Short Data Analysis Instructions for 8/16/2016

We should have 3 groups of 2 people each and one group of 3. Pick a partner, then pick a group number (1-4) that none of the other groups are using. Go ahead and get started.

Find a CFMI Macintosh computer that somebody who looks somewhat authoritative allows you to use. The computers have already been loaded with all of the materials you will need to complete this lab.

1) Navigate MATLAB

Navigate MATLAB to the bootcamp folder (e.g., `~/IPN-MRI-Bootcamp`); you can navigate via MATLAB's file selection gui on the left or by typing linux commands at the prompt (e.g. `cd ~/IPN-MRI-Bootcamp`). Make sure you see these items in your folder:

- two files ending with a "*.mat" extension,
- one empty directory names "Results",
- one directory named "Acquired-Images" that contains
    - one anatomical image named "MPRAGE.nii"
    - 122 functional images named "NBack_001.nii, NBack_002.nii, …"

a) You have just found two folders in which are stored the MRI data you've collected ("Images") and the statistical analysis you will perform ("Results"). You should see them in the current folder next to the .mat file. However, MATLAB doesn't know how to find them right now, so...

b) Click on each of the new folders in MATLAB's file selection gui while holding the CONTROL key, and then select "Add to path" from the context menu that pops up. Now MATLAB will be able to find the files in those folders.

2) Run an Analysis Script Using SPM Software

a) In MATLAB (at the >> command prompt), open SPM by typing

```
spm fmri
[enter]
```

b) After SPM "initializes", you can **check out some of your images.**

1. Hunt for the "Display" button in the SPM pop-up gui and click it.
2. Navigate to the "Images" folder if you're not already there [NB: single click only, no need for double clicking]
3. Select the anatomical image to view ("MPRAGE.nii"), click "Done"
    a. click around in the 3 image windows until you can identify front, back, top, bottom, eyes, nose, cortex, cerebellum, brain stem, corpus callosum … what else do you see? Collect bonus points for naming parts of the basal ganglia. Notice the large sinus cavities between the pons and the eyes, next to the ears, and just behind the forehead.
4. Next, check out a functional image (just pick any one of them).
    a. Try to identify some of the same landmarks. See how the 3 large sinus cavities cause distortions in the images? These pictures are terrible... why do we use these?

c) OK, let's **run the analysis script**.

1. Hunt for the "Batch" button in the SPM pop-up gui and click it.
2. In the Batch Editor window (that just popped up), click the "Load" icon (it looks like an open folder). A pop up window will demand that you "Load Job File(s)".
3. Select the ".mat" file in your group folder. Click "Done" to load the file.
4. The Batch Editor will now show a list on the left of all the steps to our data processing procedure (Slice Timing, Coregister, Segment, etc.) . If you click on an item in this list, the Batch Editor will show you specific details for that processing step on the right side of the window. You only need to enter three things to get this show running:
    a. <u>Select the fMRI images:</u>
        i. On the left list, select "Slice Timing".
            1. On the right list, select "Session" then click the "Select Files" button at the bottom of the right list.
            2. Select all your NBack images except the first two (so, select NBack_003.nii through NBack_122.nii, <u>*in numerical order*</u>, shift-click will help), 120 images total. Then click "Done" to get back to the Batch Editor.

                NB: We don't use the first two images because they are too bright and will mess up our stats (the hydrogen protons in the water of our subject's brain had not yet reached "spin saturation" from all the exciting RF pulses with which we hit them… if that makes sense to you, you're doing great). If you want to avoid clicking on the first two images by accident, you can delete them completely.
    b. <u>Select the anatomical image:</u>
        i. On the left list, select the first "Coregister:Estimate" (second item in the list).
            1. On the right list, select "Source Image" then click the "Select Files" button at the bottom of the right list.
            2. Select the anatomical image [MPRAGE] and click "done"
    c. <u>Select the output folder for your statistics:</u>
        i. On the left list, select "fMRI Model Specification". On the right list, select "Directory" then click the "Select Files" button at the bottom of the right list.
        ii. Select your "Results/" folder (the option on the right side of the dialogue box) and click "done"
5. Save your script by clicking on the picture of a disk in the Batch Editor (make up a fun name for your personalized script; funnest name gets a prize).
6. Then run your script by clicking on the green triangle in the Batch Editor.

