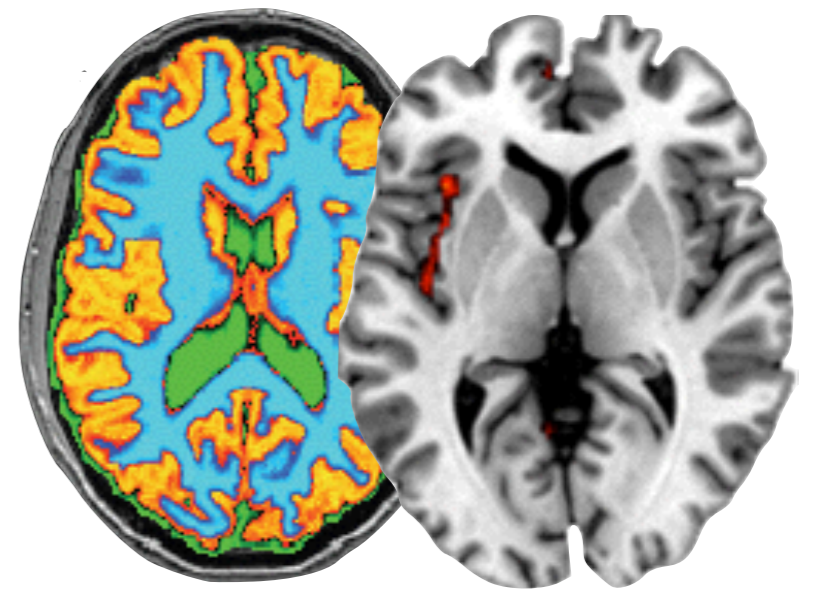


# Voxel-based Morphometry & Group level (2<sup>nd</sup>-level) analysis



K.H. Chou, Ph.D (周坤賢)

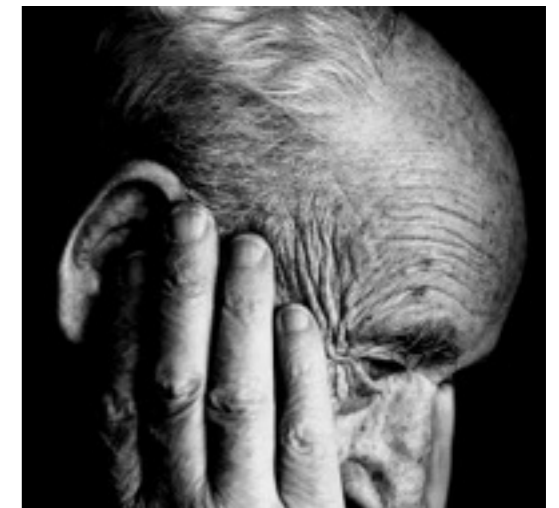
2016.07.14

Assistant research fellow / Brain Research Center / National Yang Ming University



# Today's **Mission**

My mission today is to  
teach you the basic concept of **VBM**  
and how to use **SPM12**  
to perform **basic T1-VBM analysis**  
(**with real world example**)



# The **Notice** of Today's Course !



Don't think any shortcut solutions

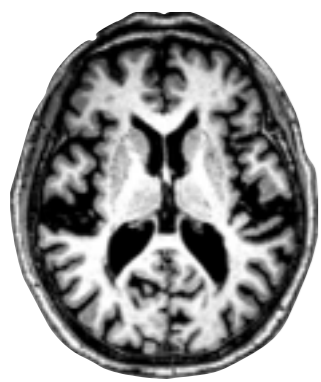


Practice, practice and practice

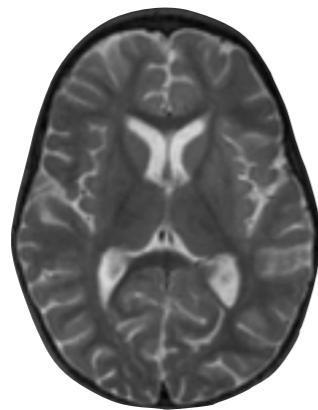


Ask me questions when we have free time

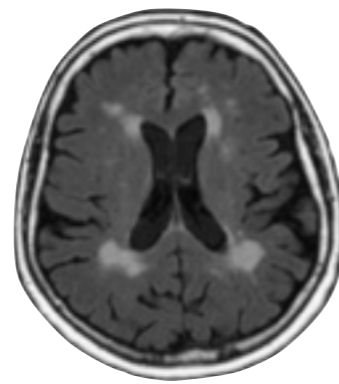
# Which Imaging Protocol We Will Focus on ?



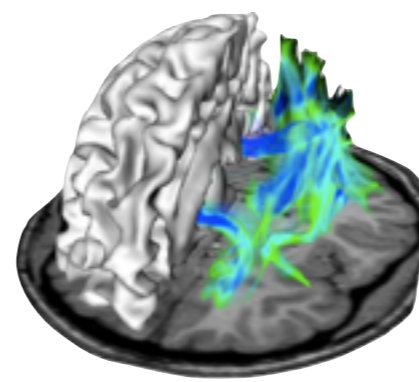
T1



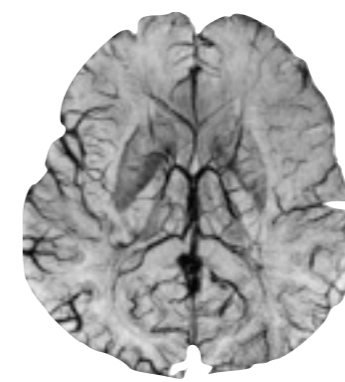
T2



FLAIR-T2



dMRI



SWI



Task / rs-fMRI

Structural MRI  
(sMRI)

Functional MRI  
(fMRI)

# The Real World Example of VBM Study (But Be Careful !)



News website header with categories: 新聞, 政治, 財經, 生活, 地方, 社會, 運動, 娛樂, 國際, 大陸, 新奇, 消費, 旅遊, 寵物, 科技. A sub-header for sports: 運動最速報, 台灣之光, 棒球, 籃球, 綜合, 足球, 趣運動. A search bar contains '頭條新聞' and '速報>>'. The main article title is '研究：朋友太少？怪你的杏仁核太小' (Study: Too few friends? Blame your small amygdala). The date is '2010年 12月 28日 00:20'. Social sharing buttons show 15萬 views, 0 likes, and 2 shares.

<http://www.nownews.com/n/2010/12/28/578022>

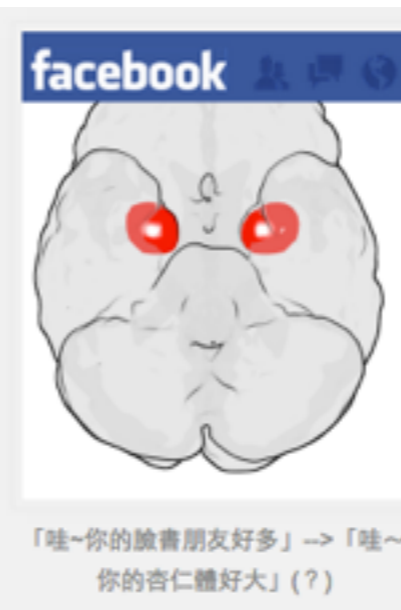
NATURE NEUROSCIENCE | BRIEF COMMUNICATION

## Amygdala volume and social network size in humans

Kevin C Bickart, Christopher I Wright, Rebecca J Dautoff, Bradford C Dickerson & Lisa Feldman Barrett

Affiliations | Contributions | Corresponding author

Nature Neuroscience 14, 163–164 (2011) | doi:10.1038/nn.2724



<http://pansci.tw/archives/468>



其他語言 > 即時新聞目錄 > 新聞內頁

國·際·新·聞 International

研究：交友廣闊 腦杏仁核也大

分享 新聞引據：中央社

時間：2010/12/27

你常花時間和很多朋友共處嗎？這可能代表你腦中有個特殊部分較大。那就是位於腦部深處的「杏仁核」(amygdala)。

一項初步研究，對58名志願者腦部進行掃描發現，杏仁核愈大，志願者的朋友及家人就越多。

「自然神經科學雜誌」(Nature Neuroscience)26日在線上刊出這項研究，作者巴瑞特(Lisa FeldmanBarrett)表示，這結果合理，因杏仁核是腦部主掌重要社交網絡核心。

[http://news.rti.org.tw/index\\_newsContent.aspx?nid=273628&id=6&id2=2](http://news.rti.org.tw/index_newsContent.aspx?nid=273628&id=6&id2=2)

# Look Into More **Details** About VBM Based Research

## 自由派或保守派 頭腦決定

AFP

法新社 - 2011年4月8日 下午7:20

-字 +字

相關內容

自由派或保守派 頭腦決定

(法新社華盛頓7日電) 每個人都知道談到世界觀, 自由派與保守派存在嚴重分歧, 然而科學家指出, 他們的腦袋構造其實不同。「當代生物」(Current Biology) 今天刊出的研究指出, 自由派的頭腦, 在與瞭解複雜事務相關的部分含有較多灰白質, 而保守派的頭腦, 腦中處理恐懼的部分較大。研究指出: 「我們發現越是傾向自由主義, 頭腦的前扣帶皮質內含較多灰白質, 反之, 越是傾向保守主義則與頭腦右側杏仁核較大有關。」其他研究顯示, 那些區域頭腦活動程度不同, 造就人們所持的政治觀點, 然而, 這個研究首度呈現頭腦相同區域, 在生理大小上存在差異。這項研究根據90位「健康的年輕人」在標示從非常自由至非常保守的1-5量表上報告他們的政治觀點, 而後同意接受腦部掃描。研究指出, 頭腦中杏仁核較大的人「對厭惡較敏感」, 且「相較於自由派, 他們以更具攻擊性的行為回應威脅情境, 對具威脅的臉部表情也更敏感」。研究指出, 自由派腦內「監視不確定性和衝突」的前扣帶皮質區域較大。(譯者: 中央社張詠晴) 1

## Political Orientations Are Correlated with Brain Structure in Young Adults

Ryota Kanai,<sup>1,\*</sup> Tom Feilden,<sup>2</sup> Colin Firth,<sup>2</sup> and Geraint Rees<sup>1,3</sup>

<sup>1</sup>University College London Institute of Cognitive Neuroscience, 17 Queen Square, London WC1N 3AR, UK

<sup>2</sup>BBC Radio 4, Television Centre, Wood Lane, London W12 7RJ, UK

<sup>3</sup>Wellcome Trust Centre for Neuroimaging, University College London, 12 Queen Square, London WC1N 3BG, UK

