Basics of fMRI Analysis: Preprocessing, First Level Analysis, and Group Analysis
Overview

• Neuroanatomy 101 and fMRI Contrast Mechanism
• Preprocessing
• Hemodynamic Response
• “Univariate” GLM Analysis
• Hypothesis Testing
• Group Analysis (Random, Mixed, Fixed)
Neuroantony

- Gray matter
- White matter
- Cerebrospinal Fluid
Functional Anatomy/Brain Mapping
Visual Activation Paradigm

Flickering Checkerboard

Visual, Auditory, Motor, Tactile, Pain, Perceptual, Recognition, Memory, Emotion, Reward/Punishment, Olfactory, Taste, Gastral, Gambling, Economic, Acupuncture, Meditation, The Pepsi Challenge, …

- Scientific
- Clinical
- Pharmaceutical
Magnetic Resonance Imaging

T1-weighted Contrast

BOLD-weighted Contrast
Blood Oxygen Level Dependence (BOLD)

- Oxygenated Hemoglobin (DiaMagnetic)
- Deoxygenated Hemoglobin (ParaMagnetic)
- Contrast Agent
- Neurons
- Lungs
- Oxygen
- CO2
Functional MRI (fMRI)

Stimulus → Localized Neural Firing → Localized Increased Blood Flow → Localized BOLD Changes

Sample BOLD response in 4D
Space (3D) – voxels (64x64x35, 3x3x5mm^3, ~50,000) 
Time (1D) – time points (100, 2 sec) – Movie

Time 1  Time 2  Time 3 …
4D Volume

64x64x35

85x1
fMRI Analysis Overview

Subject 1

Raw Data

Subject 2

Raw Data

Subject 3

Raw Data

Subject 4

Raw Data

Preprocessing
MC, STC, B0
Smoothing Normalization

First Level
GLM Analysis

Preprocessing
MC, STC, B0
Smoothing Normalization

First Level
GLM Analysis

Preprocessing
MC, STC, B0
Smoothing Normalization

First Level
GLM Analysis

Higher Level GLM

X
C

X
C

X
C

X
C

X
C
Preprocessing

• Assures that assumptions of the analysis are met
  • Time course comes from a single location
  • Uniformly spaced in time
  • Spatial “smoothness”
• Analysis – separating signal from noise
Preprocessing

• Start with a 4D data set
  1. Motion Correction
  2. Slice-Timing Correction
  3. B0 Distortion Correction
  4. Spatial Normalization
  5. Spatial Smoothing
• End with a 4D data set

• Can be done in other orders
• Not everything is always done
Motion

• Analysis assumes that time course represents a value from a single location
• Subjects move
• Shifts can cause noise, uncertainty
  • Edge of the brain and tissue boundaries
Motion and Motion Correction

- Motion correction reduces motion
- Not perfect

Raw

Corrected
Motion Correction

- Motion correction parameters
- Six for each time point
- Sometimes used as nuisance regressors
- How much motion is too much?
Slice Timing

- Volume not acquired all at one time
- Acquired slice-by-slice
- Each slice has a different delay
Effect of Slice Delay on Time Course

- Volume = 30 slices
- TR = 2 sec
- Time for each slice = 2/30 = 66.7 ms
Slice Timing Correction

- Temporal interpolation of adjacent time points
- Usually sinc interpolation
- Each slice gets a different interpolation
- Some slices might not have any interpolation
- Can also be done in the GLM
- You must know the slice order!
B0 Distortion

- Metric (stretching or compressing)
- Intensity Dropout
- A result of a long readout needed to get an entire slice in a single shot.
- Caused by B0 Inhomogeneity
B0 Map

Magnitude

Phase

Echo 1
TE1

Echo 2
TE2
Voxel Shift Map

- Units are voxels (3.5mm)
- Shift is in-plane
- Blue = P→A, Red A→P
- Regions affected near air/tissue boundaries
  - sinuses
B0 Distortion Correction

- Can only fix metric distortion
- Dropout is lost forever
B0 Distortion Correction

