AFNI Introduction (Bob Cox)

AFNI - Analysis of FUnctional NeuroImages
AFNI has become a standard largely because 1) it allows you to see all levels of the data and know what good/bad data looks like, and 2) it’s flexible and can let you do your own variations to analysis

Important Super Scripts:
\texttt{afni\_proc.py} = single subject FMRI pre-processing and time series analysis for functional activation
\texttt{uber\_subject.py} = GUI for \texttt{afni\_proc.py}
\texttt{align\_epi\_anat.py} = image alignment (registration), including anatomical - EPI, anatomical-anatomical, EPI-EPI, and alignment to atlas space (Talairach/MNI)

History: fMRI was discovered in 1991 by Kwong, et. al. He found that MRI-measurable signal increases local in the brain subsequent to increases in neuronal activity. This about 2 seconds in delay, which makes it hard to measure differences between things that are not temporarily separate to a large degree.

Understanding Regression:
- 64x64 matrix (TR=2.5 s; 130 time points per imaging run)
- Somatosensory task: 27 s “on”, 27 s “rest”
- Note that this is \textit{really} good data

\begin{itemize}
  \item \textbf{Red} - expected function to be regressed (respiratory)
  \item \textbf{Black} - real data
  \item \textbf{Blue} - real fit
\end{itemize}

\textbf{Artifact} - Something that is known and needs to be regressed out
\textbf{Noise} - Something that is unknown and cannot be well regressed
Technical Units of AFNI:
The fundamental unit of data in AFNI is the **dataset**, which is a collection of 1 or more 3D arrays of numbers. Each entry in the array is a particular spatial location in a 3D grid (a voxel, a 3D pixel). Each 3D array in a data set is called a **sub-brick** (there is only one number in each voxel in each sub-brick). **NOTE:** In AFNI, counting starts at 0, not at 1.

AFNI Storage Systems:
- `.HEAD` file folds auxiliary information
- `.BRIK` file holds all the numbers in all the sub-bricks

Different ways to view data:
- `+orig` - the way the data looks in the scanner (had have head tilt)
- `+acpc` - AC-PC view (not used so often now)
- `+tlrc` - rescaled to conform to the Talairach atlas dimensions

AFNI at NIH
AFNI can take 2D images in “realtime” from an external program and assemble them into 3D+time datasets slice-by-slice (this whole thing is done automatically on 3Ts 1.5Ts, and 7T)

fMRI Experiment Design and Analysis
**Questions you have to ask in design:**
- Event, block, or hybrid of those?
- How many types of stimuli will you have, and how will they be separated in time?
- How many subjects will you need? (Usually need about 20 in each group to get it even into review let alone published)

**Time Series Data Analysis (individual Subjects):**
- *This is what afni_proc_py does*
- assembly of images into AFNI datasets, registration of time series images (AKA motion correction), smoothing and masking of images, spatial normalization into normal space
- fit statistical model o timing+hemodynamic responses to time series data (can be fixed-shape or variable-shape response models)

**Group Analysis (Inter-Subject):**
- Depending on how much smoothing done at subject stage, may want to do more (but usually not)
- voxel-size analysis or ROI averaging
- ANOVA+ to combine and contract activation magnitudes from various subjects
Experiment Design Types:

- **Block Design:** long duration activity
- **Event-Related Design:** short duration activity
- **Hybrid Block-Event Design**

Event Related Design Types:
- **Slow** - separate activations in time so you can model the fMRI response from each separately.
- **Rapid** - Need to make inter-stimulus intervals vary if there is any potential time overlap in their fMRI response curves (if closer than 12-15 in time).

- to3d (gets images into AFNI format)

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**FMRI Data Analysis at Individual Level**

Overview

- **Basics of Linear Modeling**
  - Linear Regression is the relationship between the fMRI signal and explanatory variables (regressor)
  - there is a dependent variable and one or more explanatory (independent variable)
  - Simple Regression - fit data to a straight line
  - Assumptions
    - linearity (additivity of the effects)
    - white noise (independence) and Gaussianity
  - Various Stat Tests
    - t-test for each group
    - t-test for the linear combination f beta values MINUS the general linear test
    - F-test for composite null hypothesis
    - Omnibus or overall F-test for the whole model

- **Linear Modeling for fMRI**
  - Time series regression, assume that data y is time series from 1 voxel
  - GLM (Generalized Linear Model) - assume the same model series for every voxel in the brain, extract and model the series then do a beta analysis on those (gives activation values)
    - this is voxel-wide analysis or massively univariate method

