FMRI Data Analysis: Principles & Practice

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Kiel – 25 May 2007

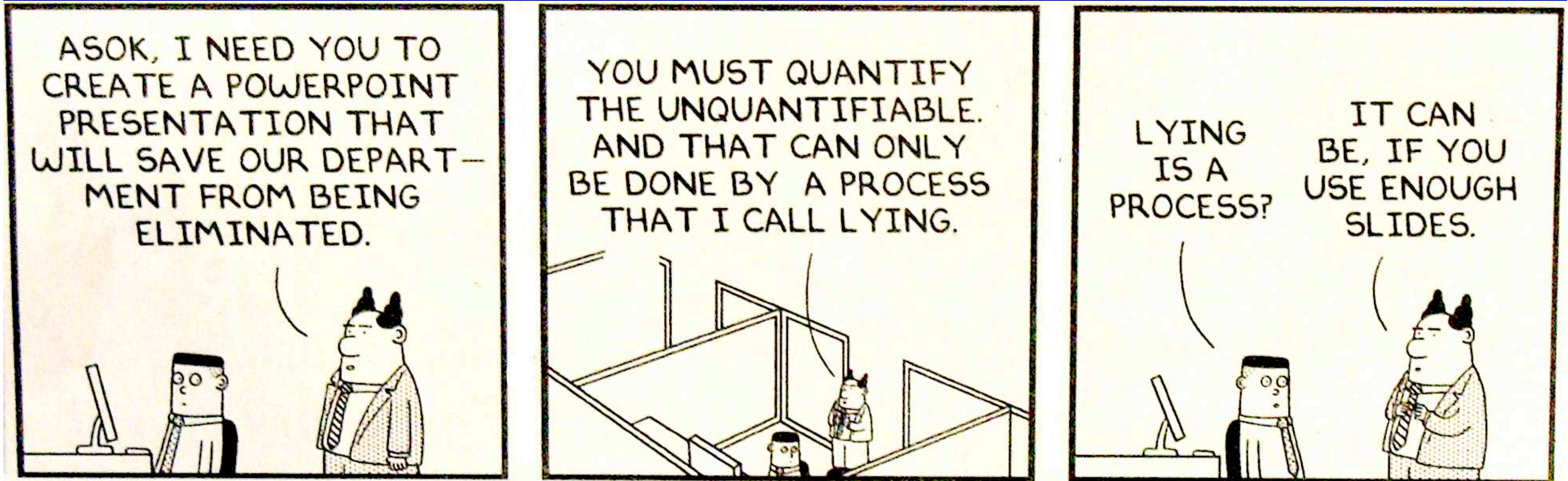
Ultimate Conclusions First

- FMRI data analysis is built upon many assumptions, arbitrary parameters, and complex software
  - Don't believe the functional activation maps blindly — check the results by "playing" with the data

- FMRI is an intricate process, from acquisition to analysis to interpretation
  - Doing it well requires a **team** of experts who work well together

Warnings & Caveats

- This talk: brief outline of a complex topic
  - I usually spend a week teaching this stuff!



- Almost everything I say herein has an exception, or a complication, or both
  - *and*, opinions differ on some of these issues

Principles: Modeling

- Data analysis always takes place in the context of a mathematical/statistical model
- Model relates the properties of the system being observed to the numbers that are actually measured
  - Sometimes the model is implicit in the analysis algorithm, rather than being explicitly stated
  - Model must take into account properties of the measurement system
- Models relating FMRI signals to neural activity are complex and tentative

Principles: Data Quality

- FMRI data are full of rubbish (Abfall):
  - Signal changes with neuronal activation are small (similar to noise magnitude)
  - MRI signal is several levels of indirection away from neuronal changes of interest
- Numerous other signal fluctuations of non-neural origin have similar or greater magnitude:
  - Ghosting, warping, small head movements, scanner imperfections, heartbeat, breathing, long-term drifts, signal dropouts, signal spikes, *et cetera*

