PLS to Study Task-Related Connectivity
NITP Summer Course 2015

An alternate, multivariate, model-free approach to task-related functional connectivity is partial least squares analysis (e.g., McIntosh & Lobaugh, 2004, Neuroimage, 23(1): S250-63; doi: :10.1016/j.neuroimage.2004.07.020). This method identifies patterns of covariation among voxels (and event TRs if desired) across task conditions or groups (rather than across the entire scan duration, which is the norm for PCA or ICA). In this lab we are going to set up a PLS analysis using the visual motion data of Friston (1997) that we used in the PPI lab. We have split the original data into 4 blocks and we are going to perform the analysis using our 4 blocks as inputs into the analysis, specifying attend/non-attend as task conditions. Our goal is to identify patterns of connectivity that distinguish attend/non-attend conditions, if these exist. *See the PLS website for a full tutorial.*

PLS SETUP
We are working in the **pls_lab** directory, so this is where you'll need to navigate to perform the lab (***cd pls_lab***). This directory contains the same data that was used for the PPI lab as well PLS results. Launch Matlab, and type **plsgui** then press enter, to launch the PLS analysis GUI. The software also performs batch processing that is very useful when working with multiple subjects.

PLS SetUp the "Datamats"

1) Click on **Blocked fMRI** tab. Notice that this software can also be used to input event-related fMRI data, PET data or EEG data (in any format – spectral, ERP etc.).
2) Click **Session profile for Block fMRI data**, to set up the profile for the input data. This will produce a data matrix that contains the input fMRI data as well specs about conditions, onsets, latencies etc.
3) From the **File** tab, click **Load**. Select **run1_BfMRIsessiondata.mat**. This is the spec file for the first run of the data. You will notice a warning in red indicating that paths have changed (which is true because you're on a different computer than the tutorial was created on). Feel free to click **Edit** and update the session path to the current working directory path if you'd like to actually perform the analyses.
4) Click on **Edit Conditions** and **Edit Runs** to understand the inputs. The former lists the condition names and a few specs about latency and reference scan. The latter lists the latency and onsets (in TRs) of each of the conditions. If you're doing this in real time, check that the path points to the current working directory and reselect the data: **snff_run1.nii** then click **Done**.
5) Next you would click **Create Datamat** to create the data matrix for this run. We've already done this so you don't have to – but do so, if you want to see it in action. It's a short step.
6) You can view and do the same for run2, 3, and 4 to confirm you understand the format.
7) The output of this process is the set of files: **runX_BfMRI_sessiondatamat.mat.** You can load these files into Matlab and explore the contents to understand what they contain – which is the data, latencies, and onsets as well as condition names.
8) Close the datamat gui when done.

PLS Run Analysis

1) Ensure you are on the **Blocked fMRI** tab.
2) Click **Run PLS Analysis on Block fMRI data**, to run the actual analysis.
3) Press **Add** and select 4 runs of data: **runX_BfMRI_sessiondatamat.mat**. You can do all four in one go. Then click **Done**.
4) Leave the centering on **mean-centering**.
5) To actually run the analysis you might set permutations to 500 (test on significance of LV), and boostrap samples to 100 (confidence intervals on LV weights at each voxel) then press **Run**. Notice that you have the option to set confidence level and specify #splits for cross-validation.
6) If you pressed Run, you can save your results with a new name (our existing results are called **run1234_BfMRIresult.mat**) or press **Cancel** – since we've already produced the results file. Close the window when done.

