Group Analysis with AFNI - Hands On

- The following sample group analysis comes from "How-to #5 -- Group Analysis: AFNI 3dANOVA2", described in full detail on the AFNI website: http://afni.nimh.gov/pub/dist/HOWTO/howto/ht05_group/html
- Brief description of experiment :
 - ♦ Design:
 - ➡ Rapid event-related
 - ♦ Independent Variable \Rightarrow "Stimulus Condition" with 4 levels:
 - → TM = Tool Movie
 - → HM = Human Movie
 - ➡ TP = Tool Point Light
 - → HP = Human Point Light



Tool Movie



Human Movie



Tool Point Light



Human Point Light

- ♦ Data Collected:
 - → 1 Anatomical (SPGR) dataset for each subject
 - ⇒ 124 sagittal slices
 - → 10 Time Series (EPI) datasets for each subject
 - ⇒ 23 axial slices x 138 volumes = 3174 volumes/timepoints per run
 - note: each run consists of random presentations of rest and all 4 stimulus condition levels
 - \Rightarrow <u>TR</u> = 2 sec; voxel dimensions = 3.75 x 3.75 x 5 mm
 - Sample size, <u>n</u>=3 (subjects ED, EE, EF)
 - note: original study had <u>n</u>=10

• Analysis Steps:

- Part I: Process data for each subject first
 - ➡ Pre-process subjects' data ⇒ many steps involved here...
 - Run deconvolution analysis on each subject's dataset --- <u>3dDeconvolve</u>
- ♦ Part II: Run group analysis
 - 2-way Analysis of Variance (ANOVA) --- <u>3danova2</u>
 - \Rightarrow i.e., Stimulus Condition (4) x Subjects (3) = 2-way ANOVA

PART I \Rightarrow **Process Data for each Subject First:**

- ♦ Hands-on example: Subject ED
- \diamond We will begin with ED's anat dataset and 10 time-series (3D+time) datasets: EDspgr+orig, EDspgr+tlrc, ED r1+orig, ED r2+orig ... ED r10+orig
 - → Below is ED's ED r1+orig (3D+time) dataset. Notice the first two time points of the time series have relatively high intensity*. We will need to

remove them later:



Images obtained during the first 4-6 seconds of scanning will have much larger * intensities than images in the rest of the timeseries, when magnetization (and therefore intensity) has decreased to its steady state value

<u>STEP 1</u>: Volume Register and time shift the voxel time series for each 3D+time dataset using AFNI program <u>3dvolreg</u>

- ♦ We will also remove the first 2 time points at this step
- Solution Strate Stra

- <u>-base</u>: Timepoint 2 is our base/target volume to which the remaining timepoints (3-137) will be aligned. We are ignoring timepoints 0 and 1
- <u>-tshift 0</u>: For each run, the slices within each volume are being time shifted prior to volume registration. This is done to correct for the different sampling times for each slice
- <u>-prefix</u> gives our output files a new name, e.g., ED_r1_vr+orig
- ED_r{\$run}+orig'[2..137]' refers to our input datasets (runs 1-10) that will be time shifted and volume registered. Notice that we are removing timepoints 0 and 1

Subject ED's newly created time shifted and volume registered datasets:

```
ED_r1_vr+orig.HEAD ED_r1_vr+orig.BRIK
... ... ...
ED_r10_vr+orig.HEAD ED_r10_vr+orig.BRIK
```

 $\diamond\,$ Below is run 1 of ED's time shifted and volume registered datasets,



ED r1 vr+orig



• <u>STEP 2</u>: Smooth 3D+time datasets with AFNI <u>3dmerge</u>

- The result of spatial blurring (filtering) is somewhat cleaner, more contiguous activation blobs
- Spatial blurring will be done on ED's time shifted, volume registered datasets:



- -1blur_rms 4 sets the Gaussian filter to have a root mean square deviation of 4mm (You decide the width of the filter)
- <u>-doall</u> applies the editing option (in this case the Gaussian filter) to all sub-bricks uniformly in each dataset

-6-

Result from 3dmerge:



Before blurring

After blurring

• **<u>STEP 3</u>**: Normalizing the Data - Calculating Percent Change

- This particular step is a bit more involved, because it is comprised of three parts. Each part will be described in detail:
 - A. Set all background values (outside of the volume) to zero with <u>3dAutomask</u>
 - B. Do a voxel-by-voxel calculation of the mean intensity value with **<u>3dTstat</u>**
 - C. Do a voxel-by-voxel calculation of the percent signal change with **<u>3dcalc</u>**

Why should we normalize our data?