- **FMRI Experiment Types**
  - Experiment Setup
    - Number of subjects
    - Number of Conditions (want 10 or more blocks per subject)
    - Sample size (repetitions) per condition
- Block, event-related, or mixed?
- Inter-stimulus Interval (ISI)

- Scanning Parameters
  - TR
  - voxel size
  - data points (volumes)
  - slice sequence (sequential or interleaved)
  - slice thickness
  - removing first few TRs

- Scanning terms
  - Run - continuous scanning, a brief break before next run
  - Session: subjects come back after long period of time
  - Experiment or Study

- Types of fMRI Experiments
  - Block (boxcar design)
  - Continuous (analyzed more like resting state)

- fMRI Data
  - Ideal Data Partition: data = Signal + Noise
  - Signal - BOLD response to stimulus
  - Noise - components in data that interfere with signal
  - Real Data = baseline + slow drift + other effects of no interest + response₁ + ... + responseₖ + noise
  - Baseline = baseline + drift + other effects of no interest
    - drift: psychological/physiological effect, thermal fluctuation
    - Data = 'baseline' + effects of interest + noise
  - baseline condition (and drift) is treated in AFNI as baseline, an additive effect, not an effect of interest
    - SPM and FSL to a high pass of the data instead
  - In AFNI baseline + slow drift is modeled with polynomials
    - longer run needs higher order of polynomials
    - with n runs, n separate sets of polynomials are needed to account for temporal discontinuities across runs
      - (m(p+1) columns for baseline + slow drift: with p-order polynomials

- Statistical Testing
  - Effects (regression coefficients) of interest
    - beta - effect relative to baseline condition by default in FNI
    - t-statistic - statistical significance
  - Pairwise Comparisons (contrasts)
    - Conditions: Beta(a) vs Beta B) (Like house vs. Face)
    - t-statistic: statistical significance
  - General Linear Test - linear combination of multiple effects
  - Composite tests - F-statistic for composite null hypotheses (a big F means that at least one of the hypotheses is probably valid, but it doesn’t tell you which one so usually must be followed by secondary analyses

- Assessing Fixed-Shape Impulse Response Function RF Approach
  - We assume that the brain responds with the same shape across 4 levels:
    - subjects
    - activated regions
- stimulus conditions
- tasks, trials
- Block design - the shape is usually not important due to accumulating effects of consecutive events
- Event-related experiment: OK most of the time

**No Constraint on IRF Shape**
- Yardstick (or TENT perspective)
  - each TENT is a basis function (just sine and cosines that you use to build up other more intricate functions)
  - Set multiple tents are various equally spaced locations in the TR

- 5 equally-spaced tent functions (yardsticks): linear interpolation between “knots” with \( TENTzero(b,c,n) = TENTzero(0,12,7) \)
  \[
  h(t) = \beta_1 \cdot T\left(\frac{t-L}{L}\right) + \beta_2 \cdot T\left(\frac{t-2L}{L}\right) + \cdots + \beta_5 \cdot T\left(\frac{t-5L}{L}\right)
  \]

- Tent parameters are easily interpreted as function values (e.g., \( L \): tent radius; \( \beta \): response (tent height) at time \( t = 2L \) after stimulus onset)
- Relationship of tent spacing \( L \) and TR \( L = n \cdot TR \), e.g., with TR=2s, \( L=2, 4s \)
- In `4d_MFMk` or `3dDeconvolve` with `TENTzero(D, n)`, specify duration \( D \) of HRF and number \( (n) \); radius \( L = D / (n+1) \) with \( (n+2) \) full tents, each tent overlaps half tent with two neighboring ones.
  
  - 3dDeconvolve - (in FSL it’s finite impulse response regression, mathematically the same)

**Intermediate Approach (SPM offers this, and AFNI can do this)**

Balance in shape flexibility and basis functions
- Constrain the HDR shape with a principal basis function
  - SPMG1 (similar to GAM in AFNI): \( e^{(a_1 t^2 + a_2 t^3)} \) where
    - \( a_1 = 0.00833333333 \) \( p1 = 5 \) (main positive lobe)
    - \( a_2 = 1.274527e-13 \) \( p2 = 15 \) (undershoot part)
- 2 or 3 basis functions: parsimonious, economical
  - SPMG1+SPMG2+SPMG3
  - SPMG2: temporal derivative capturing differences in peak latency
  - SPMG3: dispersion derivative capturing differences in peak