• Can only fix metric distortion
• Dropout is lost forever
• Interpolation
• Need:
  • “Echo spacing” – readout time
  • Phase encode direction
• More important for surface than for volume
• Important when combining from different scanners
Spatial Normalization

• Transform volume into another volume
  • Re-slicing, re-gridding
• New volume is an “atlas” space
• Align brains of different subjects so that a given voxel represents the “same” location.
• Similar to motion correction
• Preparation for comparing across subjects
• Volume-based
• Surface-based
• Combined Volume-surface-based (CVS)
Spatial Normalization: Volume

Native Space
- Subject 1
- Subject 2

MNI305 Space
- Subject 1
- Subject 2

Affine (12 DOF) Registration

MNI305 MNI152
Spatial Normalization: Surface

- Subject 1
- Subject 2 (Before)
- Subject 2 (After)

- Shift, Rotate, Stretch
- High dimensional (~500k)
- Preserve metric properties
- Take variance into account
- Common space for group analysis (like Talairach)
Spatial Smoothing

- Replace voxel value with a weighted average of nearby voxels (spatial convolution)
- Weighting is usually Gaussian
- 3D (volume)
- 2D (surface)
- Do after all interpolation, before computing a standard deviation
- Similarity to interpolation
- Improve SNR
- Improve Intersubject registration
- Can have a dramatic effect on your results
Spatial Smoothing

- Spatially convolve image with Gaussian kernel.
- Kernel sums to 1
- Full-Width/Half-max: $\text{FWHM} = \sigma / \sqrt{\log(256)}$
- $\sigma$ = standard deviation of the Gaussian

$\sigma$ = standard deviation of the Gaussian
Spatial Smoothing
Effect of Smoothing on Activation

- Working memory paradigm
- FWHM: 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20
Volume- vs Surface-based Smoothing

- 5 mm apart in 3D
- 25 mm apart on surface
- Averaging with other tissue types (WM, CSF)
- Averaging with other functional areas

14mm FWHM
Preprocessing

• Start with a 4D data set
  1. Motion Correction - Interpolation
  2. Slice-Timing Correction
  3. B0 Distortion Correction - Interpolation
  4. Spatial Normalization - Interpolation
  5. Spatial Smoothing – Interpolation-like
• End with a 4D data set

• Can be done in other orders
• Not all are done
fMRI Time-Series Analysis
fMRI Analysis Overview

Subject 1
Raw Data
- Preprocessing: MC, STC, B0
- Smoothing
- Normalization
- First Level GLM Analysis

Subject 2
Raw Data
- Preprocessing: MC, STC, B0
- Smoothing
- Normalization
- First Level GLM Analysis

Subject 3
Raw Data
- Preprocessing: MC, STC, B0
- Smoothing
- Normalization
- First Level GLM Analysis

Subject 4
Raw Data
- Preprocessing: MC, STC, B0
- Smoothing
- Normalization
- First Level GLM Analysis

Higher Level GLM
- X
- C

Graph showing data flow from raw data through preprocessing, GLM analysis, and then to higher level GLM.
Visual/Auditory/Motor Activation Paradigm

15 sec ‘ON’, 15 sec ‘OFF’
- Flickering Checkerboard
- Auditory Tone
- Finger Tapping
Block Design: 15s Off, 15s On

Voxel 1

Voxel 2

Stimulus Schedule Paradigm File
Contrasts and Inference

\[ \sigma_{ON}^2 - \sigma_{OFF}^2 = \frac{\beta_{ON} - \beta_{OFF}}{\sqrt{(N_{ON} - 1)\sigma_{ON}^2 + (N_{OFF} - 1)\sigma_{OFF}^2}} \]

Contrast = \( \beta_{ON} - \beta_{OFF} \)

Var(Contrast) = \( \frac{(N_{ON} - 1)\sigma_{ON}^2 + (N_{OFF} - 1)\sigma_{OFF}^2}{(N_{ON} + N_{OFF} - 2)^2} \)

Note: z, t, F monotonic with p

\( p = 10^{-11}, \ \text{sig} = -\log_{10}(p) = 11 \)