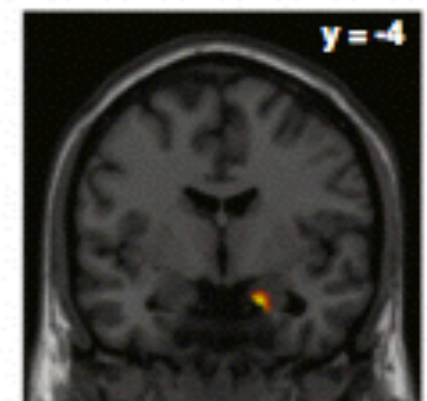
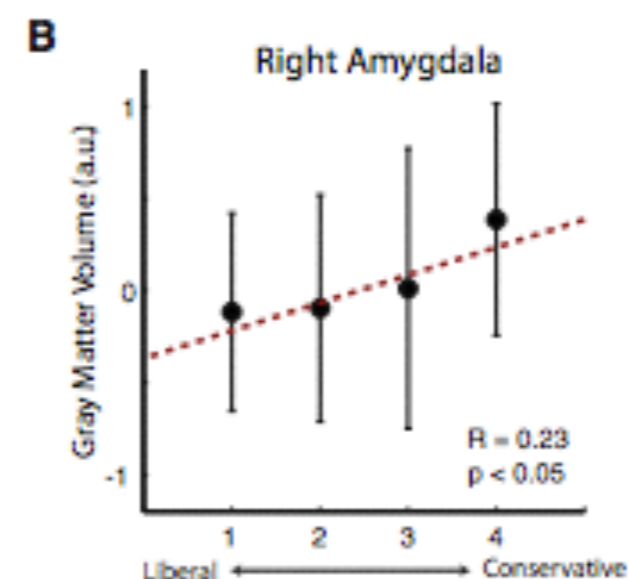
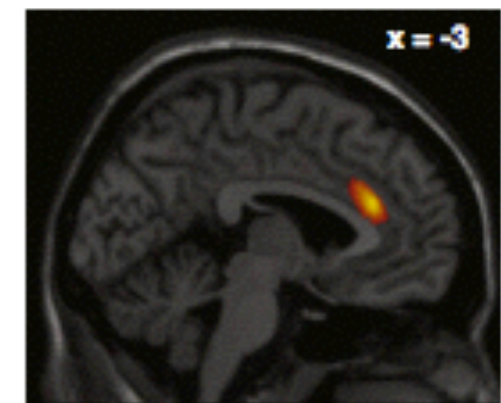
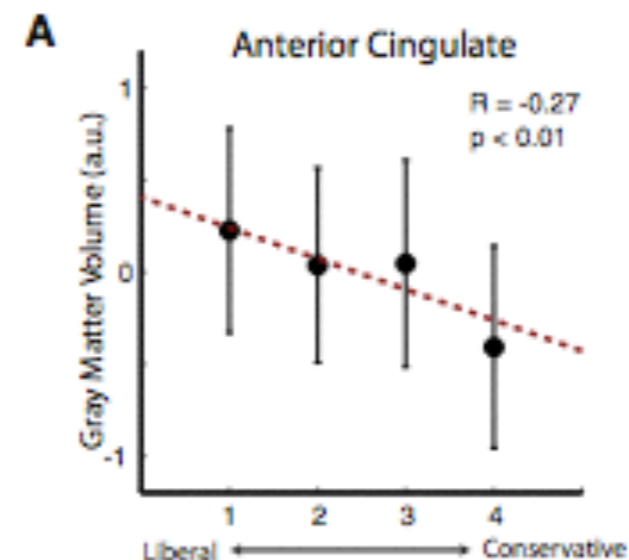
Current Biology 21, 1-4, April 26, 2011 ©2011 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2011.03.017

Tissue: GM/WM

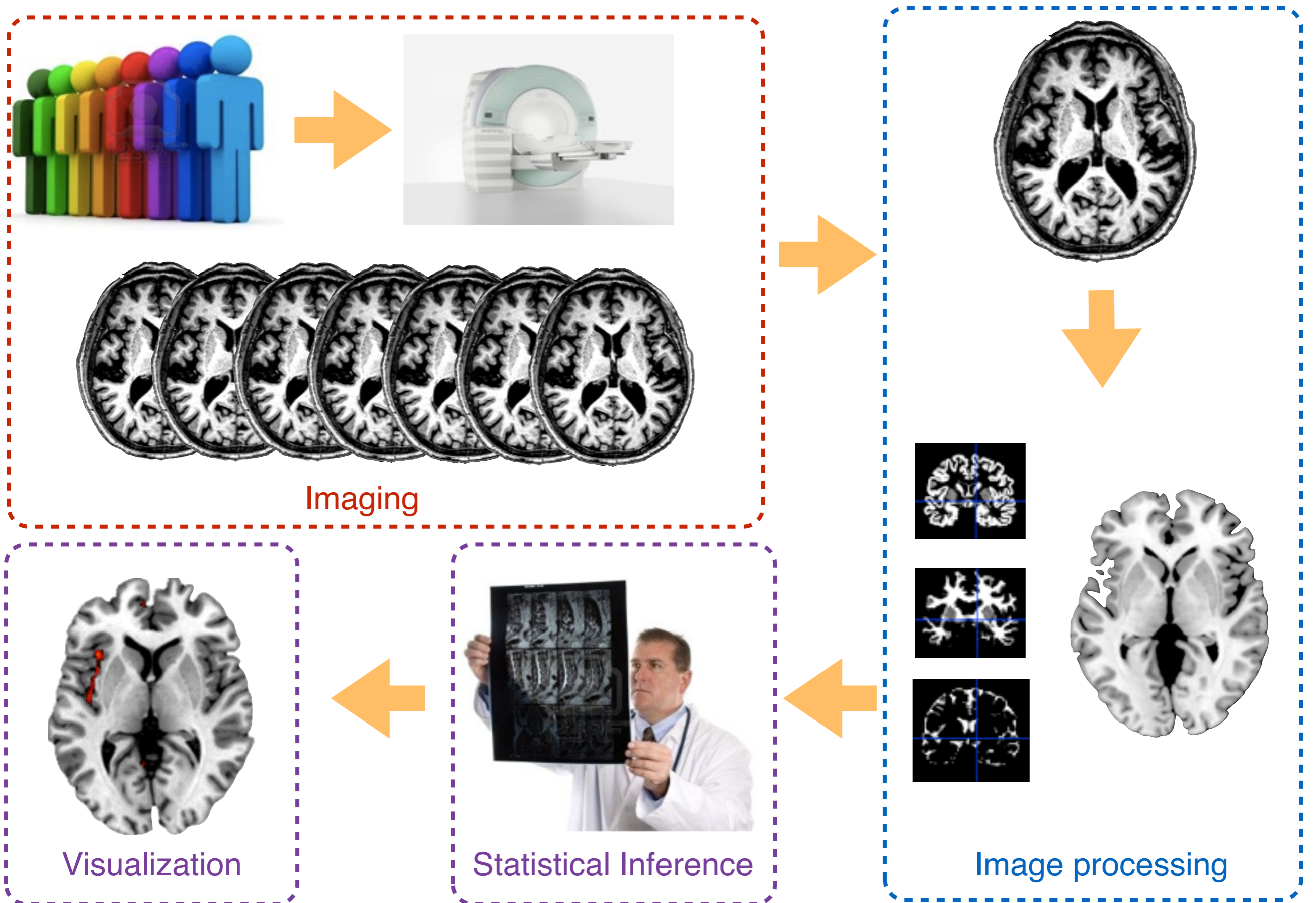
Anatomy location of brain

Statistical analysis

Many subjects



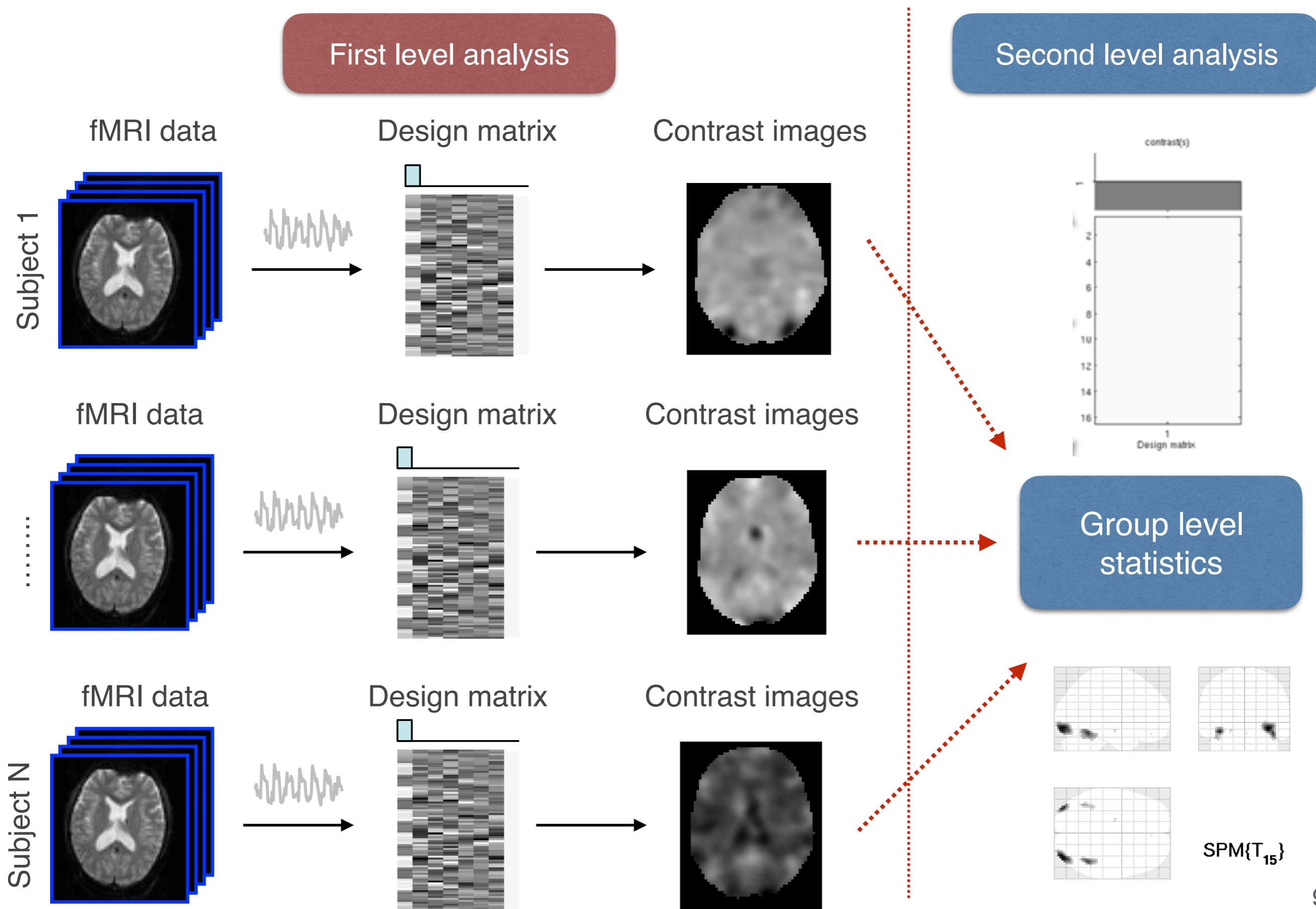
# The Possible **Flowchart** of VBM Based Research







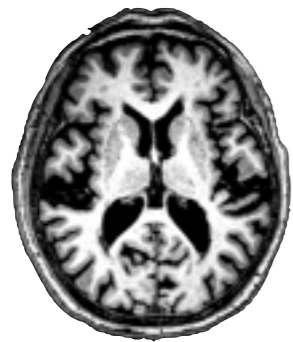
# Recall Your Memory - The **Classical** Pipeline of fMRI Analysis



