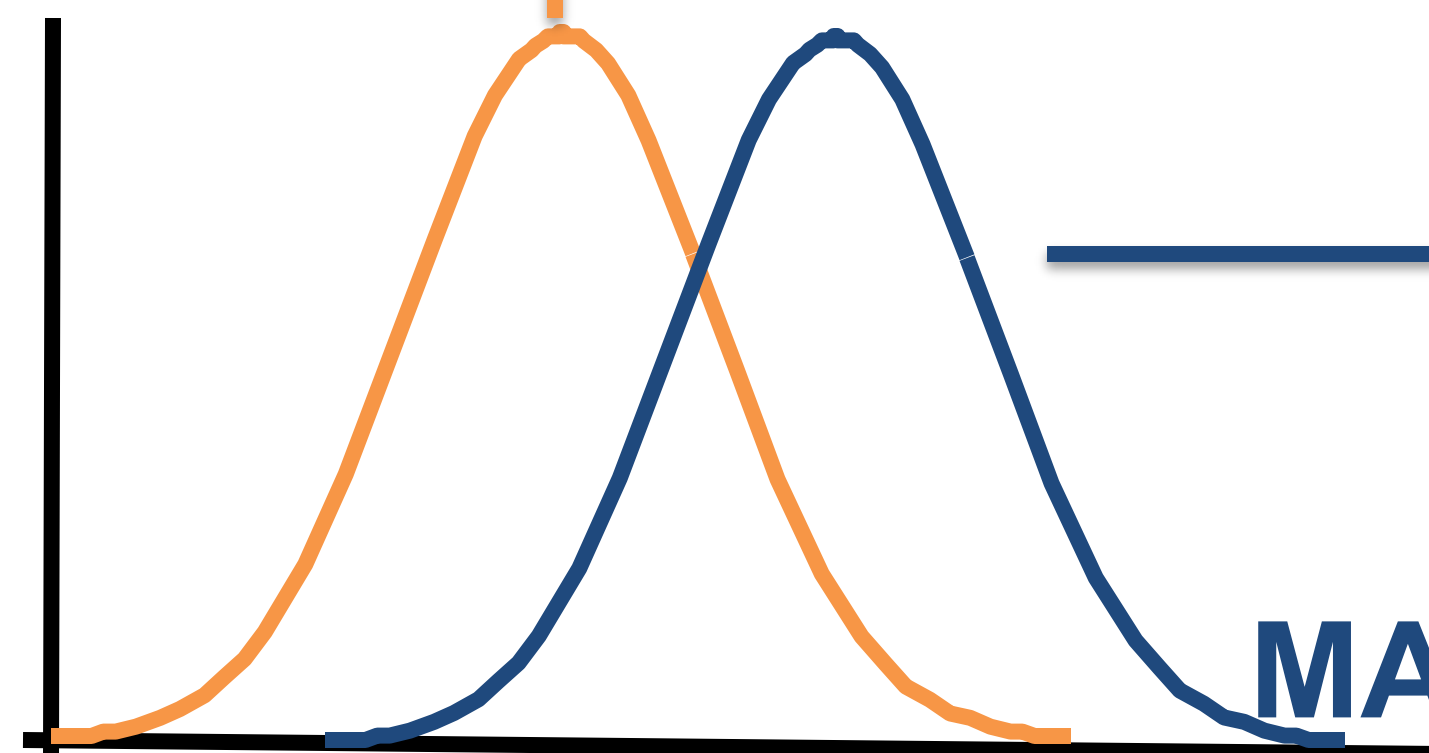
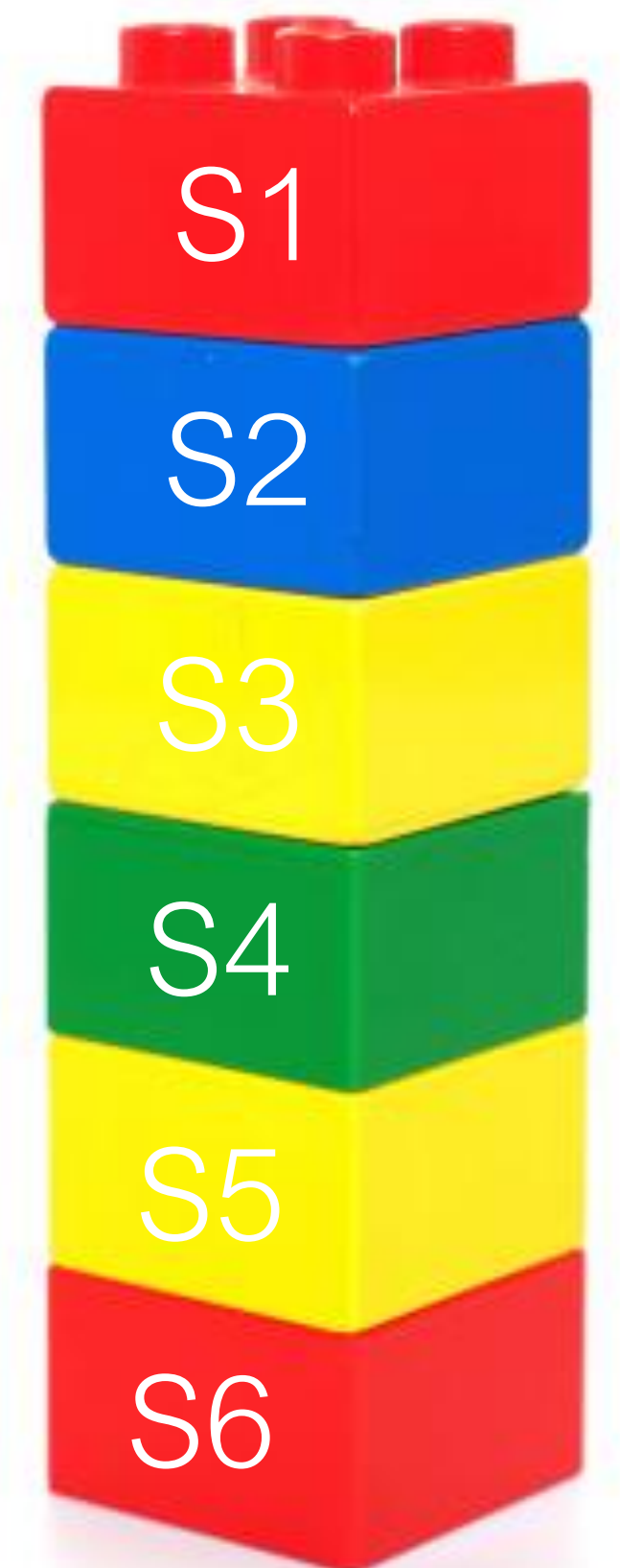
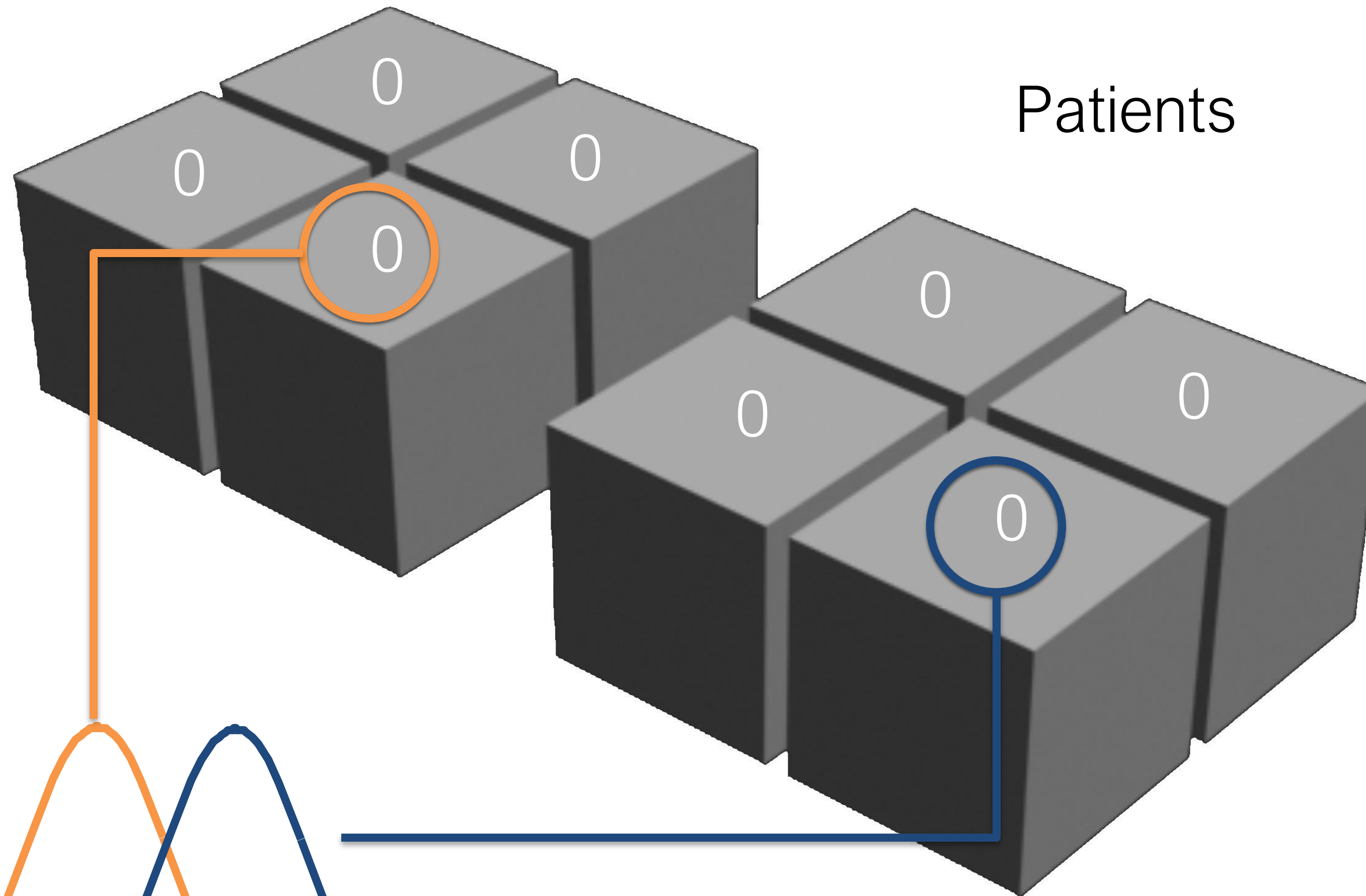