\( p = 0.10, \ \text{sig} = -\log_{10}(p) = 1 \)
Statistical Parametric Map (SPM)

Contrast Amplitude
CON, COPE, CES

Contrast Amplitude Variance
(Error Bars)
VARCOPE, CESVAR

Significance t-Map (p,z,F)
(Thresholded p<.01)
sig=-log10(p)

"Massive Univariate Analysis"
-- Analyze each voxel separately
Hemodynamics

- Delay
- Dispersion
- Grouping by simple time point inaccurate
Convolution with HRF

- Shifts, rolls off; more accurate
- Loose ability to simply group time points
- More complicated analysis
- General Linear Model (GLM)
GLM

Data from one voxel = $\beta_{\text{Task}} + \beta_{\text{base}}$

Baseline Offset (Nuisance)

$\beta_{\text{base}} = \beta_{\text{off}}$

$\beta_{\text{Task}} = \beta_{\text{on}} - \beta_{\text{off}}$

- Implicit Contrast
- HRF Amplitude
Matrix Model

\[ y = X \beta \]

Data from one voxel

Design Matrix Regressors

Design Matrix

Vector of Regression Coefficients ("Betas")

Observations
Two Task Conditions

\[ y = X \times \beta \]

\[ \beta = \begin{cases} 
\beta_{\text{Odd}} \\
\beta_{\text{Even}} \\
\beta_{\text{base}} 
\end{cases} \]

Observations

Data from one voxel

Design Matrix Regressors

Design Matrix
Working Memory Task (fBIRN)

0. “Scrambled” – low-level baseline, no response
1. Encode – series of passively viewed stick figures
   Distractor – respond if there is a face
   2. Emotional
   3. Neutral
   Probe – series of two stick figures (forced choice)
   4. Following Emotional Distractor
   5. Following Neutral Distractor

fBIRN: Functional Biomedical Research Network (www.nbirm.net)
Five Task Conditions

\[ y = X \ast \beta \]

\[ \beta_{\text{Encode}} \]
\[ \beta_{\text{EmotDist}} \]
\[ \beta_{\text{NeutDist}} \]
\[ \beta_{\text{EmotProbe}} \]
\[ \beta_{\text{NeutProbe}} \]
GLM Solution

\[ y = X \beta \]

- Set of simultaneous equations
- Each row of \( X \) is an equation
- Each column of \( X \) is an unknown
- \( \beta \)s are unknown
- 142 Time Points (Equations)
- 5 unknowns

\[ \hat{\beta} = (X^T X)^{-1} X^T y \]
Estimates of the HRF Amplitude

\[ y = X\beta + n, \quad \hat{\beta} = (X^TX)^{-1}X^Ty \]

\[ \hat{\beta}_{\text{Encode}} = \text{Hemodynamic amplitude in response to Encode} \]
\[ \hat{\beta}_{\text{EmotDist}} = \text{Hemodynamic amplitude in response to Emotional Distractor} \]
\[ \hat{\beta}_{\text{NeutDist}} = \text{Hemodynamic amplitude in response to Neutral Distractor} \]
\[ \hat{\beta}_{\text{EmotProbe}} = \text{Hemodynamic amplitude in response to Probe following Emotional Distractor} \]
\[ \hat{\beta}_{\text{NeutProbe}} = \text{Hemodynamic amplitude in response to Probe following Neutral Distractor} \]
Hypotheses and Contrasts

Which voxels respond more/less/differently to the Emotional Distractor than to the Neutral Distractor?

Contrast: Assign Weights to each Beta

\[ \gamma = c_{\text{Encode}} \hat{\beta}_{\text{Encode}} + c_{\text{EDist}} \hat{\beta}_{\text{EDist}} + c_{\text{NDist}} \hat{\beta}_{\text{NDist}} + c_{\text{EProbe}} \hat{\beta}_{\text{EProbe}} + c_{\text{NProbe}} \hat{\beta}_{\text{NProbe}} \]

