Processing, Analyzing, and Displaying Functional MRI Data

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BRCP Hawaii 2004

Shocking Truths about FMRI *!*

- **Goal**: Find and Characterize Neural "Activations" (whatever that means)
- **Shocking Revelation #1**:

FMRI data are (mostly) crap

- **But**: All other neuroimaging data are, too

  ⇒ You must know what you are doing*!*
- **Shocking Revelation #2**:

Most FMRI papers are weak on analysis

Points to Ponder & Discuss

- Field has relatively poor understanding of physiological and physics issues underlying fluctuations (both "signal" and "noise") in FMRI time series in living brain tissue
- Virtually all FMRI studies are of groups
  - Categorizing individuals (phenotyping) is **HARD**
  - Combining & contrasting multiple human brains is non-trivial (*e.g.*, align anatomies? how well?)
- Deciding what is "significant" is tricky
- Visualizing high-dimensional results at each voxel in 3D space needs more work

Caveats and Disclaimers

- Almost everything herein has an exception or complication
  - or is also the subject of ongoing research
- Special types of data or stimuli may require special analysis tools
  - *e.g.*, perfusion-weighted FMRI (via arterial spin labeling)
  - non-repeatable tasks (*e.g.*, drug challenge)
- Special types of questions may require special data *and* analyses
  - *e.g.*, relative timing of neural events

FMRI Data Acquisition & Theory

- FMRI data = scan subject's brain rapidly (2-3 s) and repeatedly (5-100 min)
  - Speed ⇒ relatively low spatial resolution (usually)
- Images are sensitized to T_2^* = sensitive to magnetic field perturbations on sub-voxel scale
  - bigger perturbations ⇒ image intensity is smaller
  - De-oxygenated hemoglobin perturbs magnetic field
  - **Result**: FMRI time series in each voxel measures how much deoxyHB is present in that voxel
  - **Observation**: less deoxyHB ⇔ more neural activity
    - ⇒ Look for signal increases correlated with tasks
    - **BOLD** = Blood Oxygenation Level Dependent imaging

Meta-Method for Data Analysis

- Develop a mathematical model relating what we **know**

  stimulus timing, behavioral measurements, image data,

  to what we **want to know**

  location, amount, timing of neural activity
- Given data, use model to solve for unknown parameters in the neural activity (*e.g.*, when, where, how much)
  - Test for statistical significance, for each task and contrasts between tasks, in individuals and groups

Why FMRI Analysis Is Hard

- Don’t know the true relation between neural “activity” and measurable MRI signal
  - What is neural “activity”, anyway?
  - What is connection between neural “activity” and hemodynamics and MRI signal?
- Noise in time series data from living subjects is also poorly characterized
  - Makes statistical assessment hard
- **Result**: There are many “reasonable” ways to do FMRI data analysis
  - And no good way to judge which are “better”

Why So Many Methods In Use?

- Different assumptions about activity-to-MRI signal connection
- Different assumptions about noise (signal fluctuations of no interest) properties and statistics
- Different experiments and questions
- **Result**: Many “reasonable” FMRI analysis methods
- Researchers **must** understand the tools*!!* (Models and software)

Temporal Models: Linear Convolution

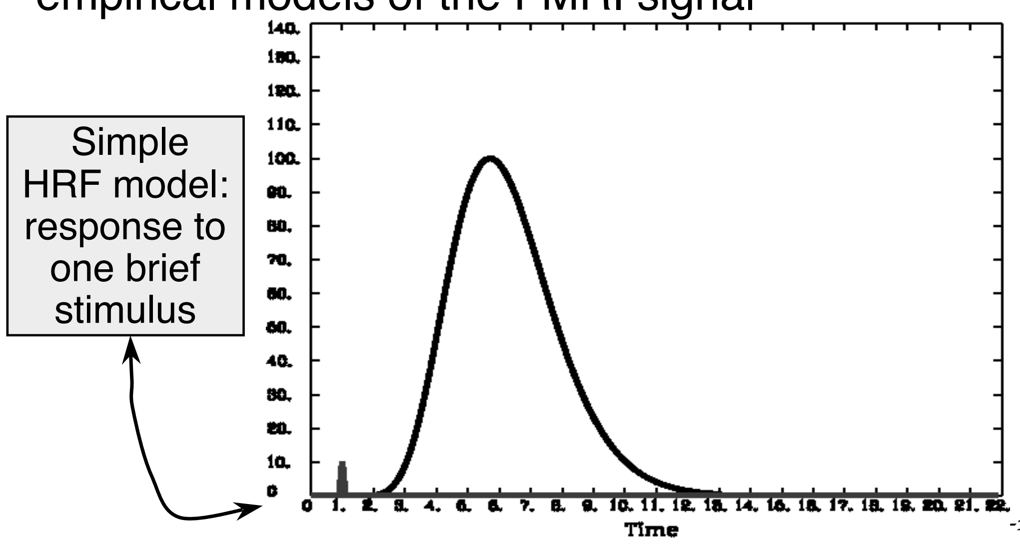
- **Central Assumption**:

FMRI (hemodynamic) response to
2 separated-in-time activations in same voxel
is the
separated-in-time sum of 2 copies of some individual task/stimulus response function

