Functional MRI Processing for Mouse Micturition Studies

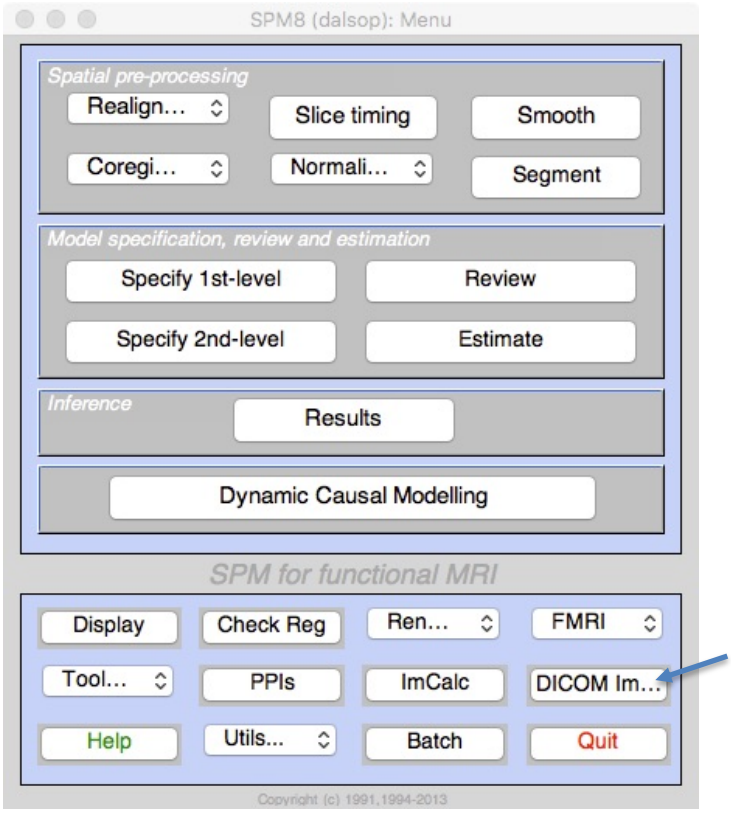
fMRI processing of our mouse micturition studies is still in development. Particular outstanding issues to resolve include

1. Motion reduction and correction
2. Accurate registration of echoplanar images to high resolution anatomic images
3. Identifying or defining anatomic location of smaller nuclei (PMC, …)
4. Converting pressure curves to key features for linear model analysis.

Processing is mostly performed within the program Statistical Parametric Mapping (SPM) from the Wellcome Institute for Cognitive Neurology. It is free software that runs within MATLAB. In addition, a toolbox called spmmouse has been downloaded for display on mouse atlases. To start SPM, you must startup MATLAB, and then type spm at the prompt. Select fMRI at the prompt.