Voxelwise
outcome
measures

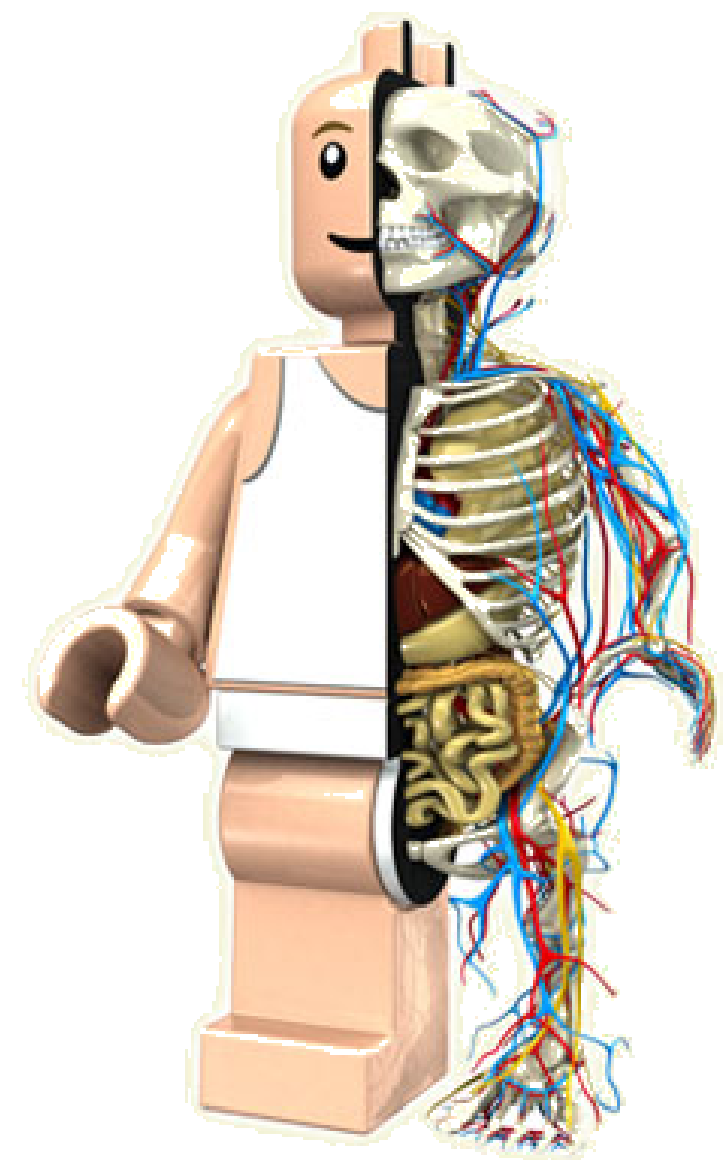


Univariate voxelwise data
regularly shaped
can use mass
univariate stats

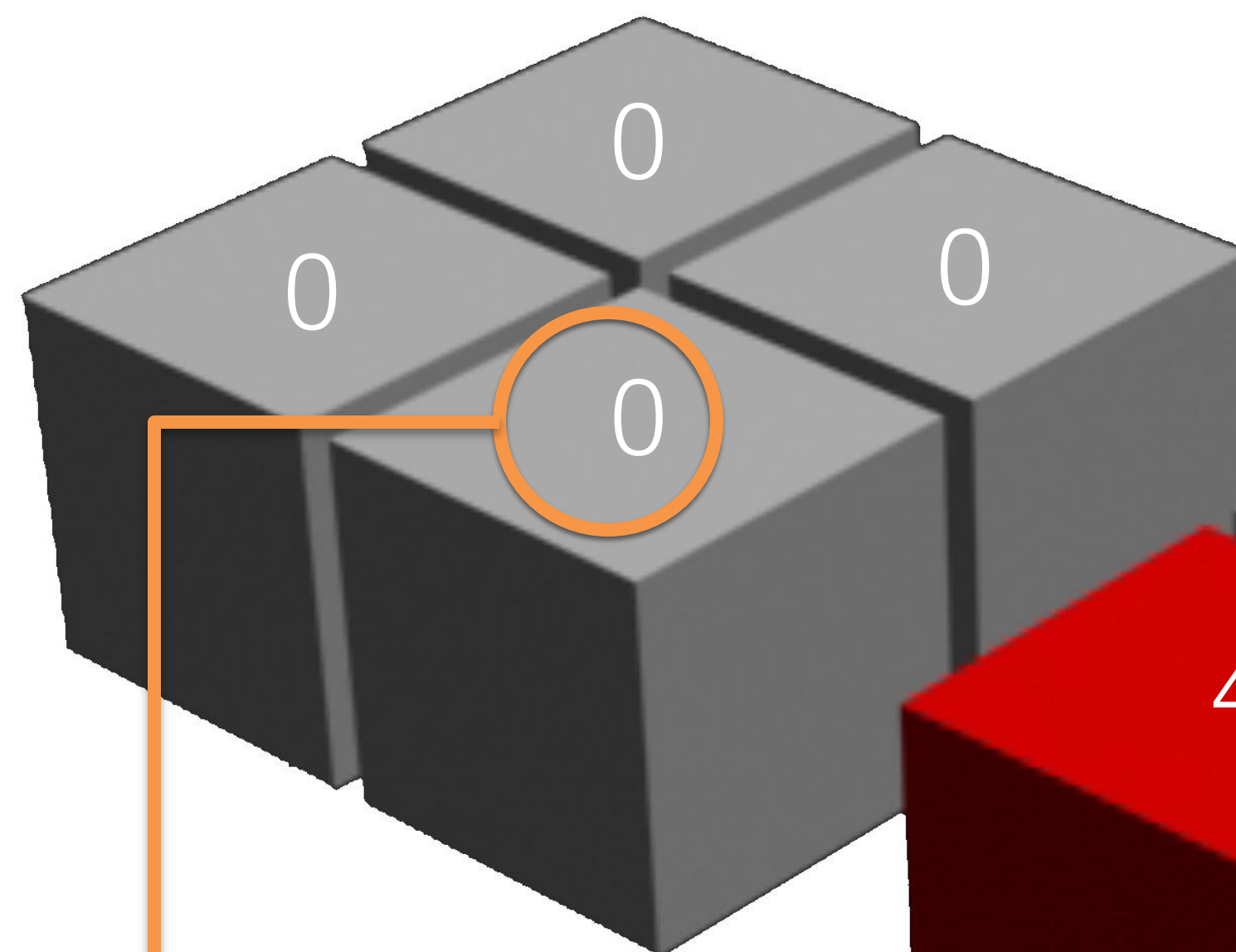
Patients



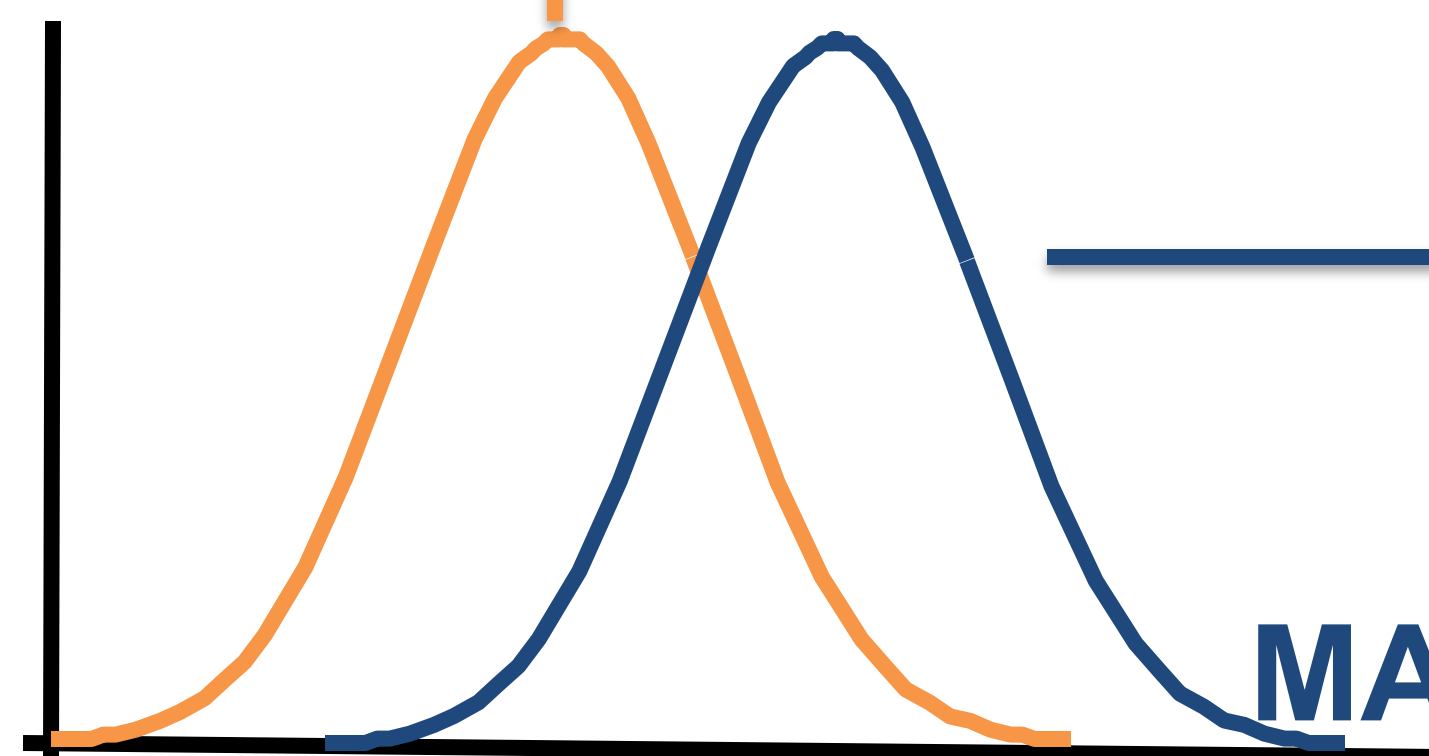
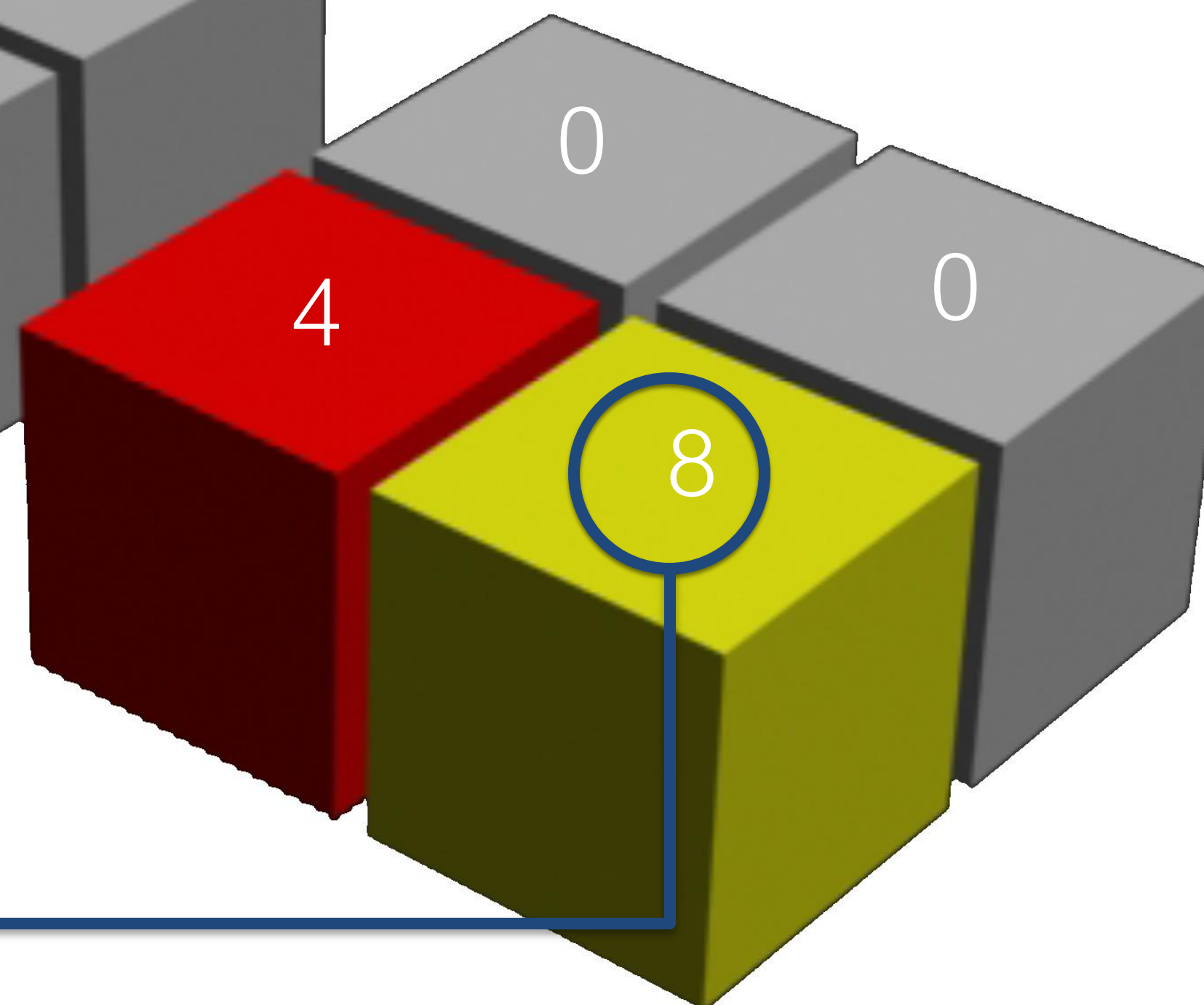
MASS UNIVARIATE TESTING FOR ALL VOXELS



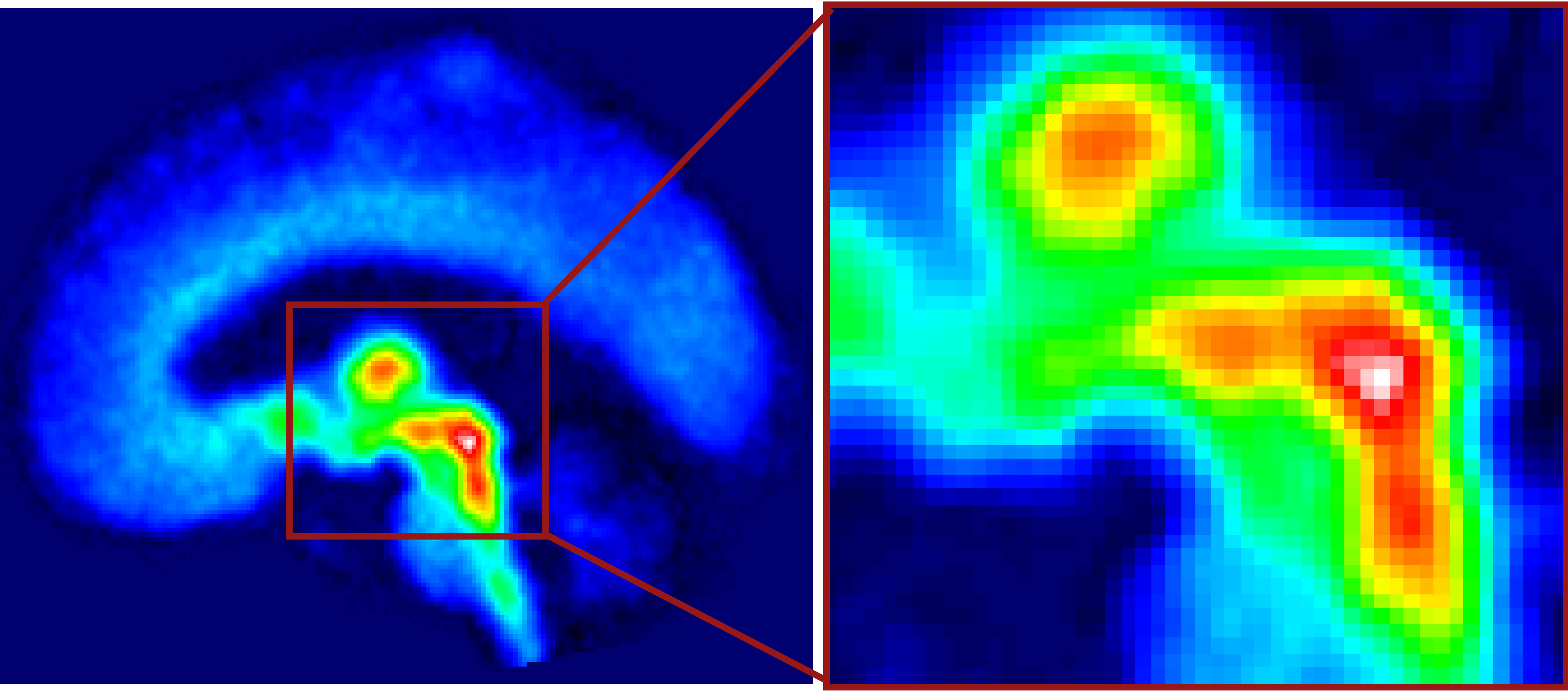
Controls



Patients



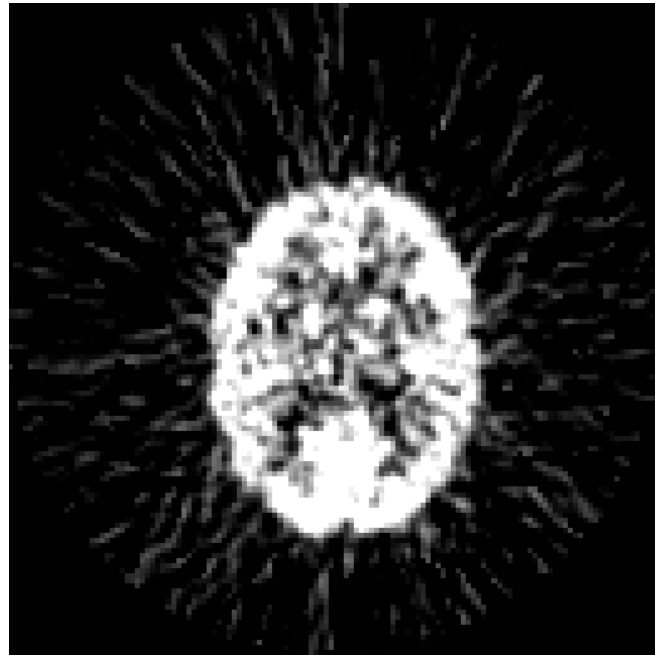
MASS UNIVARIATE TESTING FOR ALL VOXELS



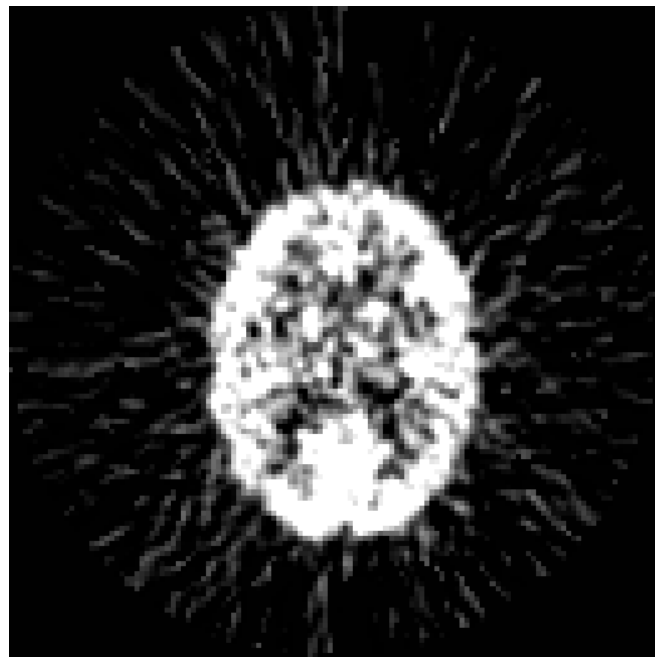
Voxel intensity = outcome measure
(BPND, contrast estimate, tissue probability)