# General **Concept** of T1 VBM



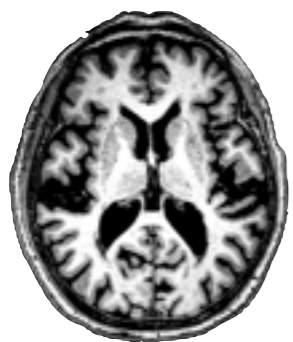
Subject 1



Subject 2

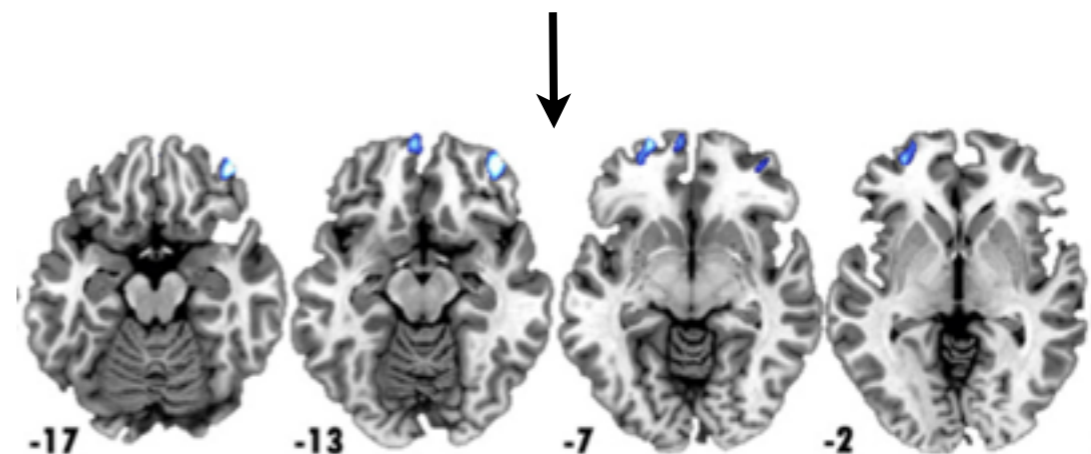
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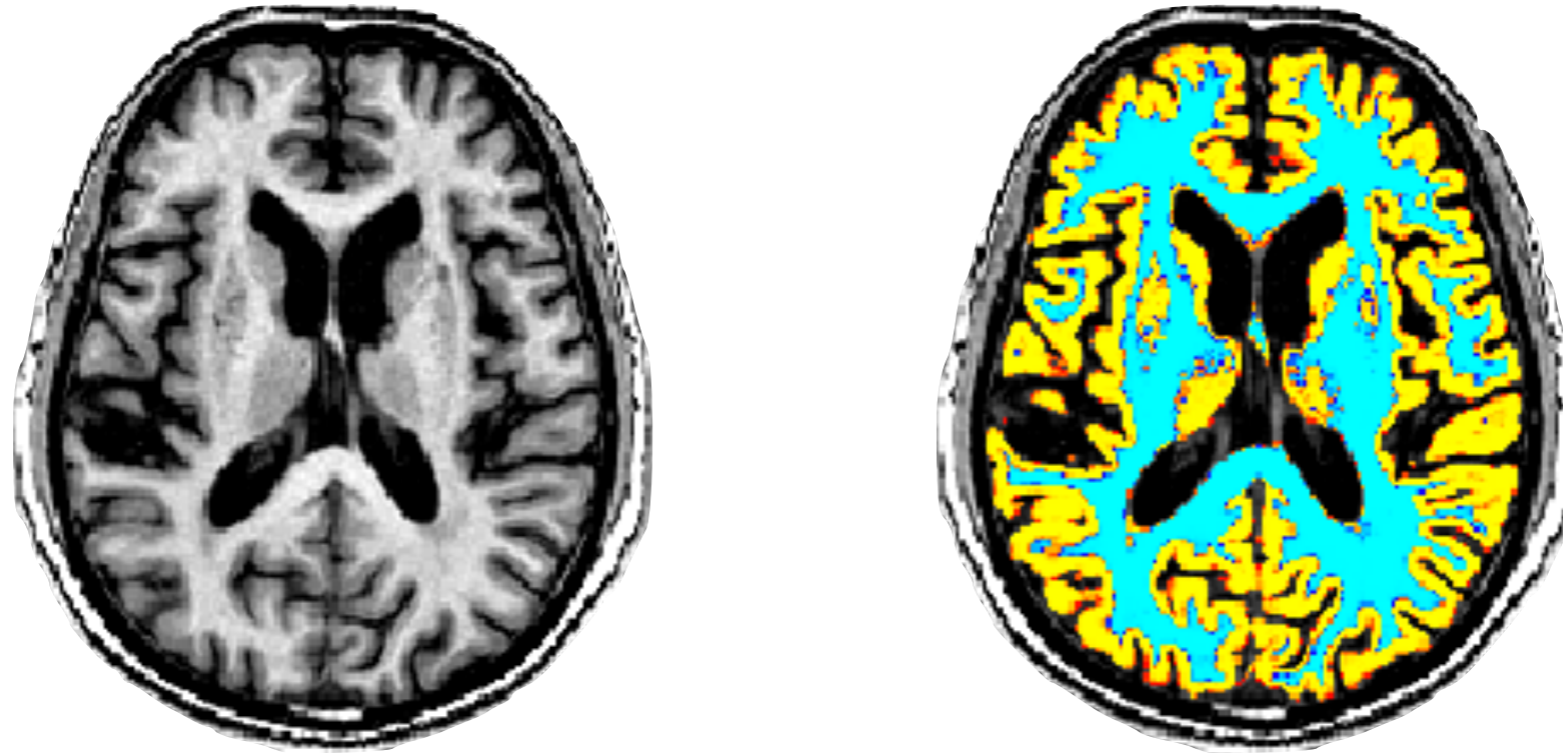


Subject N

- The core modules of VBM
1. Tissue segmentation
  2. Spatial normalization
  3. Tissue modulation (**optional**)
  4. Tissue smoothing
  5. Statistical modeling



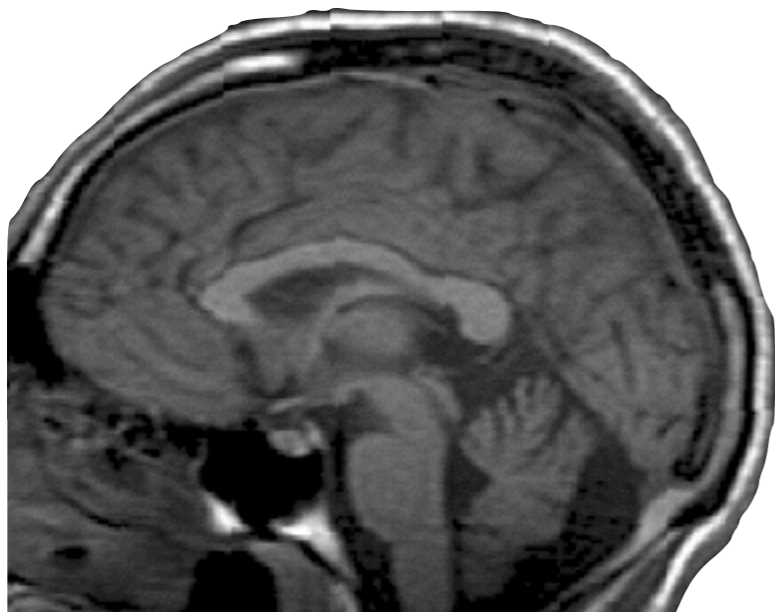
# Why Should the Tissue Need to Be Segmented ?



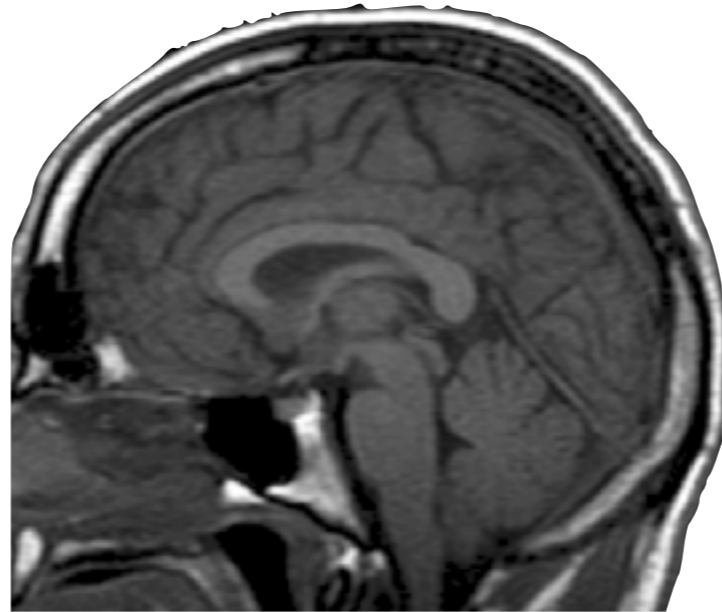
- High-resolution T1 images reveals fine structural detail in the brain, but not all of it reliable or interesting (noise, intensity-inhomogeneity, vasculature ..... )
- Intensity of T1 image is usually not **quantitatively** meaningful (related to imaging parameters)
- Regional volumes of the three main tissue types: **gray matter**, **white matter** and **CSF**, are well-defined and potentially very interesting
- We obtain tissue **probability** map after segmentation procedure

# Why We Need **Spatial Normalization** ? (1)

Shape and size of the brain can **vary** among subjects



Subject 1



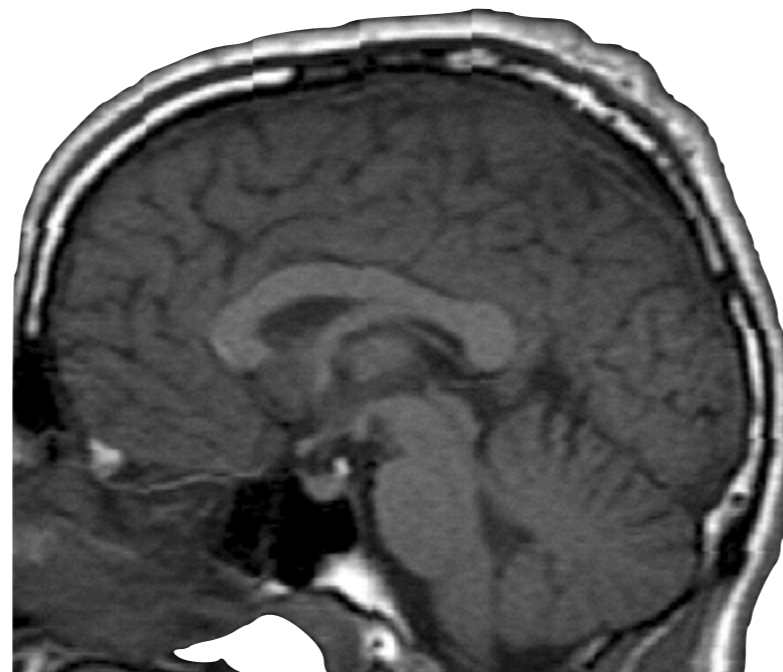
Subject 2



Subject 3



Subject 4



Subject 5



Subject 6

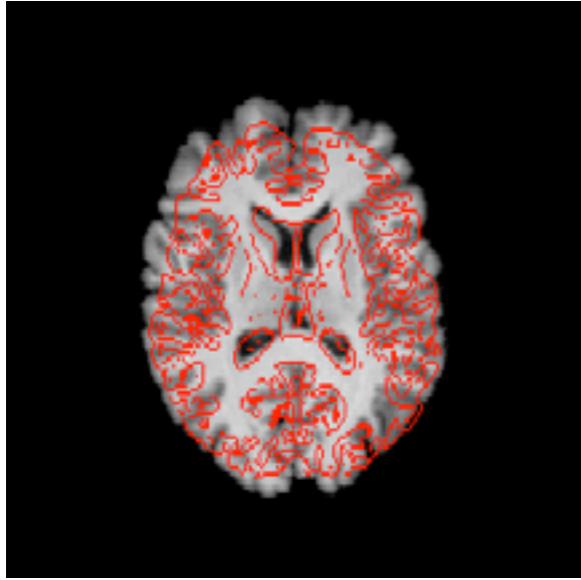
# Why We Need Spatial Normalization ? (2)

Report final result in the **standard** template space (**MNI**)