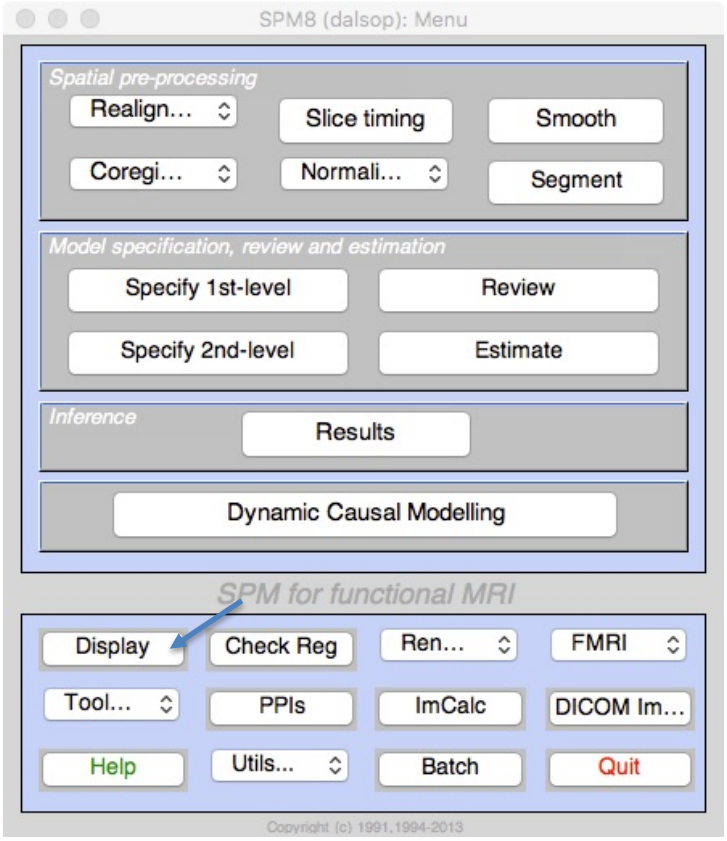
Step 1: Image conversion

Images are provided from the scanner in DICOM format. This is a standard clinical medical imaging format. The SPM software we use, however, prefers niftii format. SPM can import the images using its DICOM import tool.



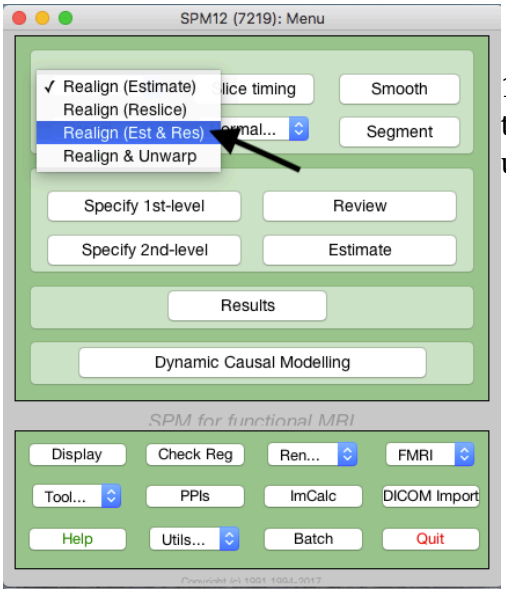
It will ask you to select the files to convert. You have to select all the files by using the shift click option. For fmri there are many files (>30000) so this can take 10-30 minutes depending on the size.

