

## AFNI Jazzercise

NB: the code snippets below will likely **not** copy+paste well into your terminal. This is not just laziness on our part, but instead it is meant to be helpfully didactic by prompting you to type the commands yourself, thereby getting used to the AFNI program syntax. And while typing, *please do* make use of the auto-completion features from both the Linux terminal and AFNI itself (→ via the “`apsearch -update_all_afni_help`” command).

Please read the following questions, and use your AFNI know-how to answer them. Hints to answering these questions are available in the “Hints” handout. The answers to these questions can be found in the “Answers” handout.

**Note these questions do not have to be done in order.** Though these questions have a very rough order in terms of complexity and general usefulness, you can do whatever interests you and skip ahead to one that you like. Some exercises require longer computation time. Start the commands for those questions, and open another terminal window to continue on to the other questions. If you get stuck on a question, check the hints. If the hints don’t help, ask for help or go ahead and look at the answers (it’s not cheating; this is not a test).

1. The dataset **AFNI\_data6/afni/func\_slim+orig** contains 7 sub-bricks of statistical data. Use **3dbucket** to create a smaller version of this dataset that contains only the sub-bricks: #0, 3-6. Name this new dataset **some\_stats**.

*Why: To understand the layout of AFNI datasets and sub-bricks. You’ll probably be interested only in specific sub-bricks and not all the sub-bricks that include baseline fit statistics.*

2. In directory **AFNI\_data6/afni** you will find two anatomical datasets: **anat+orig** and **second\_anat+orig**. These datasets are two separate anatomical scans of a single subject. They have already been aligned. Average them together into a single dataset called **anat\_mean+orig**. Notice that the result looks ‘cleaner’, since the noise has been reduced.

*Why: averaging reduces the noise.*

3. Use two of AFNI’s programs that remove non-brain data, **3dAutomask** and **3dSkullStrip**, to remove data from outside of the brain from dataset **AFNI\_data6/afni/epi\_r1+orig**. Name the output file from **3dAutomask** **epi\_auto+orig** and the output file from **3dSkullStrip** **epi\_3dSkull+orig**. Compare the two output datasets. The differences for an EPI dataset are subtle, so compare each to the original and to each other with the Underlay and Overlay displays in the AFNI GUI. Note what “non-brain” data was removed by each. Did one program do a better job at limiting the result to the brain, or are the results similar? Note **3dSkullStrip** may take a few minutes to run, so be patient. Run it in the background so you can get to the other questions while **3dSkullStrip** is running. Also note **3dAutomask** is particularly useful for EPI data, while **3dSkullStrip** is typically used for anatomical data.

*Why: Removing the skull is useful for image registration and creating a brain-specific mask.*

4. Creating and Playing with ROI Masks:

- a. The dataset **AFNI\_data6/afni/func\_slim+orig** has beta values and t-stats for 2 stimulus classes, **Vrel** and **Arel**. Use **3dcalc** to create a mask called **VA\_mask** that is **1** everywhere that both the Vrel t-stat and the Arel t-stat values are greater than 4.2, and **0** everywhere else.

*Why: Combining the results in a single mask is useful for a simple conjunction analysis.*

- b. Similar to part a, create a conjunction mask that is **1** wherever  $a > 4.2$  (from Vrel t-stat sub-brick), **2** wherever  $b > 4.2$  (from Arel t-stat sub-brick), **3** wherever both are true, and **0** otherwise. Name this dataset **VA\_mask\_4+orig** (since it contains 4 values).

*Why: Using masks with values of powers of two are easier to look at all possible combinations in conjunction analysis.*

- c. Use the afni GUI to display this mask, **VA\_mask\_4+orig**, so that each mask value gets its own color. What does each color mean?

*Why: The various combinations will each show a unique color that is easily visible and understood.*

- d. Use **3dROIstats** to store the average time series from **epi\_r1+orig** into the text file **VA\_mean.1D**, where the mean is only over the voxels in the mask (from part a), **VA\_mask+orig**.