- The FMRI response to a single activation is called the **hemodynamic response function** (**HRF**)

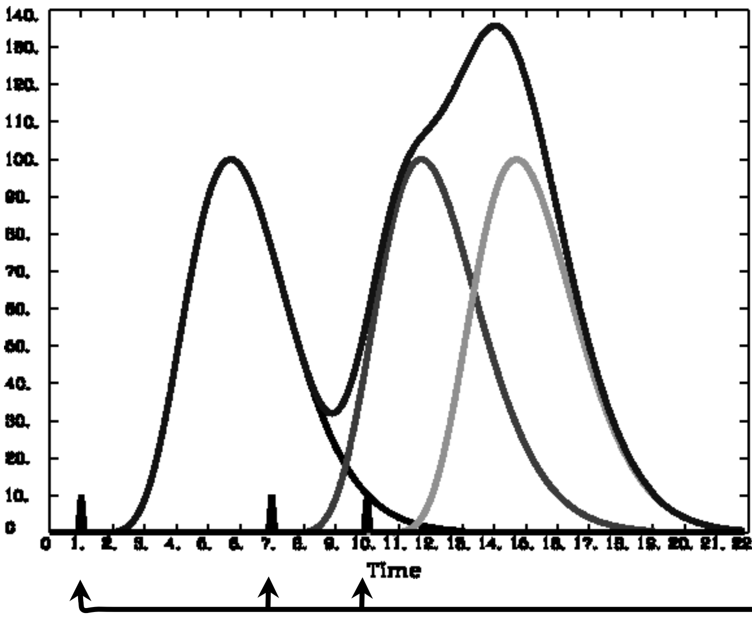
FMRI Data Analysis

- Fit data time series in each voxel to a model derived from the HRF
  - Model is based on stimulus/task timing and on empirical models of the FMRI signal



Linearity of Response

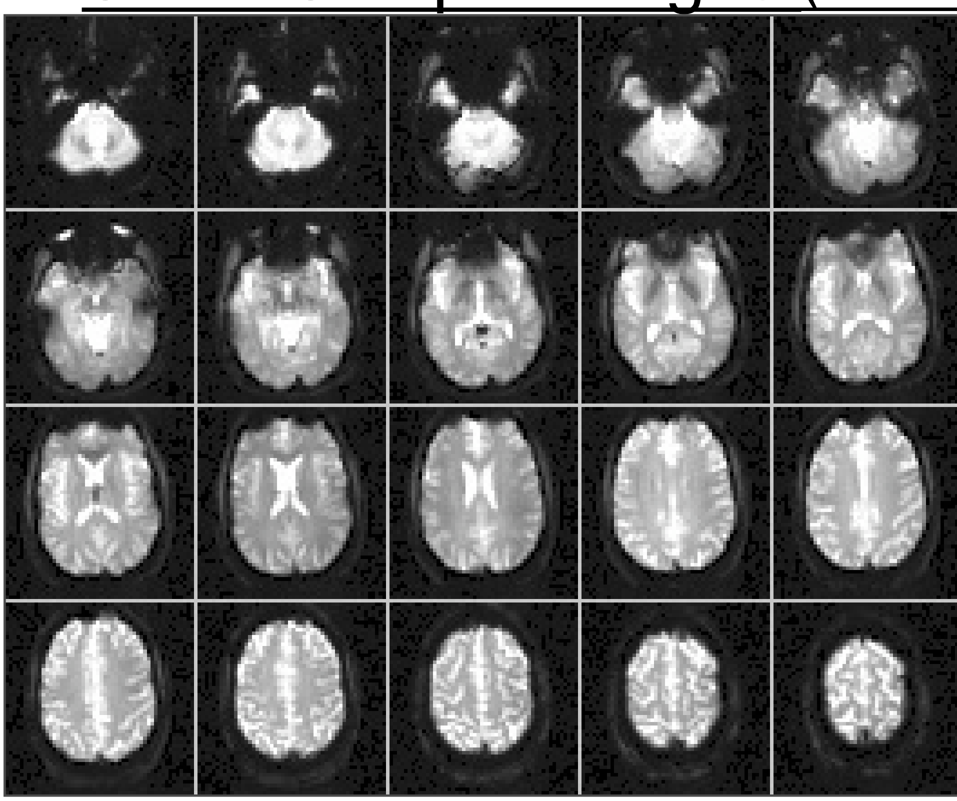
- Multiple activation cycles in a voxel:
  - Assume that overlapping responses add
  - Result = convolution of HRF with task timing



• Linearity is a good assumption
• But not perfect — about 90% correct
• Nevertheless, is widely taken to be true and is the basis for the “general linear model” (GLM) in FMRI analyses

