

1st Level Statistical Analysis of Student Collected fMRI Data

This document describes the statistical analysis steps needed to analyze your data. You can also refer to Chapter 29 from the SPM manual. This chapter describes the step-by-step analysis of a sample block fMRI experiment and includes pictures of the output from each step.

Our experiment design

Motor Task

Note: this is an extremely simple design. Each condition is repeated three times, with 16s each. So, with a TR of 2.0s that will generate 8 volumes each time the condition is on, with 7 volumes in between each task. Also, the first 3 images are excluded to avoid the T1 equilibrium effect on the functional data.

| task | Task time | Real time (s) | TR | Removing images | 3 TR -3 |
|--------------|-----------|---------------|-----|-----------------|---------|
| Dummy | 6 | 0 | 0 | - | - |
| right | 16 | 6 | 3 | 0 | 0 |
| rest | 14 | 22 | 11 | 16 | 8 |
| right + left | 16 | 36 | 18 | 30 | 15 |
| rest | 14 | 52 | 26 | 46 | 23 |
| right+left | 16 | 66 | 33 | 60 | 30 |
| rest | 14 | 82 | 41 | 76 | 38 |
| right | 16 | 96 | 48 | 90 | 45 |
| rest | 14 | 112 | 56 | 106 | 53 |
| left | 16 | 126 | 63 | 120 | 60 |
| rest | 14 | 142 | 71 | 136 | 68 |
| left | 16 | 156 | 78 | 150 | 75 |
| rest | 14 | 172 | 86 | 166 | 83 |
| right+left | 16 | 186 | 93 | 180 | 90 |
| rest | 14 | 202 | 101 | 196 | 98 |
| right | 16 | 216 | 108 | 210 | 105 |
| rest | 14 | 232 | 116 | 226 | 113 |
| left | 16 | 246 | 123 | 240 | 120 |
| rest | 14 | 262 | 131 | 256 | 128 |



Starting SPM:

Open a shell and type at the prompt matlab. In the matlab interface enter `spm fmri`.

Progress Box:

When generating the results from the statistical analysis the Progress Box adds a number of new options. The figure below shows this additional functionality, which will be used later in this tutorial. There are red boxes around some of the buttons that will be needed later on.

Graphics box:

As with the other operations, the results from a statistical analysis will be displayed in the Graphics box. A sample output is shown below. The main components of the output are the Glass Brain representation of the location of activations, the Design Matrix with the selected Contrast shown above it, and a Cluster Report.

Information needed for data analysis:

Motor Data:

Data is in your folder from the preprocessing steps. Within the subject's folder there are ANAT and MOT folders. At this point, you should have finished preprocessing the data resulting in `swraP001.*.nii` files in the MOT folder.

To perform the statistical analysis it will be essential to know exactly when each condition type of your task started and its duration in seconds. The timing of your task and each of the conditions can be extracted from the `.edat` files (Eprime logfiles) for each subject and each run. This data needs to be converted to a form that SPM can use. In particular, you will need to generate a `.mat` file with names, onsets, and durations for each condition in your task. For the purpose of this class, we will use the tables provided in the first pages of these documents.

First we need to determine the onset times of the following conditions in order: left or right. You can view the onsets and durations in the table in the preprocessing document.

**** NOTE: we did not model baseline, but you might want to do that in an experiment so you can look at the overall activation (i.e. $left > baseline$)**

Afterwards, you will need to save these values in a text file ending in .m and then in Matlab run this file to generate the new MultiConditions.mat file.

The statistical analysis of your data begins with the specification of the 1st level model. This is followed by the estimation step and then the interrogation of the results to generate figures, tables, and plots for your study. In most analyses of a group of subjects, each subject would be run through a separate 1st level model followed by a 2nd level model combining the results from each subject's 1st level models.

Specify 1st-Level (Basic Analysis):

1. Create **SPM_MOT_Results** folder where results will be saved. You have to create this folder.
2. Click on **Specify 1st Level** in the menu box.
3. Choose **SPM_MOT_Results** as **Directory**.
4. Choose **Seconds** for **Units for Design**.
5. Enter **2.0** for the **Interscan Interval**. This is your TR.
6. Add New **Subject/Session** under **Data & Design**. This will setup a fixed-effects analysis with one session per run for each of your subjects.
7. For the first Session choose the **swraP*** files from the first subject's MOT folder as the **Scans**.
8. Click on **Conditions** to create the first condition.
9. Put in the name: **Left**
10. Type in the onset time and duration based on the scan time table.
11. If you are processing the Motor task, create the condition for the move **right** task.
12. For each session add **Multiple Regressors** and choose the **rp_aP*.txt** file from the **Task folder**. This file contains 6 columns with the x, y, z translations and x, y, z rotation motion parameters calculated during realignment. By including this in the model you will be regressing out any signal that correlates with these changes. Hopefully, these changes do not overlap with timing of the conditions in your task.
13. If you have multiple sessions, **repeat steps 5-10 for each session**. For this lecture we only have one session.
14. For the **High-pass filter** you can use **128 seconds**. There is no specific formula for computing what this should be and a long cut-off period such as 128 seconds is recommended.
15. For **Global Normalization** choose **None**.