THE BASIC RECIPE

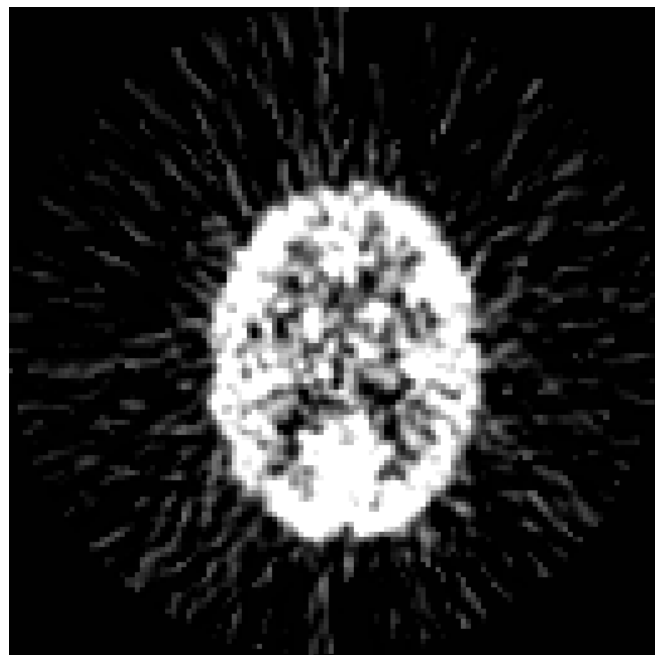
SUBJECT 1



SUBJECT 2

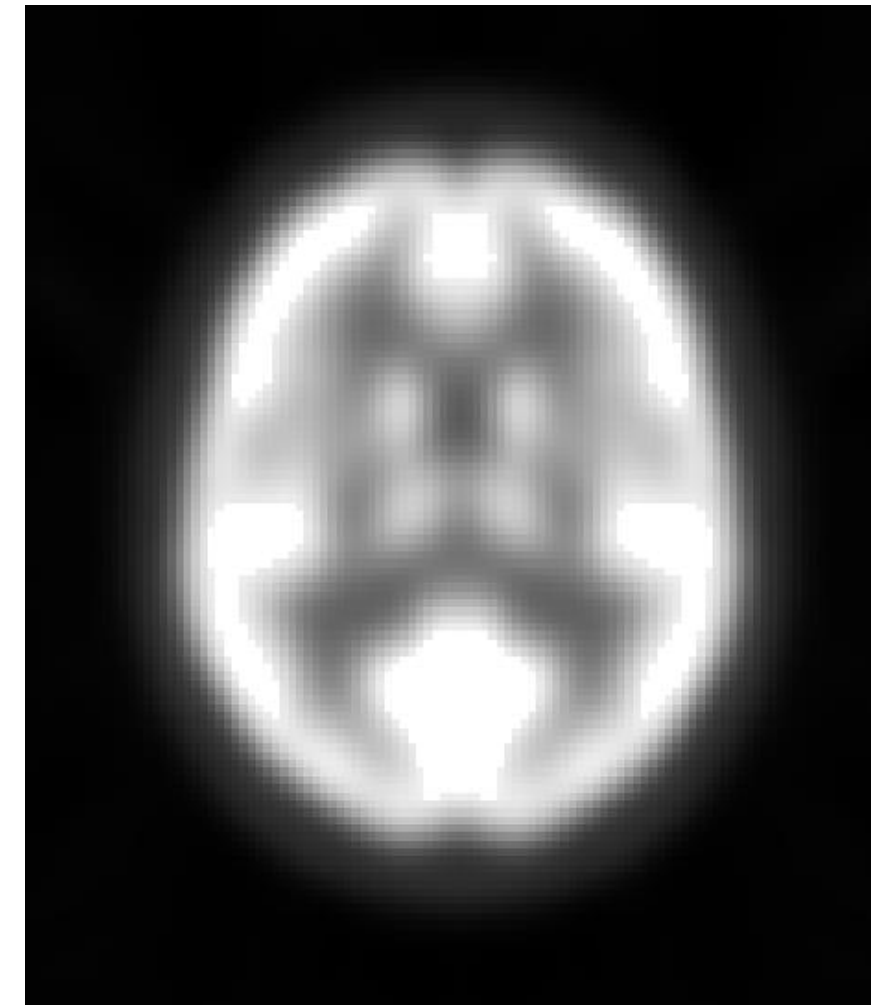


SUBJECT 3



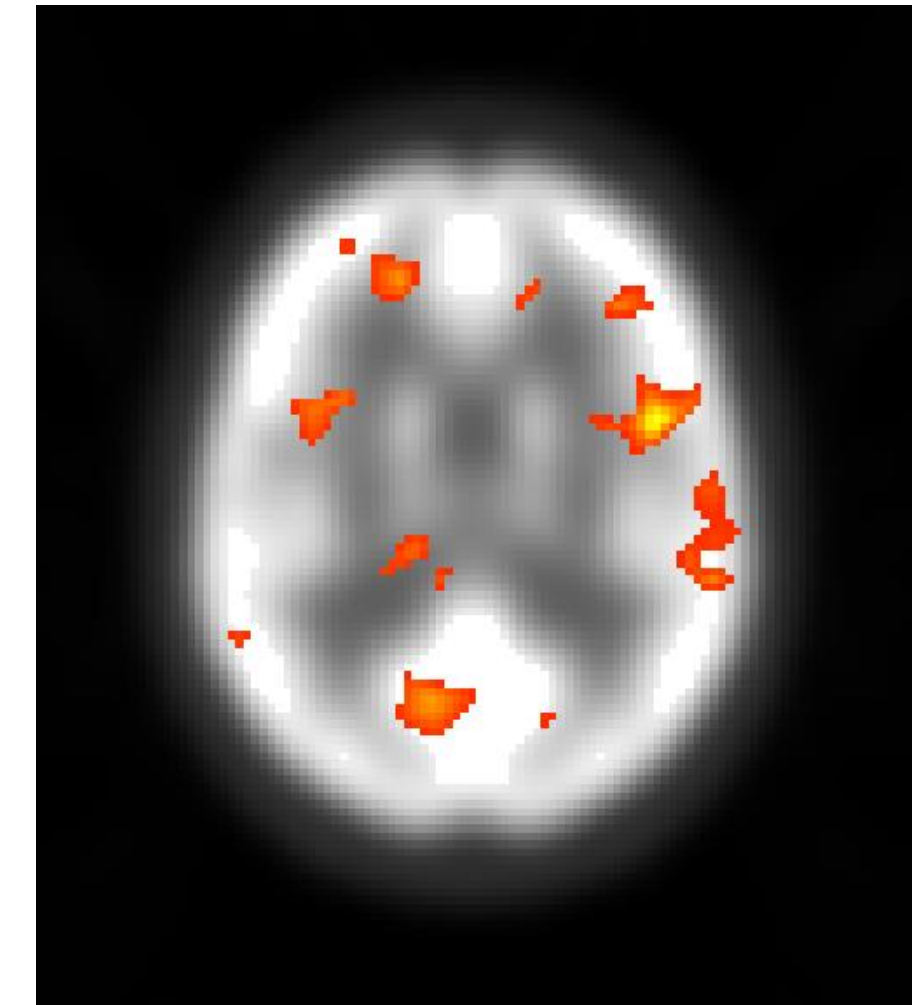
NORMALI-
ZATION

TEMPLATE



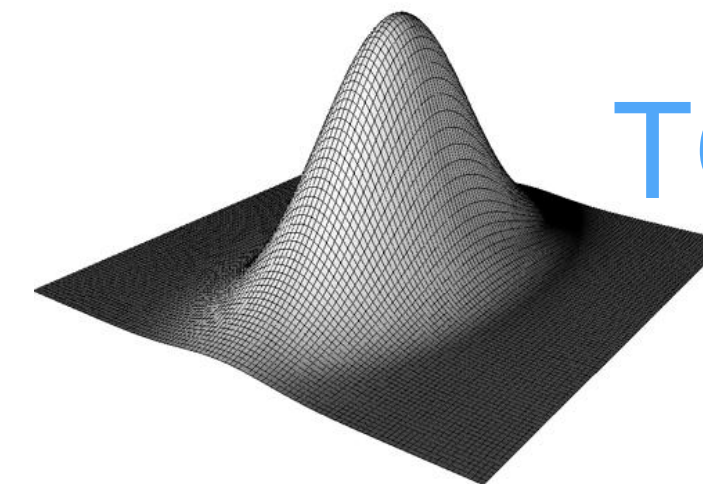
GLM

STATISTICAL
PARAMETRIC MAP



THRESHOLD
TO HIGHLIGHT

SMOOTH

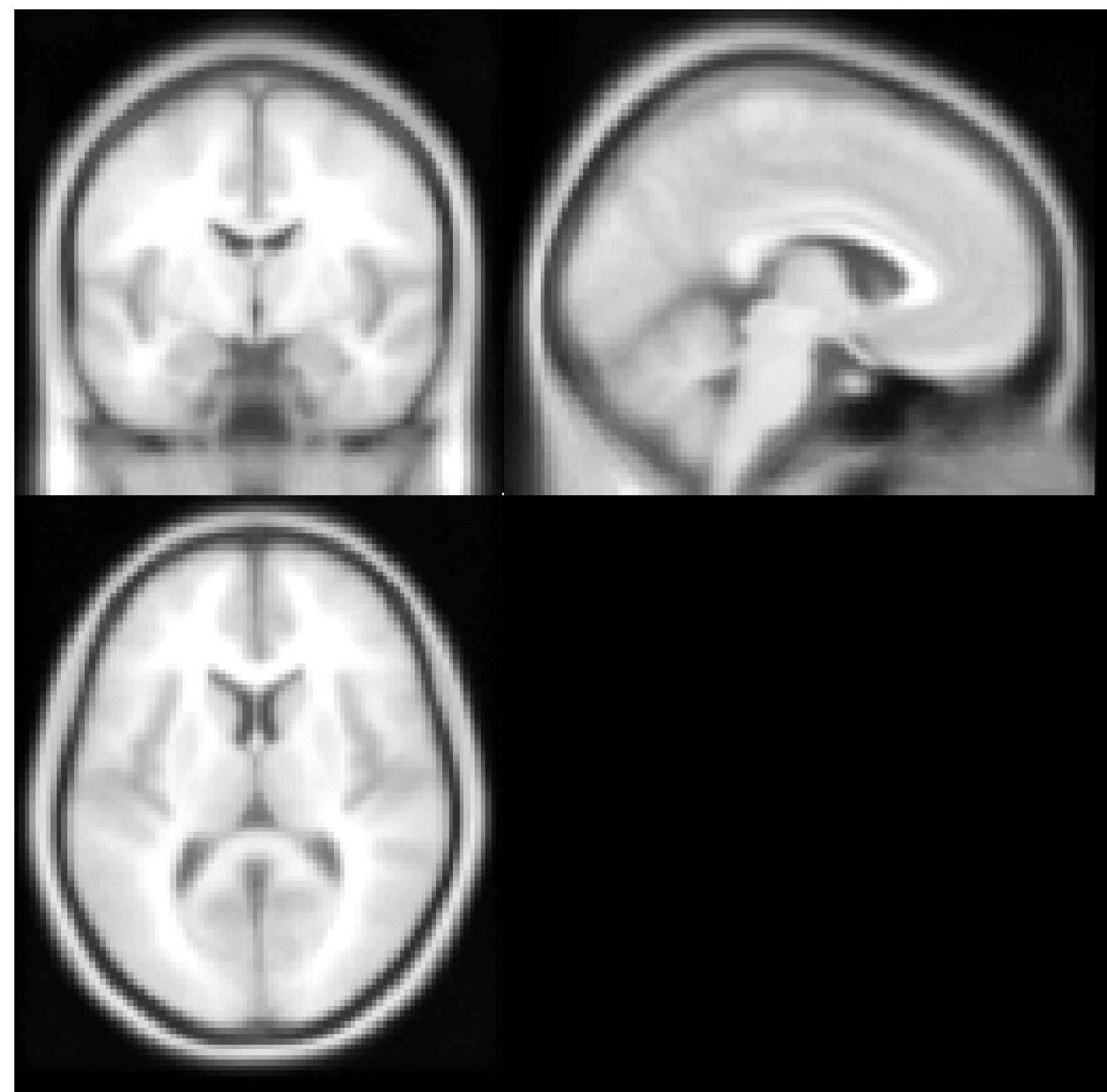


Full-volume analyses with real brains

- Basic problem: Individual brains differ in size and shape
- Solution to the problem: Make brains similar by warping them
- Problems with the solution
 - Warps distort anatomy
 - Anatomical information is not the precise anyway
 - How should we warp the brains?

The MNI space as the target

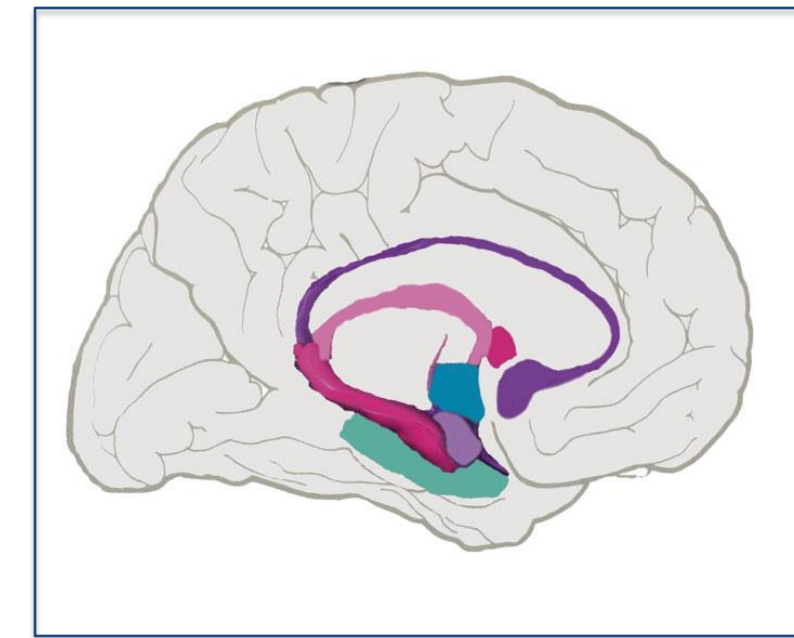
- **ICBM 152 template**
- Based on average of 152 brains that have been spatially normalized
- Statistical average of the typical western adult brain
- Problem: not necessarily representative of study sample
- In fMRI can also use e.g. spherical models



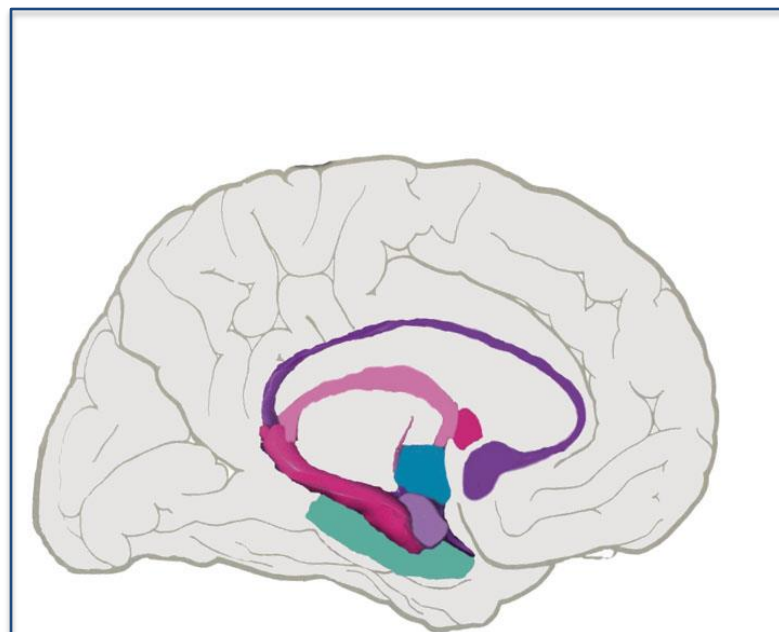
Spatial normalization in practice

1. Linear (12-parameter affine) normalization
 - Match size and position
2. Nonlinear normalization
 - Linear combinations of smooth discrete cosine basis functions