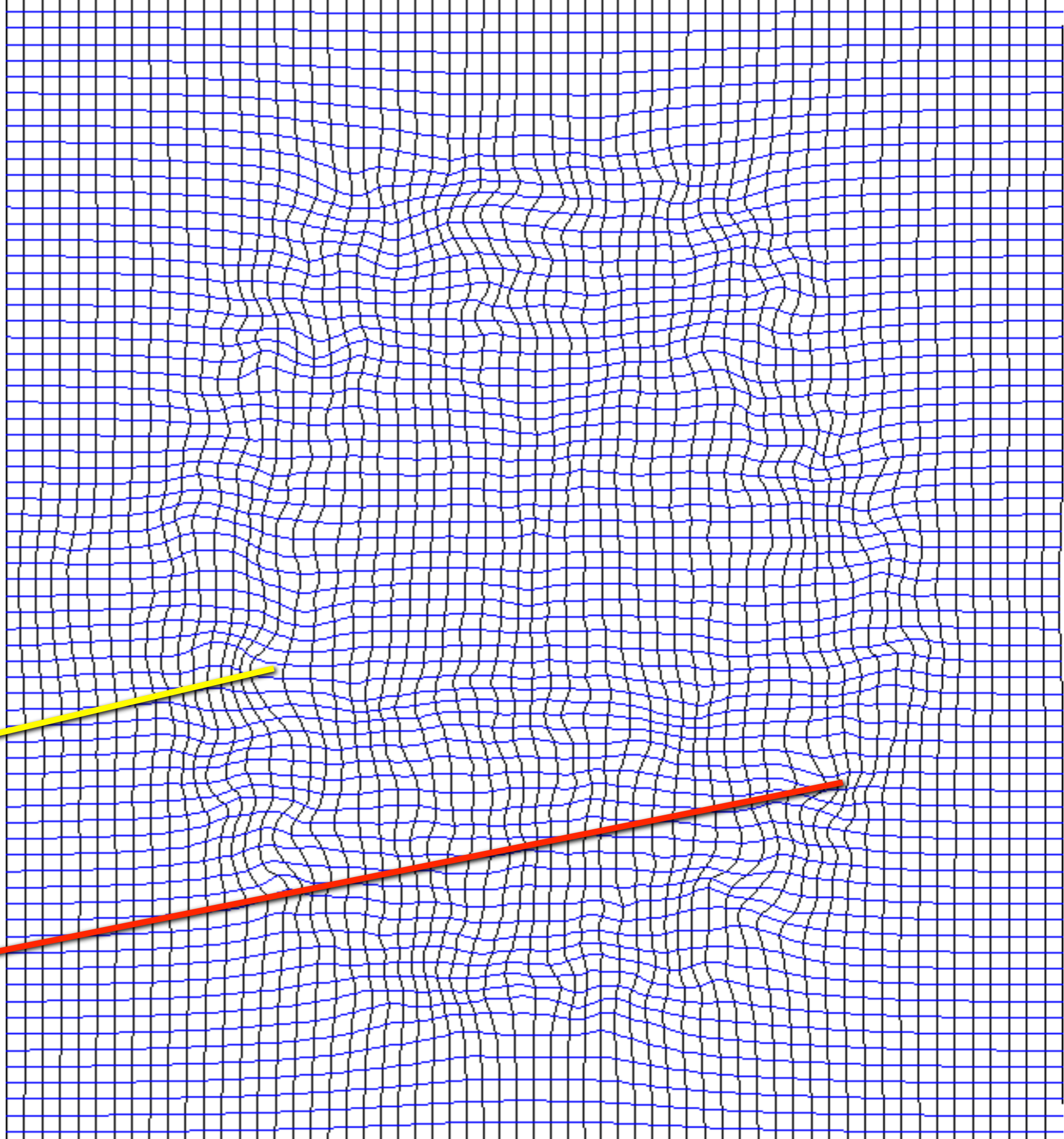
**TABLE II. Anatomical regions with significant gray matter volume reductions in patients with Parkinson's disease compared with the healthy control group**

| MNI coordinates |          |          | Cluster size | Side | Anatomical region         | Brodmann area | Z-score |
|-----------------|----------|----------|--------------|------|---------------------------|---------------|---------|
| <i>x</i>        | <i>y</i> | <i>z</i> |              |      |                           |               |         |
| -12             | -45      | -53      | 8657         | Lt.  | Cerebellar tonsil         | a             | 5.42    |
| 33              | -70      | -33      |              | Rt.  | Cerebellar tonsil         | a             | 5.11    |
| 8               | 18       | -3       | 3331         | Rt.  | Caudate                   | a             | 5.15    |
| -2              | 8        | -11      |              | Lt.  | Anterior cingulate        | BA 25         | 4.70    |
| -6              | 17       | -2       |              | Lt.  | Caudate                   | a             | 4.67    |
| -35             | -12      | -39      | 3303         | Lt.  | Parahippocampal gyrus     | a             | 4.54    |
| 51              | -9       | 4        | 2777         | Rt.  | Precentral gyrus          | BA 6          | 5.07    |
| 56              | -15      | 9        |              | Rt.  | Transverse temporal gyrus | BA 41         | 4.94    |
| -3              | -34      | 33       | 792          | Lt.  | Cingulate gyrus           | BA 31         | 4.89    |
| -48             | -24      | 12       | 691          | Lt.  | Transverse temporal gyrus | BA 41         | 4.77    |
| 59              | -40      | 4        | 552          | Rt.  | Middle temporal gyrus     | BA 22         | 4.33    |
| -18             | -70      | 12       | 519          | Lt.  | Posterior cingulate       | BA 30         | 5.13    |
| 29              | -3       | -21      | 456          | Rt.  | Parahippocampal gyrus     | a             | 4.23    |
| 41              | 45       | 24       | 423          | Rt.  | Middle frontal gyrus      | BA 10         | 4.32    |
| 15              | -72      | 7        | 339          | Rt.  | Lingual gyrus             | BA 18         | 4.25    |
| 33              | -27      | -26      | 328          | Rt.  | Parahippocampal gyrus     | BA 35         | 4.11    |
| 56              | -3       | -26      | 264          | Rt.  | Middle temporal gyrus     | BA 21         | 3.83    |
| -8              | -18      | 18       | 192          | Lt.  | Thalamus                  | a             | 3.76    |
| 24              | 62       | 1        | 166          | Rt.  | Superior frontal gyrus    | BA 10         | 4.11    |
| 47              | -33      | 62       | 164          | Rt.  | Inferior parietal lobule  | BA 40         | 3.73    |
| -45             | -34      | 40       | 125          | Lt.  | Inferior parietal lobule  | BA 40         | 4.28    |
| -51             | -13      | -41      | 122          | Lt.  | Inferior temporal gyrus   | BA 20         | 3.71    |
| 41              | -76      | 13       | 121          | Rt.  | Middle occipital gyrus    | BA 19         | 3.64    |

# Deformation Field



Encode how we map individual brain into MNI space

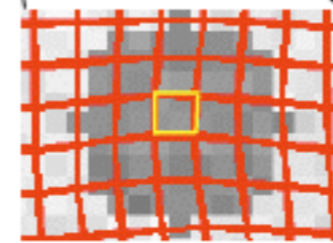
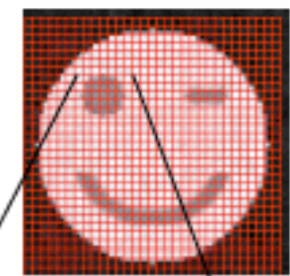
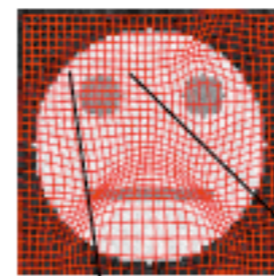
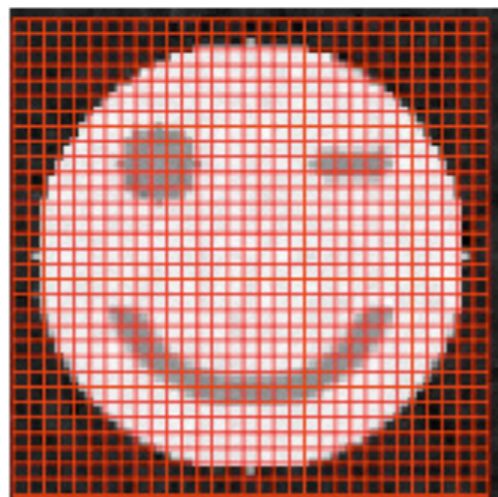
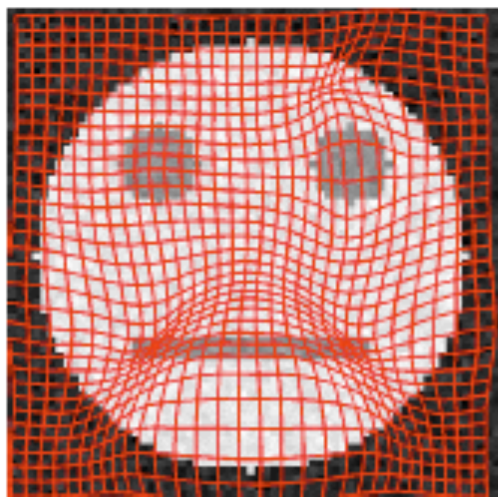


Local Expansion

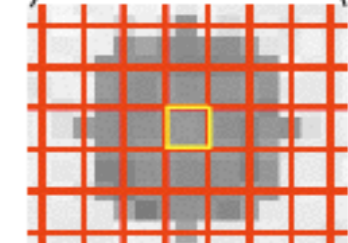
Local Shrinkage

# Do We Need Tissue **Modulation** !? (Optional)

Corrects for changes in brain **VOLUME** caused by non-linear spatial normalization

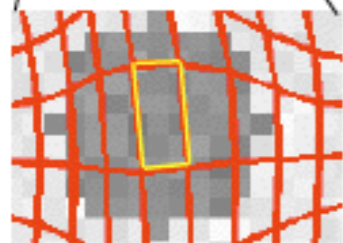
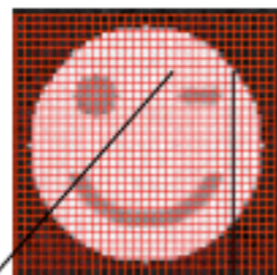
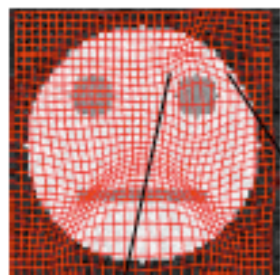


Jacobian  $\sim 1$

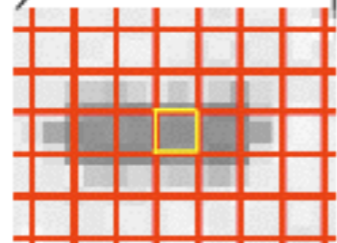


$\sim 1\text{mm}^2$  in original space

$1\text{mm}^2$  in warped space

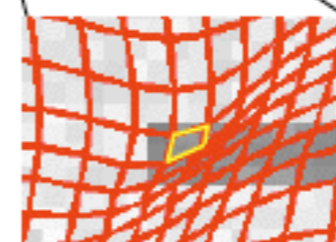
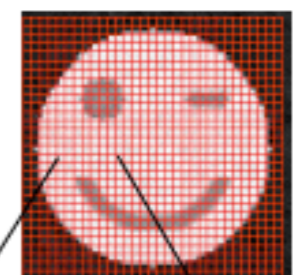
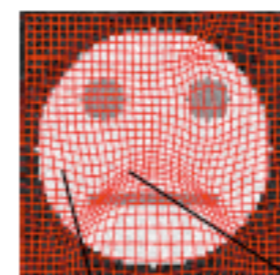


Jacobian  $\sim 3$

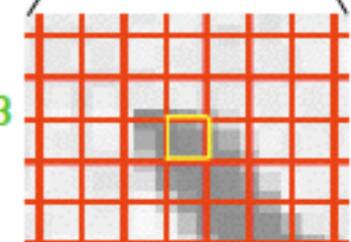


$\sim 3\text{mm}^2$  in original space

$1\text{mm}^2$  in warped space



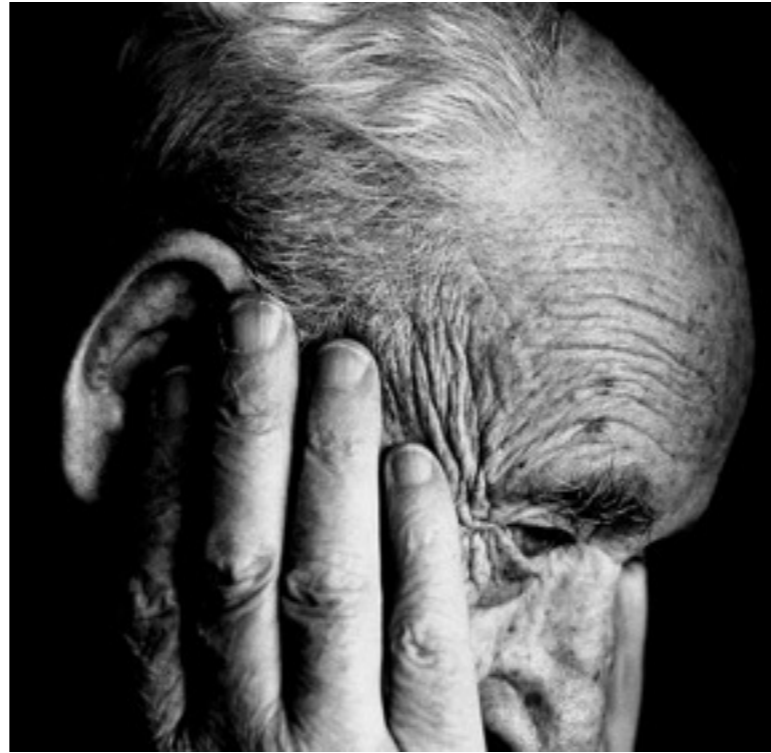
Jacobian  $\sim 1/3$



$\sim 1/3\text{mm}^2$  in original space

$1\text{mm}^2$  in warped space

# The **Material** of Today's Course



**Subjects:** [Put the demo dataset into your folder]

10 healthy controls and 10 patients with Alzheimer's disease

**Scientific question:**

Do AD patients show less gray matter volume than healthy controls ?