- Normalization becomes an important issue when comparing data across subjects, because baseline/rest states will vary from subject to subject
- The amount of activation in response to a stimulus event will also vary from subject to subject
- ➡ As a result, the baseline Ideal Response Function (IRF) and the stimulus IRF will vary from subject to subject -- we must account for this variability
- By converting to percent change, we can compare the activation calibrated with the relative change of signal, instead of the arbitrary baseline of FMRI signal

♦ For example:

Subject 1 - Signal in hippocampus goes from 1000 (baseline) to 1050 (stimulus condition)

Difference = **50** IRF units

Subject 2 - Signal in hippocampus goes from 500 (baseline) to 525 (stimulus condition) Difference = 25 IRF units

- ♦ Conclusion:
 - Subject 1 shows *twice* as much activation in response to the stimulus condition than does Subject 2 --- WRONG!!
 - If ANOVA were run on these difference scores, the change in baseline from subject to subject would add variance to the analysis
 - We must control for these differences in baseline across subjects by somehow normalizing the baseline so that a reliable comparison between subjects can be made

-9-

- ♦ Solution:
 - ➡ Compute Percent Signal Change
 - i.e., by what percent does the Ideal Response Function increase with presentation of the stimulus condition, relative to baseline?
 - Percent Change Calculation:
 - $\Rightarrow If \mathbf{A} = \underline{Stimulus IRF}$
 - \Rightarrow If **B** = <u>Baseline IRF</u>

Percent Signal Change = (A/B) * 100%

-10-

-11-

(1050/1000) * 100% = 105% or <u>5% increase in IRF</u>

♦ Subject 2 -- Stimulus (A) = 525, Baseline (B) = 500

(525/500) * 100% = 105% Or <u>5% increase in IRF</u>

- ➡ Conclusion:
 - Both subjects show a 5% increase in signal change from baseline to stimulus condition
 - Therefore, no significant difference in signal change between these two subjects

<u>STEP 3A</u>: Ignore any background values in a dataset by creating a mask with <u>3dAutomask</u>

- Values in the background have very low baseline values, which can lead to artificially large percent signal change values. Let's remove them altogether by creating a mask of our dataset, where values inside the brain are assigned a value of "1" and values outside of the brain (e.g., noise) are assigned a value of "0"
- This mask will be used later when the percent signal change in each voxel is calculated. A percent change will be computed only for voxels inside the mask
- A mask will be created for each of Subject ED's time shifted, volume registered, and blurred 3D+time datasets:

Output of 3dAutomask: A mask dataset for each 3D+time dataset:

```
mask_r1+orig, mask_r2+orig ... mask_r10+orig
```

- Now let's take those 10 masks (we don't need 10 separate masks) and combine them to make one master or "full mask", which will be used to calculate the percent signal change only for values inside the mask (i.e., inside the brain).
- <u>3dcalc</u> -- one of the most versatile AFNI programs -- is used to combine the 10 masks into one:



- Output: full_mask+orig:



-13-

- <u>STEP 3B</u>: Create a voxel-by-voxel mean for each timeseries dataset with <u>3dTstat</u>
 - For each voxel, add the intensity values of the 136 time points and divide by 136
 - The resulting mean will be inserted into the "B" slot of our percent signal change equation (A/B*100%)

- Unless otherwise specified, the default statistic for 3dTstat is to compute a voxel-by-voxel mean
 - Other statistics run by 3dTstat include a voxel-by-voxel standard deviation, slope, median, etc...