*Why: Mean time series curves for each ROI are useful for further analysis and display with other AFNI programs or with external applications like Excel and Matlab.*

## 5. Understanding the regression matrix:

Answer the following questions based on plotting the regression matrix **X.xmat.1D** created during single subject analysis with subject FT. Evaluate the matrix plot as if you have no idea about the experiment design or analysis used. The file is from the single subject analysis of subject FT, and might be under **subject\_results/group.horses/subj.FT/FT.results**, for example. Start by plotting the regression matrix using **"1dplot -sepsc1 X.xmat.1D"**, and note that graphs are plotted from the bottom up.

- a. How many runs were there in the analysis?
- b. What -polort was used (degree of baseline polynomial)?
- c. How many regressors of interest were there?
- d. What type of experimental design was used (block or event)?
- e. How many TRs were analyzed per run?
- f. Were there any significant subject movements?

## 6. Fun with 1D files:

- a. Create three 1-column files with the numbers 1-10 in one column of the first file, 11-20 in the second file, and 21-30 in the third file. (note: you might use 2 different AFNI programs to create each of these files, but it can be done with only one)

- b. Concatenate these 3 files into one 3-column file. Call this 1D file **3\_cols.1D**.
- c. Create a new file that contains columns 1, 2, 3, 3, 2,1, from part b (i.e., there will be a total of 6 columns in this new 1D file). Call this new 1D file **6\_cols.1D**.
- d. Now take the 6 columns from the previous question and average them together to create a new file with a single column. Call that new file **ex\_mean.1D**.

*Why: AFNI 1D programs can help to combine and analyze data from multiple voxels or masks.*

## 7. Fun with the AFNI GUI:

- a. Open **AFNI\_data6/afni/anat+orig** and in any one of the views (sagittal, axial, or coronal), change the gray-scale intensity range to be 300 minimum and 1200 maximum.
- b. Open **AFNI\_data6/afni/func\_slim+orig** and set the Full-F as the Olay and Threshold. Set the Threshold to F=8.0. Show only Positive values and set the color scale to show only 8 colors. Edit the color scale so that F-values between 45 and 90 are shown in lime green.
- c. View the above settings you created from part b in a sagittal slice. Make a jpeg file from sagittal slice #107 and name it **cool\_slide**.
- d. Switch to Talairach view and go to the location for the **right cuneus**.
- e. Change the display to show 6 sagittal slices all at once, in a 3x2 montage.
- f. Can you find the AFNI Mission statement hidden in the AFNI GUI?

*Why: The AFNI GUI has the flexibility to look at your data, and it's fun once you learn how.*

## 8. Doing Calculations in AFNI:

- a. Determine what type of data (short, float, etc) makes up dataset **AFNI\_data6/afni/func\_slim+orig**.

*Why: It's important to know how to find information about your dataset.*

- b. Calculate  $22.3 * 44.5$  using the simple calculating program in AFNI.

*Why: AFNI provides calculation tools including simple ones that are useful in scripts.*

- c. Plot the function  $\sin(x)/(1+x)$  for  $x=0$  to 20 using command line AFNI programs.

## 9. Aligning data:

Align the anatomical dataset, **AFNI\_data6/anat+orig**, with the EPI dataset, **AFNI\_data6/epi\_r1+orig** using the `align_epi_anat.py` script. Using the `-AddEdge` option for the script, examine the differences

between before and after alignment in the AFNI GUI. Note this script can take a few minutes, so start it and continue with the other questions while the script is running.

*Why: Alignment is important for knowing exactly where functional activation occurs. The quality of alignment is not always obvious and requires careful visual examination.*

#### 10. Image Filtering:

- a. Smooth **AFNI\_data6/afni/epi\_r1+orig** with a 8mm FWHM filter. Name the output file **ex\_blur8**.
- b. Enhance **AFNI\_data6/afni/anat+orig** by emphasizing the minimum-valued voxels across +/- 3 voxels in the sagittal (z) direction. Name the output dataset **ex\_minz3**. Note the effects on each slice direction.
- c. Enhance dataset **ex\_minz3+orig** from part b by removing the noise with program **3danisosmooth**. Name the output dataset **ex\_aniso**. Use the **-viewer** option in this program to select the number of noise-removing iterations.