**Canonical HRF**

```
  Temporal derivative
  Dispersion derivative
```

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Hands-On AFNI Regression Analysis

- Voxel-wise regression model:
  \[ y = X\beta + \varepsilon \]
  - Voxel-wise regression model: \( y = X\beta + \varepsilon \)
    - \( y \): signal (time series) at a voxel – different across voxels
    - \( X \): explanatory (independent) variables (regressors) – same across voxels
    - \( \beta \): regression coefficients (response strength) – different across voxels
    - \( \varepsilon \): residuals (anything we can’t account for) – different across voxels

- Graph Mode
  - Most common motion is nodding, so you should look at the sagittal image for motion the most
  - hitting A will auto scale all of your voxels to the regressor as you move around the brain

Alignment and Atlases (Reynolds)

- Alignment goals and tools in AFNI
  - EPI data across time in a single run or across runs to a base image!
  - 3dvolreg – motion correction (rigid), align data to template
  - 3dWarpDrive, @auto_tlr – align similar volumes (affine) even across subjects!
  - 3dQwarp, auto_warp.py – align similar volumes nonlinearily to template, align images across modalities – EPI to anat!
  - 3dAllineate – align different or similar volumes
  - align_epi_anat.py – general alignment script to align EPI with anatomical data!
  - Correct for motion between two volumes by aligning in two dimensions using corresponding slices!
    - @2dwarper.Allin – non-linear alignment of slices!
    - @2dwarper, 2dimreg limit alignment to specific plane!
  - Nudge plug-in - visually align two volumes, rotate by known amount between volumes!
  - 3drotate – moves (shifts and rotates) volumes!
  - 3dWarp – make oblique, deoblique to match another dataset!
  - Put centers of data from outside sources in roughly the same space!
    - @Align_Centers, 3dCM – put centers or centers of mass of dataset in same place
    - 3dTagalign, tagset plugin – place and align volumes using corresponding fiducial marker points
    - imreg – align two 2D images!
  - Measuring the Error between the Transform and Target Image
    - 3dvolreg is designed to run very fast for EPI to EPI registration with small movements (a few seconds)
- **3dWarpDrive** is slower, but it can have up to 12 parameters affine transformation

- **Looking for Motion**
  - Seeing Motion afni GUI
    - Image - do video (click v while on graph) r use arrow keys
    - Graph - spikes in the voxel data
  - Motion in Results
    - activation and deactivation occurs in high contrast around edges of brain

- **Mitigating Motion**
  - Can’t have paradigms where there is movement in the scanner during the responses (for example, someone had participants doing sign language or talking, and this made so much noise it was pretty much untouchable)
  - Do you have a high and low moving population that could make your group effects mostly motion? (kids vs. adults, some clinical populations vs. TDs)
  - Look at your data **before and after** data correction, there are things that can go wrong and it’s a good way of knowing where your data effects might be coming from

- **Cross Modality Registration**
  - lpc - Local Pearson Correlation (looking for the negative correlation). It targets the difference in CSF that is bright in EPI and dark in (structural?)
  - **align_epi_anat.py**
    - combines deoblique, motion correction, alignment and talairach transformations into a single transformation. Also does slice time correction and applies transformations to “child” datasets
  - **@AddEdge display** - cyan is structural, blue is epi, and red is the right overlap
    - should look at it in 3 planes, look around insula, general cortex, mid-sagittal (definitely both hemispheres)

- **Different Ways to Align Visualization in AFNI**
  - **Graph and image** – travel through time for motion correction or for a thousand datasets in a row.
  - **Multiple controllers and crosshairs** – up to ten datasets at a time, quick and rough.
  - **Overlay display** – opacity control, thresholding. A single pair – good for different or similar datasets.
  - **Overlay toggle, Underlay toggle** – wiggle, good but a little tricky (‘o’ and ‘u’ keys in image viewer)
  - **Checkerboard Underlay** – two similar datasets in underlay but must be virtually identical.
  - **Edge display for underlay** – effective pairwise comparison for quick fine structure display and comparison with overlay dataset with opacity. One dataset should have reliable structure and contrast. Now with ‘e’ toggle
- **AddEdge** – single or dual edges with good contrast for pairwise comparison.