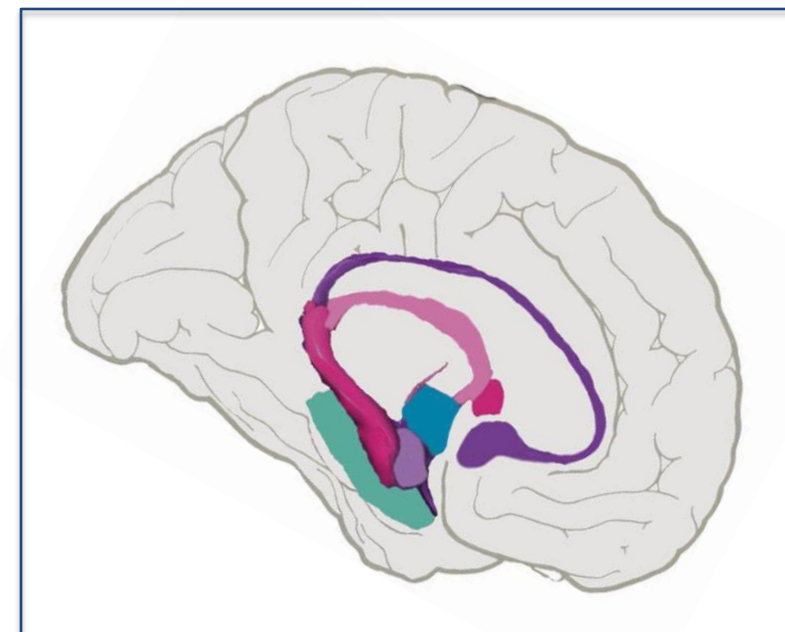
NATIVE



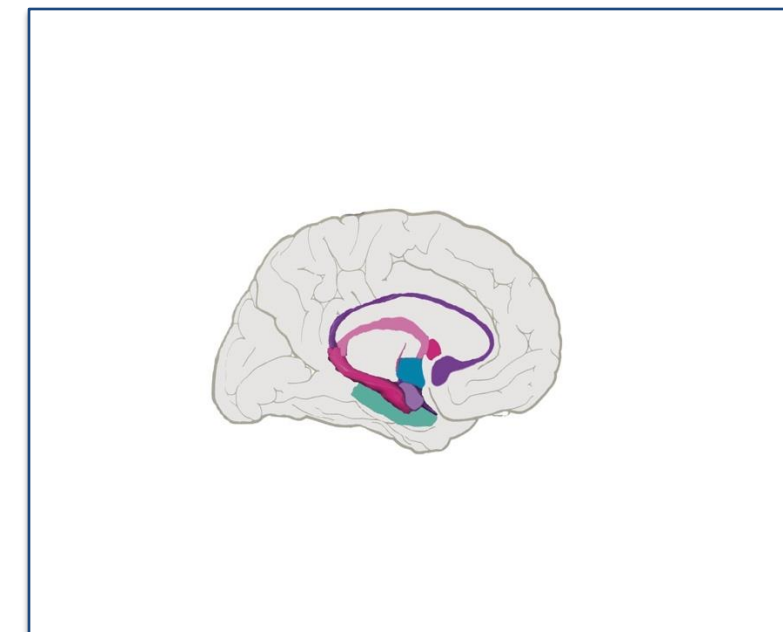
TRANSLATION



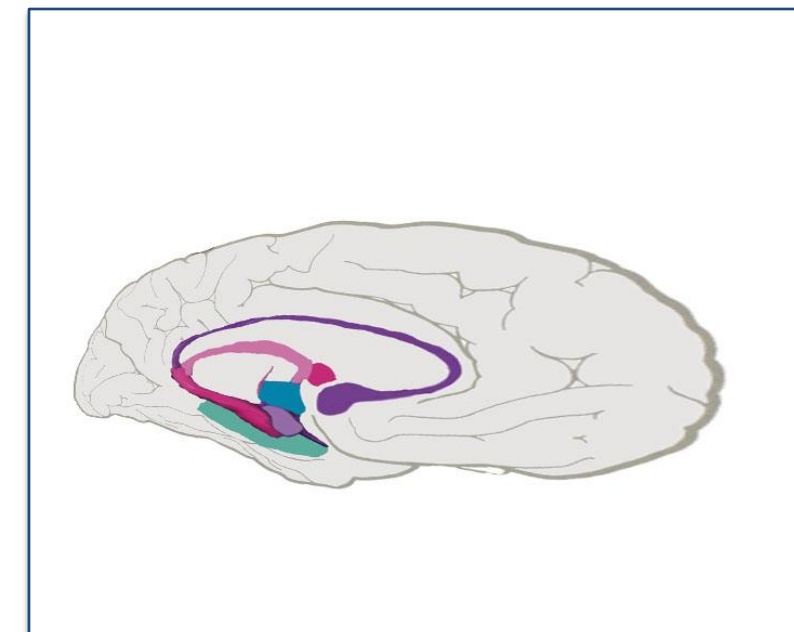
ROTATION



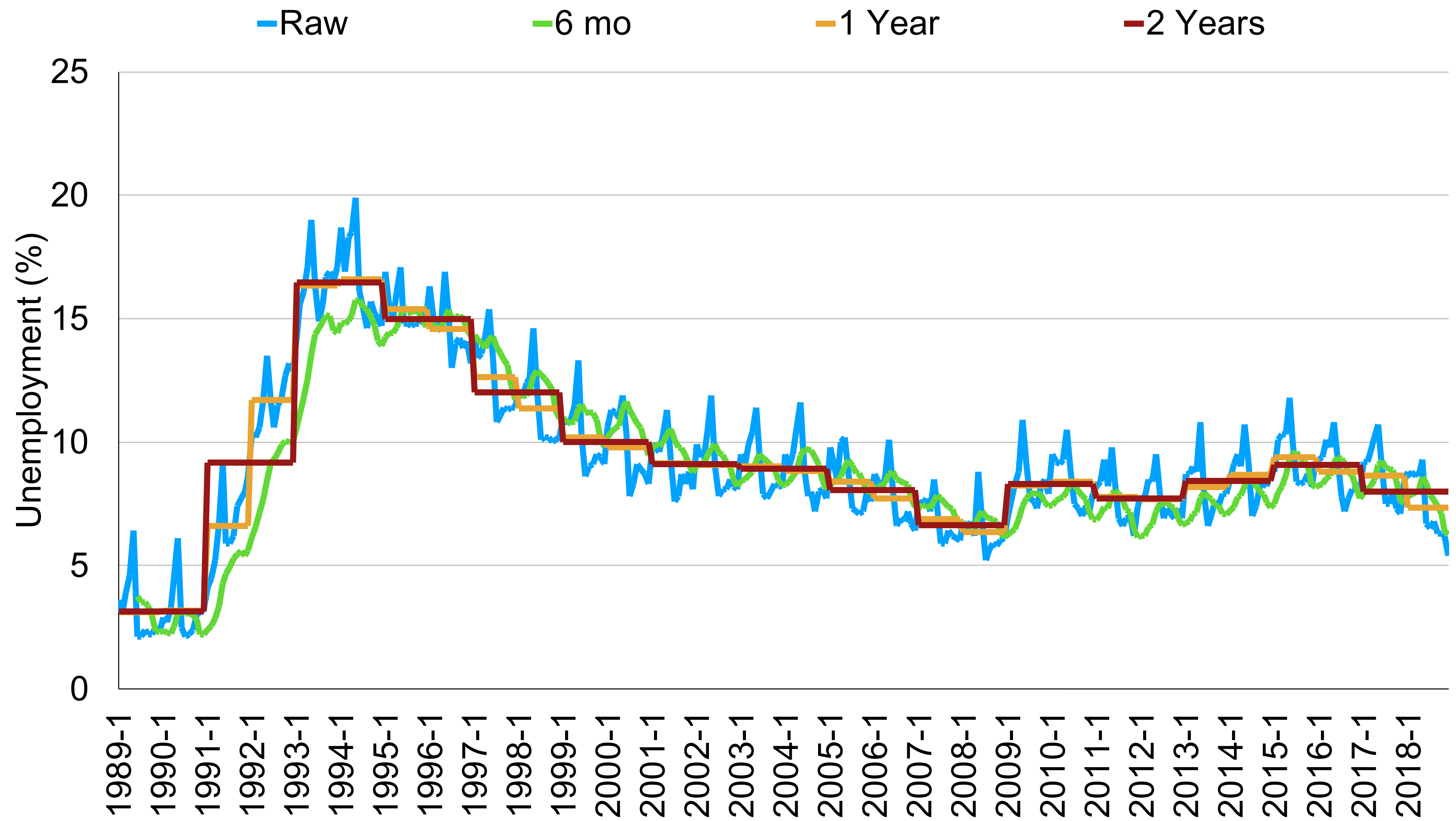
ZOOM



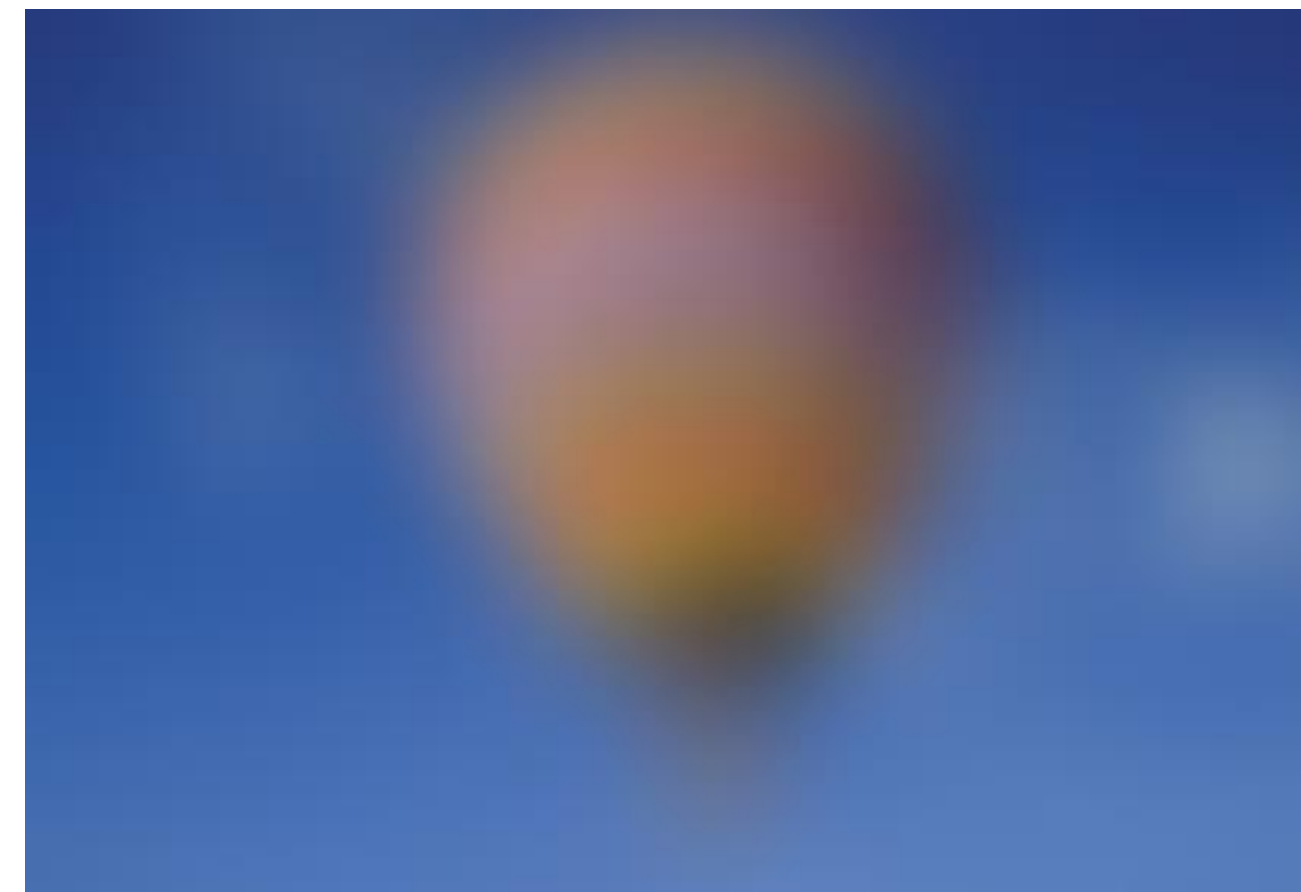
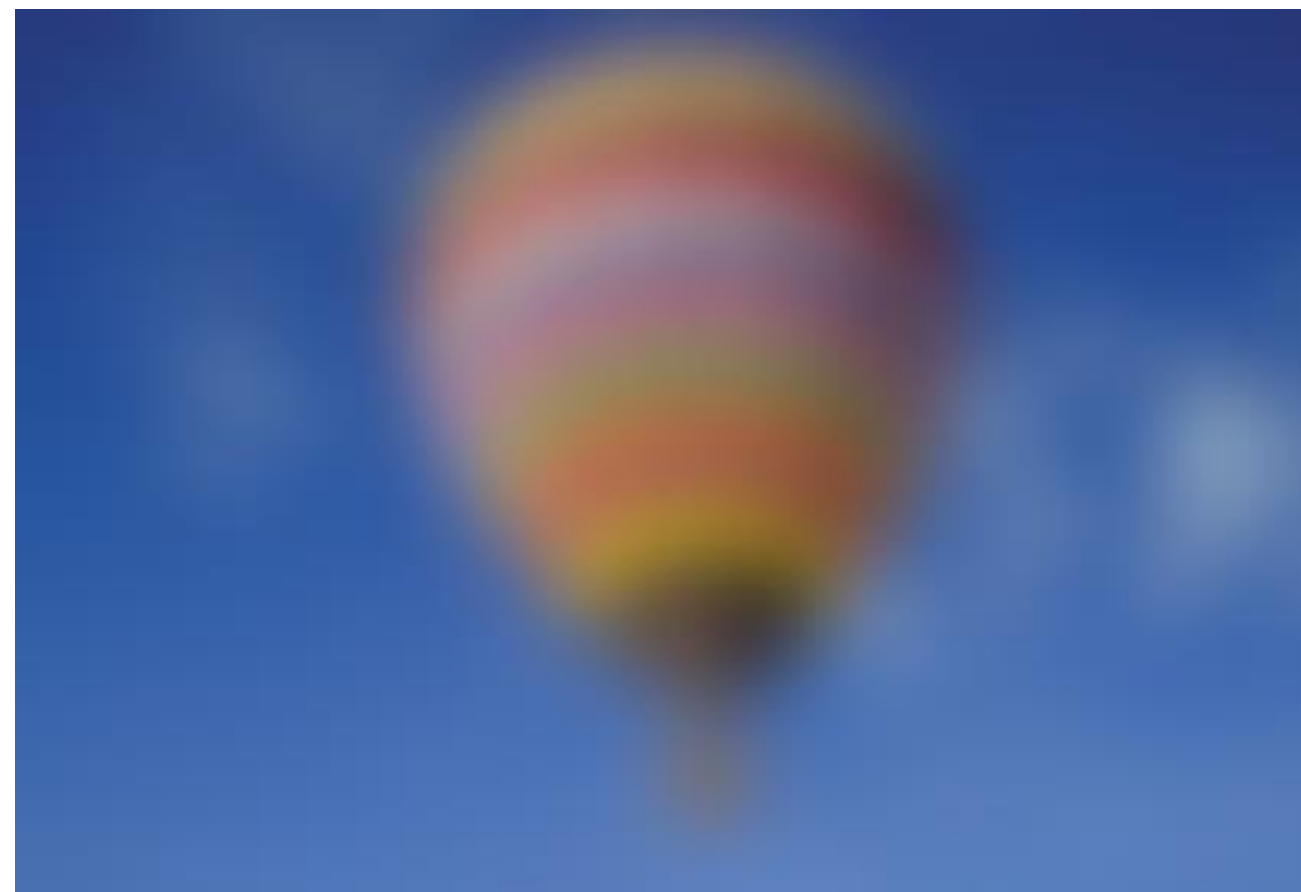
SHEAR