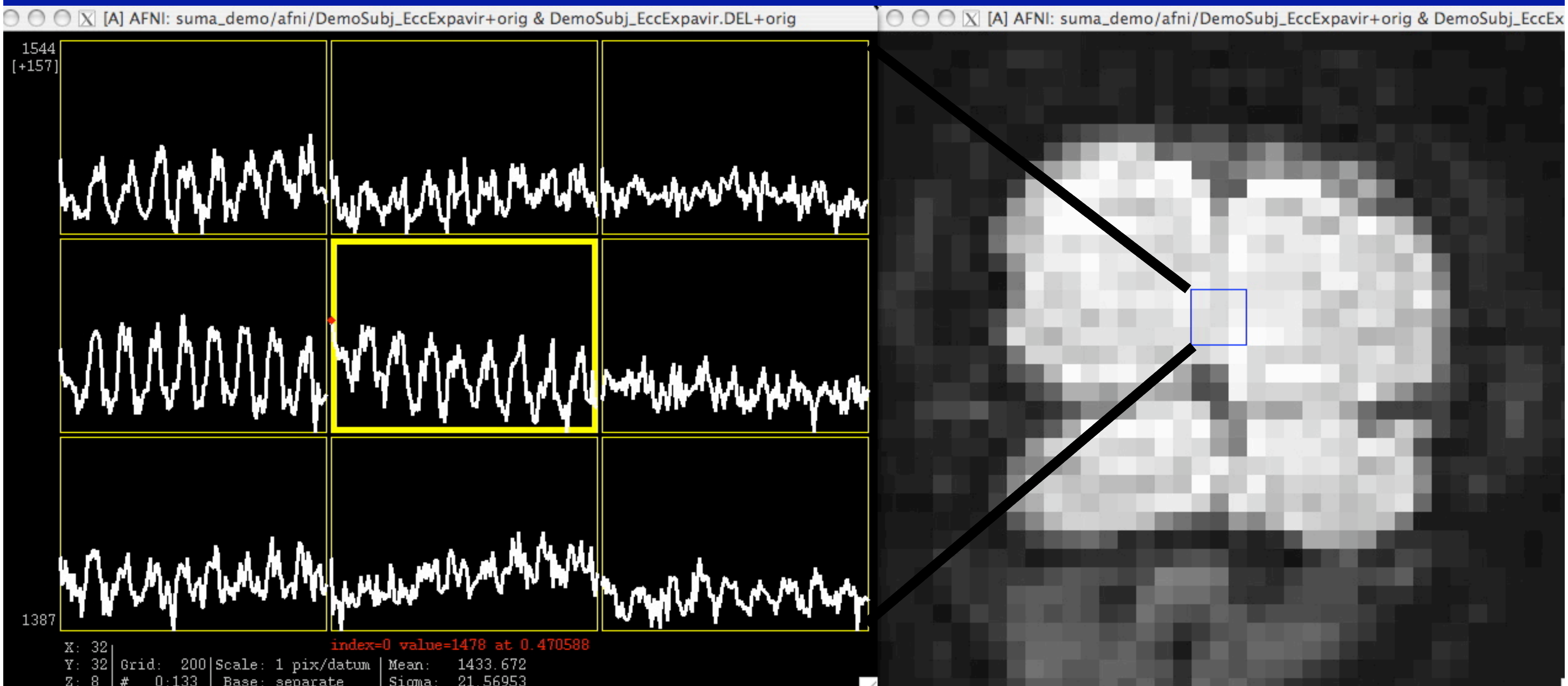
Conclusions from Principles

- It is better to state the mathematical model rather than implicitly rely on an algorithm
  - To understand what is being computed
- It is important to try to reduce the rubbish in the data
  - Reduce it at the source *and* in the analysis
  - More data is better (to average out the rubbish)
- It is important to examine the processed data visually at each step in the analysis, to ensure that nothing bad has happened
  - You should understand the process and results

The Data

- 10,000..50,000 image voxels inside brain (resolution ≈ 2-3 mm)
- 100..1000+ time points in each voxel (time step ≈ 2 s)
  - Some of which may be heavily contaminated by subject movement
- Also know timing of stimuli delivered to subject (*etc*)
  - Behavioral, physiological data?
- Hopefully, some hypothesis
  - What are you looking for?

Sample Data: Visual Area V1



Graphs of 3×3 voxels through time

One slice at one time; Blue box shows graphed voxels

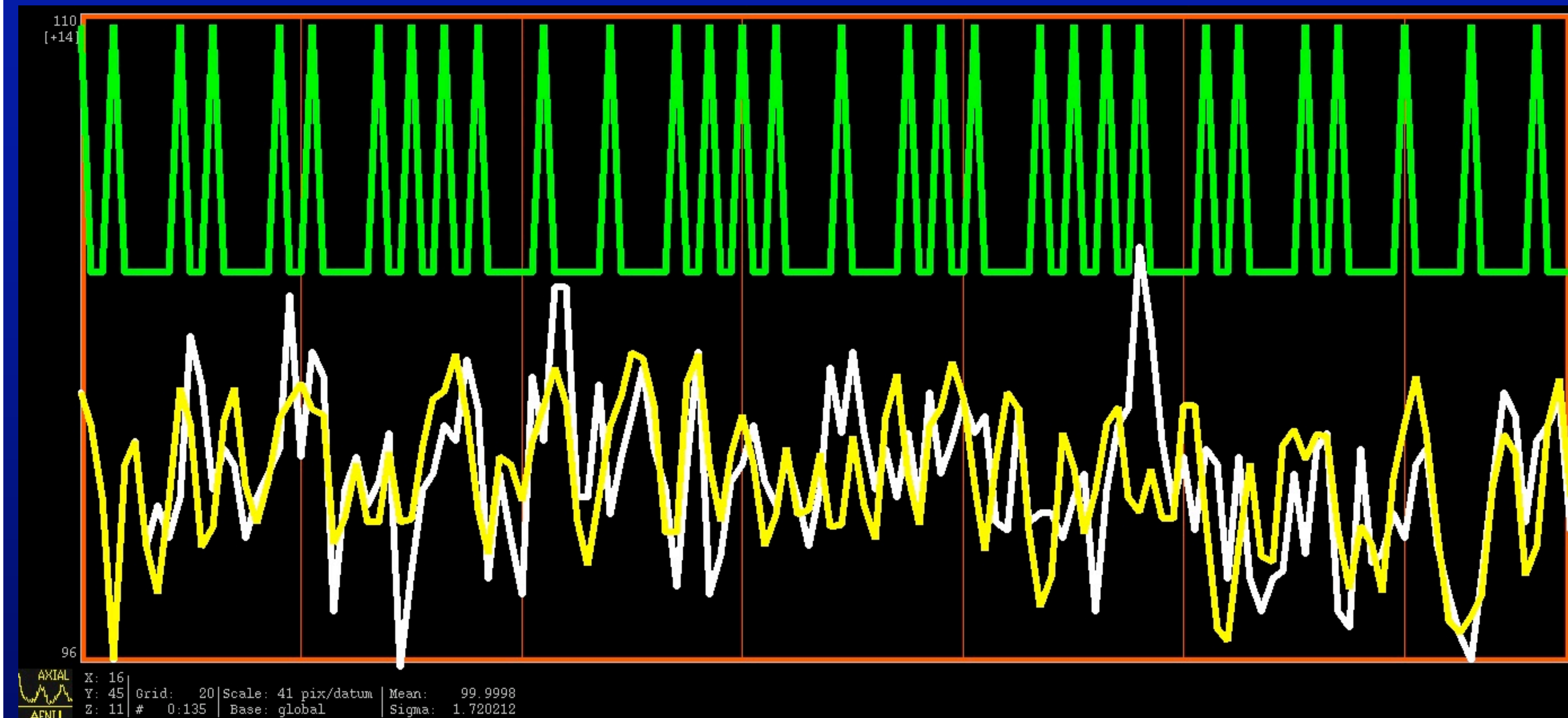
Same Data as Last Slide

Blowup of central time series graph: about 7% signal change with a **very** powerful periodic neural stimulus

Block design experimental paradigm: visual stimulation

Event-Related Data

Four different visual stimuli



- White curve = Data (first 136 TRs)
- Orange curve = Model fit (R^2=50%)
- Green = Stimulus timing

Very good fit for ER data (R^2=10-20% more usual).

Noise is as big as BOLD!