- **Transformation Chain**

  ![Transformation Chain Diagram]

  - **NOTE:** for alignment across 2 sessions, go to:
    

- **Atlases**
  - Atlases have segmentation information
    - examples: TTatlas+tlrc, TT_N27_EZ_ML+tlrc
  - Stage 1: Alignment to acpc coordinates (automatic, but can be done manually, see below)

  ![Atlas Diagram]

  - Stage 2: Scaling to Talairach-Tournoux (tlrc)
    - Talairach comes from a French woman’s brain
Templates you can use in @auto_TLC

- **TT_N27+tlrc**: AKA “Colin brain”. One subject (Colin) scanned 27 times and averaged. (www.loni.ucla.edu, www.bic.mni.mcgill.ca) Has a full set of FreeSurfer (surfer.nmr.mgh.harvard.edu) surface models that can be used in SUMA (link).
  - Is the template for cytoarchitectonic atlases (www.fz-juelich.de/ime/spm_anatomy_toolbox) For improved alignment with cytoarchitectonic atlases, I recommend using the TT_N27 template because the atlases were created for it. In the future, we might provide atlases registered to other templates.


- **TT_EPI+tlrc**: EPI template from spm2, masked as TT_avg152T1. TT_avg152 and TT_EPI volumes are based on those in SPM's distribution (www.fil.ion.ucl.ac.uk/spm/)

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### Start to Finish - How to Analyze Data in AFNI

- **Step 1**: CD into the drive and check on motion
- **Step 2**: run **ubersubject.py**
  - this opens a grey box, enter in subject ID and group ID
  - **for rest**: lick on the analysis initialization and change type to rest
    - this will initialize to going bandpass (which is standard but he doesn’t recommend)
    - better to put bandpass in the regressor model and not do it separately
  - **anatomical dataset**: click browse anat, then put in the anat HEAD file
  - **EPI datasets**: click browse and add ALL epi HEAD files
  - **stimulus timing files**: browse stim and add the txt files (usually a visual and audio file)
    - under, there is init basis funcs: offers common ones or can be typed in
  - **Symbolic GLTs**: Click on init with examples
  - **Expected Options**:
    - remove first 2 TRs (or more, depending on protocol)
- volume registration base:
  - can click choose and choose MIN_OUTLIER
- **blur size**: make 6.0
- **motion censor limit**: (for adult .3 is good, for populations like children, might go up to 1., but that will have a big impact on the BOLD signal)
- **extra regress options**: if you have more CPus you can make that bigger to speed up processing
- Once all aspects are entered, **click top left button on the GUI** (looks like piece of paper)
  - this will create a processing script and open it on the screen (afni proc command)
  - terminal will show where it saved the afni_proc.py command (should be the terminal you opened into)
- **click on the top middle button**, will open an output text from afni_proc.py
- **click on green button** and it will start the analysis (this is the button form of tcsh)

- **Step 4**: Outcount_rail.1D - look at this graph (motion) and you should really consider censoring points that are above .1
- **Step 5**: 1dplot -sepscl motion_demean.1D
- **Step 6**: Set overlay to Stats (at bottom), underlay is EPI

**NOTE:** Sometimes you’ll see the biggest BOLD effect outside of the brain, that could be a draining vein pushing blood out of the cortex.

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**Advanced Features of AFNI**

- **Amplitude Modulated (AM) Regression**
  - have some extra data measured about each response to stimulus that might be modulating the BOLD response
    - ex: reaction time, Galvanic skin response, emotional valence
  - Auxiliary Behavioral Information (ABI)
    - AM regression is continuous with ABI levels
    - 3dDeconvolve is a linear program, so must make the assumption that the change in BOLD as ABI changes is linearly proportional
  - Need 2 regressors:
    - The mean fMRI response (usual -stim_times analysis)
      - yields standard activation map
    - variations in the fMRI response as the ABI data varies
      - yields second activation map of places whose BOLD response changes with ABI

- **Addressing Noise Issues**
  - Somewhat known noise come from:
    - MR thermal noise (known and removable)
    - cardiac and respiratory (partly understood)
      - regressed by RETROICOR program
    - scanner fluctuations (hardware, timing errors)
    - small subject head movements (within 10-100 mm)
    - very low frequency fluctuations (longer than 100 seconds)
- **3dREMLfit**
  - gives an estimate correlation structure of noise and also an estimate of beta fit parameters using more efficient “generalized least squares”
  - The outputs are similar to those in 3dDeconvolve
  - Below is an example of how it gets rid of noise outside of the brain

  ![AFNI_3dREMLfit examples](image)