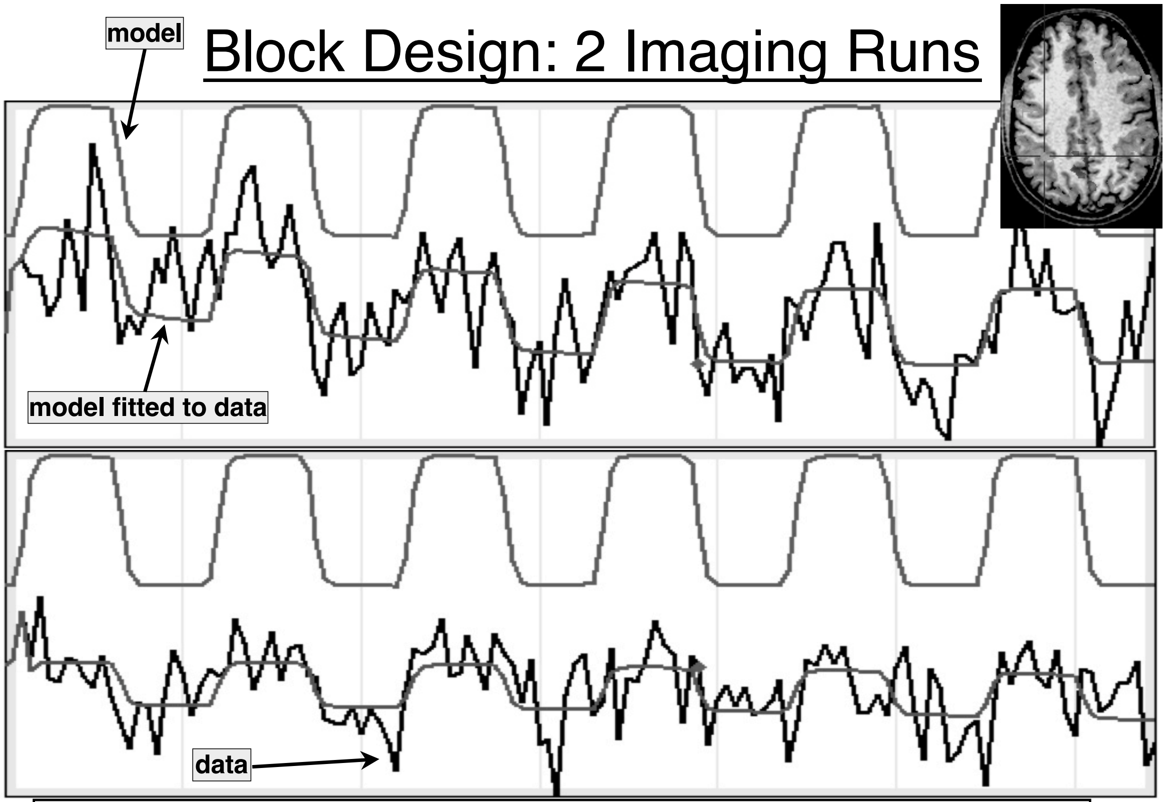
3 Brief Activations

Some Sample Images (1 volume)



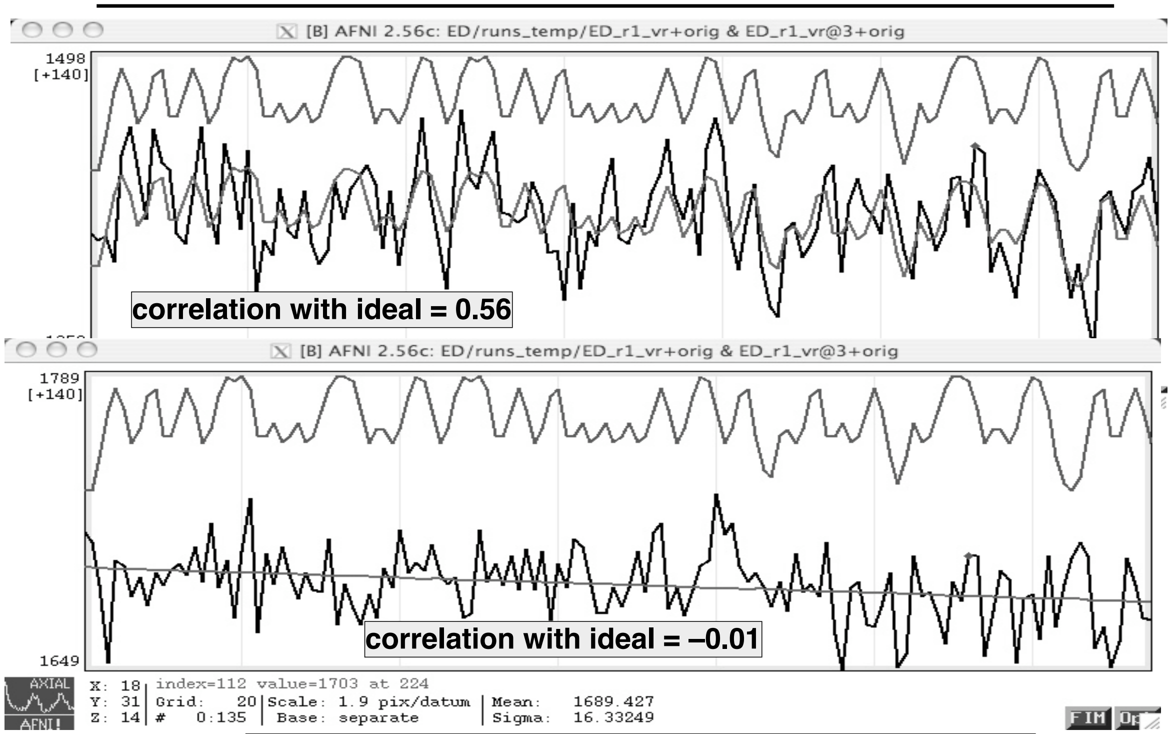
Next slides: some voxel time series graphs