How FMRI Experiments Are Done

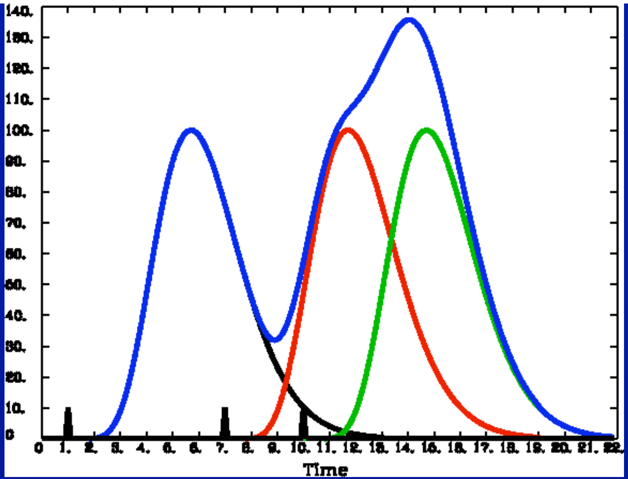
- Alternate subject's neural state between 2 (or more) conditions using sensory stimuli, tasks to perform, ...
  - Can only measure relative signals, so must look for *changes* in the signal between the conditions
- Acquire MR images repeatedly during this process
- Search for voxels whose signal time series (up-&-down) matches stimulus time series pattern (on-&-off)
- Signal changes due to neural activity are small
  - Need about 1000 images in time series (in each slice) ⇒ takes about 1 hour to get fully reliable activation maps
    - Must break image acquisition into shorter "runs" to give the subject and scanner some break time
  - Other small effects can corrupt the results ⇒ postprocess the data to reduce these effects & be careful

FMRI Experiment Design and Analysis

All on one slide !

- FMRI experiment design
  - Single subject or group study? Event-related, block, hybrid event-block?
  - How many types of stimuli? How many of each type? Timing (intra- & inter-stim)?
  - Will experiment show what you are looking for? (Hint: bench tests)
  - How many subjects do you need for group analysis? (Hint: answer does not have 1 digit)
- Time series data analysis (individual subjects)
  - Assembly of images into 4D datasets; Visual & automated checks for bad data
  - Registration of time series images (attempt to correct for subject motion)
  - Smoothing & masking of images; Baseline normalization; Censoring bad data
  - Catenation of imaging runs into one big dataset
  - Fit statistical model of stimulus timing+hemodynamic response to time series data
    - Fixed-shape <u>or</u> variable-shape response models
  - Segregation into differentially active blobs
    - Thresholding on statistic + clustering <u>and/or</u> Anatomically-defined ROI analysis
  - Visual examination of maps and fitted time series for validity and meaning
- Group analysis (inter-subject)
  - Spatial normalization to Talairach-Tournoux atlas (or something like it)
  - Smoothing of fitted parameters
    - Automatic global smoothing + voxel-wise analysis <u>or</u> ROI averaging
  - ANOVA to combine and contrast activation magnitudes from the various subjects
  - Visual examination of results (usually followed by confusion)
  - Write paper, argue w/ co-authors, submit paper, argue with referees, publish paper, …

Experiment Design - Blocks



- Hemodynamic (FMRI) response
  - peak = 4-6 s after neural activation
  - width = 4-5 s for brief (< 1 s) activation
  - ⇒ two separate activations less than 12-15 s apart will have their responses overlap and add up (approximately)
- Block design experiments: Extended activation, or multiple closely-spaced (< 2-3 s apart) activations
  - Multiple FMRI responses accumulate ⇒ big response
  - But: can't distinguish separate but closely-spaced activations
    - Stimulus = "**subject sees a face for 1 s, presses button #1 if male, #2 if female**"; faces every 2 s for a 20 s block, then 20 s of "**rest**", *etc*.
    - What to do about trials where the subject makes a mistake?
    - Neurally different than correct trials, but there is no way to separate out the activations when the hemodynamics blurs so much in time.

Experiment Design - Event-Related

- Separate activations in time so can model FMRI response from each separately, as needed
- Need to make inter-stimulus gaps vary ("jitter") if there is any time overlap in their FMRI response curves: if events are closer than 12-15 s in time
  - Otherwise, tail of event #x always overlaps head of event #x+1 in same way ⇒ amplitude of response in tail of #x can't be told from response in head of #x+1
- You cannot treat every single event as a distinct entity whose response is to be calculated separately!
  - You must group events into classes, and assume that all events in the same class evoke the same response.
  - Approximate rule: 25**+** events per class (with emphasis on the '**+**')
  - There is just too much noise in FMRI to be able to get an accurate activation map from a single event!

Experiment Design - Block/Event

- Long "blocks" are situations where you set up some continuing condition for the subject
- Within a block, multiple distinct events; *Example*:
  - Event stimulus is a picture of a face
  - Block condition is instruction on what the subject is to do when he sees the face:
    - Condition A: press button #1 for male, #2 for female
    - Condition B: press button #1 if face is angry, #2 if face is happy
- Event stimuli in the two conditions may be identical
  - It is the instructional+attentional modulation between the two conditions that is the goal of such a study
  - Perhaps you have two groups of subjects (patients and controls) which respond differently in bench tests
  - You want to find neural substrates for these differences

3D Individual Subject Analysis

Assemble images into 4D datasets (e.g., NIfTI-1)

to3d
OR
can do at NIH scanners

Check images for quality (visual & automatic)

afni + 3dToutcount + 3dDespike

Register (realign) images

3dvolreg
OR
3dWarpDrive

Smooth images spatially

3dmerge
OR
3dBlurToFWHM
(optional)

Mask out non-brain parts of images

3dAutomask + 3dcalc (optional)

Normalize time series baseline to 100 (for %-izing)

3dTstat + 3dcalc
(optional: could be done post-fit)

Fit stimulus timing+hemodynamic model to time series

- Catenates imaging runs, removes residual movement effects, computes response sizes and inter-stimulus contrasts

3dDeconvolve

Segregate into differentially "activated" blobs

Alphasim + 3dmerge
OR
Extraction from ROIs

Look at results, and **think** (e.g., play with thresholds)

afni
AND
your personal brain

… to group analysis (next page)

Group Analysis: in 3D or on folded 2D cortex models

Datasets of results from individual subject analyses

Construct cortical surface models

Normalize datasets to Talairach "space"