The end result will be a dataset consisting of a single mean value in each voxel. Below is a graph of a 3x3 voxel matrix from subject ED's dataset mean_r1+orig:



• <u>STEP 3C</u>: Calculate a voxel-by-voxel percent signal change with <u>3dcalc</u>

- Take the 136 intensity values within each voxel, divide each one by the mean intensity value for that voxel (that we calculated in Step 3B), and multiply by 100 to get a percent signal change at each timepoint
- This is where the A/B*100 equation comes into play

Output of 3dcalc: 10 normalized datasets for Subject ED, where the signal intensity value at each timepoint has now been replaced with a percent signal change value

```
scaled_r1+orig, scaled_r2+orig ... scaled_r10+orig
```



- ♦ E.g., Timepoint #18 above shows a percent signal change value of 101.7501
- i.e., relative to the baseline (of 100), the stimulus presentation (and noise too) resulted in a percent signal change of 1.7501% at that specific timepoint

 <u>STEP 4</u>: Concatenate ED's 10 normalized datasets into one big dataset with <u>3dTcat</u>



- The output from 3dTcat is one big dataset -- ED_all_runs+orig -- which consists of 1360 volumes (i.e., 10 runs x 136 timepoints). Every voxel in this large dataset contains percent signal change values
- This output file will be inserted into the <u>3dDeconvolve</u> program

• **STEP 5**: Perform a deconvolution analysis on Subject ED's data with <u>3dDeconvolve</u>

- What is the difference between regular linear regression and deconvolution analysis?
 - With <u>linear regression</u>, the hemodynamic response is already assumed (we can get a fixed hemodynamic model by running the AFNI waver program)
 - ➡ With <u>deconvolution analysis</u>, the hemodynamic response is not assumed. Instead, it is computed by 3dDeconvolve from the data
 - Once the HRF is modeled by 3dDeconvolve, the program then runs a linear regression on the data
 - To compute the hemodynamic response function with
 3dDeconvolve, we include the "minlag" and "maxlag" options on the command line
 - The user (you) must determine the lag time of an input stimulus
 - $1 \log = 1 \text{ TR} = 2 \text{ seconds}$
 - In this example, the lag time of the input stimulus has been determined to be about 15 lags
 - As such, we will add a "minlag" of 0 and a "maxlag" of 14 in our 3dDeconvolve command

```
3dDeconvolve -input ED all runs+orig -num stimts 4
 -stim file 1 all stims.1D'[0]' -stim label 1 ToolMovie
       -stim minlag 1 0 -stim maxlag 1 14 -stim nptr 1 2
 -stim file 2 all stims.1D'[1]' -stim label 2 HumanMovie
       -stim minlag 2 0 -stim maxlag 2 14 -stim nptr 2 2
 -stim file 3 all stims.1D'[2]' -stim label 3 ToolPoint
       -stim minlag 3 0 -stim maxlag 3 14 -stim nptr 3 2
 -stim file 4 all stims.1D'[3]' -stim label 4 HumanPoint
       -stim minlag 4 0 -stim maxlag 4 14 -stim nptr 4 2
 -glt 4 contrast1.1D -glt label 1 FullF
 -glt 1 contrast2.1D -glt label 2 HvsT
 -glt 1 contrast2.1D -glt label 3 MvsP
 -glt 1 contrast2.1D -glt label 4 HMvsHP
 -glt 1 contrast2.1D -glt label 5 TMvsTP
 -glt 1 contrast2.1D -glt label 6 HPvsTP
 -glt 1 contrast2.1D -glt label 7 HMvsTM
 -iresp 1 TMirf -iresp 2 HMirf -iresp 3 TPirf -iresp 4 HPirf
 -full first -fout -tout -nobout -polort 2
 -concat runs.1D
 -progress 1000
 -bucket ED func
```

- -iresp 2 HMirf
- -iresp 3 TPirf
- ♦ -iresp 4 HPirf
 - These output files are important because they contain the estimated Impulse Response Function for each stimulus type
 - The percent signal change is shown at each time lag
- ♦ Below is the estimated IRF for Subject ED's "Human Movies" (HM) condition:



- Focusing on a single voxel (from ED's HMirf+orig dataset), we can see that the IRF is made up of 15 time lags (0-14). Recall that this lag duration was determined in the <u>3dDeconvolve</u> command
- ♦ Each time lag consists of a percent signal change value:



✤ To run an ANOVA, only one data point can exist in each voxel

- ➡ As such, the percent signal change values in the 15 lags must be averaged
- → In the voxel displayed below, the mean percent signal change = 1.557%



-23-

- Before averaging the values in each voxel, one option is to remove any "uninteresting" time lags and average only those time lag points that matter
 - In this example, "uninteresting" refers to time lags whose percent signal change value is close to zero
 - If we included these uninteresting time lags in our mean calculation, they would pull the mean closer to zero, making it more difficult to pick up any statistically significant results in the ANOVA
- In this example, the IRF is fairly small from time lags 0-3, but begins to spike up at time lag 4. It peaks at time lag 7, declines at 9, drops sharply by 10,

and decreases steadily from there.