Block Design: 2 Imaging Runs



27 s "on" / 27 s "off"; Δt=2.5 s; 130 points/run; 9 runs/subject

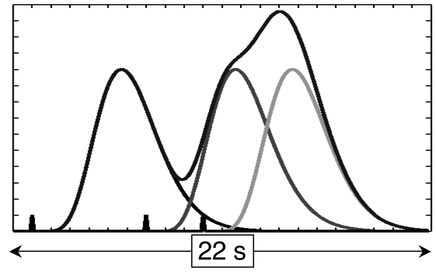
Event-Related FMRI: 2 Different Voxels

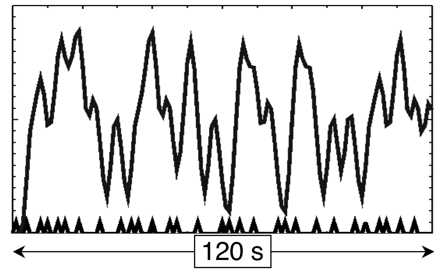


Strong activation is not obvious via casual inspection!

Convolution Signal Model

- FMRI signal we look for in each voxel is taken to be sum of individual trial HRFs
  - Stimulus timing is assumed known (or measured)
  - Resulting time series (**blue** curves) are called the ***convolution*** of the HRF with the stimulus timing
- Must also allow for baseline & baseline drifting
  - Convolution models only the FMRI signal **changes**





- Real data starts at and returns to a nonzero, slowly drifting baseline

Time Series Analysis on Voxel Data

- Most common forms of FMRI analysis involve fitting the activation+BOLD model to each voxel's time series **separately** (AKA "univariate" analysis)
- Result of model fits is a set of parameters at each voxel, estimated from that voxel's data
  - *e.g.*, activation amplitude, delay, shape
  - "SPM" = statistical parametric map
- Further analysis steps operate on individual SPMs
  - *e.g.,* combining/contrasting data among subjects

FMRI Activation Amplitude

- Amplitude of activation (in one voxel, in one subject) = amplitude of model fitted to data
  - Usually fitted to all imaging runs simultaneously
  - Usually normalized to be in units of percent signal change from baseline (based on deoxyHB theory)
- Commonly have more than one category of stimulus/task
  - *e.g.*, Image Viewing: Working Memory vs. Labeling
  - Each category gets its own time series model
  - All models fitted at once using multiple regression
  - Each stimulus/task gets assigned its own amplitude

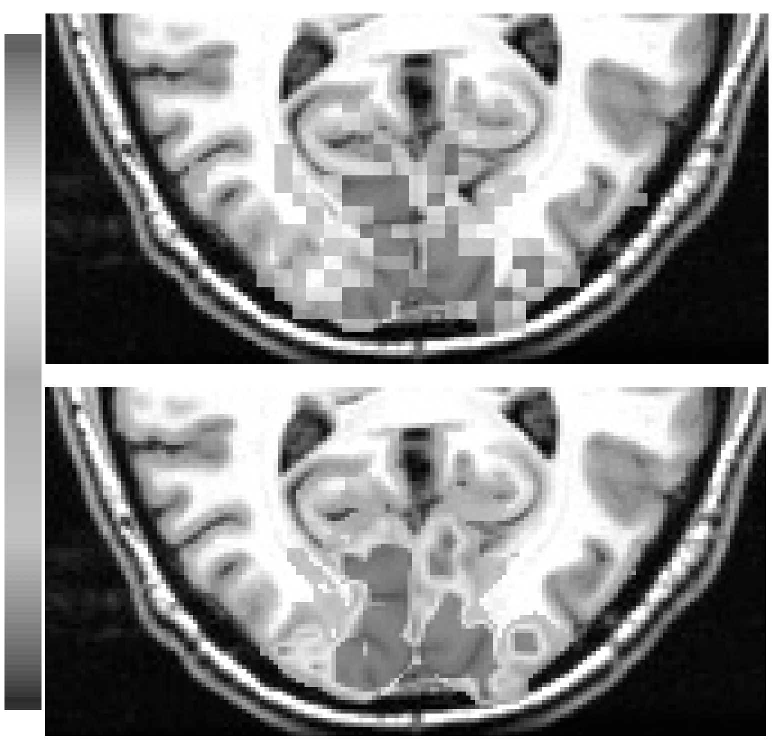
Multiple Stimuli = Multiple Regressors

- Usually have more than one class of stimulus or activation in an experiment
  - *e.g.*, "face activation" vs "house activation"
- Model each separate class of stimulus with a separate response function $r_1(t)$, $r_2(t)$, $r_3(t)$, …
  - Each $r_j(t)$ is based on the stimulus timing for activity in class number j
  - Calculate β_j amplitude = amount of $r_j(t)$ in voxel data time series $Z(t)$
  - Contrast βs to see which voxels have differential activation levels under different stimulus conditions
    - *e.g.*, statistical test on $\mathbf{\beta_1 - \beta_2 = 0}$ **?**