AFFINE NORMALIZATION: 4*3 PARAMETERS



Smoothing



FWHM = spatial extent of the filter

Example on smoothing brain-PET images

UNSMOOTHED



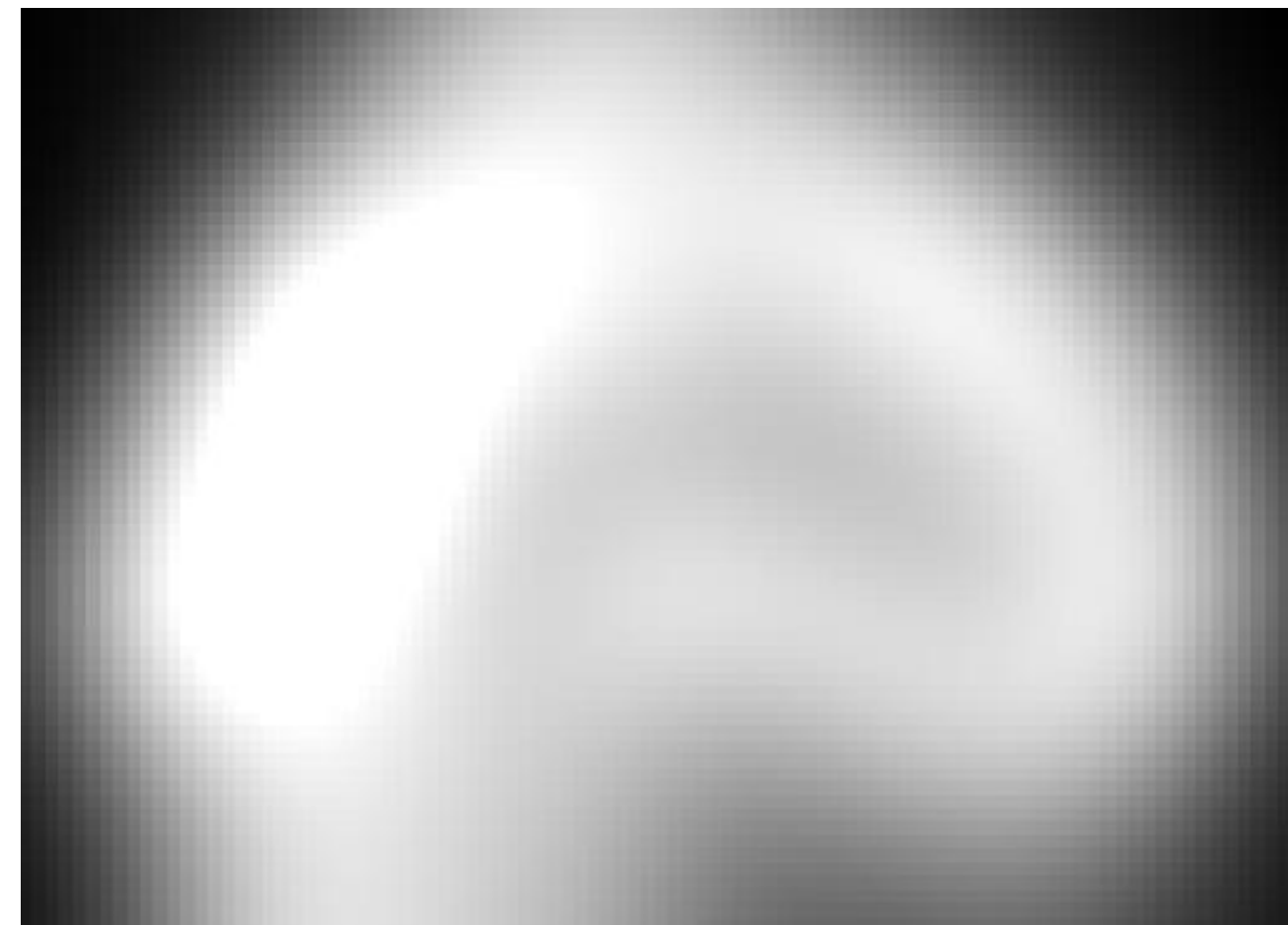
12mm FWHM



16mm FWHM



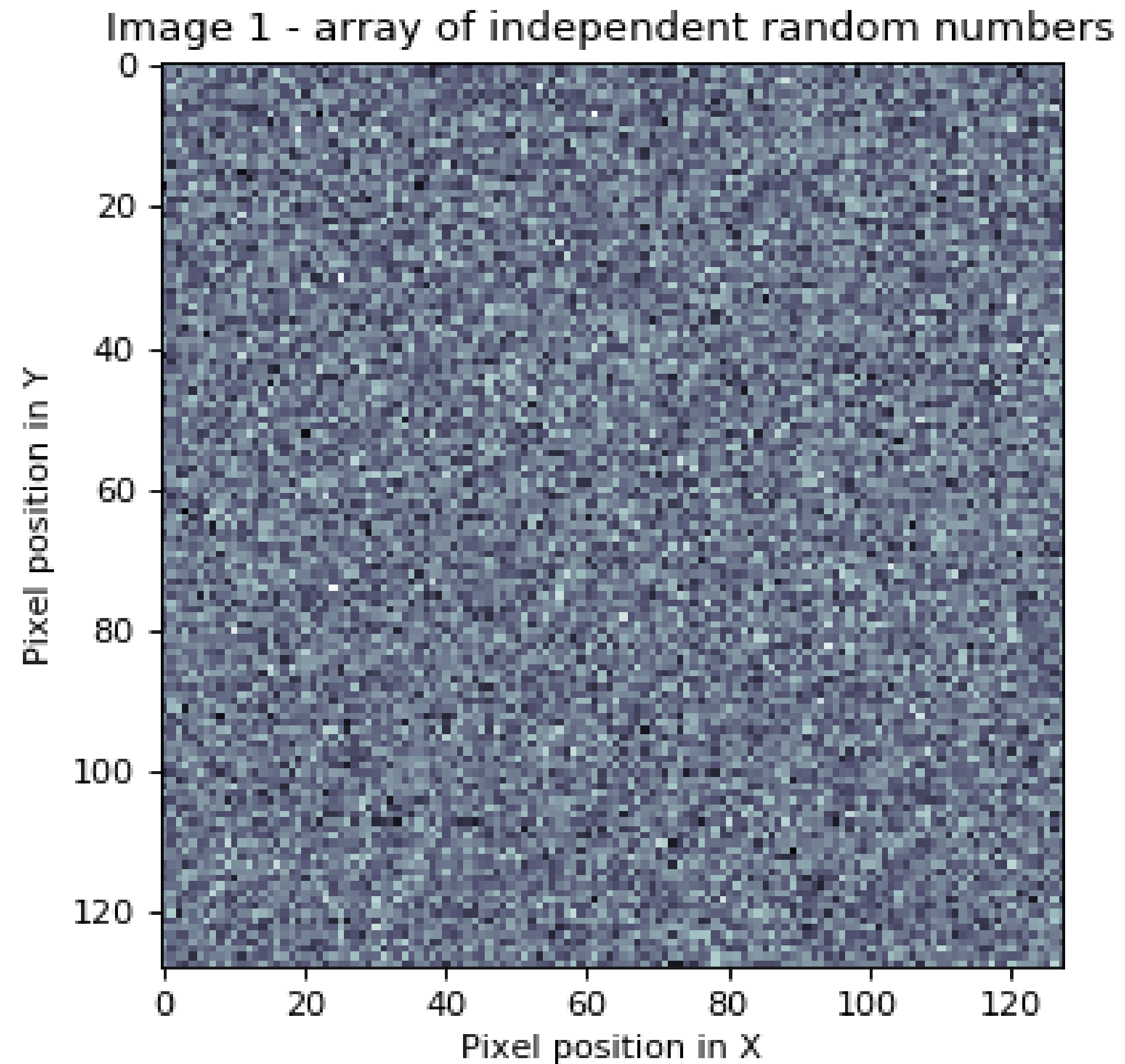
32mm FWHM



Why smooth?

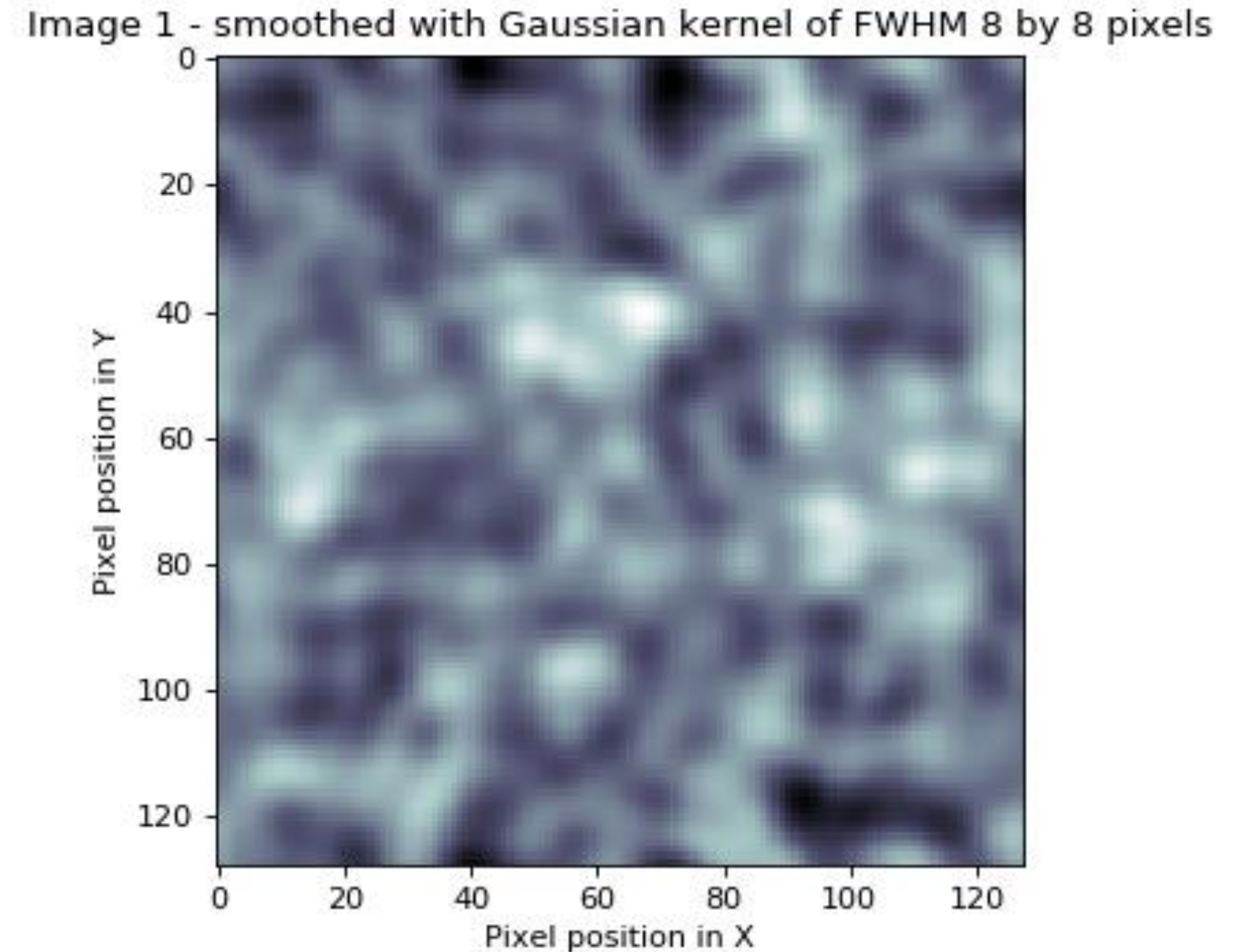
- Smoothing neuroimaging data: reduces noise and anatomical discrepancies
- Assumption: error terms are roughly Gaussian; FWHM greater than voxel size
- Enables hypothesis testing and dealing with multiple comparison problem in functional imaging
- However introduces problem of how to correct for multiple comparisons

Raw data: 16384
independent numbers



**8 by 8
square
smooth**

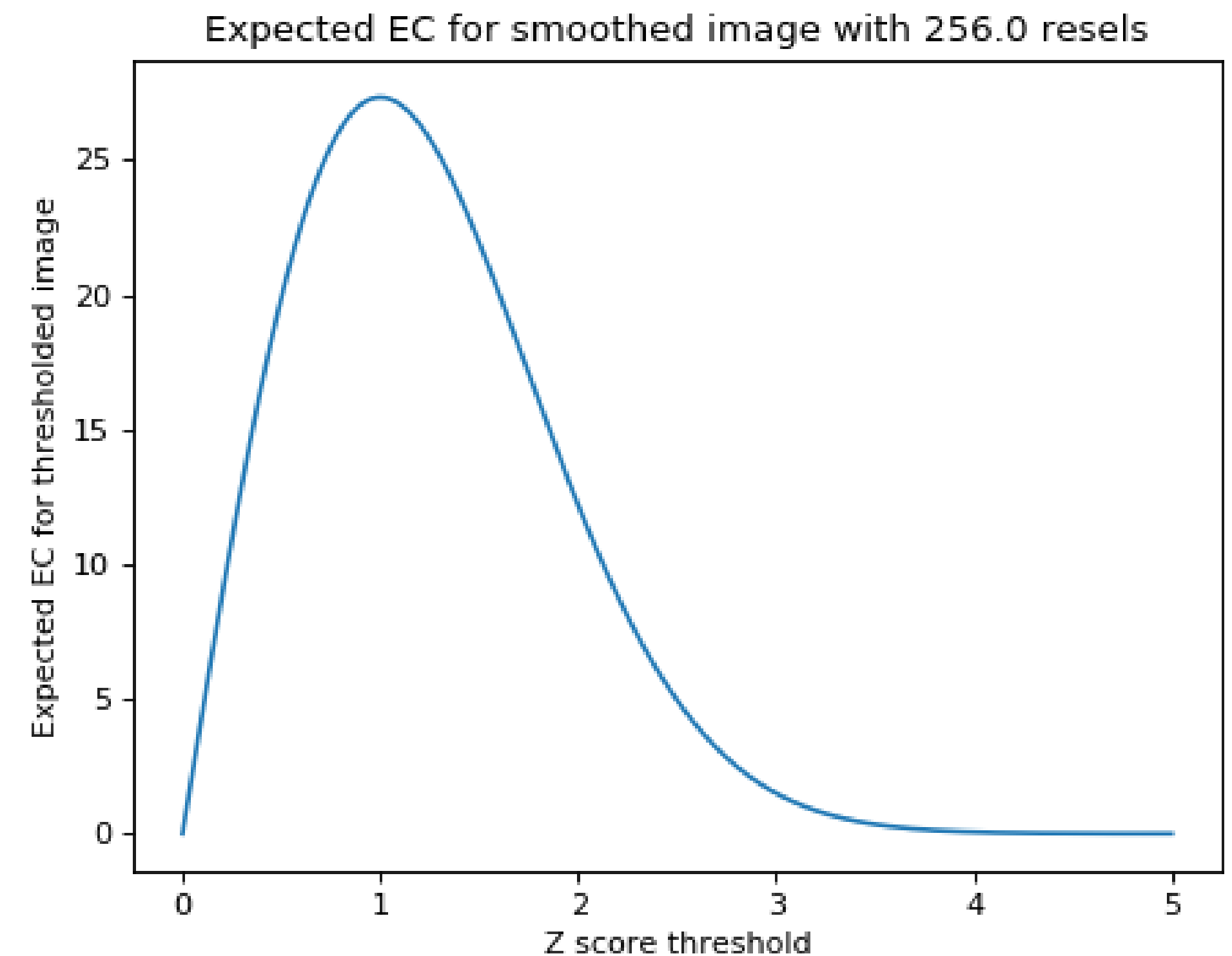
Kernel-based smoothing
How many independent numbers?



Problem with kernel-based smoothing: How many numbers are independent?

Random Field Theory in nutshell

- Estimate the number of resels in the image
 - Resel= block of pixels / voxels of the same size as the FWHM of the smoothness of the image. Depends on both image size and FWHM
- Work out the Euler characteristic (EC) of the image
 - Property of the image after it has been thresholded.
Roughly number of blobs in image after thresholding
- Resels and EC are linked: when Z thresholds increases and EC drops the expected EC approximates the probability of observing one or more blobs at that threshold.



What sort of voxelwise model to fit?

GLM

ANOVA, ANCOVA, linear regression...

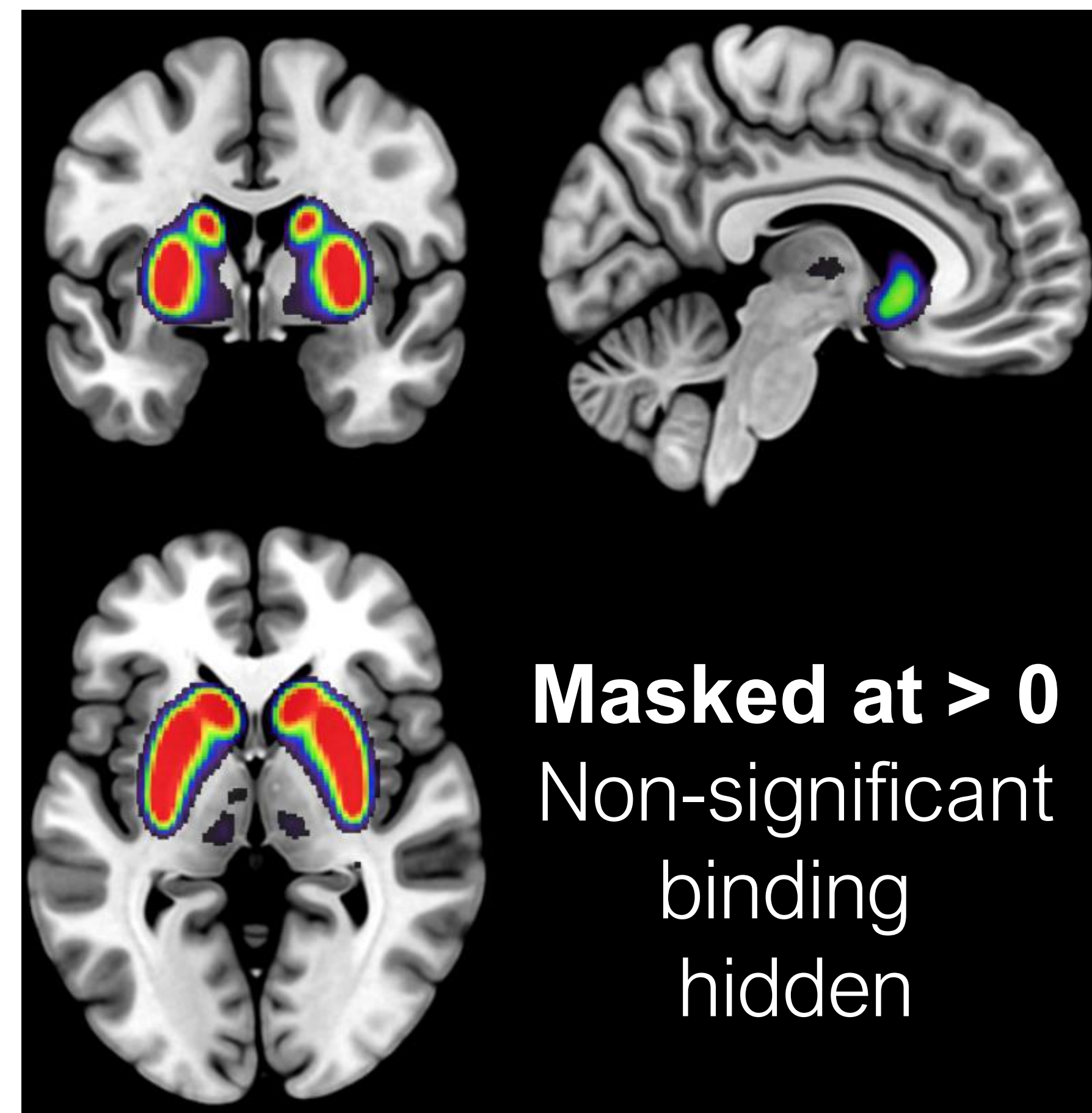
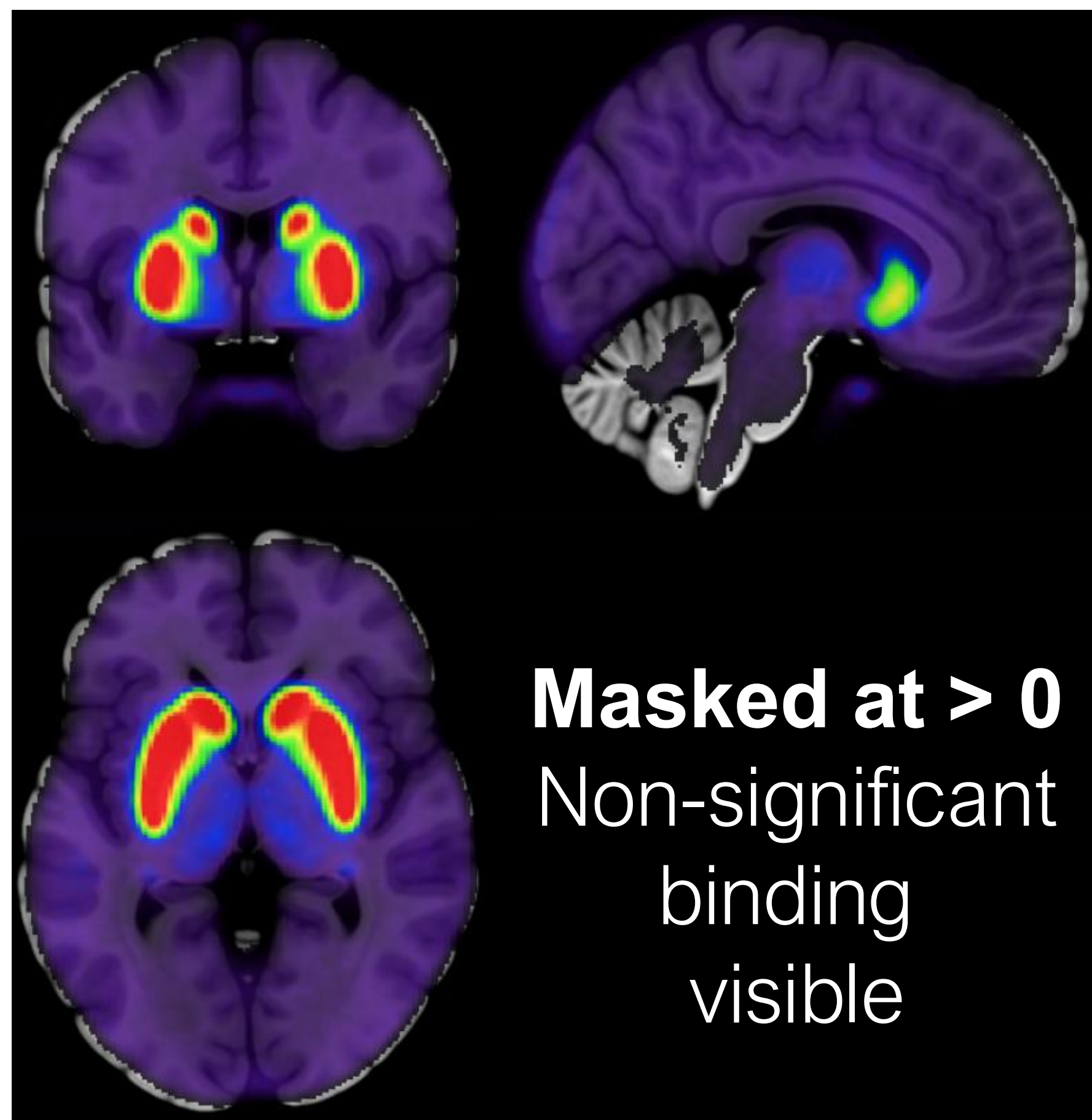
The diagram illustrates the General Linear Model (GLM) equation: $Y_i = \beta_0 + \beta_1 X_i + \epsilon_i$. Each term is labeled with an arrow: Y_i is the 'Dependent Variable'; β_0 is the 'Population Y intercept'; β_1 is the 'Population Slope Coefficient'; X_i is the 'Independent Variable'; and ϵ_i is the 'Random Error term'. Below the equation, two blue curly braces group the terms: the first brace under $\beta_0 + \beta_1 X_i$ is labeled 'Linear component', and the second brace under ϵ_i is labeled 'Random Error component'.