- **Nonlinear Regression**
-  

- **Spatial Models of Activation**
  - General Idea
    - smooth data in space before analysis
    - Average data across anatomically-selected regions of interest ROI (before and after analysis)
    - Reject isolated small clusters of above-threshold voxels after analysis

- **Multi-Voxel Statistics (Spatial Clustering and False Discovery Rate: “Correcting the Significance”)**
  - Need to be able to make a basic decision about each voxel about whether it is active (about 50-200k voxels in each scan)
  - Family-Wise Error (FWE)
    - with N novels, what is the chance to make a false positive error (type 1) in one or more voxels?
    - NOTE: The constraint on the per-voxel (“uncorrected”) p-value is so stringent that we would end up rejecting a lot of true positive (type 2 errors)
  - Multiple testing problem in fMRI
    - 3 occurrences of multiple tests: Individual, Group, and Conjunction (group analysis is the most severe situation)

- **Two Approaches to Multiple Comparisons**
  - Control FEW to keep expected total number of false positives below 1
  - 1) Typically, the Bonferroni correction is used, but it’s made with bad assumptions for fMRI
  - 2) FWE control in AFNI: 3dClustSim
- 3dClustSim does Monte Carlo simulations with a random set of 10,000 variables
  - this outputs the simulated overall significance level and the corresponding minimum cluster size

- **False Discovery Rate**
  - FDR - accept the fact that there will be multiple erroneous detections when making lots of decisions
    - control the fraction of positive detections that are wrong (or at least control the expected value of this reaction)
  - Uncorrected p-value of h is the probability that F > h when the null hypothesis is true
    - the "corrected p-value is the probability that any voxel is above the threshold in the case that they are unactivated
  - FDR q-value of h is the fraction of these false positives expected when we set the threshold to h

**Basic Ideas Behind FDR q**

- *If all the null hypotheses are true, then the statistical distribution of the p-values will be uniform*
- Deviations from uniformity at low p-values → true positives
- Baseline of uniformity indicates how many true negatives are hidden amongst the low p-value region

**How q is Calculated from Data**

- 1) compute p values for each statistic
- 2) sort them from least to greatest
- 3) by tracking where in the voxels each p came from, can put 1-values back into image (this is what 3dFDR does)
- 4) by keeping track of the stats values for each p and where it came from, create a curve of the threshold h vs. z(q)
- **FDR Statistical Issues**
  - FDR is conservative (q-values are really big) when voxels are positively correlated (from spatially smoothing)

**Group Analysis in AFNI**

- **Basic Concepts**
  - Why the two tere step (individual then group)
    - we don’t do them at once because there is typically heterogeneity in data or experimental design across subjects

- **Group Analysis Approaches**
  - **Group Analysis Caveats**
    - conventional - voxel-wise (brain) or node-wise (surface), you need to be sure that you have the proper model to account for cross and within-subject variability
  - Results - two components on AFNI (OLay +Thr)
    - effect estimates have unit and physical meaning
    - their significance is relative (response to house significantly > face)

- **Terminology - Explanatory Variables**
  - **Response/Outcome variable** - (HDR) regression coefficients
  - **Factor** - categorical, qualitative, nominal or discrete variable
    - within subject (repeated measures) factors
    - Subject-Group Meaning - (sex, normal/patients)
    - Between-subjects factor
    - gender, patients/controls, genotypes
    - subject - random factor measuring deviations

- **Quantitative Covariate**
  - Three usages of covariate - quantitative, variable of no interest (scanner, sex, handedness), or explanatory variable (like IQ, reaction time)

- **Fixed-Effects Factor** - categorical (qualitative or discrete)
  - treated as a constant in the model
  - all levels of a factor are of interest (main effect, contrasts among levels)
  - Fixed in the sense that they apply only to specific levels of the factor (discrete like human, tool) and thus cannot be generalized

- **Random Effects**
  - random variable in the model - exclusively subject in fMRI

- **Omnibus tests** - main effect and interaction
  - main effect - any difference across levels of a factor
  - interactions - with more than two factors, interaction may exist
  - Solutions: reduction
    - pairwise comparison
    - Plotting ROI
    - Requires sophisticated modeling: AN(C)OVA: 3dANOVAx, 3dMVM, 3dLME
    - there can be nonlinear effects
- **Models at Group Level**
  - Better to take both effect estimates and t-statistic
    - t-statistic contains precision information about effect estimates
    - each subject is weighted based on precision of effect estimate

- **Group Analysis in Neuroimaging: Why Big Models?**
  - t-test: one, two-sample, and paired
  - ANOVA - one or more categorical explanatory variables (factors)
  - GLM: AN(C)OVA
  - LME

- **Choosing a program**
  - Two Perspectives
    - data structure
    - ultimate goal: list all the tests you want to perform
  - Most analyses can be done with 3dMVM and 3dLME
    - BUT this is computationally inefficient and should only be used as a last resort
  - T-tests
    - 3dttest++ and 3dMEMA
    - Better to use ESM for FSM?