*Why: Image filtering provides some powerful ways to enhance the data. Data can be changed in radically different ways. Use this power wisely and carefully.*

#### 11. Random Exercises with AFNI Datasets:

- a. Open dataset **AFNI\_data6/afni/anat+orig** dataset and find the spatial storage order (i.e., xyz-orientation). Re-orient it to LPI orientation and name the new output dataset **exLPI**.

*Why: Data may be required by specific programs to match other data or to match a specific orientation.*

- b. Open dataset **AFNI\_data6/afni/func\_slim+orig** and create 2 separate datasets: one with sub-brick 3 only and one with sub-brick 4 only. Call the former dataset **ex\_arel\_coef** and the latter **ex\_arel\_tstat**.

*Why: Extracting specific data is useful for exporting to other software or for making the data fit in memory more easily if it's a large dataset.*

- c. Combine **ex\_arel\_coef+orig** and **ex\_arel\_tstat+orig** from part b into a single dataset called **ex\_fneg**, having the fneg Coef for sub-brick 0, and fneg t-stat for sub-brick 1.

*Why: Datasets can be manipulated to include only what you're interested in.*

- d. Convert dataset **AFNI\_data6/afni/func\_slim+orig** to Talairach coordinates with a 4mm<sup>3</sup> resolution. Use dataset **anat+tlrc** in the same directory as the data parent to perform the transformation on **func\_slim+orig**. Name the output file **func\_slim4mm**.

*Why: Talairach data will be 1mm<sup>3</sup> by default. This resolution is often not necessary because it doesn't reflect the resolution of the EPI data. It makes processing slower and taxes memory too.*

- e. Locate the maximum “Full-F” stat voxel value in dataset `func_slim4mm+tlrc` and find the name of the Talairach atlas region that corresponds to that voxel’s position.

*Why: Finding maximum activation can be scripted or searched interactively. Atlas regions should be used as a guide. The AFNI GUI includes various atlases that cite the regions associated with any specific voxel.*

- f. Dataset `AFNI_data6/afni/anat+orig` was acquired sagittally and contains 124 slices. Create a new dataset that contains only slices 40-90 of `anat+orig`. Provide the new dataset with the prefix name `anat_40_90`.

*Why: If memory or processing speed is a constraint, you can work with only part of the data.*

## 12. Volume Rendering:

- a. Use the volume rendering plug-in to render the Talairached anatomical dataset.
- b. Add an overlay of the `func_slim` dataset’s full F-stat (resampled to `tlrc` space) thresholded to a value of 10. Use the dataset created in 10d (or write out a new dataset from the GUI if you have skipped ahead).
- c. Find an ROI that corresponds to the maximum F-stat location from the Talairach daemon atlas found in 10e (or eyeballed if you have skipped ahead).

*Why: Volume rendering is good for getting a good idea of 3D locations and for making cool presentations.*

## 13. Simple statistics:

- a. The dataset, `rall_func+orig` under `~/AFNI_data6/afni/`, is the output from the hands-on regression analysis, and it contains the t-statistic for the null hypothesis of "visual-reliable effect = auditory-reliable effect". Find out all the processing steps/scripts (including pre-processing and regression) that lead to this output, `rall_func+orig`.
- b. Find out in which sub-brick this t-statistic is stored in the output file. What is the range of these t-values and the degrees of freedom?
- c. What are the two-sided (or two-tailed) significance level ( $p$ -value) and FDR  $q$ -value corresponding to the t-statistic value of 4.25 with the degrees of freedom from question b? What is the one-sided (or one-tailed) significance level ( $p$ -value) for the alternative hypothesis of "visual-reliable effect < auditory-reliable effect"?
- d. What are the t-statistic and FDR  $q$ -value corresponding to a two-sided significance level ( $p$ -value) of 0.0001 with the degrees of freedom from question b? If only a one-sided alternative hypothesis (e.g., "visual-reliable effect < auditory-reliable effect") is considered but with the same significance level ( $p$ -value of 0.0001), what is the t-statistic and FDR  $q$ -value? Which one is more lenient or stringent, and which one should be adopted: one-sided or two-sided?

*Why: Finding out where your statistics are and what they mean are vital for understanding and reporting your results.*