$$Y_i = \beta_0 + \beta_1 X_i + \epsilon_i$$

Labels and components:

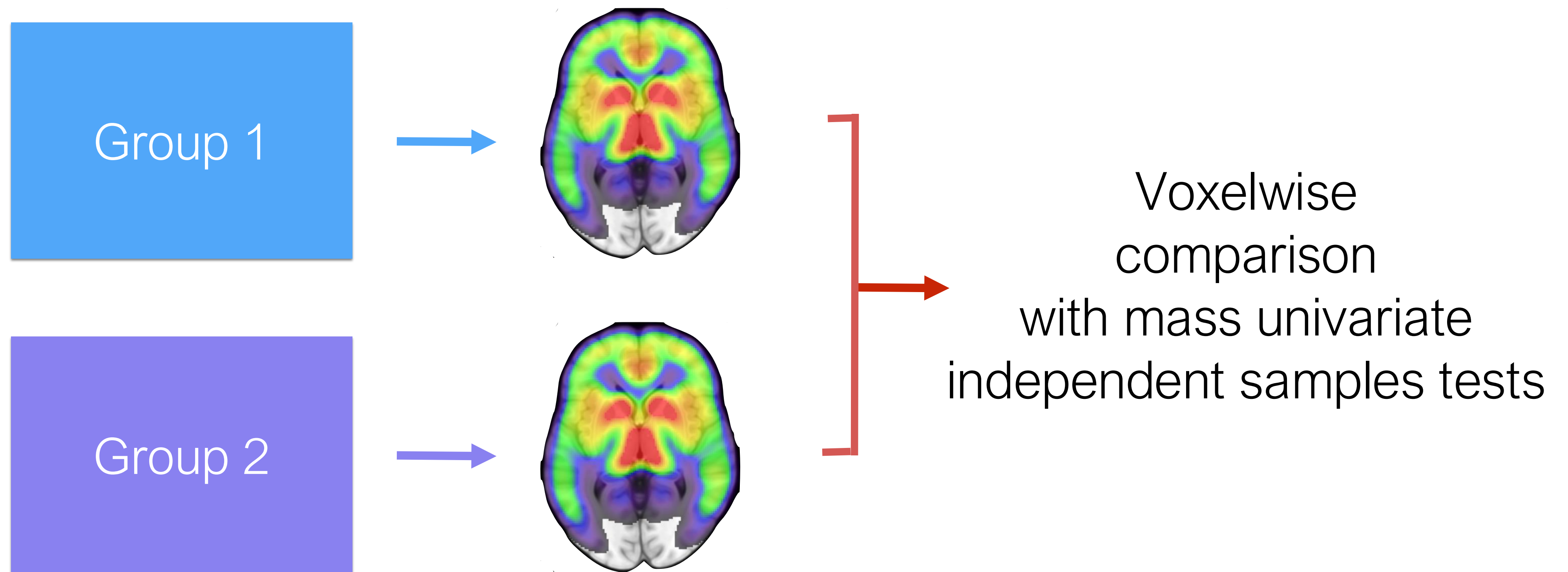
- Dependent Variable: Y_i
- Population Y intercept: β_0
- Population Slope Coefficient: β_1
- Independent Variable: X_i
- Random Error term: ϵ_i
- Linear component: $\beta_0 + \beta_1 X_i$
- Random Error component: ϵ_i