OR

Project 3D /results to cortical surface models

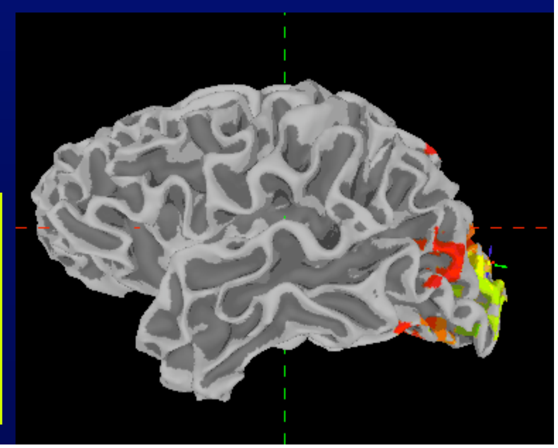
Smooth fitted response amplitudes

OR

Average fitted response amplitudes over ROIs

Use ANOVA to combine + contrast results

View and understand results;
Write paper;
Start all over



Fundamental Principles Underlying Most FMRI Analyses (esp. GLM): HRF ⊗ Blobs

- **H**emodynamic **R**esponse **F**unction
  - Convolution model for *temporal* relation between stimulus and response

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- Activation **Blobs**
  - Contiguous *spatial* regions whose voxel time series fit HRF model
  - *e.g.*, Reject isolated voxels even if HRF model fit is good there

Temporal Models: Linear Convolution

- **Additivity Assumption**:
  - Input = 2 separated-in-time activations
  - $\Rightarrow$ Output = separated-in-time **sum** of 2 copies of the 1-stimulus response
  - Additivity: approximately true, and improved by caffeine! (Tom Liu, ISMRM 2007)

- FMRI response to single stimulus is called the **H**emodynamic **R**esponse **F**unction (**HRF**)
  - Also: **I**mpulse **R**esponse **F**unction (IRF)

Hemodynamic Model

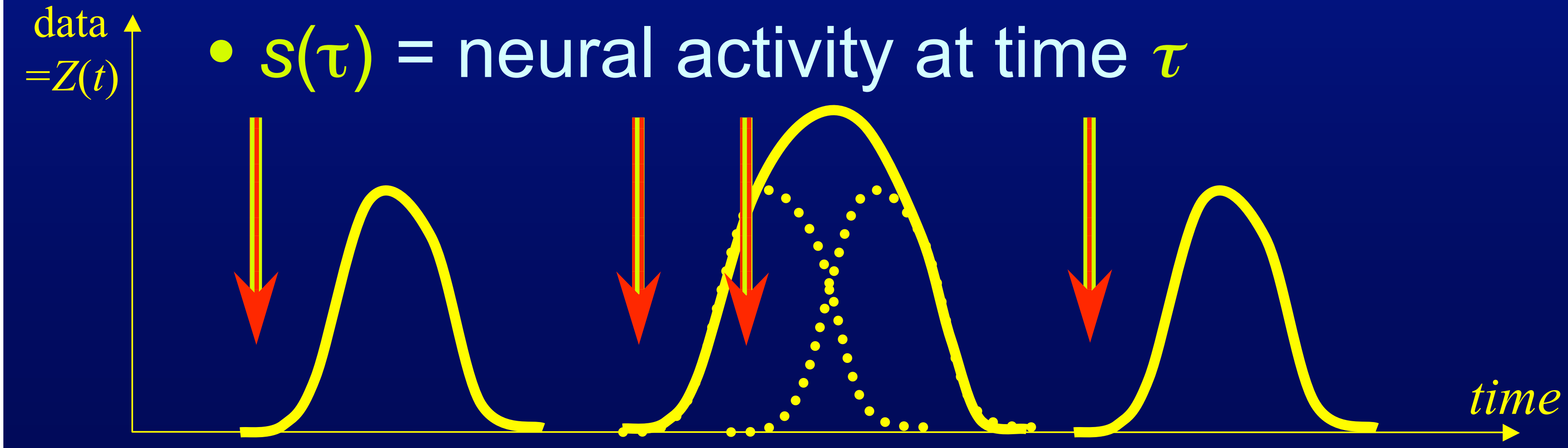
- Measured MRI value in each voxel is sum of:
  - Slowly drifting baseline
  - Hemodynamic response that is linearly proportional to "neural activity", delayed and blurred in time
  - Non-neural physiological "noise" due to respiration and blood flow pulsations through the cardiac cycle
  - Residual effects from uncorrectable subject motion and unmeasured scanner hardware fluctuations
  - White noise from random (thermal) currents in the body and the scanner
- Imaging is assumed perfect (no rubbish)
  - Or at least is fixed up in preprocessing steps