- **Choosing a Template**
  - same population type (adults, children, monkeys) and same modality (T1 to T1 for example)
  - Must have the relevant atlas segmentation
  - Individual or group template
  - OR make our own template

- **MNI vs TLRC**
  - there isn't much difference. MNI is just a little bit bigger
  - the original TLRC does not have a template, but N27 template is put into it

- **Going Between TLRC and MNI:**
  - Approximate equation - used `whereami` and 3dWarp
  - Manual TLRC transformation of MNI template to LRC - used `whereami`, based on N2
  - Where am I from GUI
    - right click on location on image and click on "where am i", this will show where you are in all of the respective atlases
  - AFNI-ATLAS_COLORS
  - Defining your own ROI by hand
Go to Define Data Mode > Plug-IN > Draw ROI
Set the dataset for coping
choose ROI type
middle click and drag to draw on image

- **TTEST**
  - cd into file
  - uber_ttest.py
    - must define script output

**SUMA Introduction**
- We tend to look at voxels, 3d pixels with 6 neighbors
- **SUMA - Surface Mapping with AFNI**
  - AFNI works with data defined over volumes
  - SUMA works with data defined over surfaces
- **PreSUMA (setup phase)**
  - collect and align (on GE 3T, the 2-4 MPRAGE is good for this
  - correct image non-uniformity
  - 3dUNIFORMIZE (or N3 normalization tool are good)
    - can use FreeSurfer, SureFit, or BrainVoyager
- **CircumSUMA**
  - create standard-mesh version of surface models and align surface to experimental data
  - use @SUMA_AlignToExperiment

**A: Preparing surface models for SUMA**

- High-Res. Anatomical MRI data
- Create Surface Models (FreeSurfer, Caret, etc.)
- @SUMA_Make_Spec_-
- SurfVol AFNI format Surface Volume that is aligned with surface models
- Spec File ASCII file defining relationships between different surfaces

- Things tend to go wrong in Occipital areas and the inferior temporal

- **Steps**
  - 1. run @SUMA_Make_Spec_-
  - 2) Scroll through volume to make sure surfaces are accurate
  - 3) cd into freesurfer surfaces
  - 4) press ‘t’ in SUMA window to ‘talk’ to AFNI
    - this will show you the surface lines n your AFNI anatoidal image
    - you can change the colors and node box visibility from the Control Surface button in AFNI
- **Basic Suma Viewer Functions**
  - Left Button - been down while moving mouse left and right (Y axis)
- can also use arrow keys
- CNTL + SHIFT + arrow keys it will give you the 90 angle sides
- CNTL + left click should open up the brain and let you see inside
- To pry open from the top and bottom instead of right and left:
  - FUNCTION + SHIFT + F10
- Double click CNTRL to get back to original view
- CNTRL + SHIFT to get it facing coronal again

- **Saving an Image in SUMA**
  - SNAPSHOT - To snapshot an image, hit the ‘r’ key while on the image. This should bring up a snap shot of that image
  - SAVE - to save an image, hit CNTL+r, and it will create it in the same directory that you are running SUMA from
  - If you want a higher resolution image, you have to physically make the image bigger then save or snapshot

- **Help in SUMA**
  - CNTL + H, then you can type whatever you are trying to find or do in suma
  - Whelp button in that viewer gives you web help

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### Interactive SUMA

- **Mapping Data**

- **Aligning Surface with Experiment Data**
  - **align_epi_anat.py** - will align anatomical to EPI (surface Volume is aligned to experiment’s anatomical volume with rigid-body or affine transformation)
  - **@SUMA_AlighnToExperiment** - simplifies the above step (and rarely fails)
    - if this doesn’t work, use **3dTagalign**
  - **Mapping fMRI Data onto Surface** Choosing Mapping Optins - Consider whether to do surface or shell intersection. The cortex is 3d, not 2d, if you only take surface volume you may be loosing some of the computational information
- **Looking at Time Series**
  - CNTL + S or go to view > object controller