**Analysis approach:**

Whole brain gray matter volume voxel based morphometry analysis

**Statistical approach:**

(1) 2 sample T-test with covariate of non-interest (**Analysis of covariance test ; ANCOVA**)



# The **Interface** of Statistical Parametric Mapping (1)

The screenshot displays the SPM12 software interface, divided into two main windows: 'SPM12 (6685): Menu' and 'SPM12 (6685): Graphics'.

**SPM12 (6685): Menu** window features a green-themed interface with several sections:

- Top section:** Includes dropdown menus for 'Real...', 'Norm...', 'Core...', and 'Smooth'. The 'Smooth' and 'Segment' buttons are highlighted with a purple dashed border.
- Basic models section:** Contains buttons for 'Basic models', 'Review', 'Estimate', and 'Bayesian'.
- Results section:** Contains a 'Results' button.
- Dynamic Causal Modelling section:** Contains a 'Dynamic Causal Modelling' button.
- SPM for PET/SPECT section:** Contains buttons for 'Display', 'Check Reg', 'Re...', 'PET', 'To...', 'PPIs', 'ImCalc', 'DICOM Import', 'Help', 'Util...', 'Batch', and 'Quit'.

**SPM12 (6685): Graphics** window features a white background with a menu bar (File, Edit, View, Insert, Tools, Desktop, Window, SPM Figure, Help) and a central graphic area. The graphic area displays a brain diagram with a network of colored lines (yellow, blue, red, green, pink, brown) representing neural pathways or connections. Below the graphic, there is a 'Welcome to SPM12' message and a paragraph of text:

Please refer to this version as "[SPM12](#)" in papers and communications.

The SPM12 [Manual](#) and [Release Notes](#) are available as PDF documents in the *man* directory of your SPM installation.

[Updates](#) will be made available from time to time and advertised on the [SPM mailing list](#). You can also check for updates by clicking [here](#).

We would love to hear your comments or bug reports – please contact us at [<fil.spm@ucl.ac.uk>](mailto:fil.spm@ucl.ac.uk).

SPM is developed under the auspices of the Functional Imaging Laboratory (FIL), the Wellcome Trust Centre for Neuroimaging (WTCN), in the Institute of Neurology at University College London (UCL), UK.

SPM is free software; you can redistribute it and/or modify it under the terms of the [GNU General Public Licence](#) as published by the Free Software Foundation; either version 2 of the Licence, or (at your option) any later version.

Image Preprocessing

# Obtain Normalized Modulated / Unmodulated Tissue Segments in **One Step**

Current Module: Segment

Help on: Segment

Data

- Channel
- Volumes
- Bias regularisation
- Bias FWHM
- Save Bias Corrected

Tissues

- Tissue
- Tissue probability map
- Num. Gaussians
- Native Tissue
- Warped Tissue
- Tissue
- Tissue probability map
- Num. Gaussians
- Native Tissue
- Warped Tissue
- Tissue
- Tissue probability map
- Num. Gaussians
- Native Tissue
- Warped Tissue

Warping & MRF

- MRF Parameter
- Clean Up
- Warping Regularisation
- Affine Regularisation
- Smoothness
- Sampling distance
- Deformation Fields

20 files  
light regularisation (0.001)  
60mm cutoff  
Save Field and Corrected

/Users/PaulSmith/Research\_Software/spm12/tpm/TPM.nii,1  
1  
Native + Dartel Imported  
Modulated + Unmodulated

/Users/PaulSmith/Research\_Software/spm12/tpm/TPM.nii,2  
1  
Native + Dartel Imported  
Modulated + Unmodulated

/Users/PaulSmith/Research\_Software/spm12/tpm/TPM.nii,3  
2  
Native + Dartel Imported  
Modulated + Unmodulated

1  
Light Clean  
1x5 double  
ICBM space template - East Asian brains  
0  
3  
Inverse + Forward

## Volumes:

Input dataset

## Output file:

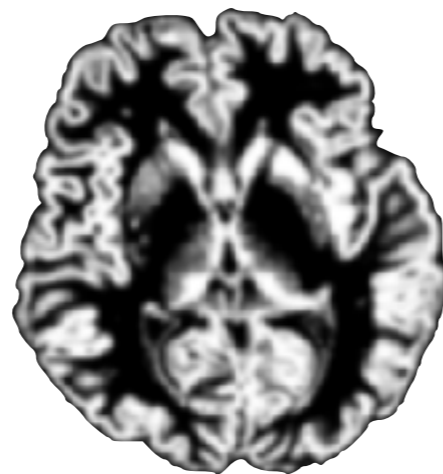
- (1) Bias field (BiasField\_\*.nii) and bias field corrected (m\*.nii) native space T1 image
- (2) Native (c1~c3) and DARTEL imported (rc1~rc3) tissue segments - **Optional**
- (3) **Modulated (mwc1~mwc3) and unmodulated tissue segments (wc1~wc3)**
  - **The most important output files**
- (4) Deformation field of each participants (y\_\*.nii and iy\_\*.nii)

# Why Do We Need to Smooth Data ?

- Primary reason:
  - Make data become more gaussian distributed (for **parametric** statistical analysis)
  - Compensates for **inaccuracies** in spatial normalization procedure
- Recommendation : **6 ~ 12** mm



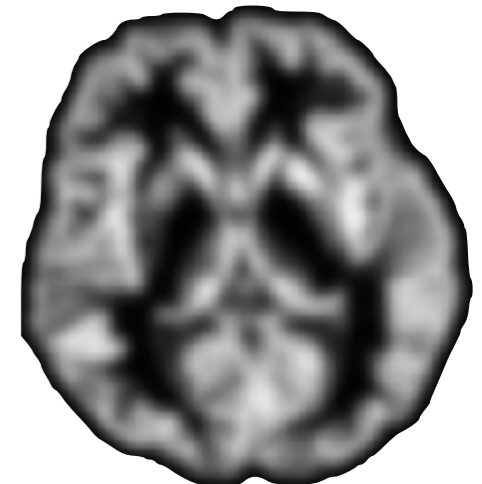
Original data



2mm smooth



4mm smooth



6mm smooth



8mm smooth



12mm smooth

# Smoothing Data with SPM12

Current Module: Smooth

Help on: Smooth  
Images to Smooth  
FWHM  
Data Type  
Implicit masking  
Filename Prefix

20 files  
[8 8 8]  
SAME  
No  
s8

## Image to Smooth:

Input dataset : Modulated gray matter segments (**mwc1\*.nii**)

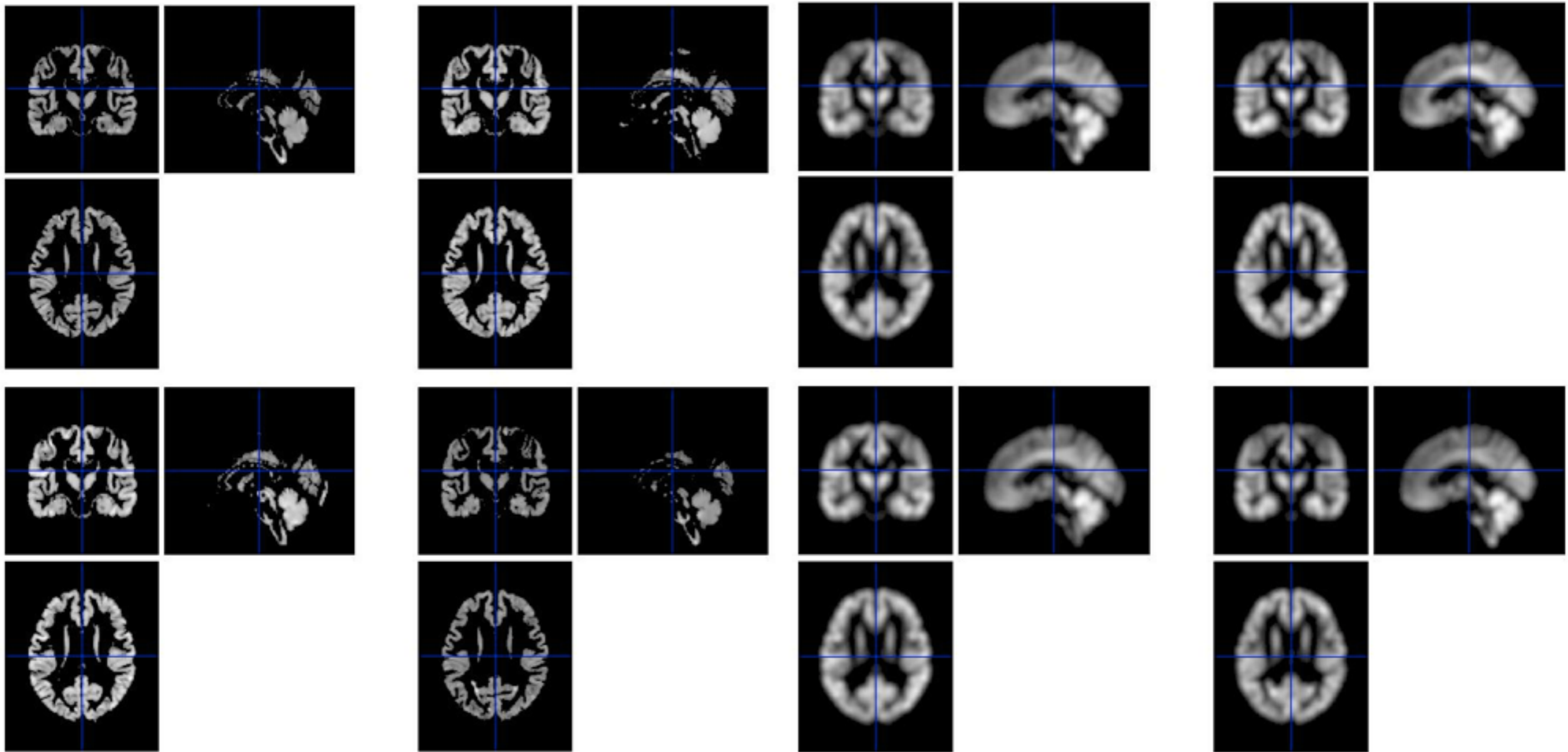
## FWHM:

The FWHM of Gaussian smooth kernel in mm

## Filename Prefix:

The prefix of output file: **s8**mwc1\*.nii

# The **Result** You Obtained After VBM Image Preprocessing

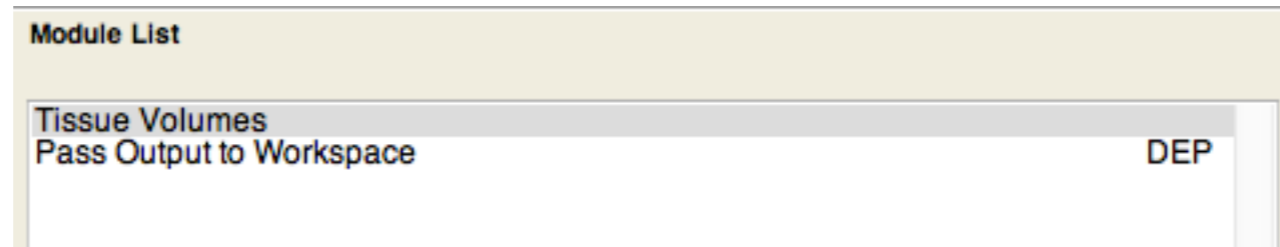


Normalized and modulated  
GM segments

Normalized, modulated and smoothed  
GM segments

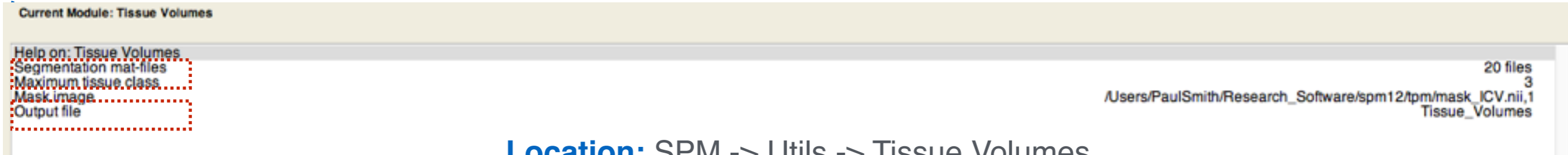
# Calculate **Global** Tissue Volume With SPM12

Using two modules in a single batch



Practice in 15 mins

1st module



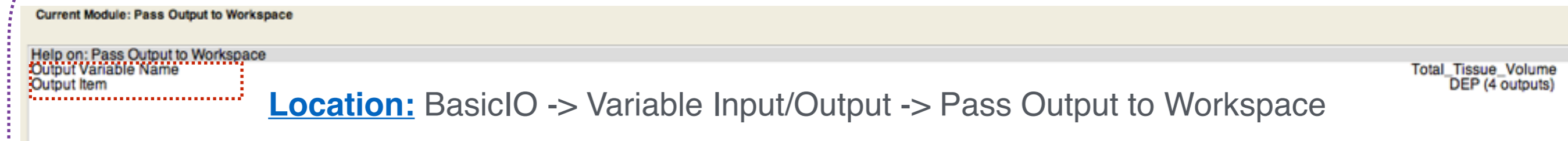
**Location:** SPM -> Utils -> Tissue Volumes

**Segmentation mat-files:** Input files (\*\_seg8.mat)

**Maximum tissue class:** 3 (GMV, WMV and CSFV) or 2 (GMV and WMV)

**Output file:** filename of output CSV file

2nd module

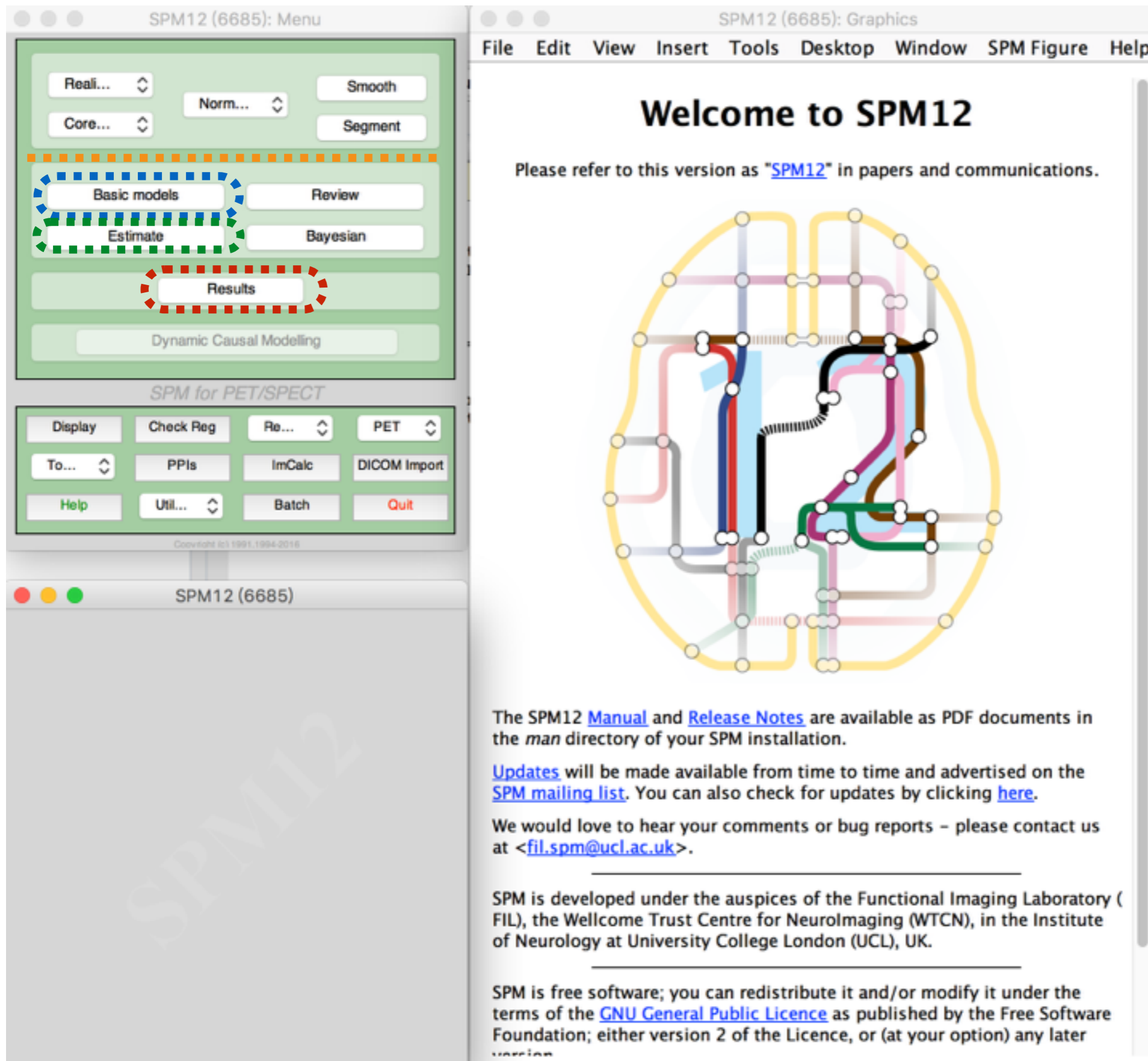


**Location:** BasicIO -> Variable Input/Output -> Pass Output to Workspace

**Output Variable Name:** Name of output variable

**Output item:** Use “Dependency” option [related to the option of 1st module]

# The Interface of Statistical Parametric Mapping (2)

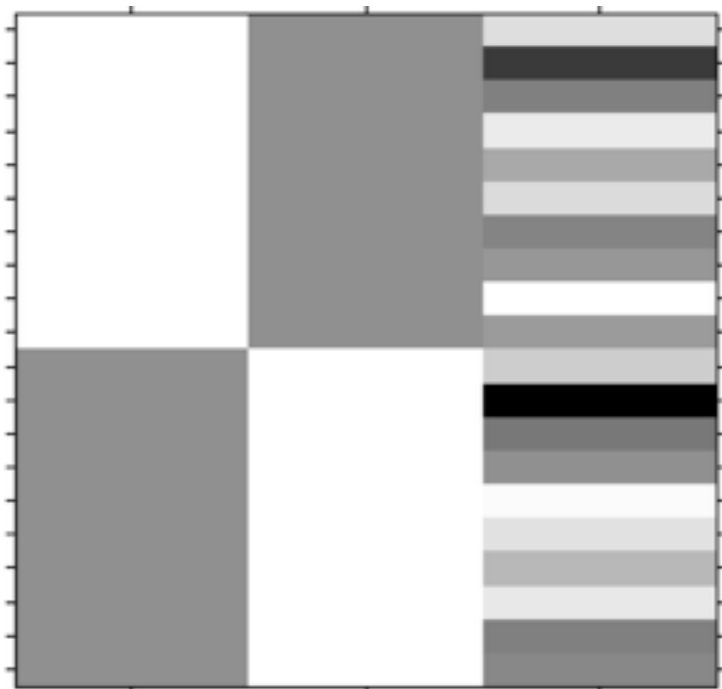


Model Construction

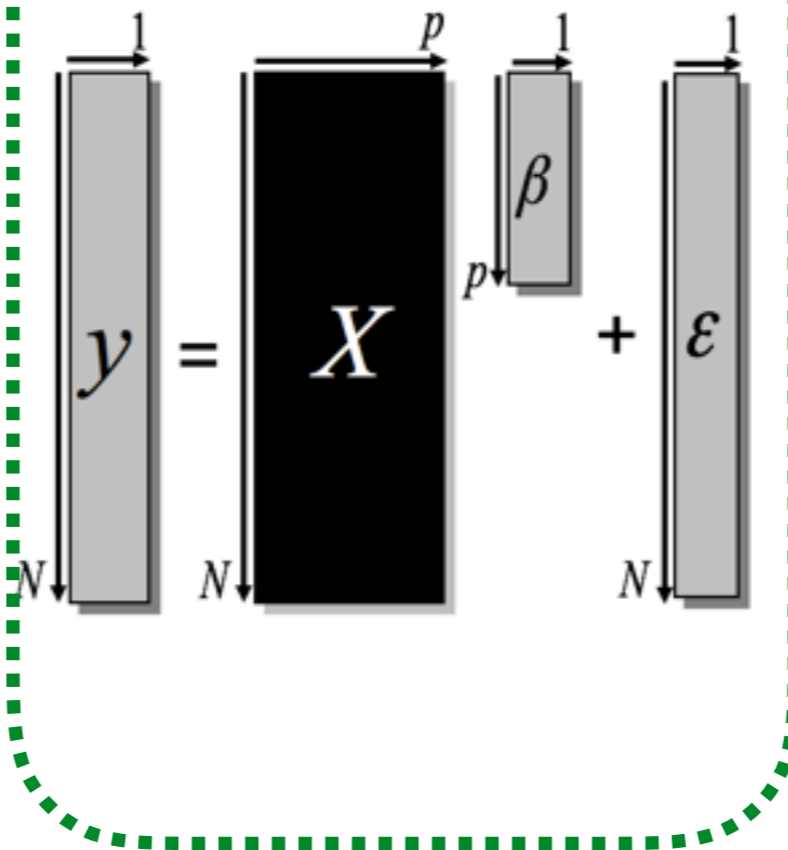
Model Estimation

Statistical Inference

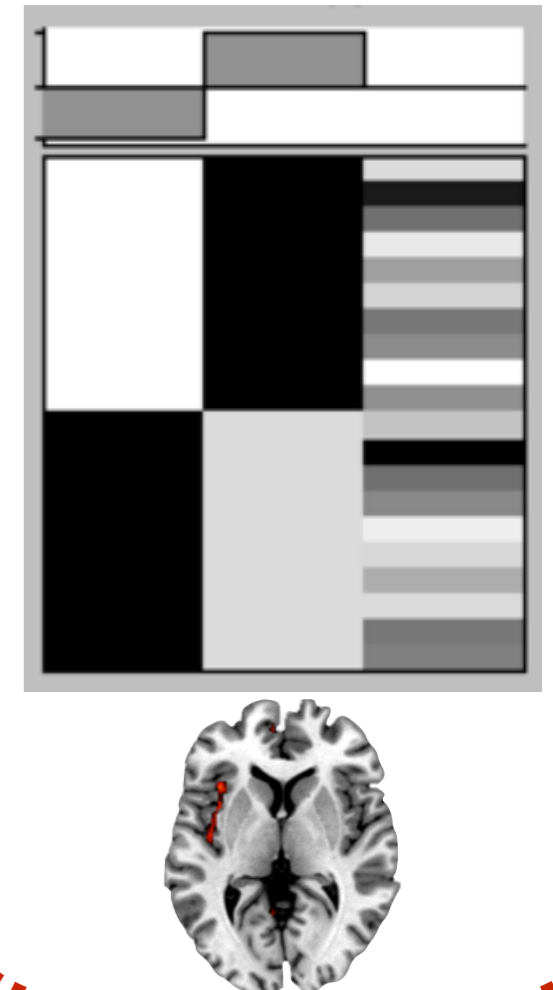
# The Basic Statistical Pipeline of SPM



Model Construction



Model Estimation



Statistical Inference



# General Linear Model - Modeling Tissue Segments

**Why?** Make inferences about effects of interest

**How?**

- Decompose data into effects and error
- Form statistic using estimates of effects and error

$$Y = X^x \beta + E$$

Dependent Variable

(What you are measuring)