Once the images are converted, you can look at them with the DISPLAY button



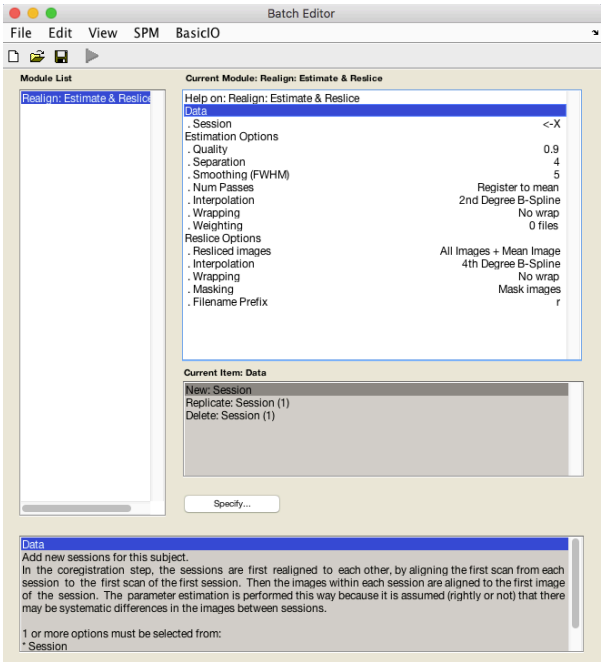
Step 2: Realignment

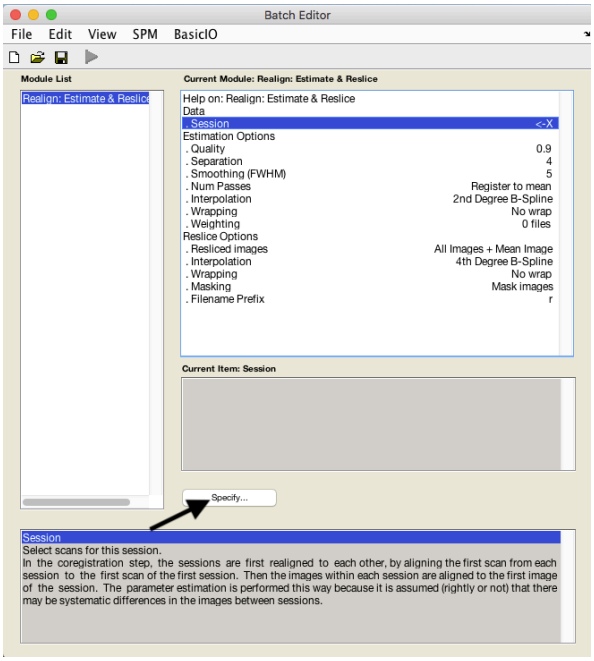
Images will need to be realigned and resliced to create a mean image. This is done under the "realign" drop down menu.



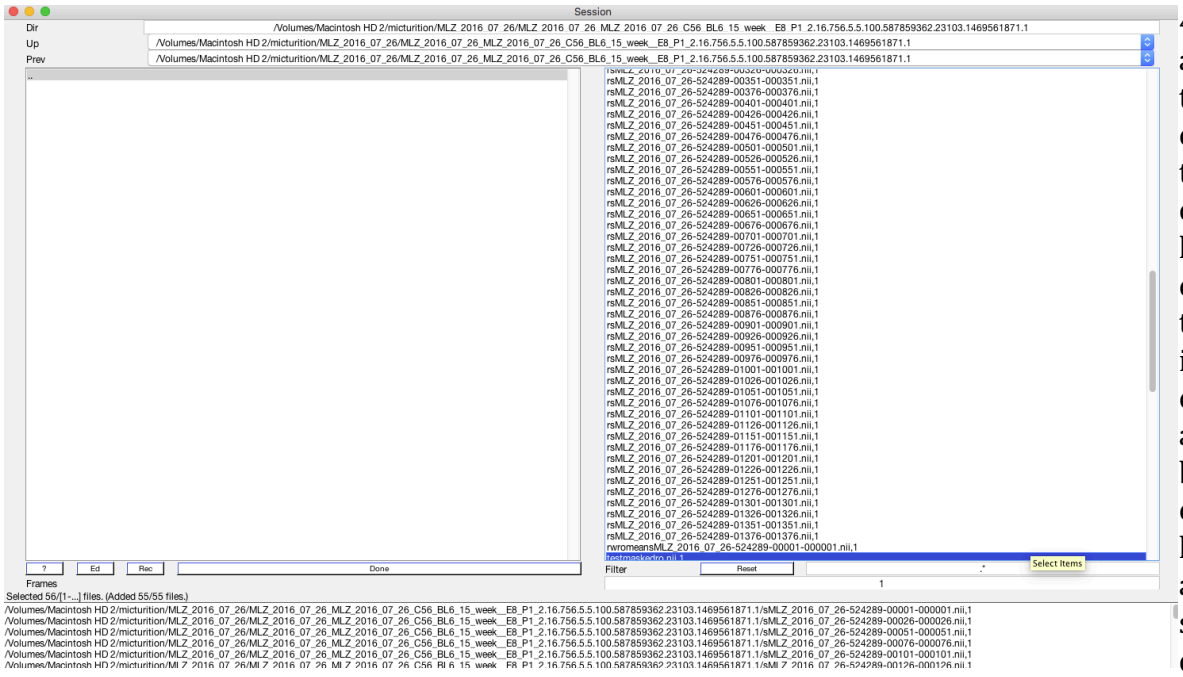
1.click the realign tab and select "Realign (Est & Res)" to open the batch editor. The batch editor will allow you to run modules on multiple sets of data at the same time.

2.Click the "Data" tab. Then click on the "Session" option. A new session will then be added.