PLS Results

1) Ensure you are on the **Blocked fMRI** tab.
2) Click **Show PLS Result on Block fMRI data**, to run view the results. Select **run1234_BfMRIresult.mat** to see the tutorial results.
3) You will see a window with some red and blue blobs. Click on **File** and **Load Background Image**, change the file filter (at bottom) to *.nii, and select **mean_func.nii**. Press **Select**. This makes the subject's functional image, the background.
4) You'll notice that the window shows **LV1.** This means that by default it's showing the first latent variable. ***Recall that PLS analysis produces weights for the task dimension and also for the voxel/brain dimension, and these together with the singular values form the "LV". Note:*** *What you're viewing by default here are the weights for the voxel/brain dimension. We will first examine the weights for the task dimension and the singular values to appreciate what the brain dimension means.*
5) To better understand what's going on click on **Window** and select **Design Scores & LV Plot**. This shows you a bar graph, for LV1, which represents the condition contrast identified in this LV (i.e., quite literally these bar graph values are the "weights" for each condition on the latent variable). In this case we see a difference between attend and non-attend conditions. Note that the non-attend is negative (for later). Close this, then, from **Window,** look at **Task PLS Brain Scores with CI,** you'll see the same plot but with confidence intervals showing that the weights in this contrast differ from zero, and thus are meaningful. ***The first LV thus discriminates between attend and non-attend conditions.*** Close this window.
6) Again go to **Window** and click **Singular Values Plot**. This shows the %covariance accounted for and probability of each LV (assessed via permutations). In this case we don't see any as significant, but that's ok. Close this window.
7) So now you can interpret the results in the main window, which shows the bootstrapped LV weights for each voxel**.** This is the first LV, and the interpretation is driven by that bar graph you viewed (since the bar graph and this image are correlated). Recall that the attend condition was negative and the non-attend conditions was positive. This means that the regions with a negative value here are more strongly co-active in attend than in non-attend, and vice versa for positively weighted values. Set the Bootstrap threshold (upper left) to 10 for positive to mask out those values, and -3 for negative. See anything interesting?

8) Ok great. This is not the best way to view the output. You can export the bootstrap ratio to an image file (from the File menu) and view elsewhere. We've done this for you, so close the GUI and move on to the next step.

PLS vs PPI in standard-brain space.

1.) Stay in the **pls_lab** directory. You can close Matlab if you'd like. From here, type **fslview &** and press enter to open the FSL viewer.
2.) Press **File,** then **Open**, and navigate to **./pls_lab/snff_convert.feat/reg/** and select **standard.nii.gz** (this just loads a standard image as background).
3.) Then, from **File,** click **Add** and **add 21_11_14_sphere_mask_normed.nii.gz** (the mask for our seed for the PPI analysis). Check it out – surprise, it's not where we thought it was. It's actually a seed in the cerebellum so all this time you've been studying the functional connectivity of the cerebellum. That's ok.
4.) Now **Add zstat3_normed.nii.gz**. This is the result of the [a-na] PPI. It is the same file you viewed previously but in standard space. You can change the color by selecting this file in the low right corner and pressing the little **"I"** button for specs on the file. Notice that a little superior to the seed in the cerebellum there is a little blob that we previously thought was the PPI correlate. That's ok. Inspect the result and notice some other interesting things – like the hippocampus connectivity for instance.
5.) Ok so now let's see what PLS tells us. Add the file **run1234_BfMRIbsr_lv1_normed.nii.gz** and change color if desired. Set the thresholds to Min=-3 and Max=-5. Recall that we want the negative values because these indicate connectivity that's stronger in attend than non-attend. These values are bootstrap values – thus you can think of them as z-scores. First scroll through the cerebellar seed and notice that PLS also identifies a few regions here. Then scroll up to hippocampus and notice that PLS also identifies connectivity here. Feel free to explore the image. Note that these results are very messy since they're not significant, single subject results. But notice both similarities and differences between the PPI and PLS results, appreciating that PLS found some strong similarities without the need to specify a seed or a model.
6.) Note that the PLS results interpretation is: "these regions are more coactive in attend than in non-attend condition." Thus they form a task-related functional network. The PPI interpretation is: "these are the regions that correlate with the seed(a-na) interaction term", thus are likely to show a stronger correlation with the seed in the attend versus non-attend condition (independent of seed or n-na main effects).*