Independent Variable

(What you are manipulating)

Relative Contribution

(These need to be estimated)

Error

(The difference between the observed data and that which is predicted by the model)

Aim: To explain as much of the variance in Y by using X, and thus reducing E

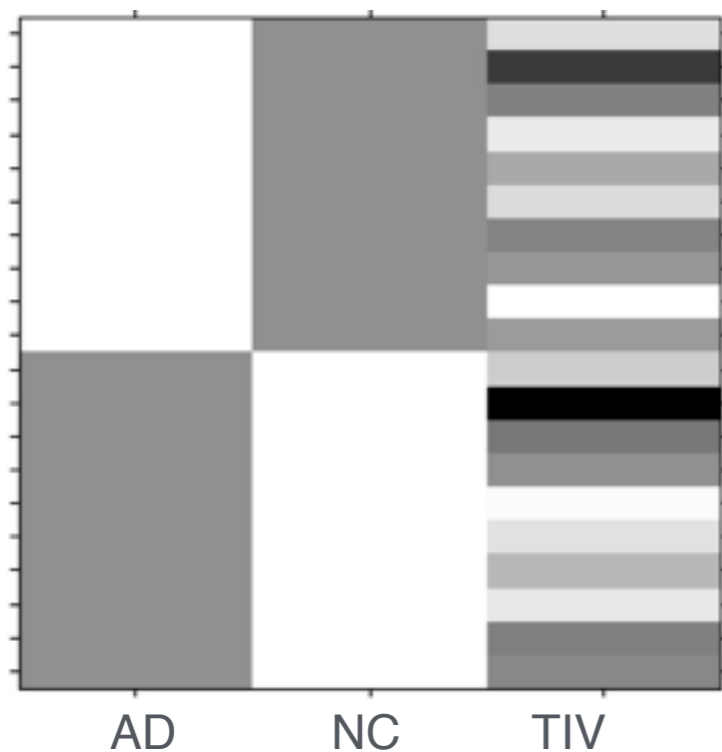
More than 1 EV ?  $Y = X_1\beta_1 + X_2\beta_2 + \dots X_n \beta_n \dots + E$

Univariate analysis !!

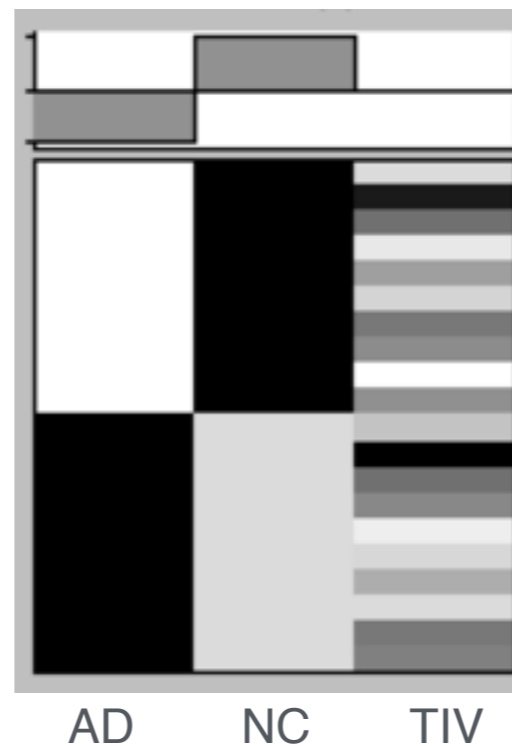
# Gray Matter Volume **Atrophy** in Patients with Alzheimer's Disease

*“Is there significantly lower gray matter volume in the patients with Alzheimer disease than in the healthy controls after adjusting total intracranial volume ? (2 sample T-test )”*

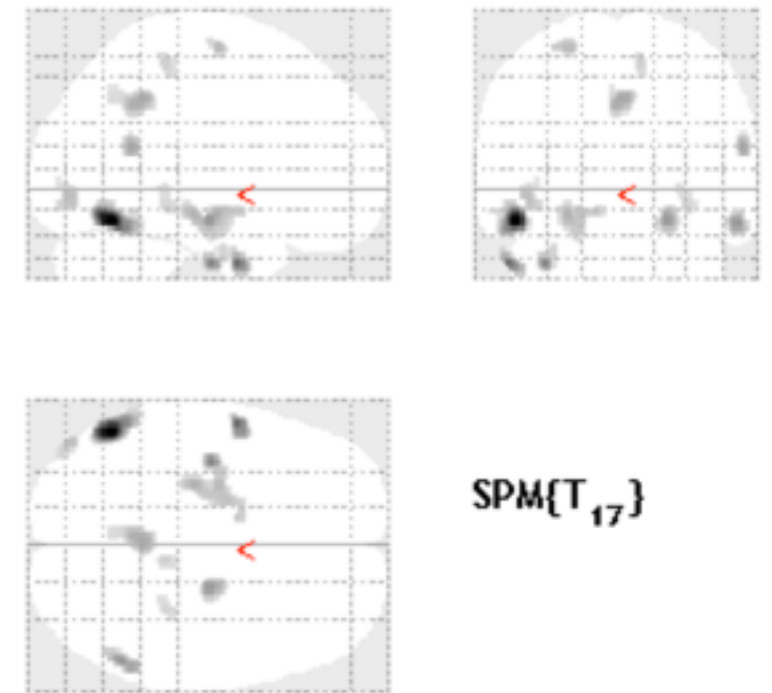
$$Y = \beta_1(\text{AD}) + \beta_2(\text{NC}) + \beta_3(\text{TIV}) + \varepsilon$$



Model construction



Statistical inference



Result visualization

## 2 Sample T-test with Covariate of Non-interest (Model Construction)

Help on: Factorial design specification

Directory /Users/PaulSmith/Analyze\_Field/SPM12\_VBM\_Pipeline\_NTU\_Simple\_Version/5\_Step\_4\_Statistical\_Modeling

|                               |              |
|-------------------------------|--------------|
| Design                        |              |
| .. Two-sample t-test          |              |
| .. Group 1 scans              | 10 files     |
| .. Group 2 scans              | 10 files     |
| .. Independence               | Yes          |
| .. Variance                   | Unequal      |
| .. Grand mean scaling         | No           |
| .. ANCOVA                     | No           |
| Covariates                    |              |
| .. Covariate                  |              |
| .. Vector                     | 20x1 double  |
| .. Name                       | TIV          |
| .. Interactions               | None         |
| .. Centering                  | Overall mean |
| Multiple covariates           |              |
| Masking                       |              |
| .. Threshold masking          |              |
| .. Absolute                   |              |
| .. Threshold                  | 0.2          |
| .. Implicit Mask              | Yes          |
| .. Explicit Mask              |              |
| Global calculation            |              |
| .. Omit                       |              |
| Global normalisation          |              |
| .. Overall grand mean scaling |              |
| .. No                         |              |
| .. Normalisation              | None         |

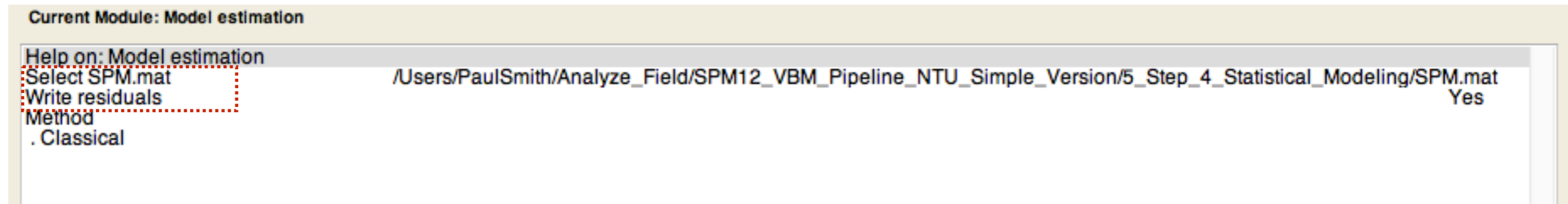
**Directory:** The output directory of your statistical model