SPM will go crazy running all your math for you, about 10 minutes or so, showing you charts of your progress at each step. Please review the other hand out, *What's going on in my fMRI processing?*, so that you have some sympathy for what SPM is going through right now. Imagine for a second doing this math with a slide rule… at each of the 172,032 voxels you have collected. Yay, computers! Lots of black writing will show up in the workspace of the main MATLAB window as the analysis goes on; this is totally normal and can be somewhat interesting. If red writing shows up, then

there is an error and it is time to troubleshoot.

Good Luck!!!

3) Visualize Your Results

1. Once your analysis has stopped running, there should be a report page showing a "glass brain" of voxels significantly activated more during 2-Back than 1-Back, using a Familywise Error correction for multiple comparisons at $p<0.05$. Check it out.
2. Next, check out interactive results by clicking on the "Results" button in the SPM window on the left (half-way down the same window in which you found the "Batch" button).
    a. In the pop-up window, navigate into your "Results" folder and select "SPM.mat" (this is the main output file containing your statistical results).
    b. In the pop-up window that asks you to "Select Contrasts...", highlight the "2Back-1Back" item from the list, then click "Done".
    c. Now turn your attention to the small window on the bottom left, and choose:
        i. "apply masking?" click "None"
        ii. Accept the default title
        iii. whether or not you want to correct for multiple comparisons (you're doing over 150k comparisons, so using FWE correction can help you minimize false positives)
        iv. your desired p-value (for corrected data, 0.05; for uncorrected 0.001)
        v. and the number of consecutive voxels that you decide to call "significant" for your results (maybe use the defaults for your first try; standard value to try = 10).
    d. When the glass brain pops up, click on "Whole Brain" in the left lower window to show results for significant voxel activations.
    e. Try running a couple different thresholds for p-value and cluster extent to see how different choices change the look of your results. *If you were publishing these data, which thresholds would you choose?*

4) Make some pretty slice pictures:

1. While you are looking at your results, drag the red arrow to an interesting place in the brain
2. click on the "Overlay" button in the lower-left SPM pop-up window (the same one in which you found the "Whole Brain" button).
3. In the drop-down menu, select "Slices", and when a pop-up asks you for an "image for rendering on" choose
   `/Applications/MATLAB_R2016a.app/toolbox/spm12/canonical/`
   `/single_subject_T1.nii`
4. Try this again selecting "Sections" instead of "Slices".
5. Finally, make some cortical surface pictures by selecting "Render" instead of "Sections" and select as your template:
   `/Applications/MATLAB_R2016a.app/toolbox/spm12/rend/render_single_subj.mat`

Answer a couple questions in the lower-left window, and voila! You made blobs.

6. Save some of the better images into your Results folder by clicking “File” then “Save As” in the window with the pictures (save as a .jpg or .tif or .png). We’ll be passing these on to the IPN brass to show them what you've done today, so make them pretty and name them well.
    a. Save two images: results at one significance/cluster threshold combination and results at another significance/cluster threshold combination.

4) What are your conclusions?

Statistical parametric maps (that’s really what SPM stands for) are based on hypothesis tests.
Think:

1. *What is the null hypothesis that you have tested?*
2. Based on the results from your analysis, what conclusions are you comfortable making?
3. Think about the two images you saved. Do you think that if you choose different thresholds for your statistical significance and cluster extent (and, therefore, report different locations of significant activity in your published results) that this should make a difference in your reported conclusions?

Thanks for coming down to play at CFMI, and have a great weekend!