- **To Draw ROI**
  - on SUMA image, click Tools > Draw ROI
  - right click and drag to draw

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**PsychoPhysiological Interactions (PPIs)**

- **PPIs are interactions between tasks (psychology) and deconvolved BOLD signal (physiology)**
  - PPI betas are measured above and beyond the main (average)
  - PPI betas are also measured above overall seed effects
  - want to look at interaction of tasks within seed values

- **Advantage of Betas as measure of Effect (rather than using r)**
  - meaning is more like a task effect
  - not affected by relative duration of stimulus classes
  - should have more reasonable distribution
  - can compute betas for all task conditions at once
  - *could temporally partition seed per task, except that the seed contains task (physiology) convolved with IRF*
  - Allows for non-TR-locked events
  - durations are not TR-locked
  - Decon/Recon steps are almost inverses

- **Overview of Processing Steps**
  - Generate seed time series - ROI average of errts from original regression
  - Deconvolve seed TS to “neural” timing
    - cannot cross run breaks
    - deconvolve using consistent basis function (block?)
  - Partition neuronal data per ask
    - Yields one neural time series per task (like visual or auditory)
      - same as time series put zero out the time series that are not for your class of interests, should give you what is happening instantaneously from the class that is of interest
  - Re-convolve with basis
    - still per run, then concatenate
- Add PPI regressors with seed to regression model
  - you'll have for example an audio and a visual PPI, and you'll have the seed. May not make sense to put the seed back in but that's how it's don't right now

- Running PPI
  - using the results in cd subject_results/group.horses/FT.results
  - afni
  - generate seed time series by going to stats.FTin overlay(?) then choose the OLay contrast (on the far right)
  - Clusterize, then on that hit plot. You will have the voxel fluctuation for that ROI.
  - you can click save on that screen and it will save a 1d file (time series in text file)

- Converting this to PPI
  - TR is 2, but upsample the TRnp (PPI) so it's nearly continuous
  - timing_tool.py will make a neural timing file (???)

- Partition the time series
  - upsample ROI time by factor of 20
  - should look exactly like it did before (look at it in 1dplot seedfilename.1D

- Deconvolve
  - deconvolved time series should be compressed around zero and less expanded over time

- Partitioning into Classes
- Using ReBOLDed Time series
  - not separated in BOLD time, but separated in neural time (the theoretical difference between them is fixation period
  - downsample back ot TR 2
  - concatenate across all runs

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AFNI InstaCorr

- Basic Ideas
  - In resting state we don’t have timing of a task to link parts of the data to
  - Instead, we are looking at correlations
  - InstaCorr - instantaneous correlation map of resting state data with interactively selected seed level

- Steps
  - setup phase - preps data for correlations (takes about 10 or more seconds)
    - For setup, you should mask: user selected or Automask
    - Bandpass and other filtering of voxel time series
    - Burring inside mask
  - Correlations phase - select seed voxel, correlation map appears (by magic)
    - correlate selected seed voxel time series with all other prepared voxel time series
    - make new data set, if needed, to store results
    - save seed time series for graphing
    - Redisplay color overlay
    - Optional: compute FDR curve for correlations
      - this calculation is slow, so FDR is not turned on by default

- To get instacorr running in AFNI
  - go to define overlay
  - Set InstaCorr (top right corner)
  - Setup Icorr
    - Star, end (set 2, to get rid of start voxel peak)
- Blur (set to 5 for raw data)
- Automask
- Set Bandpass
- Despike - Yes
- Global Orts (optional, extra time series to be removed from the data, usually motion)
- SeedRad - Possibly best to match to blur
- Setup+Keep (saves) Setup+Quit (RUNS it)
- Right click and do InstaCorr Set on Image

- **Bandpassing**
  - in resting state we are looking at the BOLD effect, which isn’t fast. Fluctuations that are fast are assumed not to be BOLD effect, so even if they are correlated they aren’t interesting (artifacts, like heart rate)
  - highest frequency they currently allow is .1, which is an oscillation in about 10 second

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### Anaticor

- How Anaticor works
  - Move ball (radius = 30mm) around a grey matter voxel and then it makes a voxel-specific regressor (via 3dTproject)
  - Eroded WM time courses - most coherent structure in the white matter is artifact, so we regress it out of the data (along with motion, bandpassing, physio)
  - **Anaticor does this nuisance regressor process for every single voxel in the brain**

- Resting state can only describe the relationships between brain regions
  - Interpret correlation strength as proxy for brain function coupling between reed regions

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The Trouble with Resting State

- We have no model for signal, and no good models for noise
- Effect size (s correlation) is a spatially varying function of noise

**Physiological Recording**

- Physio is recorded at a much more rapid rate than the TRs, and must be transformed to those TR Rates
Also, some clinical populations have different breathing rates (see this easily for anxiety, but also in things like ASD). Need to be aware of this while looking at group level.