**Design:** The statistical model you want to use (**Two sample T-test**)

**Covariates:** The effect you want to adjust (TIV)

**Masking:** The region you want to do statistical inference

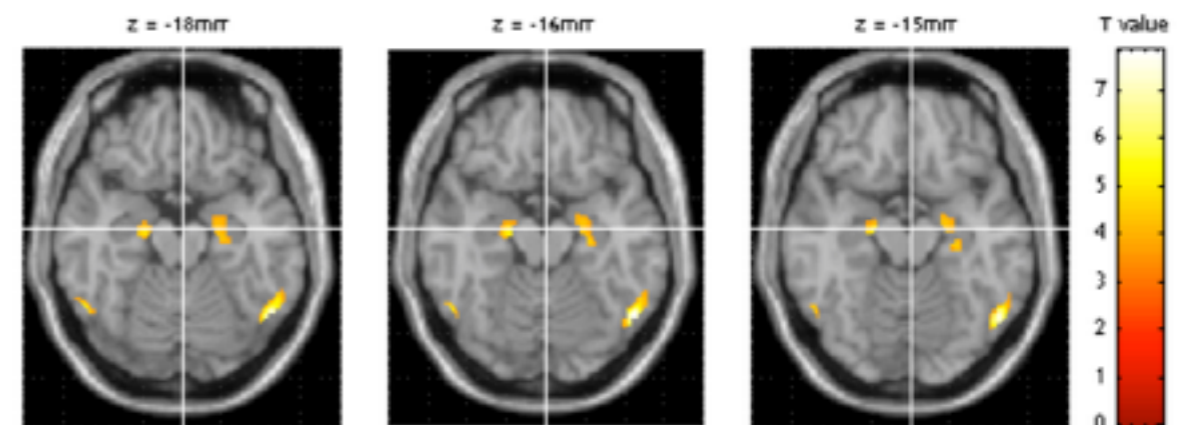
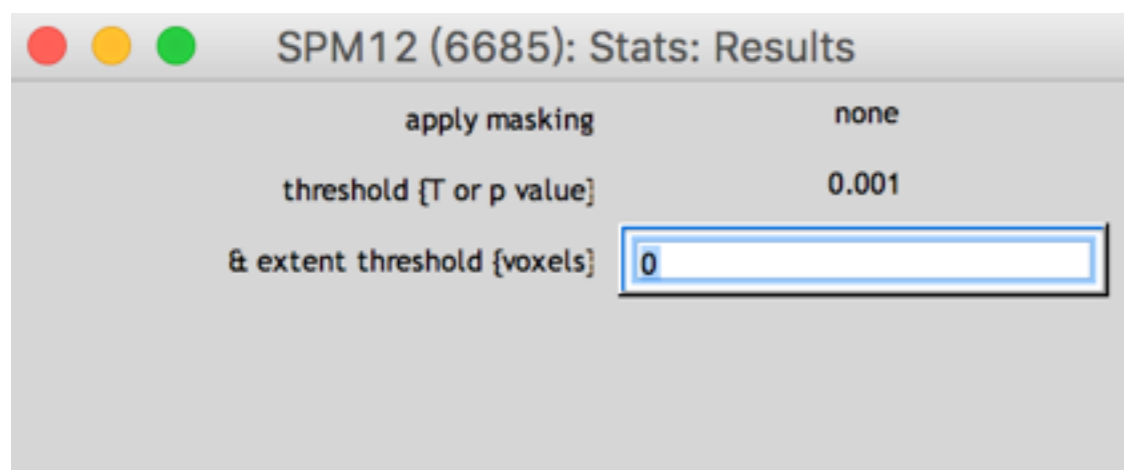
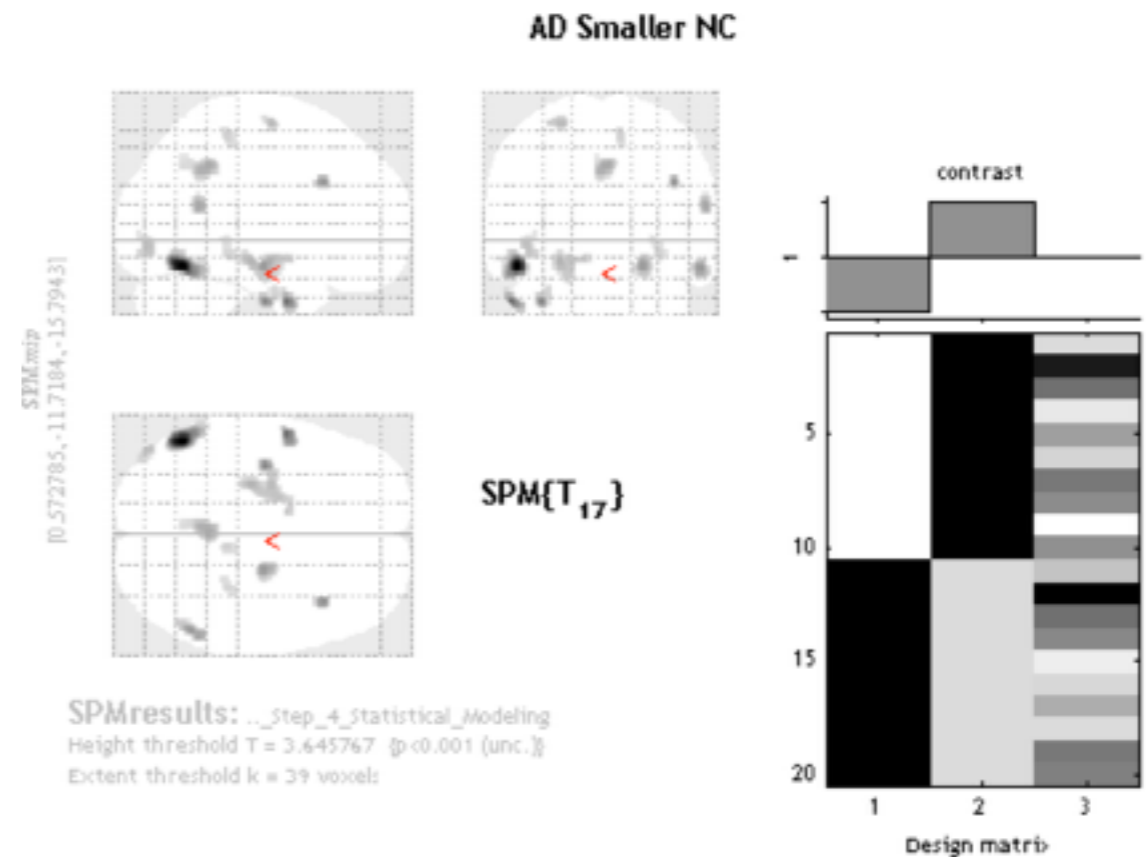
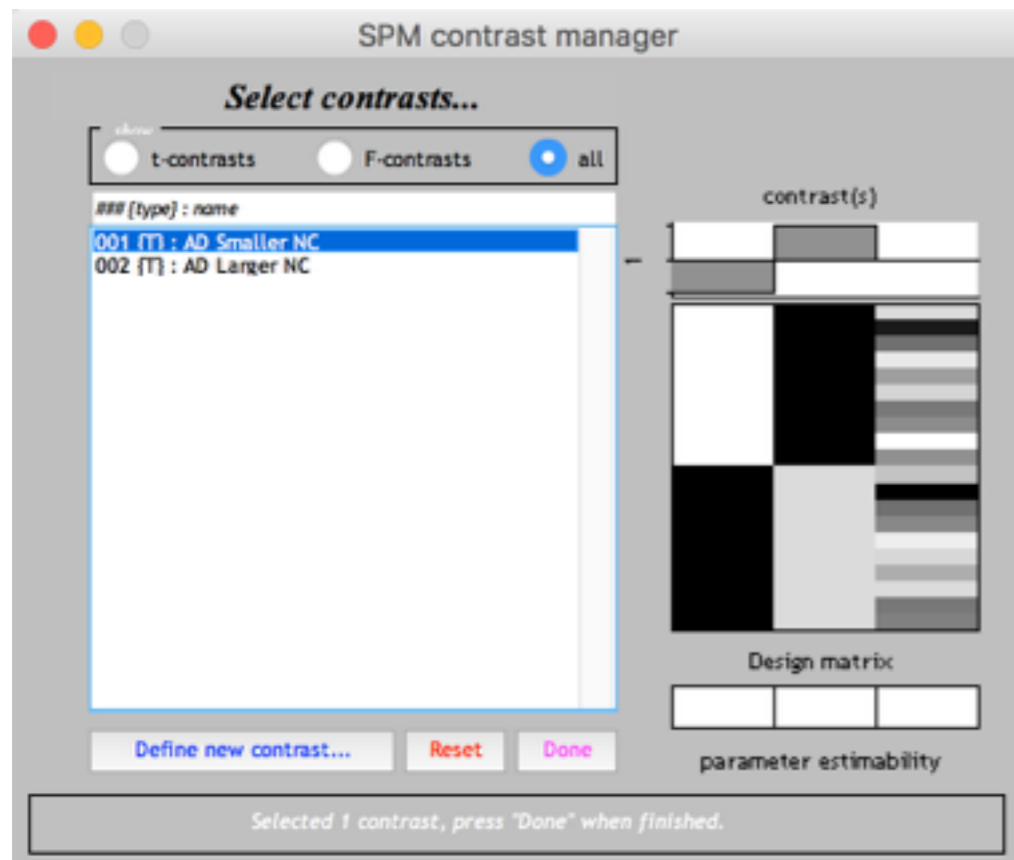
## 2 Sample T-test with Covariate of Non-interest (Model Estimation)



**Select SPM.mat:** The statistical model file of your design (SPM.mat)

**Write residuals:** Save the residual file of GLM estimation

# 2 Sample T-test with Covariate of Non-interest (Statistical Inference & Result Visualization)



Statistical criteria:

Cluster location:

Uncorrected voxel  $p < 0.001$  with 39 extended voxels

$(x,y,z) = (21, -15, -16)$

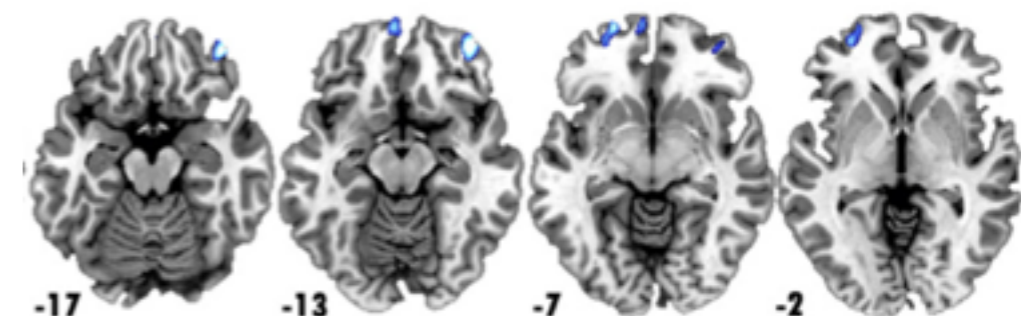
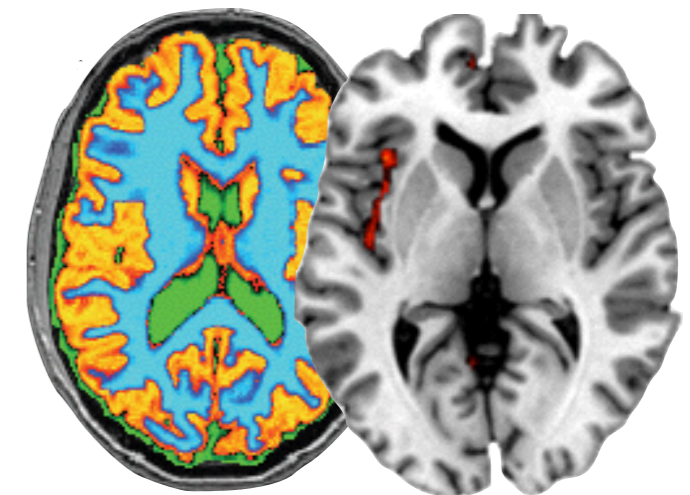
Practice in 10 mins

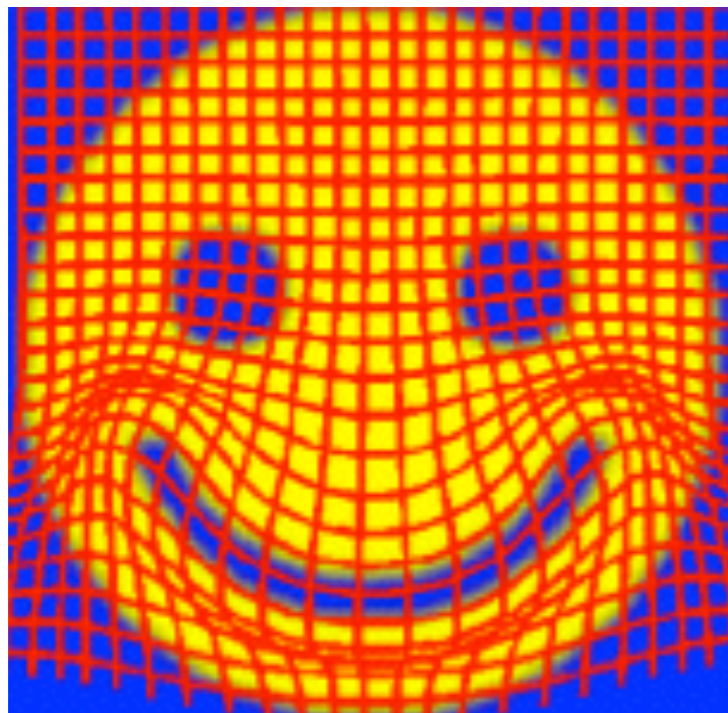
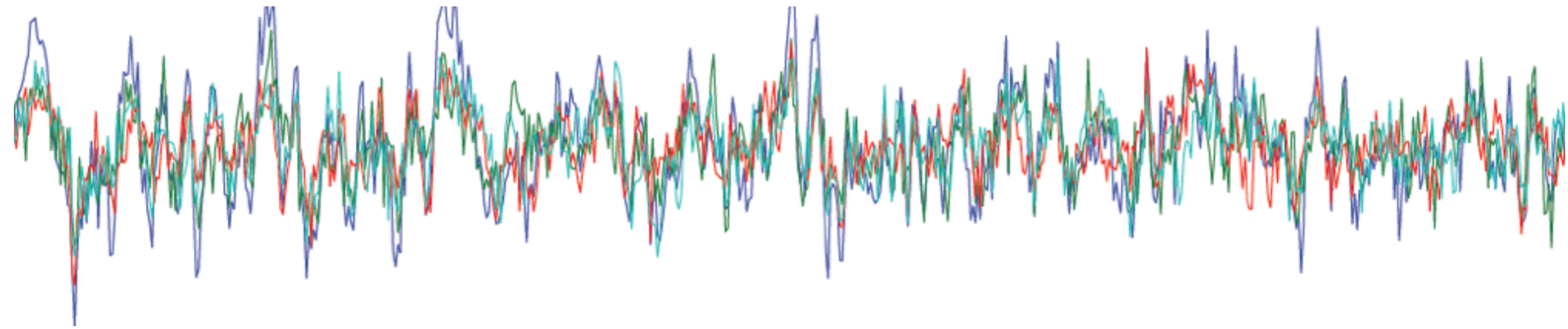
# Take Home Messages

Select the appropriate **MR modality** to served as input for VBM analysis

## The core modules of VBM

1. Tissue segmentation
2. Spatial normalization
3. Tissue modulation (**optional**)
4. Tissue smoothing
5. Statistical modeling





Analyzing Your  
Structural Data for **Fun** !!

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