Masking the data

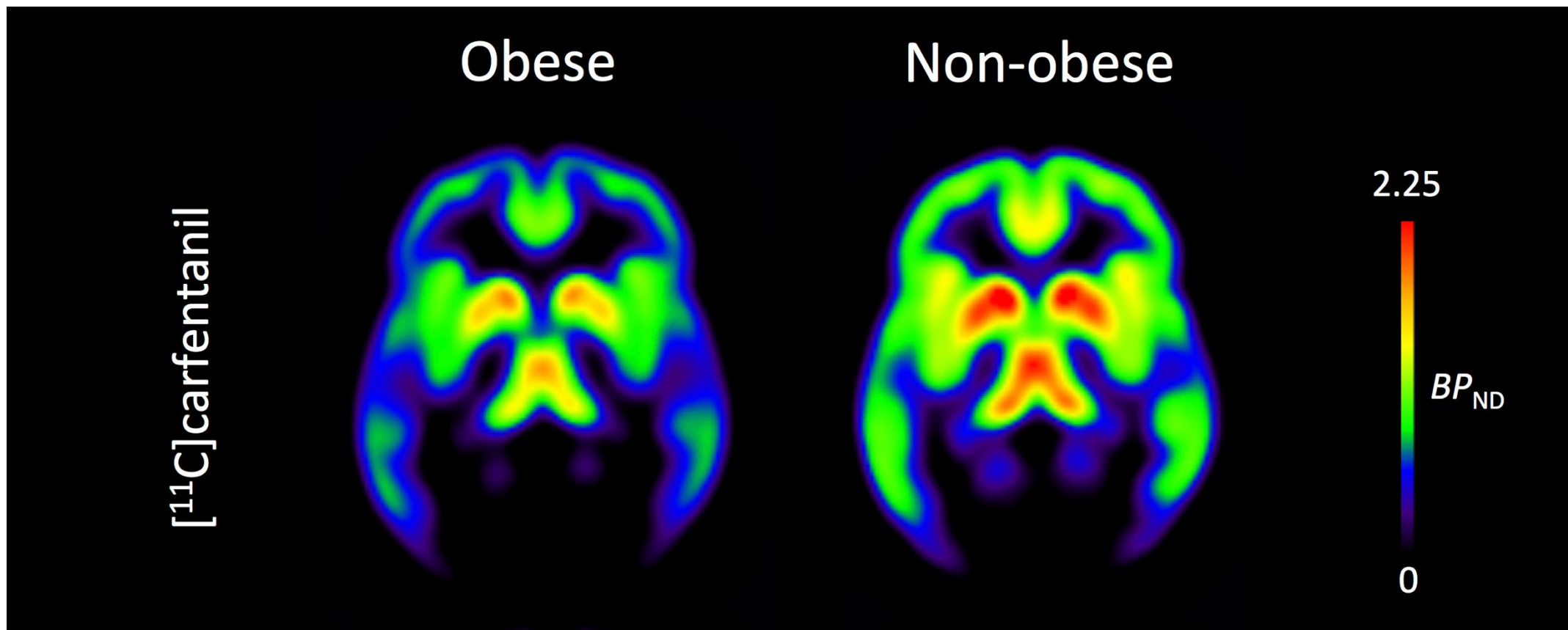


Applying explicit / threshold mask is necessary to avoid fitting model to noise

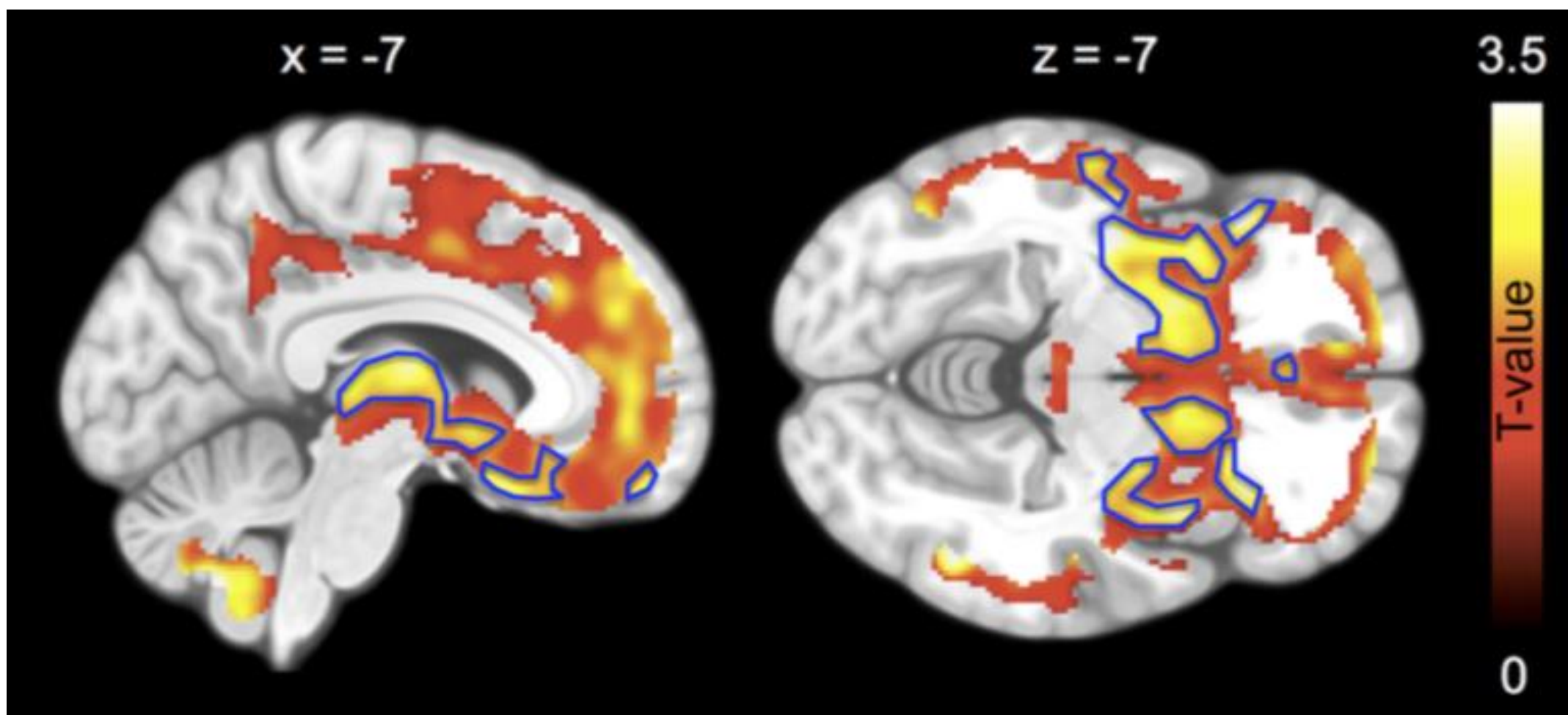
Between-groups design



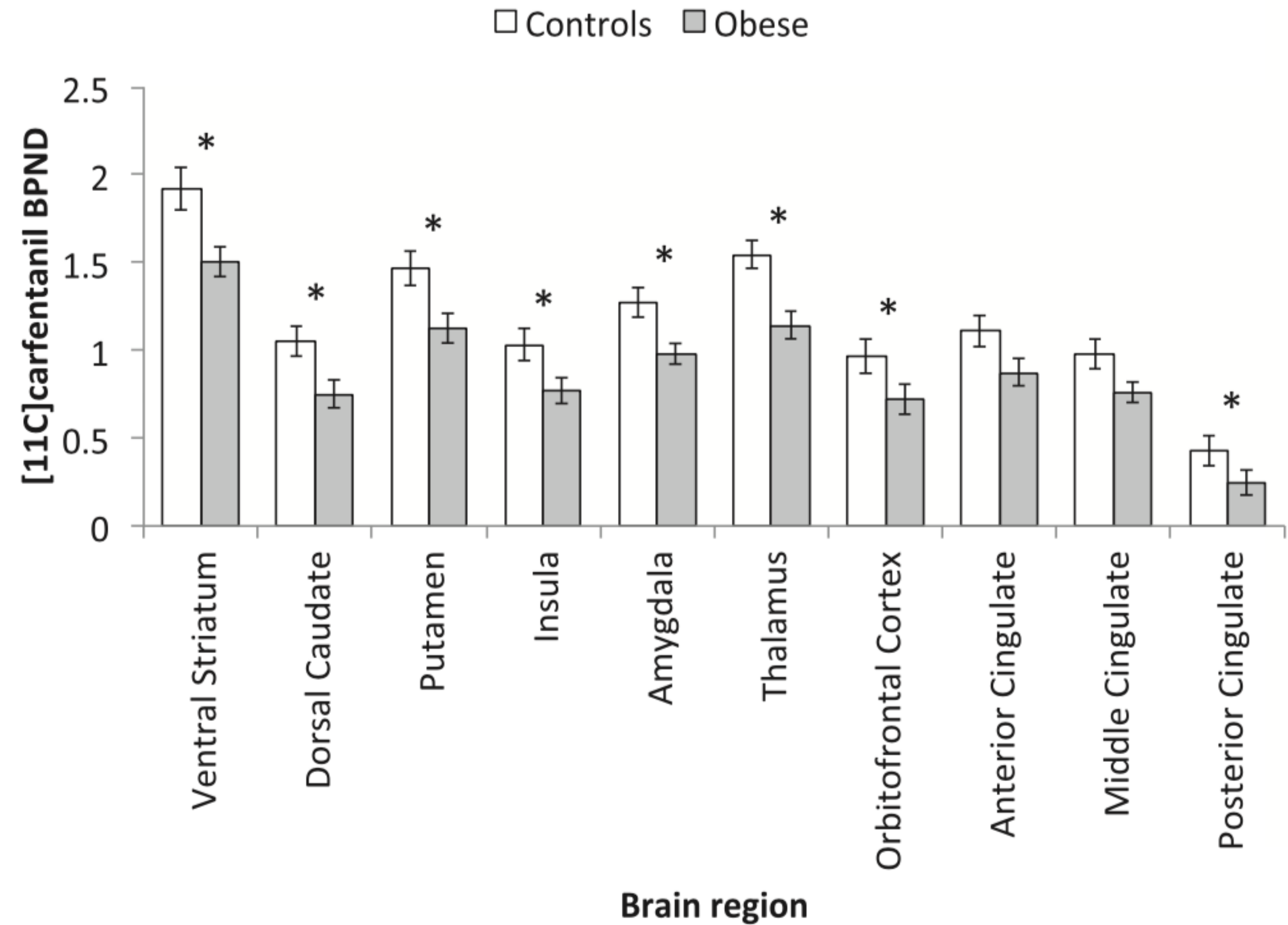
1) Mean images for each group



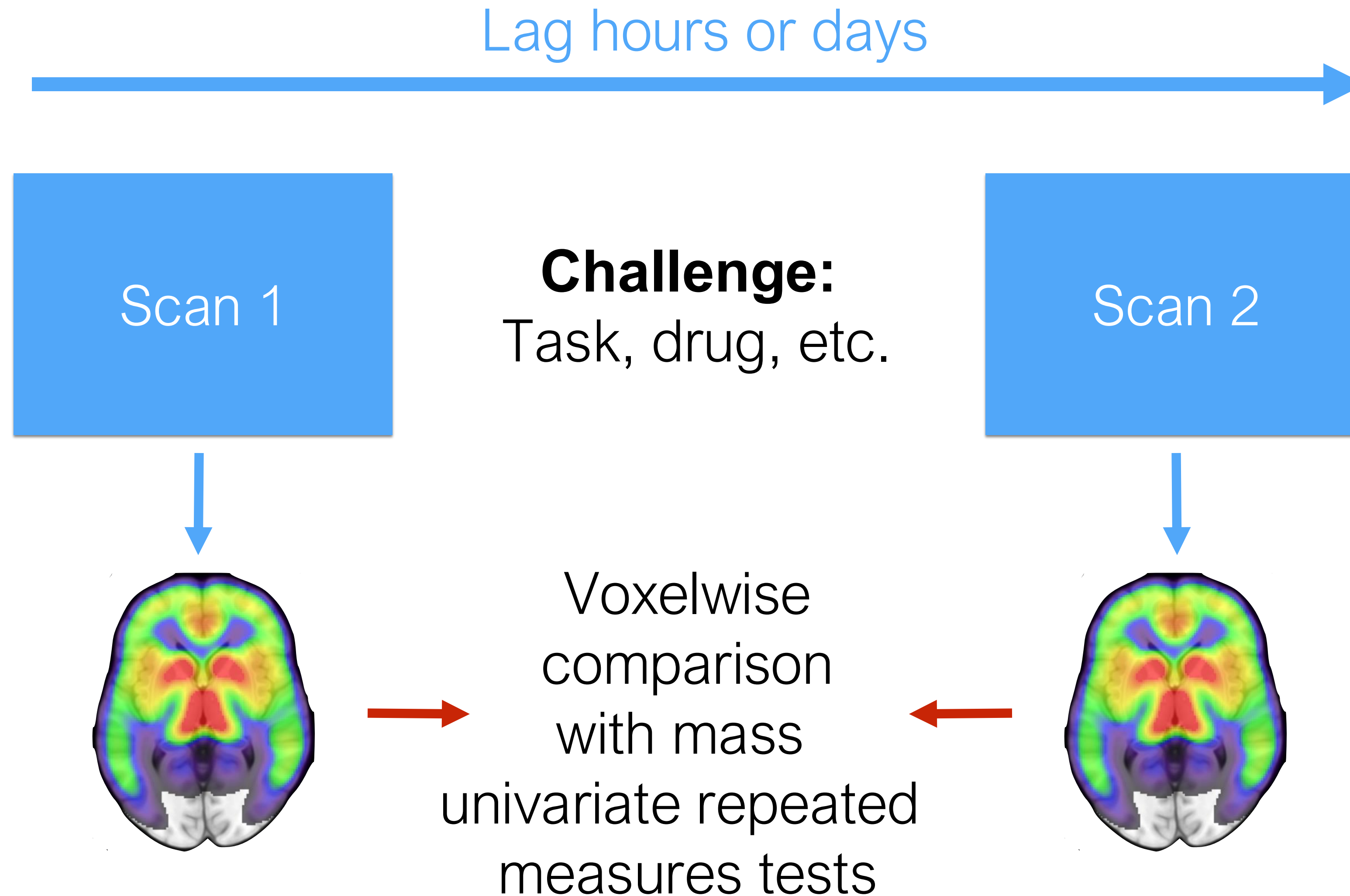
2) Statistical differences (t-map)



3) Region-of-interest data



Challenge / longitudinal design

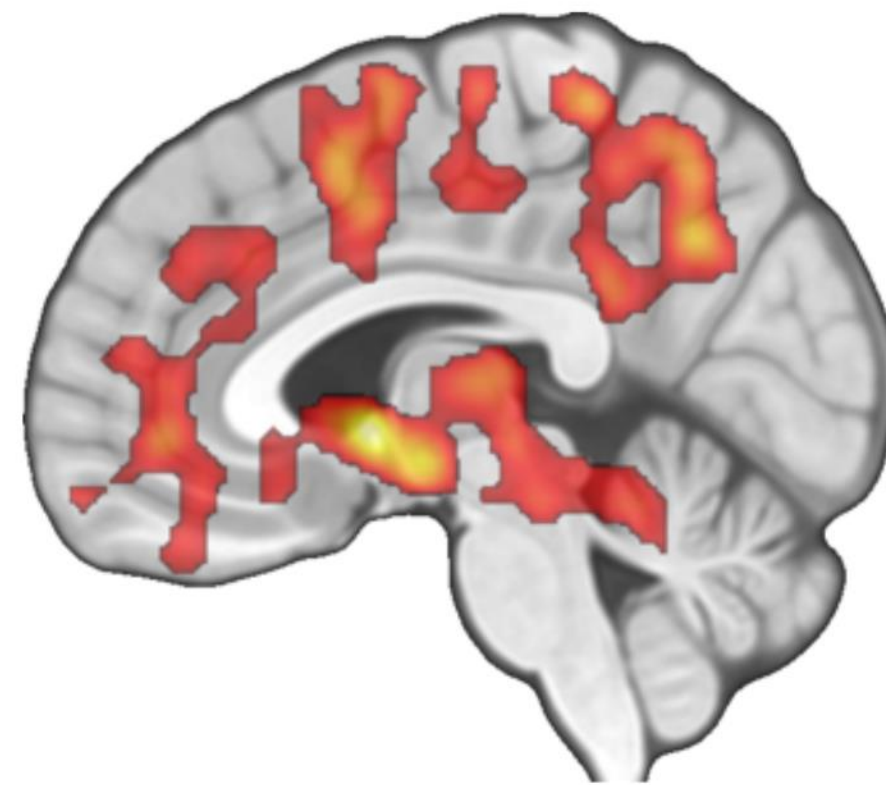
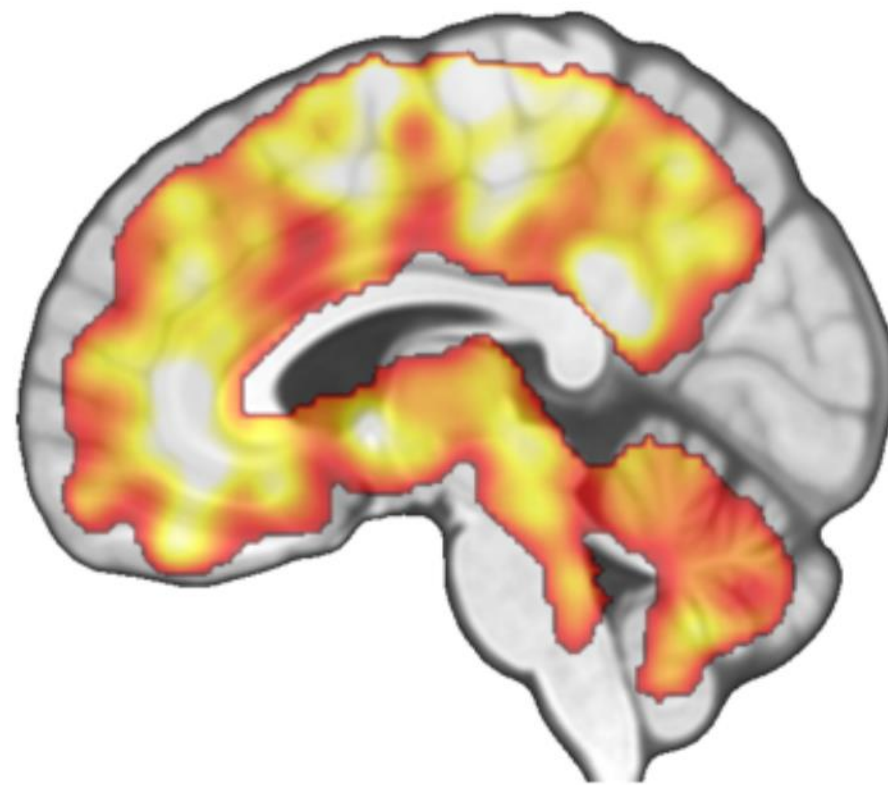


Fast vs.
Non-palatable

Fast vs.
Palatable

□ Non-palatable meal ■ Palatable meal ■ Fast

X = 4

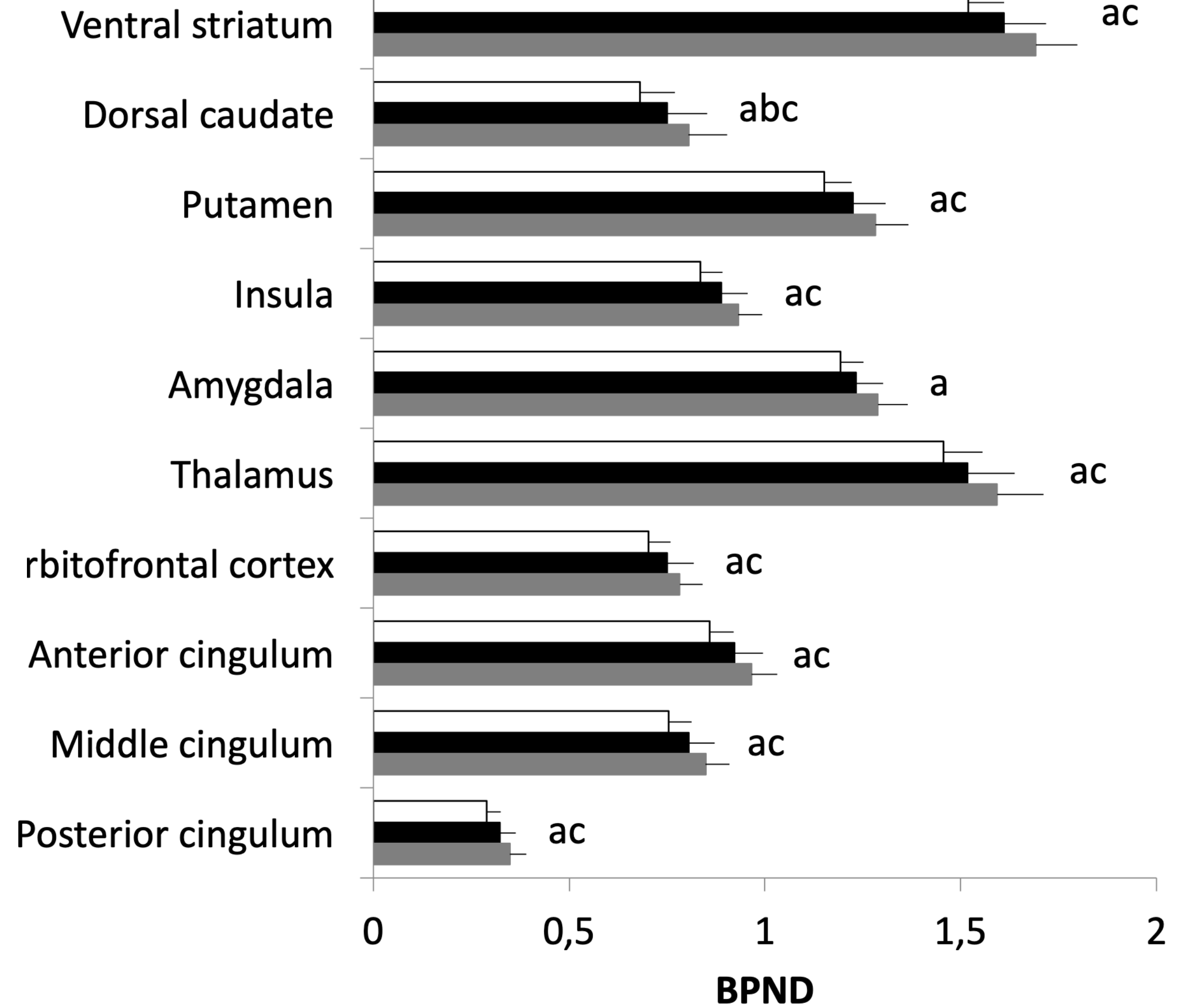
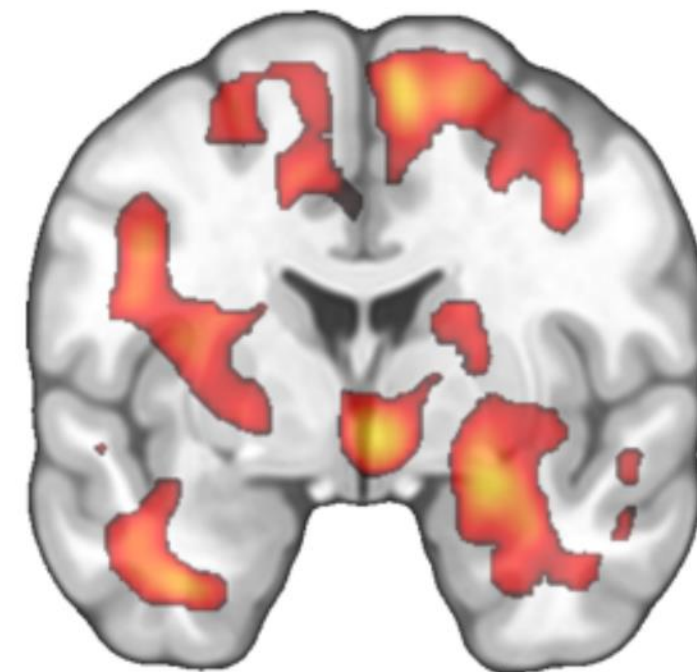
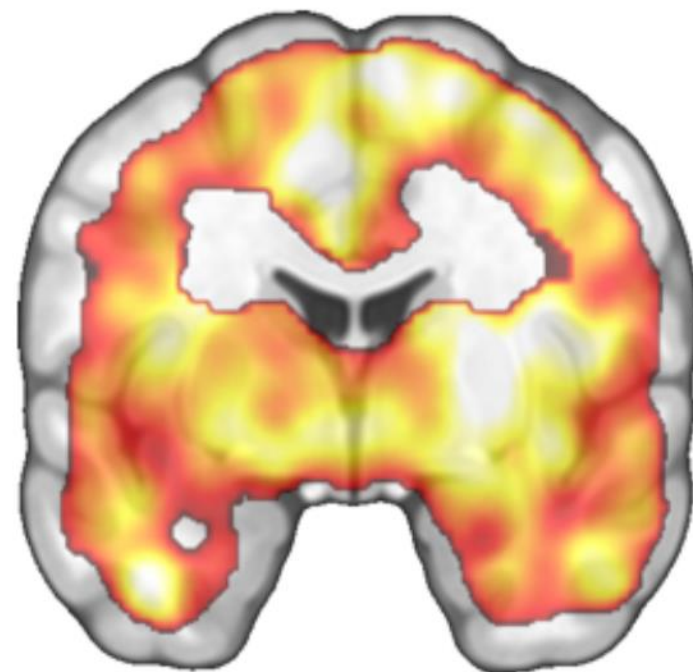