- \( c_{\text{Encode}} = 0 \)
- \( c_{\text{EDist}} = +1 \)
- \( c_{\text{NDist}} = -1 \)
- \( c_{\text{EProbe}} = 0 \)
- \( c_{\text{NProbe}} = 0 \)

\[
C = \begin{bmatrix}
0 & +1 & -1 & 0 & 0
\end{bmatrix} \]

Contrast Matrix

\[
\hat{\beta}_{\text{EDist}} \succ \hat{\beta}_{\text{NDist}}
\]

\[
\hat{\beta}_{\text{EDist}} \prec \hat{\beta}_{\text{NDist}}
\]

\[
\hat{\beta}_{\text{EDist}} \neq \hat{\beta}_{\text{NDist}}
\]

\[
\gamma = \hat{\beta}_{\text{EDist}} - \hat{\beta}_{\text{NDist}} > 0, < 0, \neq 0
\]
Hypotheses

• Which voxels respond more to the Emotional Distractor than to the Neutral Distractor?
• Which voxels respond to Encode (relative to baseline)?
• Which voxels respond to the Emotional Distractor?
• Which voxels respond to either Distractor?
• Which voxels respond more to the Probe following the Emotional Distractor than to the Probe following the Neutral Distractor?
Which voxels respond more to the Emotional Distractor than to the Neutral Distractor?

- Only interested in Emotional and Neutral Distractors
- No statement about other conditions

Condition: 1 2 3 4 5
Weight: 0 +1 -1 0 0

Contrast Matrix
$C = \begin{bmatrix} 0 & +1 & -1 & 0 & 0 \end{bmatrix}$
Contrasts and the Full Model

\[ y = X\beta + n, \quad y = s + n, \quad n \sim N(0, \sigma_n^2) \]

\[ \hat{\beta} = (X^T X)^{-1} X^T y \quad \text{Parameter Estimates} \]

\[ \hat{\sigma}_n^2 = \frac{\hat{n}^T \hat{n}}{DOF} \quad \text{Residual Variance,} \quad \hat{n} = y - X\hat{\beta} \]

\[ \hat{\gamma} = C\hat{\beta} \quad \text{Contrast} \]

\[ \hat{\sigma}_{\gamma}^2 = \hat{\Sigma}_{\gamma} = \frac{1}{J} \left( C(X^T X)^{-1} C^T \right) \hat{\sigma}_n^2 \quad \text{Contrast Variance Estimate} \]

\[ J = \text{Rows in C} \]

\[ t_{DOF} = \frac{\hat{\gamma}}{\hat{\sigma}_{\gamma}} = \frac{C\hat{\beta}}{\sqrt{\left( C(X^T X)^{-1} C^T \right) \hat{\sigma}_n^2}} \quad \text{t - Test (univariate)} \]

\[ F_{DOF, J} = \hat{\gamma}^T \hat{\Sigma}_{\gamma}^{-1} \hat{\gamma} \quad \text{F - Test (multivariate)} \]
First Level GLM Outputs

Contrast Amplitude
CON, COPE, CES

Contrast Amplitude Variance
/Error Bars/
VARCOPE, CESVAR

Significance t-Map \((p,z,F)\)
(Thresholded \(p<.01\))
\(\text{sig}=-\log_{10}(p)\)
Time Series Analysis Summary

- Correlational
- Design Matrix (HRF shape)
- Estimate HRF amplitude (Parameters)
- Contrasts to test hypotheses
- Results at each voxel:
  - Contrast Value
  - Contrast Value Variance
  - p-value (Volume of Activation)
- Pass Contrast Value and Variance up to higher level analyses
fMRI Group Analysis
fMRI Analysis Overview

Subject 1
Raw Data
Preprocessing MC, STC, B0
Smoothing Normalization
First Level GLM Analysis
X C

Subject 2
Raw Data
Preprocessing MC, STC, B0
Smoothing Normalization
First Level GLM Analysis
X C

Subject 3
Raw Data
Preprocessing MC, STC, B0
Smoothing Normalization
First Level GLM Analysis
X C

Subject 4
Raw Data
Preprocessing MC, STC, B0
Smoothing Normalization
First Level GLM Analysis
X C

Higher Level GLM
X C
Overview

• Goal of Group Analysis
• Types of Group Analysis
  – Random Effects, Mixed Effects, Fixed Effects
• Multi-Level General Linear Model (GLM)
Spatial Normalization, Atlas Space

Native Space

Subject 1

Subject 2

MNI305 Space

Subject 1

Subject 2

MNI305

Affine (12 DOF) Registration
Inter-Subject Averaging

Subject 1
Native
Subject 2
Native

Surface-to-Surface
Surface-to-Surface

Spherical
Spherical

GLM
Demographics

Surface-to-Surface

cf. Talairach
Is Pattern Repeatable Across Subject?