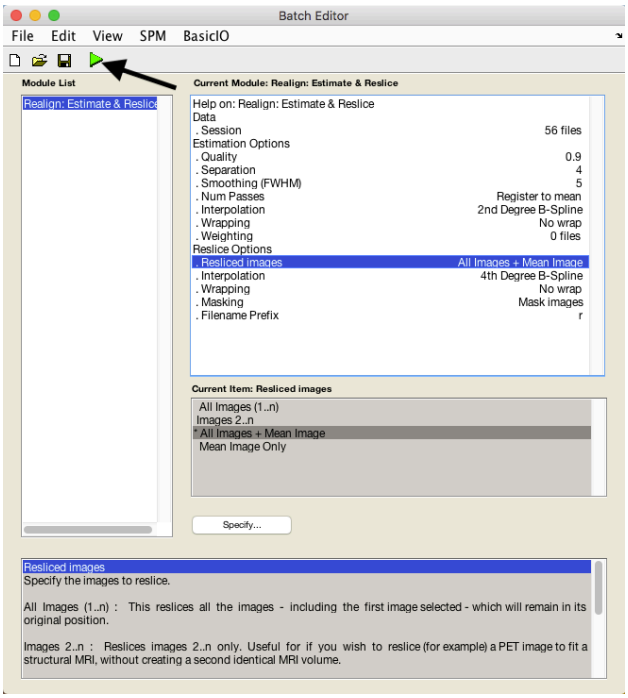


3. After highlighting “session” under current modules, click specify to navigate to the data you want aligned.



4.Navigate to all of the data to be processed. More than one file can be selected at once. Images that are going to be processed will appear in the bottom box once selected. When all images are selected click done.

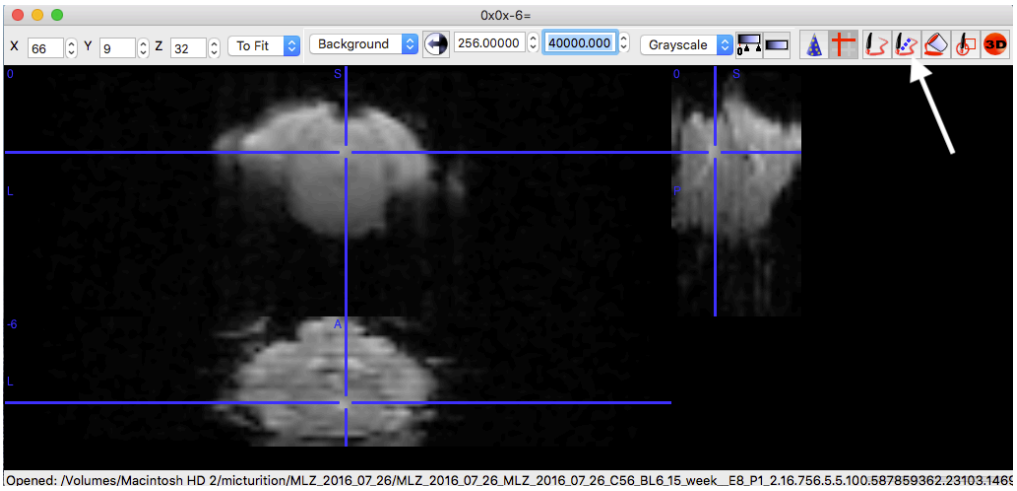
5. Make sure “All images + Mean Image” is selected under resliced images. If so hit the “run batch” button to run the realignment. Realigned files will be labeled with an “r” prefix. The Next step will be to rotate the realigned images to match a reference image.