4

T-score

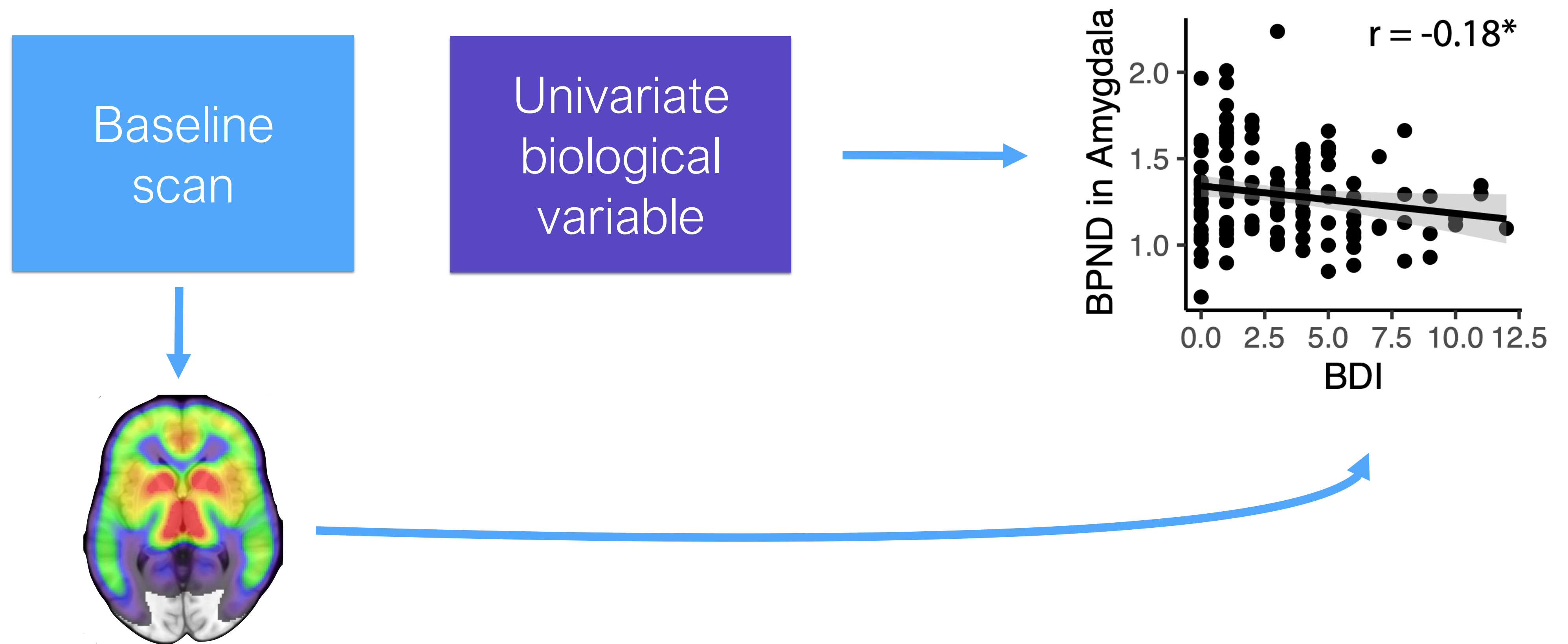
FDR

Y = -1

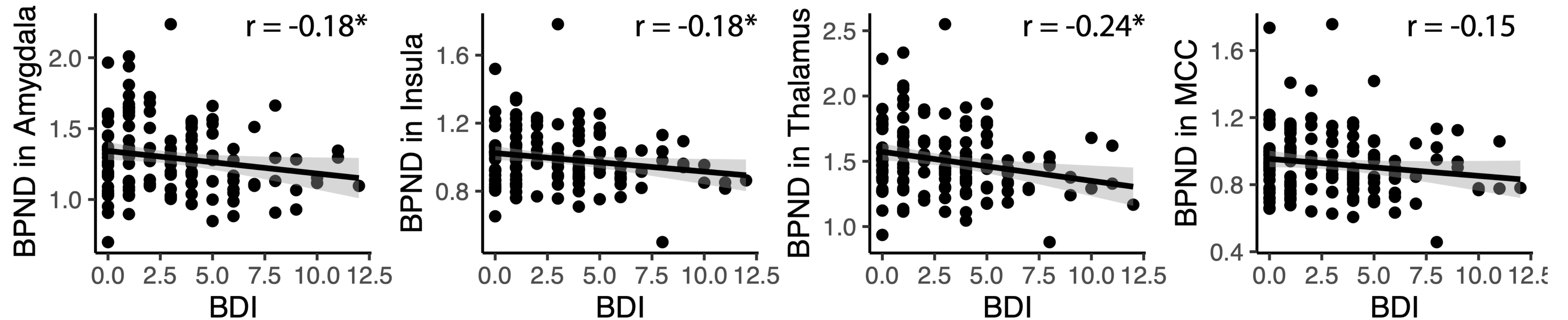
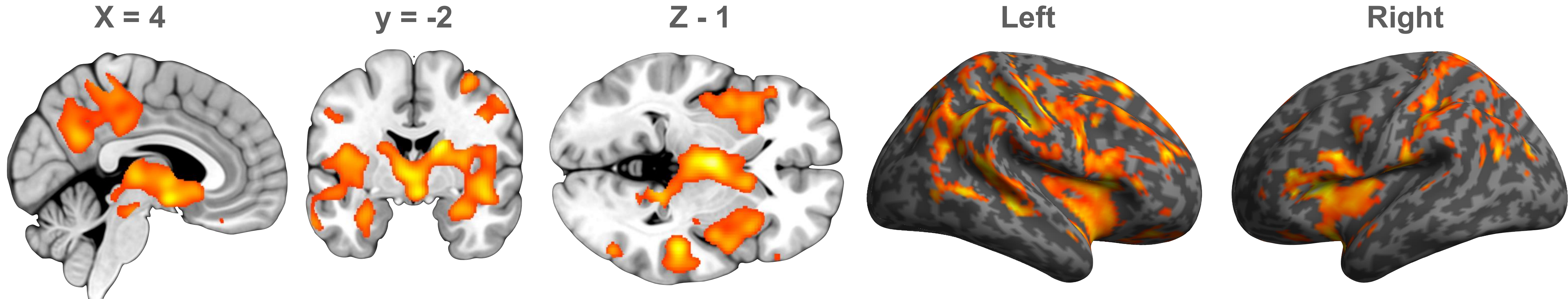


Tuulari et al (2018 J Neurosci)

Correlational design



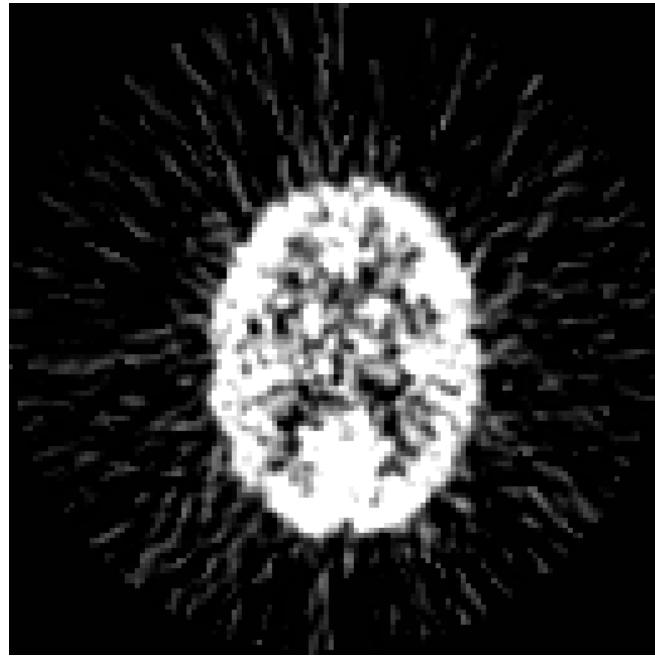
Lowered mu-opioid receptor levels in subclinical depression



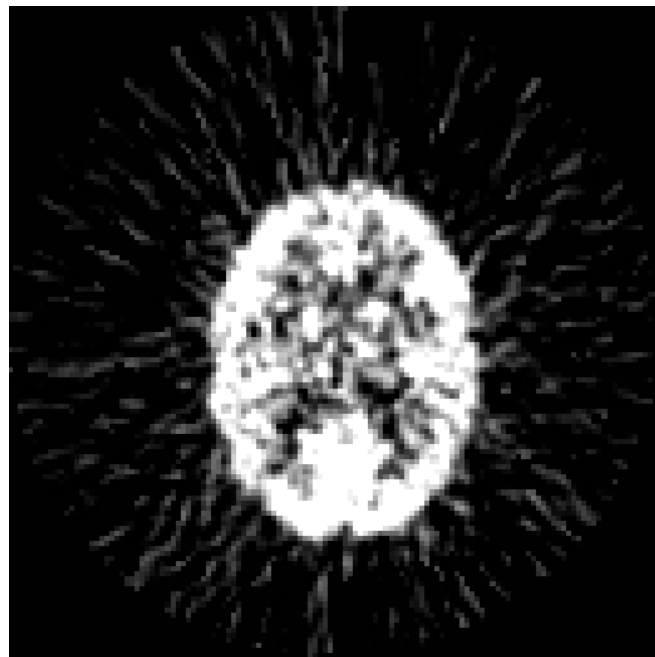
FDR T-score 4

Voxel intensity = outcome measure
(BPND, contrast estimate, tissue probability)

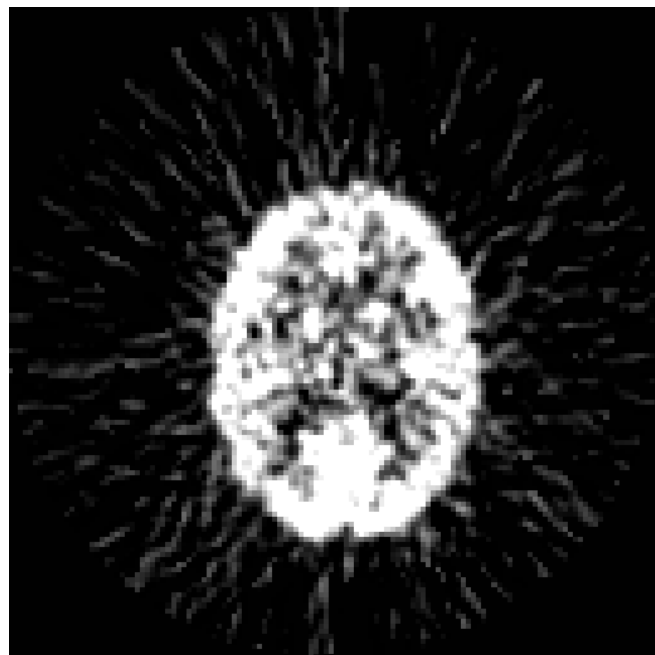
SUBJECT 1



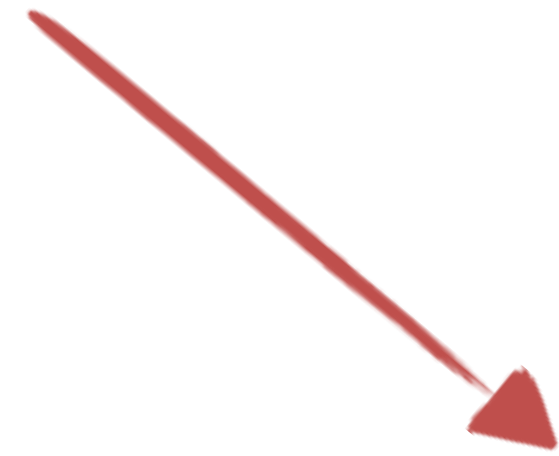
SUBJECT 2



SUBJECT 3

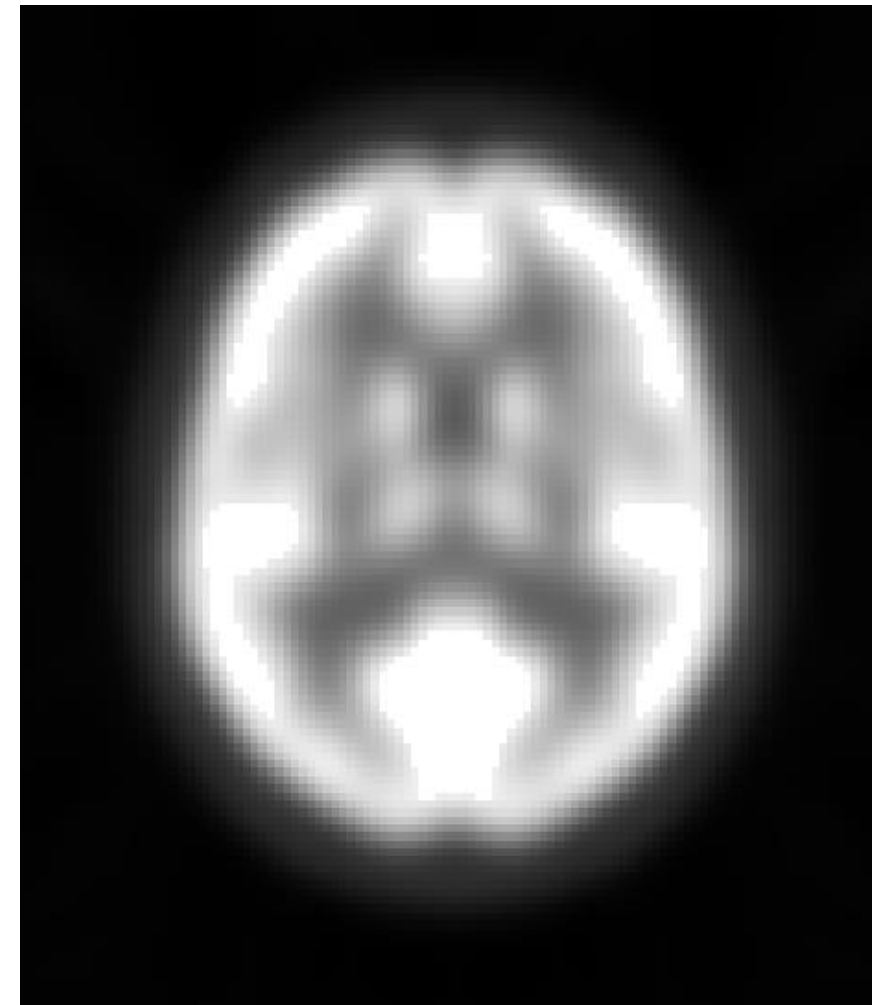


NORMALI-
ZATION



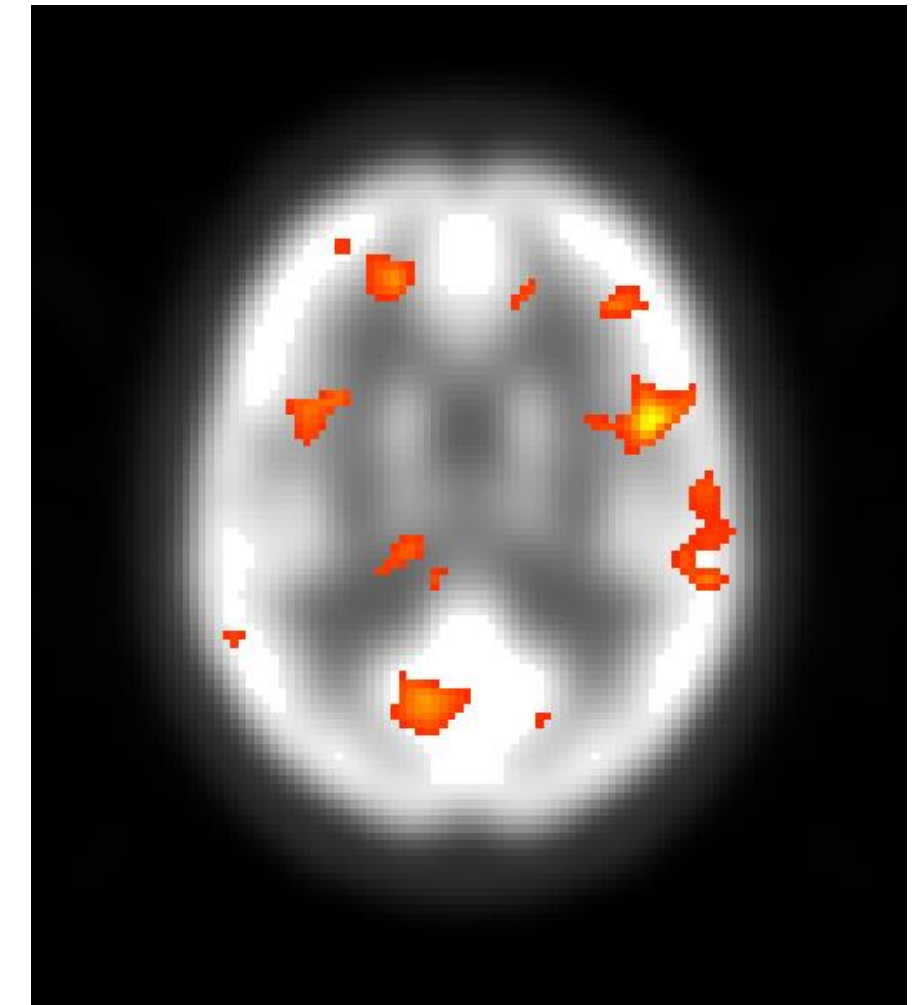
THE BASIC RECIPE

TEMPLATE



STATISTICAL
PARAMETRIC MAP

GLM



SMOOTH