 Given this information, one option is to compute the mean percent signal change for time lags 4-9 only



 If the mean is computed only for time lags 4-9, then mean percent signal change in the voxel below increases dramatically, from 1.557% (all lags) to 3.086%:



• STEP 5: Compute a voxel-by-voxel mean percent signal change -including only time lags 4-9 -- with AFNI <u>3dTstat</u>

- The following 3dTstat command will focus only on time lags 4-9, and compute the mean for those lags
- This will be done on a voxel-by-voxel basis, and for each IRF dataset, of which we have four: TMirf, HMirf, TPirf, HPirf

The output from 3dTstat will be four irf_mean datasets, one for each stimulus type. Below are subject ED's averaged IRF datasets:

```
ED_TM_irf_mean+orig ED_HM_irf_mean+orig
ED_TP_irf_mean+orig ED_HP_irf_mean+orig
```

Each voxel will now contain a single number (i.e., the mean percent signal change). For example:



- **STEP 6**: Resample the mean IRF datasets for each subject to the same grid as their Talairached anatomical datasets with <u>adwarp</u>
 - For statistical comparisons made across subjects, all datasets -- including functional overlays -- should be standardized (e.g., Talairach format) to control for variability in brain shape and size

♦ The output of adwarp will be four Talairach transformed IRF datasets.

ED_TM_irf_mean+tlrc	ED_HM_irf_mean+tlrc
ED_TP_irf_mean+tlrc	ED_HP_irf_mean+tlrc

 We are now done with Part 1-- Process Individual Subjects' Data -- for Subject ED

♦ Go back and follow the same steps for Subjects EE and EF

• We can now move on to Part 2 -- RUN GROUP ANALYSIS (ANOVA)

• PART 2 \Rightarrow Run Group Analysis (ANOVA2):

- In our sample experiment, we have two factors (or Independent Variables) for our analysis of variance: "Stimulus Condition" and "Subjects"
 - → IV 1: STIMULUS CONDITION \Rightarrow 4 levels
 - Tool Movies (TM)
 - Human Movies (HM)
 - Tool Points (TP)
 - Human Points (HP)
 - → IV 2: SUBJECTS \Rightarrow 3 levels
 - Subject ED
 - Subject EE
 - Subject EF
- ♦ The mean IRF datasets from each subject will be needed for the ANOVA:

ED_TM_irf_mean+tlrc	EE_TM_irf_mean+tlrc	EF_TM_irf_mean+tlrc
<pre>ED_HM_irf_mean+tlrc</pre>	EE_HM_irf_mean+tlrc	EF_HM_irf_mean+tlrc
<pre>ED_TP_irf_mean+tlrc</pre>	EE_TP_irf_mean+tlrc	EF_TP_irf_mean+tlrc
ED_HP_irf_mean+tlrc	EE_HP_irf_mean+tlrc	EF_HP_irf_mean+tlrc

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-acontr 1	1 1	1 AllAct	١		
-acontr -1	1 -1	1 HvsT	١		
-acontr 1	1 -1 -	1 MvsP	١		
-acontr 0	1 0 -	1 HMvsHP	١		
-acontr 1	0 -1	0 TMvsTP	١		
-acontr 0	0 1 -	1 TPvsHP	١		
-acontr -1	1 0	0 TMvsHM	١		
-fa StimEffect \					
-bucket AvgANOVAv1					

- <u>-type 3</u>: Tells **3dANOVA2** what type of ANOVA model is to be used. Type 3 refers to a mixed effects model (a = fixed, b=random)
 - ▷ Type 1 = fixed effects model (a and b are fixed)
 - ▷ Type 2 = random effects model (a and b are random)
- <u>-alevels 4</u>: Our first IV "Stimulus Condition" has 4 levels --TM,HM,TP,HP
- → <u>-blevels</u> 3: Our second IV "Subjects" has 3 levels -- ED, EE, EF
- -dset a b filename: Sets up all the factor level combinations
 - Subject (b) ED EE EF 1,2 TΜ 1,1 1,3 Stim Cond (a) 2,1 2,2 2,3 HM TΡ 3,1 3,2 3,3 HP 4,1 4,2 4,3