16. We will use the **default Canonical HRF** for the **Basis Function**. This HRF model is the one we have discussed in class.
17. Save the job as **Basic_StatsModel.mat** in your result folder and then press Run
18. The results for this operation will be a display of your Design Matrix. There will be a separate column for each of the conditions followed by 6 columns for the motion parameters. This will be replicated for each of the sessions in a stair-step fashion. Each row corresponds to a scan starting from the first scan of the first session at the top to the last scan of the last session at the bottom. The gray tone in each column indicates the weighting for the particular condition type or motion parameter. The scaling is relative within each column with white being the highest value and black being the lowest value. Check the columns for each condition type to ensure there is a whitish coloring during the period of the given condition type.
19. A separate row below the design matrix, labeled “Parameter Estimability” shows how independent each of the conditions and factors are from one another. If these boxes are not all white that indicates there is a degree of overlap in between that component and others in your design. This should be addressed before proceeding with the analysis. In most cases, the overlap might occur with the motion parameters and one or more conditions. If this were to occur, I would recommend taking the Multiple Regressors out of the design.
20. You will want to save this figure for future reference. Select File -> Save As from the Graphics Box menu bar; be sure to change the format to PDF.

Estimate:

1. Click on **Estimate** in the menu box.
2. Choose the **SPM.mat** file that was generated in your **SPM_MOT_Results** folder and then press Run.
3. There are no displayed results for this operation.

Results:

1. This is where you will perform specific statistical comparisons. Statistical tests are made by specifying contrasts, which are defined by entering a weight for each column in the design matrix.
2. Click on the **Results** button to start this process.
3. A pop-up will open. Choose the **spm.mat** (**SPM_MOT_Results** folder) for your design. After, the Contrast Manager will appear.
4. Click on the **Define New Contrast** button.

5. The order is left, right, left+right, rest.
6. To compare the activity of **Left > Right** enter the following contrast weights: **1 -1 0 0 0 0 0 0 0 0**. This is effectively telling SPM to weight the columns corresponding to the Left with a 1 and the columns corresponding to the right conditions with a -1. The next eight zeros are for left+right, rest and the motion parameters and are needed to tell SPM not to include both conditions in this particular statistical test. Then submit.
7. Do the same, but now for **Right > Left**. Remember to change the contrast weights.
8. Do the same, but now for Left > Rest. Remember to change the contrast weights.
9. Do the same, but now for Right > Rest. Remember to change the contrast weights.
10. Click on the OK button and then the Done button.
11. The Progress Box now becomes the Results box and you are asked a number of questions. To start with enter the following:
 - a. Apply masking: None
 - b. p-value adjustment to control: none (Later on you can come back and select one of the corrections. For now see what the results are without the correction as the correction may remove all activations).
 - c. Threshold {T or p-value}: 0.05 (This is to get a sense of how much activation there is and later it will probably be necessary to use a more stringent cut-off).
 - d. Extent Threshold {voxels}: 10 (Later on you may need to raise this)

The Graphics box will show a Glass Brain representation of the activations, your Design Matrix, and the contrast being viewed.

At this point, you might need to re-threshold the results with a more stringent p-value cut-off. This is done by clicking on the Results button in the Menu box. Since the contrast is already defined you will only need to select this contrast and click on the Done button. Answer the questions in a. – e. above but use your more stringent cut-off.

Generating a Table of Results:

In any neuroimaging paper, you will need to list the areas that were activated in your study for various comparisons. After completing the above steps for a given contrast and arriving at a set of thresholds that look satisfactory click on the Whole Brain button.

1. A table will appear under the Glass Brain and Design Matrix listing by row information about each of the clusters of activation. From right-to-left, the columns are
 - a. The location of the peak activation in MNI coordinates
 - b. The uncorrected p-value
 - c. The Z-score for the peak activation
 - d. The t-statistic
 - e. Followed by p-values based on different corrections for multiple comparisons. The bold rows correspond to the location of peak (the largest t-statistic within the cluster).
 - f. The non-bold rows are the local maximum that are at least 8 mm away from the peak but still within the cluster.
2. Clicking on the x, y, z coordinates will move the cursor in the Glass Brain to that location. An untruncated printout of any p-value can be obtained by clicking on the p-value in the table. The result is printed in the matlab window where SPM was started.
3. This information will need to be included in your paper. It will be necessary to transform the MNI coordinates to Talairach coordinates. In addition, you will want to look-up the anatomical label for each of the major areas of activation (e.g. left middle frontal gyrus, etc).

Generating Figures:

In any neuroimaging paper, you will almost always see a figure showing the location of the activations overlaid on some representation of the anatomy. In general, cutting-and-pasting the Glass Brain into the paper is a clear sign of someone who does not know what they are doing. There are a number of ways to display the results.

1. Axial Slices:
 - a. After completing the above steps for a given contrast click on the Overlays... menu in the Results box and choose Slices
 - b. Choose the image to overlay results onto. If you did the optional step in the preprocessing lab and applied the Spatial Normalization to the subjects own SPRG, you can select the wPXXX.ANAT.nii. If not choose one of the files in the canonical directory of the SPM distribution: spm12/canonical.
 - c. Three axial planes centered on where the cursor is located in the glass brain are shown.

2. Sections (orthographic views):

- a. After completing the above steps for a given contrast click on the Overlays... menu in the Results box and choose Sections
- b. Choose the image to overlay results onto. If you did the optional step in the preprocessing lab and applied the Spatial Normalization to the subjects own SPRG, you can select the wPXXX.ANAT.nii. If not choose one of the files in the canonical directory of the SPM distribution: spm12\canonical.
- c. All three cross-sections are shown (axial, coronal, and sagittal).
- d. In this view, you can also click on any of the sections to move around to get different locations.

3. Renderings:

- a. After choosing a contrast click on the Overlays... menu in the Results box and choose Render
- b. Choose one of the files in the rend directory of the SPM distribution: spm12\rend.
- c. Choose New for the Old/New option, Slightly for the Brighten option, and RGB for the Colors option.
- d. 3D renderings of your data will appear in the Graphics box from 6 different angles.

Additional Contrasts:

For your study, you will want to compare other conditions and possible combinations of conditions. As described above, the basic comparison of two condition types is done using a 1 -1 weighting of those conditions with zeros for the other conditions and factors.