Fixed Shape HRF Analysis

- Assume a fixed shape $h(t)$ for the HRF
  - *e.g.*, $h(t) = t^{8.6} \exp(-t/0.547)$ [MS Cohen, 1997]
  - Convolved with stimulus timing, get model response function $r(t)$
- Assume a form for the baseline
  - *e.g.*, $a + b \cdot t$ for a constant plus a linear trend
- In each voxel, fit data $Z(t)$ to curve of form $Z(t) \approx a + b \cdot t + \beta \cdot r(t)$
  - a, b, β are unknown parameters to be calculated in each voxel
  - a,b are "nuisance" parameters
  - β is amplitude of $r(t)$ in data = "how much" BOLD

Sample Activation Map

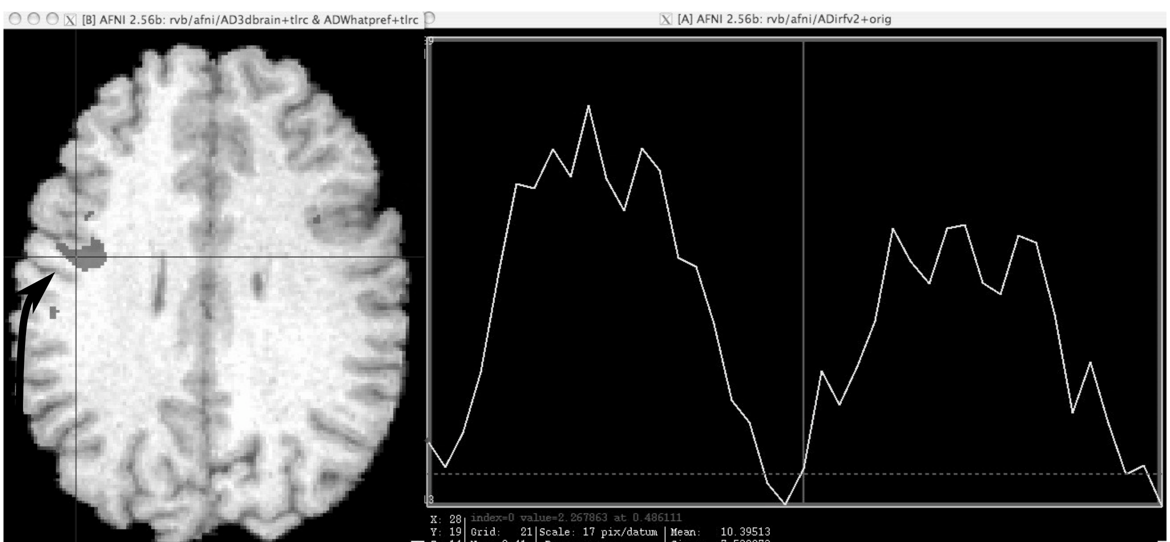


- Threshold on significance of amplitude
- Color comes from amplitude
- Upper Image: color overlay at resolution of EPI
- Lower Image: color overlay interpolated to resolution of structural image

Variable Shape HRF Analysis

- Allow shape of HRF to be unknown, as well as amplitude (deconvolution of HRF from data)
- **Good**: Analysis adapts to each subject and each voxel
- **Good**: Can compare brain regions based on HRF shapes
  - *e.g.*, early vs. late response?
- **Bad**: Must estimate more parameters
  - ⇒ Need more data (all else being equal)
- Usually extract some parameters from shape for inter-task and inter-subject comparisons

Sample Variable HRF Analysis



- What-vs-Where tactile stimulation
- <u>Red</u> ⇒ regions with $\beta_{What} > \beta_{Where}$

Noise Issues in Time Series

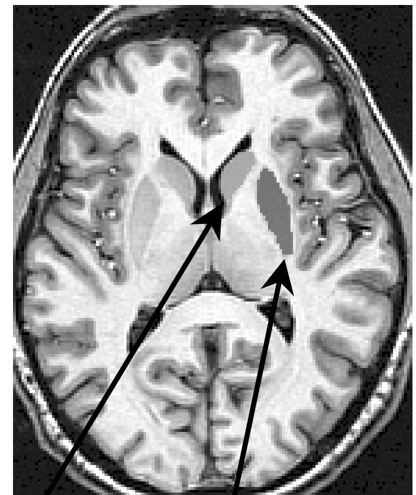
- Subject head movement
  - Biggest practical annoyance in FMRI
- Physiological noise
  - Heartbeat and respiration affect signal in complex ways (*e.g.*, correlation in time and space)
- Magnetic field fluctuations
- **Poorly understood** and hard to correct:
  - Sometimes see $\pm 5\ \sigma$ spikes in data with no apparent cause
  - Very slow signal drifts make long term experiments (*e.g.*, learning, adaptation) difficult

Inter-Subject Data Alignment

- Cortical folding patterns are (at least) as unique as fingerprints
- Inter-subject comparisons requires some way to bring brain regions into alignment
  - So that SPMs can be averaged and contrasted in various ways
- Solutions: **Brain Warping** and **ROIs**

ROIs = **R**egions **O**f **I**nterest

- Manually draw anatomically defined brain regions on 3D structural MRIs
  - Can be tediously boring
- Use ROIs to select data from each subject
- Combine averages from ROIs as desired
  - *e.g.*, ANOVA on signal levels



- Issue: Are anatomical ROIs the “right” thing to do?