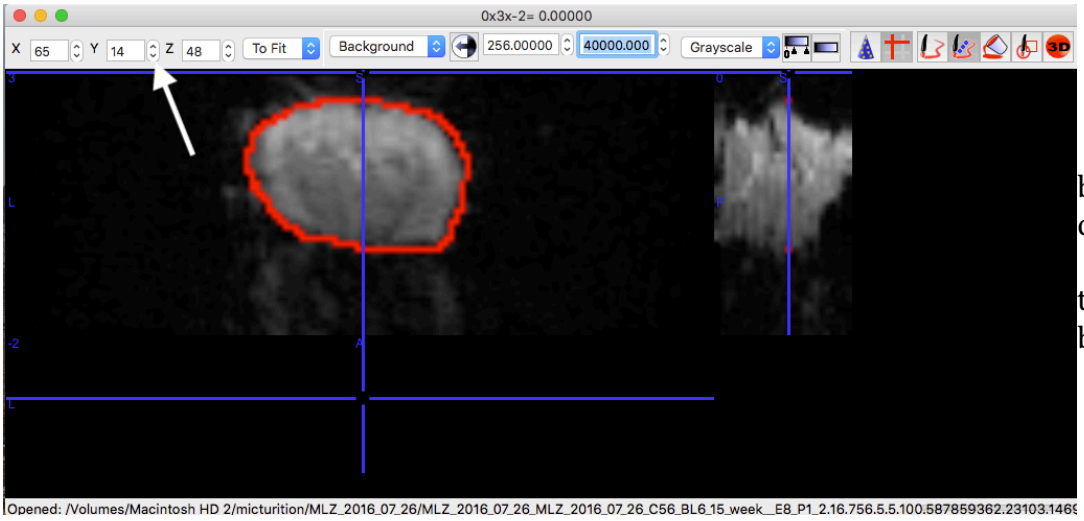


Step 3:Mask image

Before normalizing the images it is helpful to mask the scans to help the program’s algorithms run smoothly. Masking is done in the MRIcron program.

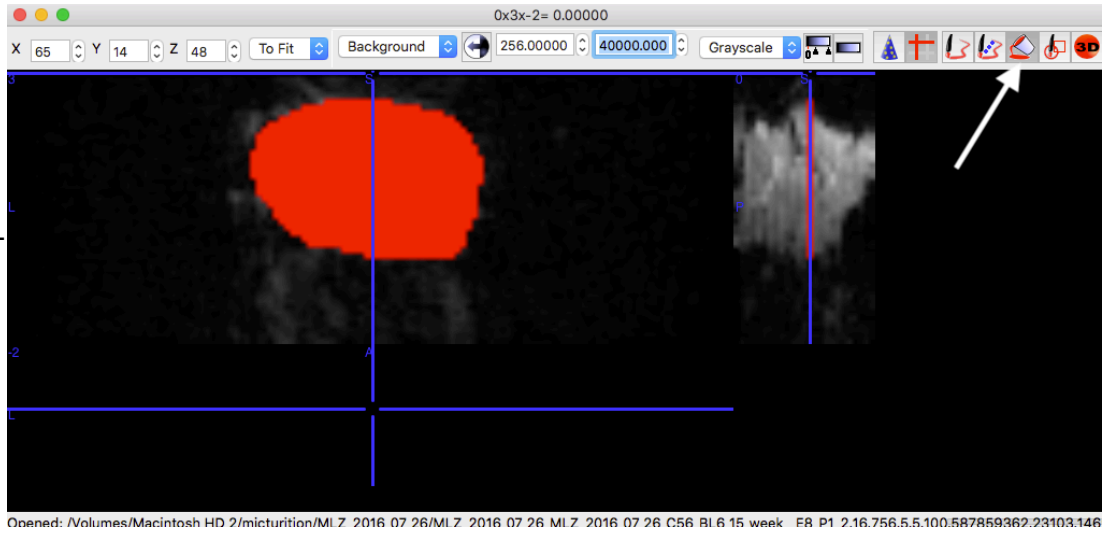
1.open MRIcron from the Applications list
2. open the reoriented image
3. click on the autoclose pen tool