- can be seen most easily when you see that the ventricles are more activated in one group than another

- **Anatomical Bias**
  - If concerned about systematic differences in anatomy, consider:
    - surface-based analysis with smoothing on the surface, or smooth within gray matter mask nly
    - ROI-based analysis with ROIs restricted to grey-matter voxels in each subject
  - **Check the EPI to T1 by eye to be sure (really should do this)**

- **Tissue-Based Nuisance Regressors**
  - Avoid Projecting Fluctuations f Interest
    - OK to sample nuisance signals from regions whose fluctuations are not correlated with the fluctuations of interest in the regions of interest
    - Should not project out time series containing aggregates of fluctuations of interest, even if they contain contribution from noise
    - saggital sinus voxels might allow sampling of aliased heart rate, BUT they also exhibit BOLD fluctuations of interest from the regions being modeled
  - And Why Not?
    - because you end up differentially biasing the correlation matrices of your groups, and considerably distorting group differences

- Why not GSReg?
So when you look at the difference between the two, then some people argue that it makes the differences more clear. The problem with this comes from when you go to group analysis because the change (or bias) in correlation after GSR is dependent on the unnormalized global correlation matrix.

- Suppose you had two groups where you thought there was a difference in this global correlation matrix
  - But if the global structure is difference between the two groups, then the GSR regressed matrix will be dependant on the group you are in, and not really with regards to the intra-group differences

- **Regional pair dependent biasing is OK is:**
  - Not interpreting correlations between regions as those between the sampled BOLD signals and by extension neuronal signals
    - Not just about interpretability of negative correlations
    - Two strongly correlated regions after GSReg DOES NOT imply regions were strongly correlated before GSReg

**An illustrative model**

**Comparing Groups**
- Same Holds with Empirical Data

Interactive AFNI Daniel G
- Make sure you are cd’ed into the file that you’ll be pulling from before you open AFNI
- Click on any of the slice images and press ‘v’ on keyboard to get a video view of them, you can stop it at any time by pressing any key
- REI and SPM have different XYZ order (in top left corner of AFNI), you can right click on this to change that order
- **BHelp** - (left bottom corner), click on this and makes it into a “hand” cursor, then you can click on anything and it will tell you what those buttons mean in AFNI
- **AFNI Tips** -
  - Click on brain slice images themselves, and if you drag up/down it changes brightness, drag right/left and it changes contract. You can always just click norm on the right top to make it go back to original view.
  - **Z** on right of slice screen is the zoom button (upper Z zooms in, lower Z zooms out)
  - Pan will let you move around the image (panoramic)
  - **L** on keyboard can switch from left being left to left being right view
  - 2% gets mapped black, 98% gets mapped white, everything else gets made a contrast in between
    - hit M on the slice and you can make it relative contrast scaling
    - each slice is scaled separately
  - To save an image file, click on the bottom middle button **Sav1.ppm**, right click on it and can make it a jpeg, etc. (should save in your data cd)
  - To the right of that, **Mont** will give Montage of slices (whole list of images throughout brain slices). Let you see the whole volume at once.
  - If you open two AFNIs (A and B controller, for examples) they will be locked together. You can undo this by going to Define Datamode > Lock

Within Time Series
- On AFNI, click Graph (next to image on axial, sagittal coronal box)
- click shift+M on the graph and it will make the set of voxels bigger
- **For Overlay**
  - There is a hidden menu in the color bar, you can right click and change the color scale there
  - click on clusterize and you can change the range for the clusters and their respective colors

**New Overlay Tricks**
- If you click 4, then you can toggle within the image window and see the structural toggle with functional (underlay and overlay)
- If you click 6, then you can get a gradient that will pull away the underlay
- If you click 3, then you can get a checkerboard to compare alignment of two images

- In Clusterize, if you click bi-sided then it will choose the negative and positive clusters separately
- RPT button to the right of Clusterize will show exactly where the clusters are and what their values are