Easy Brain Warping

- Align brain volume so that inter-hemispheric fissure is vertical (*z*), and Anterior-Posterior Commissure line is horizontal (*y*)
- Stretch/shrink brain to fit **Talairach**-Tournoux Atlas dimensions
- Use (*x*,*y*,*z*) coordinates based at AC=(0,0,0)
- **Accuracy**: Not so good (≈5-15 mm)
  - FMRI analysts often spatially blur data or SPMs to adapt to this problem

Hard Brain Warping (3D)

- Nonlinearly distort (warp, morph, transform) brain volume images in 3D to match sulcus-to-sulcus, gyrus-to-gyrus
- Very computationally intensive
- **Accuracy**: hard to gauge, since method is not widely used
  - Good software for this is not readily available
- **Issue**: Very large inter-subject variability even in existence and shape of many sulci

Hard Brain Warping (2D)

- **Idea**: Warp brain only along cortical sheet (triangulated 2D surface model) rather than general 3D transformation
  - Goal is still to align sulci and gyri (*e.g.*, by matching brain convexities)
  - Then create a new "standard" surface model, where nodes from all subjects are aligned
    - Does not deal with non-cortical structures
- **Hope**: 2D is a little easier than 3D and may be more anatomically meaningful
- Not widely used at present
  - Software is available: FreeSurfer and SureFit

Inter-Subject Analyses

- Current methodologies are based on some sort of ANOVA (after alignment)
  - Alternative: PCA (etc) is not much used in FMRI
- Important to treat intra-subject and inter-subject variance separately
  - *e.g.*, paired and unpaired *t*-tests, and their generalizations in random-effects ANOVA
  - This point is not always appreciated
- Multi-way ANOVA is a method for structuring hypotheses and tests
  - Supplement with continuous covariates (*e.g.*, age)?
  - A proper analysis will need to be more general

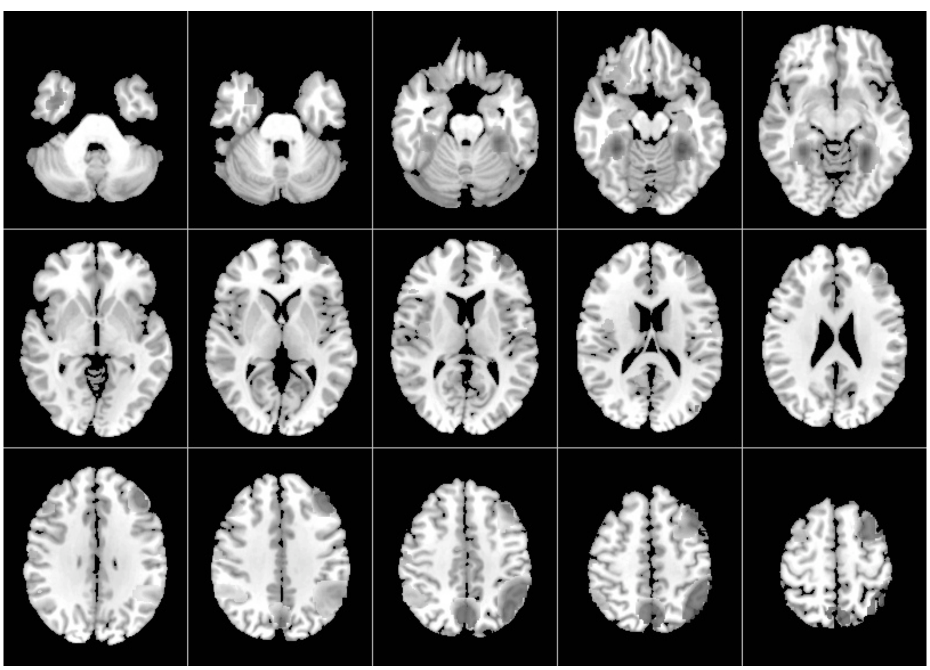
5 Types of 4-Way ANOVA Being Used

$A_F \times B_F \times C_F \times D_F$ All factors fixed; fully crossed	**A**,**B**,**C**,**D**=stimulus category, drug treatment, etc. All combinations of subjects and factors exist; Multiple subjects: treated as repeated measures; One subject: longitudinal analysis
$A_F \times B_F \times C_F \times D_R$ Last factor random; fully crossed	**A**,**B**,**C**=stimulus category, etc. **D**=subjects, typically treated as random (more powerful than treating them as repeats) Good for an experiment where each fixed factor applies to **all** subjects;
$B_F \times C_F \times D_R(A_F)$ Last factor random, and nested within the first (fixed) factor	**A**=subject class: genotype, sex/gender, or disease **B**,**C**=stimulus category, etc. **D**=subjects nested within A levels
$B_F \times C_R \times D_F(A_F)$ Third factor random; fourth factor fixed and nested within the first (fixed) factor	**A**=stimulus type (*e.g.*, repetition number) **B**=another stimulus category (*e.g.*, animal/tool) **C**=subjects (a common set among all conditions) **D**=stimulus subtype (*e.g.*, perceptual/conceptual)
$C_F \times D_R(A_F \times B_F)$ Doubly nested!	**A**, **B**=subject classes: genotype, sex, or disease **C**=stimulus category, etc. **D**=subjects, random with two distinct factors dividing the subjects into finer sub-groups (*e.g.*, **A**=sex $\times$ **B**=genotype)