Subject 1  Subject 2  Subject 3  Subject 4  Subject 5
Group Analysis

Contrast Amplitudes

Contrast Amplitudes Variances (Error Bars)

Does not have to be all positive!
“Random Effects (RFx)” Analysis

\[
t = \frac{\beta_G}{\sigma_{\beta_G}}, \quad \sigma_{\beta_G}^2 = \frac{\sigma_G^2}{N_G}
\]

\[
\sigma_G^2 = \frac{\sum (\beta_G - \beta_i)^2}{(N_G - 1)}
\]

\[
DOF = N_G - 1
\]

Random Effects (RFx) Analysis
“Random Effects (RFx)” Analysis

• Model Subjects as a Random Effect
• Variance comes from a single source: variance across subjects
  – Mean at the population mean
  – Variance of the population variance
• Does not take first-level noise into account (assumes 0)
• “Ordinary” Least Squares (OLS)
• Usually less activation than individuals
“Mixed Effects (MFx)” Analysis

- Down-weight each subject based on variance.
- Weighted Least Squares vs (“Ordinary” LS)
“Mixed Effects (MFx)” Analysis

- Down-weight each subject based on variance.
- Weighted Least Squares vs (“Ordinary” LS)
- Protects against unequal variances across group or groups (“heteroskedasticity”)
- May increase or decrease significance with respect to simple Random Effects
- More complicated to compute
- “Pseudo-MFx” – simply weight by first-level variance (easier to compute)
“Fixed Effects (FFx)” Analysis

\[ t = \frac{\beta_G}{\sqrt{\sigma_{\beta_G}^2}} \]

\[ \sigma_{\beta_G}^2 = \sum \sigma_i^2 \left( \frac{N_G}{2} \right)^2 \]

\[ \text{DOF} = \sum \text{DOF}_i \]
“Fixed Effects (FFx)” Analysis

- As if all subjects treated as a single subject (fixed effect)
- Small error bars (with respect to RFx)
- Large DOF
- Same mean as RFx
- Huge areas of activation
- Not generalizable beyond sample.
fMRI Analysis Overview

Subject 1
Raw Data
Preprocessing MC, STC, B0 Smoothing Normalization
First Level GLM Analysis
X₁ C₁

Subject 2
Raw Data
Preprocessing MC, STC, B0 Smoothing Normalization
First Level GLM Analysis
X₁ C₁

Subject 3
Raw Data
Preprocessing MC, STC, B0 Smoothing Normalization
First Level GLM Analysis
X₁ C₁

Subject 4
Raw Data
Preprocessing MC, STC, B0 Smoothing Normalization
First Level GLM Analysis
X₁ C₁

Higher Level GLM
X₆ C₆
Higher Level GLM Analysis

\[ y = X \ast \beta \]

Observations (Low-Level Contrasts)

Data from one voxel

Design Matrix (Regressors)

\begin{bmatrix}
1 \\
1 \\
1 \\
1 \\
1
\end{bmatrix}

Vector of Regression Coefficients ("Betas")

Contrast Matrix:

\[ C = [1] \]

Contrast = \[ C \ast \beta = \beta_G \]

One-Sample Group Mean (OSGM)
Summary

- Preprocessing – MC, STC, B0, Normalize, Smooth
- First Level GLM Analysis – Design matrix, HRF, Nuisance
- Contrasts, Hypothesis Testing – contrast matrix
- Group Analysis
  - Random, Mixed, Fixed
  - Multi-level GLM (Design and Contrast Matrices)