Hemodynamic Model

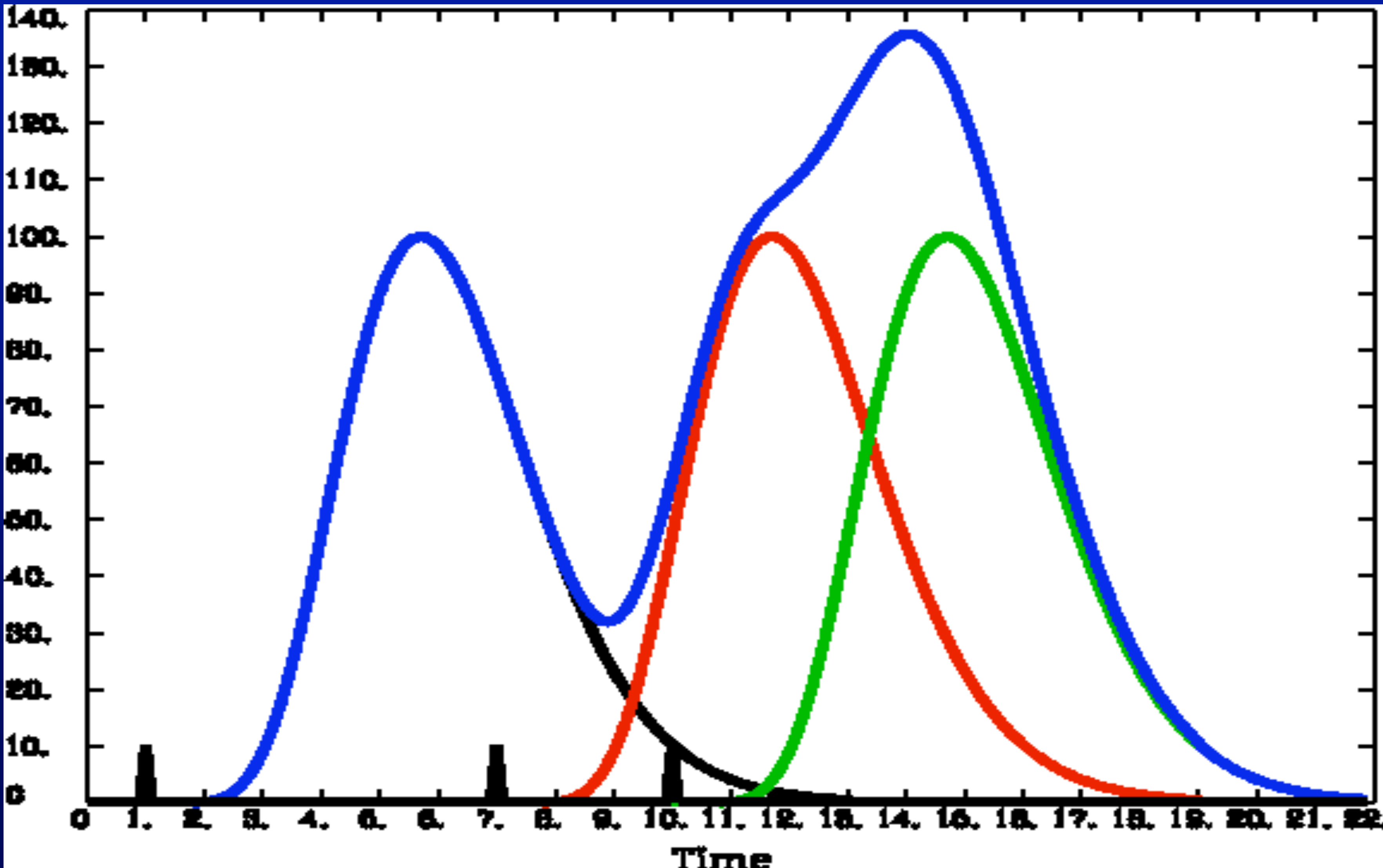
- Linear shift-invariant model for single voxel time series:

$$\text{data} = Z(t) = \text{baseline}(t) + \sum_{\tau=0}^{t} h(t-\tau)s(\tau) + \text{noise}(t)$$

  - $h(t)$ = hemodynamic response at time t after neural activity
  - $s(\tau)$ = neural activity at time τ



HRF Model Response to 3 Separate Brief Activations



Ways to Use This Model

- Assume $s(t)$ is known, and then
  - Assume $h(t)$ is known except for amplitude $\Rightarrow$ correlation method or fixed shape regression
  - Assume shape of $h(t)$ is also unknown $\Rightarrow$ deconvolution (variable shape) method
  - Assume several different classes of $s(t)$'s and correspondingly several different $h(t)$'s $\Rightarrow$ generic linear model (GLM)

- Assume $h(t)$ is known, and find $s(t)$

  $\Rightarrow$ inverse FMRI
- Try to find both $h(t)$ and $s(t)$

  $\Rightarrow$ blind deconvolution

FMRI as Pattern Matching

- HRF = mathematical model relating what we **know** (stimulus timing and image data) to what we **want to know** (location, amount, *…*, of neural activity)
- Given data, use this model to solve for unknown parameters in the neural activity (*e.g.*, where, how much, *…*)
  - Solving: via multivariate regression
- Then test for statistical significance
- The basis for most published FMRI

HRF Model Equations

$$h(t) = a \cdot t^b e^{-t/c}$$

Simplest model: fixed shape
Unknown = a [b & c fixed]

$$h(t) = a_0 \cdot t^b e^{-t/c} + a_1 \cdot \frac{d}{dt}\left[t^b e^{-t/c} \right]$$

Next simplest model: derivative allows for time shift
Unknowns = a_0 and a_1 [b & c fixed]

$$h(t) = \sum_{q=1}^{Q} w_q \Phi_q(t)$$

Expansion in a set of fixed basis functions $\{\Phi_q(t)\}$ (e.g., Splines, sines, …);
Unknowns = $\{w_q\}$

Multiple Stimulus Classes

- Need to calculate HRF (amplitude or amplitude+shape) separately for each class of stimulus
- Novice FMRI researcher pitfall: try to use too many stimulus classes
- Event-related FMRI: need 25**+** events per stimulus class
- Block design FMRI: need 10+ blocks per stimulus class

Spatial Models of Activation

- 10,000..50,000 image voxels in brain
- Don’t really expect activation in a single voxel (usually)
- Curse of multiple comparisons:
  - If have 10,000 statistical tests to perform, and 5% give false positive, would have 500 voxels “activated” by pure noise — way **way** too much!
- Can group voxels together somehow to manage this curse

Spatial Grouping Methods

- Smooth data in space before analysis
  - Apply threshold based on smoothness
- Average data across anatomically-selected regions of interest ROI (before or after analysis)
  - Labor intensive (*i.e.*, send more postdocs)
- Reject isolated small clusters of above-threshold voxels after analysis

Spatial Smoothing of Data

- Reduces number of comparisons
- Reduces noise (by averaging)