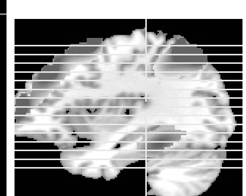
Standard FMRI Visualizations

- 2D Grayscale anatomicals with functional activation percent change overlaid in color
- 3 orthgonal 2D projections of activation maps
  - The SPM “glass brain” — very common in journal papers
- 3D volume rendering
- 3D rendering of cortical surface models
  - Analysis can also be performed directly on time series data projected to the cortical surface model — initial results are promising

2D Slice Array

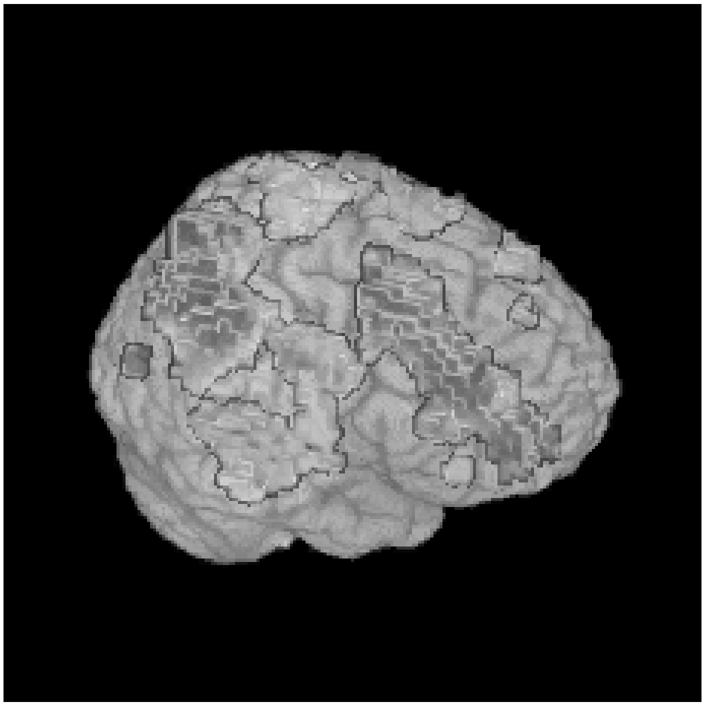


- 84 subj
- 4 way ANOVA:
  Gender
  × CogTask
  × Valence
  × Subject
- WM–Lab



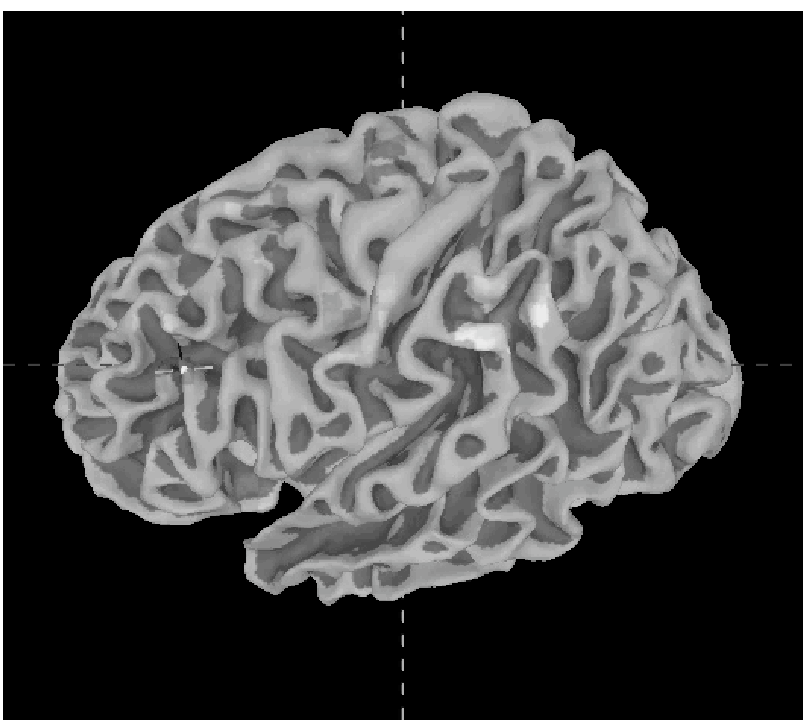
Commonly used in journal articles

3D Volume Rendering



- "Show Through" rendering: Color overlay above statistical threshold is projected outward to brain surface
- 3D structure becomes apparent from rotation of viewpoint

Cortical Surface Models



- Color overlay above statistical threshold is intersected with surface model
- Surface model can be inflated to see into sulci