▷ For example:

- <u>-amean</u>: Estimates the mean for every level of factor 'a' (collapsed across factor 'b') and provides a t-statistic for the null hypothesis, H_o: mean = 0
 - In this example, the mean percent signal change for each stimulus condition type is averaged *across* subjects on a voxel-by-voxel basis
 - ⇒ For example:

Mean Percent Signal Change at Voxel "x"				
	Subject (b)			
		<u>ED</u>	<u>EE</u>	<u>EF</u>
	<u>TM</u>	4.1%	3.8%	4.5% ⇒ <u>M</u> = 4.13%
Stim Cond (a)	<u>HM</u>	3.2%	2.5%	2.8% ⇒ <u>M</u> = 2.83%
	<u>TP</u>	4.6%	4.1%	4.9% ⇒ <u>M</u> = 4.53%
	<u>HP</u>	1.7%	2.0%	1.1% ⇒ <u>M</u> = 1.60%

 Allows you to determine if the percent signal change in a voxel is significantly different from zero. If so, appears as colored activation blobs

cd AFNI_data2/group_data

- the group_data/ directory contains a script called @anova2 that will run 3dANOVA2
- This script can be viewed with a text editor, e.g., emacs @anova2

./@anova2

execute the ANOVA script from the command line

ls

result from ANOVA script is a bucket dataset AvgANOVAv1+tlrc, stored in the group_data/ directory

afni &

- launch AFNI to view the results
- The output from 3dANOVA2 is bucket dataset <u>AvgANOVAv1+tlrc</u>, which contains 24 sub-bricks of data:
 - i.e., means and t-tests for each level of factor A (i.e., "Stimulus Condition"), contrasts between the different levels of factor A, and a main effect of factor A

Brain areas corresponding to "Human Movies" A second second

→ -amean 2 HM



-35-

- <u>-acontr</u>: Estimates contrasts among levels of factor 'a'
 - E.g., t-tests are conducted among levels of the factor "Stimulus Condition" to determine if their percent signal changes differ significantly from each other throughout the brain
 - ⇒ Here are some contrasts we performed:

<u>TM</u>	<u>HM</u>	<u>TP</u>	<u>HP</u>		voxel-by-voxel t-tests:
0	1	0	-1	\Rightarrow	Human Movies vs. Human Points
1	0	-1	0	⇒	Tool Movies vs. Tool Points
0	0	1	-1	⇒	Tool Points vs. Human Points
1	-1	0	0	⇒	Tool Movies vs. Human Movies
-1	1	-1	1	⇒	Tools vs. Humans
1	1	-1	-1	\Rightarrow	Movies vs. Points

- For simple contrasts, you could also use the -adiff option (e.g., -adiff 2 4)
- Tools vs. Humans contrast collapses across Animation Type (i.e., Movies and Points)
- Movies vs. Points contrast collapses across Object Type (i.e., Tools and Humans)

♦ Brain areas corresponding to "Movies" (reds) vs. "Point-light displays" (blues)

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→ -acontr 1 1 -1 -1



→ <u>-fa</u>: Produces a main effect for factor 'a'

- In this example, -fa determines which voxels show a percent signal change that is significantly different from zero when any level of factor "Stimulus Condition" is presented
- ▷ -fa StimEffect:



- Many thanks to Mike Beauchamp for donating the data used in this lecture and in the how-to#5
- For a full review of the experiment described in this lecture, see Beauchamp, M.S., Lee, K.E., Haxby, J.V., & Martin, A. (2003). FMRI responses to video and point-light displays of moving humans and manipulable objects. Journal of Cognitive Neuroscience, <u>15:7</u>, 991-1001.
- For more information on AFNI ANOVA programs, download Doug Ward's paper on Analysis of Variance for FMRI data, located on the AFNI website at:

<u>http//afni.nimh.gov/pub/dist/doc</u> \Rightarrow **3dANOVA.pdf** or **3dANOVA.ps**