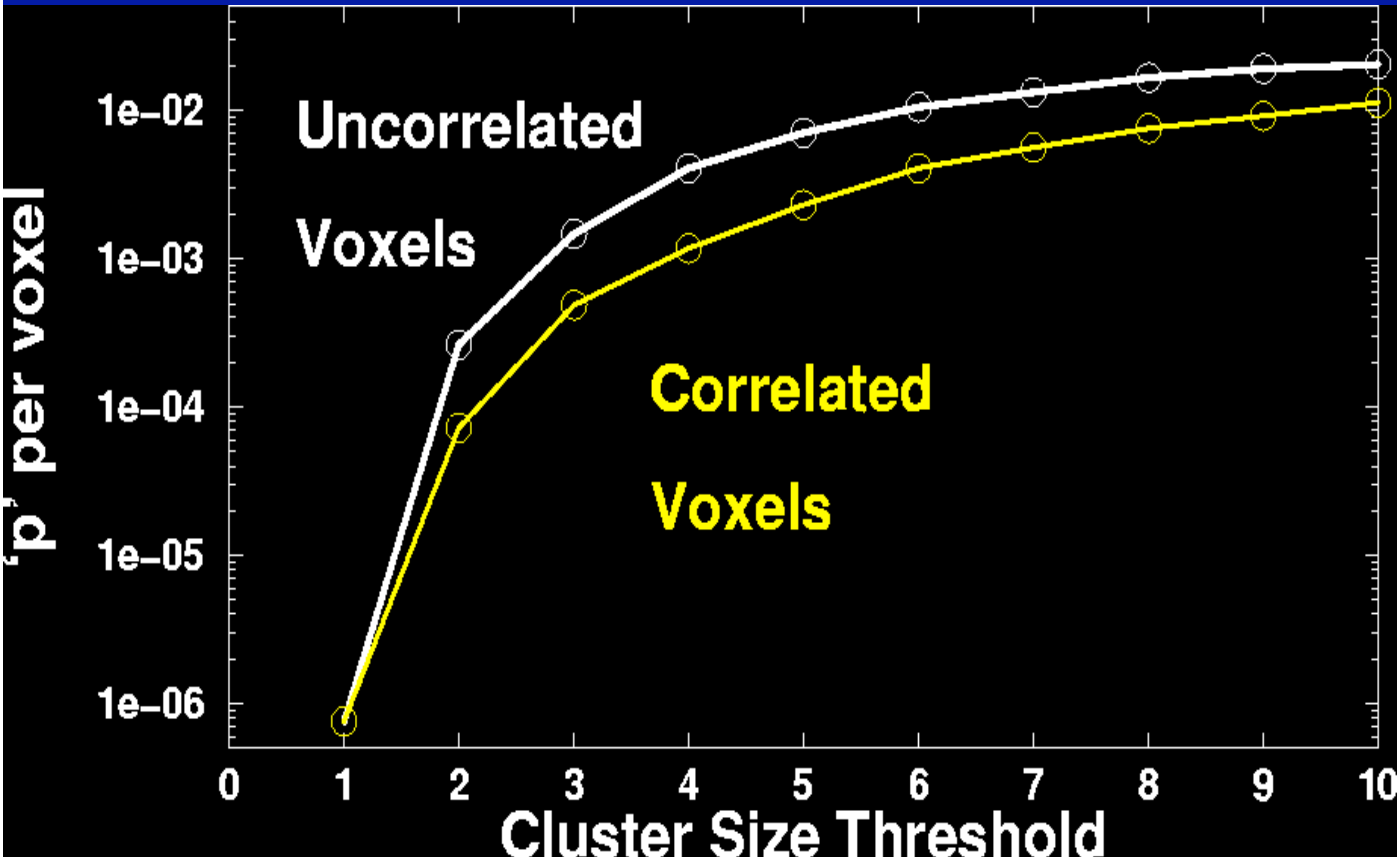
Good things

- Reduces spatial resolution
  - Can make FMRI results look PET-ish
  - In that case, why bother gathering high resolution MR images?
- Smart smoothing: average only over nearby brain or gray matter voxels
  - Uses resolution of FMRI cleverly
  - Or: average over selected ROIs
  - Or: cortical surface based smoothing

Spatial Clustering

- Analyze data, create statistical map (*e.g.*, ***t*** statistic in each voxel)
- Threshold map at a lowish ***t*** value, in each voxel separately
- Threshold map by rejecting clusters of voxels below a given size
- Can control false-positive rate by adjusting ***t*** threshold and cluster-size thresholds together

Cluster-Based Detection



Allowing for "Noise"

- Physiological "noise"
  - Heartbeat & respiration affect signal
  - Can monitor and try to cancel out

- Subject head movement
  - After realignment, some effects remain
  - Can include in regression model to reduce effects
  - Task-correlated motion: clever design can help …

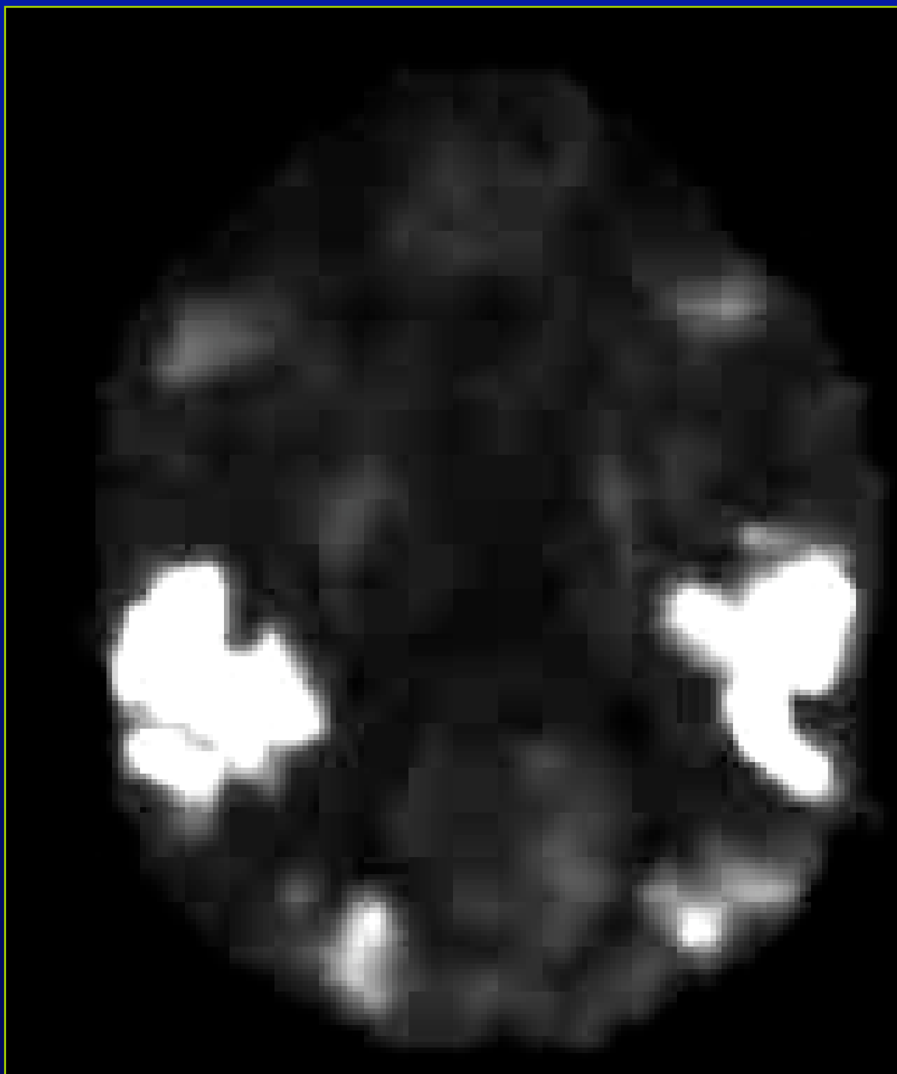
- Low frequency drifts ($\leq$ 0.01 Hz)
  - Need to include in baseline model