Software Tools

- Several widely used packages
  - In order of popularity; ♦ principal authors

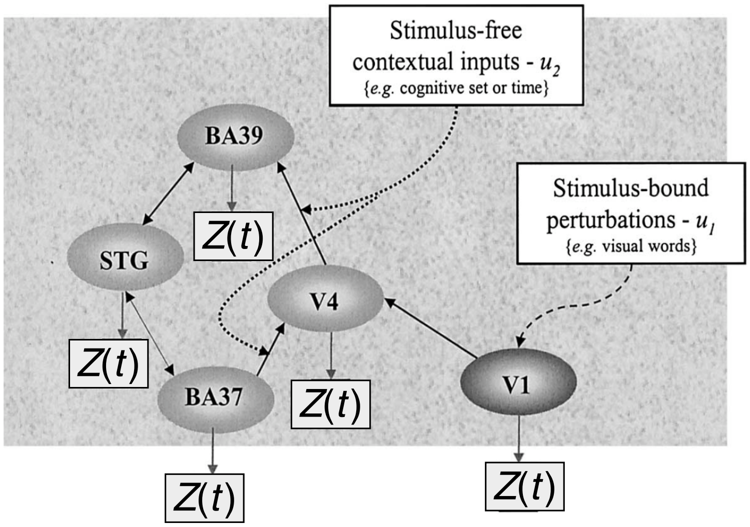
1) **SPM** - Wellcome Institute/London
   - ♦ John Ashburner
2) **AFNI** - NIMH IRP/Bethesda
   - ♦ Robert Cox (your humble servant)
   - ♦ Includes a module for realtime image analysis
3) **FSL** - FMRIB/Oxford
   - ♦ Steve Smith
4) Homegrown and/or pastiche

Points for Discussion & Comment

- Variations on standard FMRI time series analyses
- Directions in FMRI analysis research
- Things that are hard to do with FMRI
- Origins of fluctuations in FMRI activation amplitude
  - And what to do about them?
- Visualization issues

FMRI Analyses: Variations

- Spatial smoothing and spatial clustering
- Data-driven analyses (“components”)
- Inter-region connectivity:



- Analyze data for correlations amongst activation amplitudes in different brain ROIs

FMRI Analysis Research

- Many “reasonable” space+time series analyses
  - Need methodologies for comparing them
- Combining data from multiple scanners/centers
- Closer integration of analysis to neural-level hypotheses
  - Cognitive models; signaling networks
  - Understand physiology better!
- “Brainotyping”: methods for grouping and discriminating among brain maps
  - Application to individual patients?
  - Combining with X-omic data (X=gene, protein, …)?

fMRI-DC
fBIRN

Some Things That Are Hard in FMRI

- Measuring neural effects that take a long time to occur (ten minutes or more)
  - Learning, adaptation; Effects of some drugs
- Measuring neural effects associated with tasks that require big subject movements
  - Continuous speech; swallowing; head movement
- Distinguishing neural events closer than ~500 ms in time
- Measuring activation in brainstem nuclei
- Measuring differences in timing or strength of neural activity between brain regions
- Characterizing individual subject phenotypes

FMRI Amplitude Fluctuations

- Task type (often the principal concern)
- Subject type (concern? or confound? or both?)
  - Disease status, genotype, sex, age, …
- Subject task performance (behavior, attention)
- Neural "activation" level (whatever that is)
- Physiological noise (heartbeat, breathing)
- Task-related noise
  - Movement artifacts, breathing changes, …
- Subject's hemo-response
  - Different shapes, OEFs, vasculature, …
- Subject monitoring and calibration?

Simple Model for Fluctuations

- Little has been done to systematically model inter-subject signal variablility
- In each voxel separately, *after* time series analysis estimates the FMRI signal $\boldsymbol{y}$:

$$\underbrace{y_{ij}}_{\substack{\text{FMRI}\\ \text{signal for}\\ \text{task } \#i \text{ in}\\ \text{subject } \#j}} = \underbrace{a_{ij}}_{\substack{\text{neural}\\ \text{"activation"}\\ \text{for task } \#i\\ \text{in subject } \#j}} \cdot \underbrace{h_j}_{\substack{\text{hemodynamic}\\ \text{scaling for}\\ \text{subject } \#j}} + \underbrace{\varepsilon_{ij}}_{\substack{\text{various}\\ \text{noises}}}$$

  - Depending on experiment and hypotheses, will break down tasks and subjects into various categories
  - To do statistics, need parametric models for activation $\boldsymbol{a}$, hemo-response $\boldsymbol{h}$, and noise $\boldsymbol{\varepsilon}$

Issues in Visualization

- Regions below statistical threshold:
  - translucency? topographically? animation?
- Multi-subject data - beyond averages?
- Connectivity maps - inter-regional correlations? Dynamic Causal Modeling?
- High dimensional patterns that activate much of the brain
  - *e.g.*, Watching a movie
- **Basic problem**: even after filtering out much of the crap, are left with high-dimensional info at each place in a 3D space

Finally … Thanks

- The list of people I should thank is not quite endless …

MM Klosek. JS Hyde. JR Binder. EA DeYoe. SM Rao.
EA Stein. A Jesmanowicz. MS Beauchamp. BD Ward.
KM Donahue. PA Bandettini. AS Bloom. T Ross.
M Huerta. ZS Saad. K Ropella. B Knutson. J Bobholz.
G Chen. RM Birn. J Ratke. PSF Bellgowan. J Frost.
K Bove-Bettis. R Doucette. RC Reynolds. PP Christidis.
LR Frank. R Desimone. L Ungerleider. KR Hammett.
A Clark. DS Cohen. DA Jacobson. JA Sidles. EC Wong.
Et alii …