- Scanner glitches can produce gigantic ($\geq$10 σ) spikes in data
  - Can try to automatically "squash" these

Rubbish: Things to Look For

- Errors in setting up the scans
  - Be consistent if scanning same subject on multiple days (*e.g.*, same FOV, slice thickness)
- Large head movements
  - More than a few mm or few degrees
  - Stimulus correlated motion: brain “cap”
- Spikes in the data time series
- Scanner drifts
  - Short term: During long imaging runs
  - Long term: Hardware slowly degrading
    - Set up an FMRI quality check system!
- Palliative: real-time image acquisition

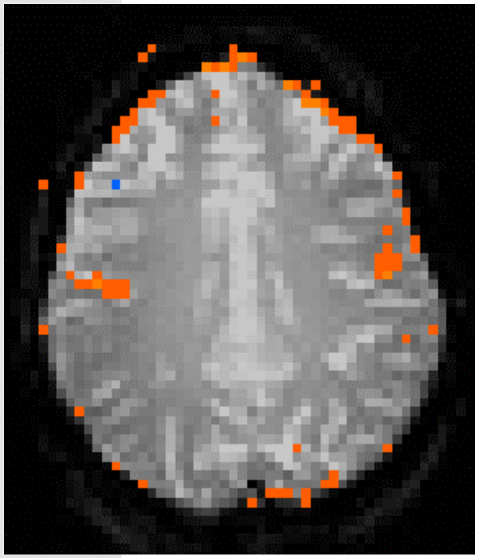
Playing with Your Results

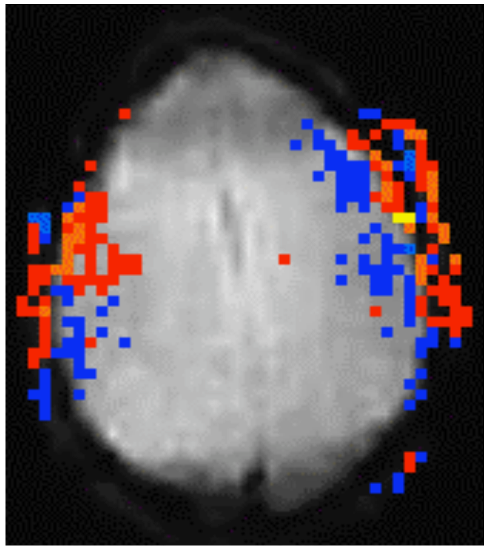


- Unthresholded *F*-statistic in grayscale
- Animation: loops from very strict threshold to very non-strict
  - No spatial clustering

Correcting for speech-related motion

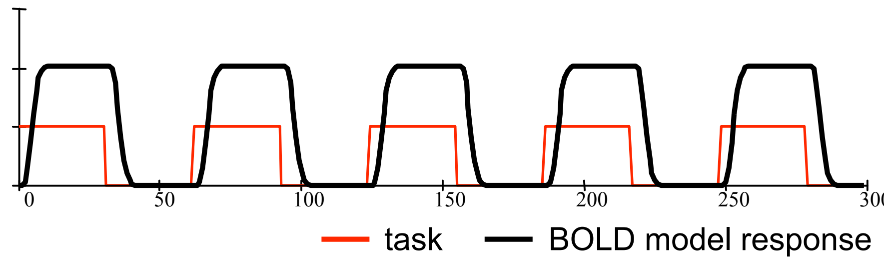
Overt Speech – 2 block design experiments





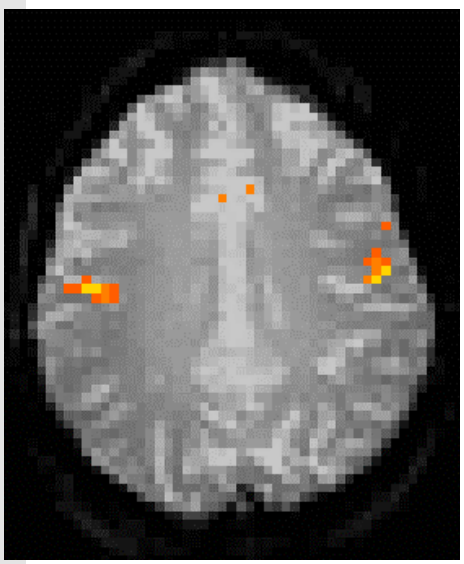
Overt speech results in large task-related motion artifacts…

(30s task / 30s rest) (motion highly correlated)

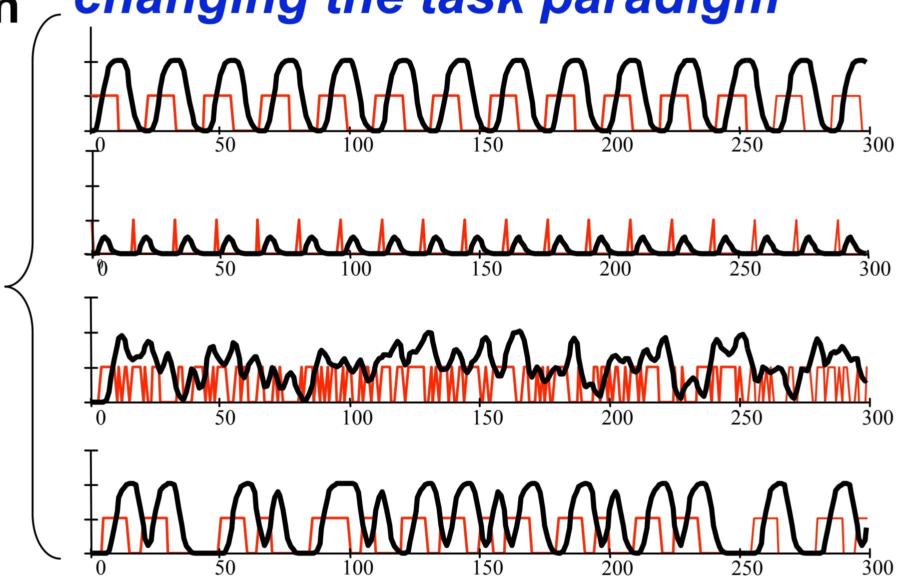


…These can be reduced by changing the task paradigm

Overt Speech – event-related design



Blocked / Event-Related (low correlation with motion)



R.M. Birn

Differential activation of frontal and temporal cortex by phonemic and category fluency

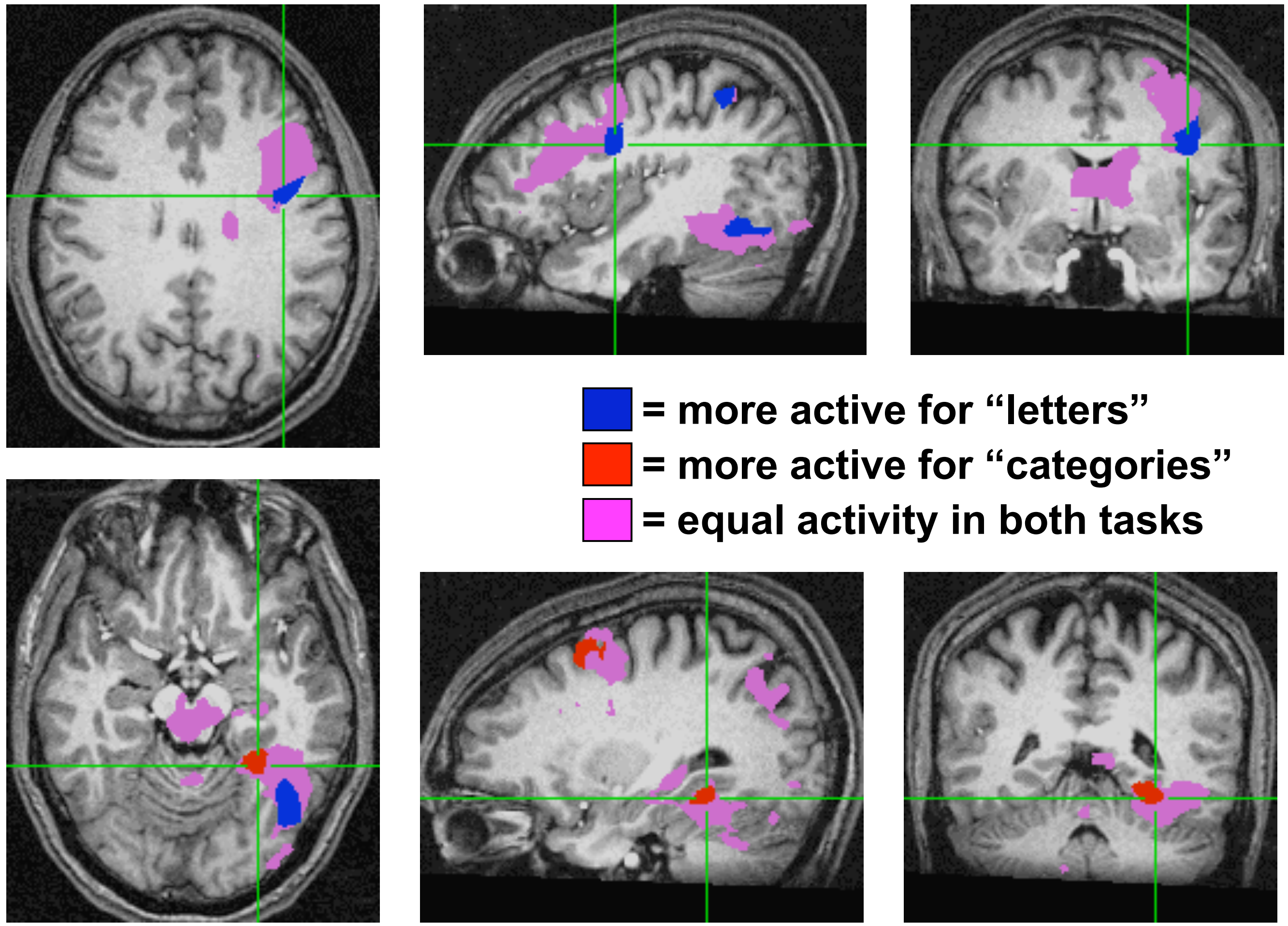
A self-paced overt response fMRI study

S

"Name words that start with the letter S"

Animals

"Name as many animals as you can"



Task-related motion artifacts reduced by using 10s ON/10s OFF block design

Software Tools

- What package to use?
  - Sociological answer: the one your neighbors are using (so you can ask them for help)
  - Having a support system in place is crucial*!*
- SPM: most widely used at present
- *AFNI*: flexible, customizable
  - and has the coolest logo
- FSL: solid package from Oxford
- Numerous other good packages out there
  - Mix-and-match with NIfTI-1 common data format
- Commercial products: MedX, Brain Voyager

Second Set of Conclusions

- FMRI data contain features that are about the same size as the BOLD signal *and* are poorly understood

- **Thus**: There are many "reasonable" ways to analyze FMRI data
  - Depending on the assumptions about the brain, the signal, and the noise

- **Conclusions**: **Understand what you are doing & Look at your data**
  - Or you will do something stupid

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. DS Cohen. DA Jacobson. EC Wong. D Glen.

And* YOU, *the audience …

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