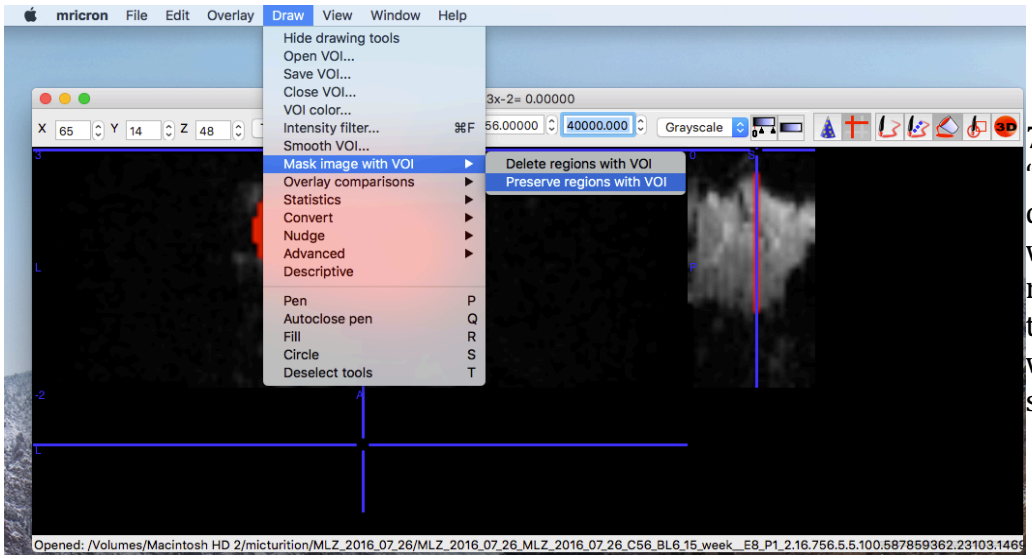


4.draw around the brain sections for each coronal slice. Filter through the Y-axis until all regions of the brain are outlined.

5.click on the fill tool and fill in the ROIs for each slice but clicking within the outlined region of the brain slice.

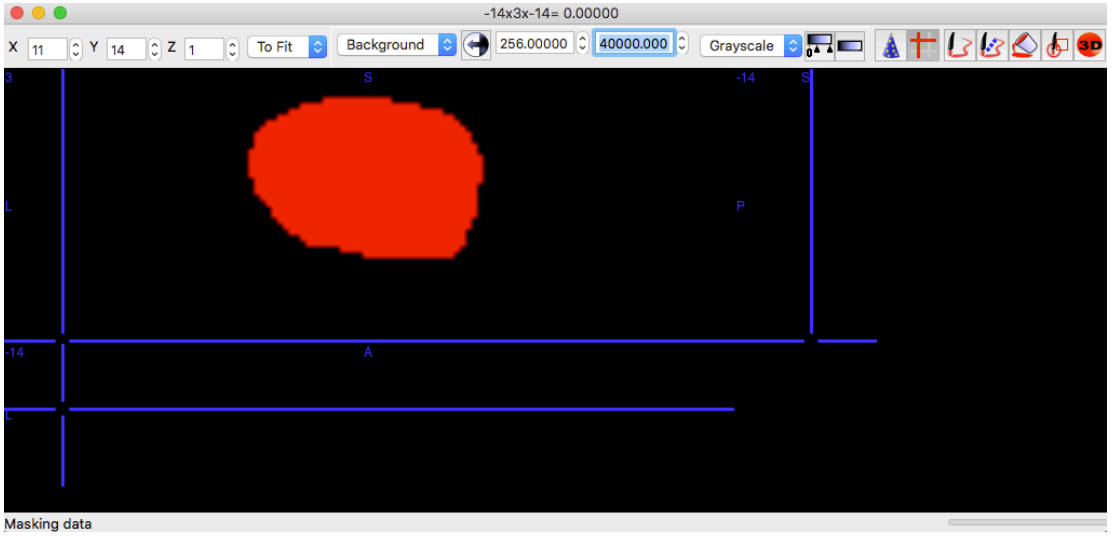


6.Once all slices are filled in, the VOI can be saved under the“Draw “ dropdown menu.



7.To mask the image, select “mask image with VOI” under the draw menu. Since we are trying to remove signal from areas that are not the brain, “preserve regions with VOI” will be selected to signal outside the VOI.

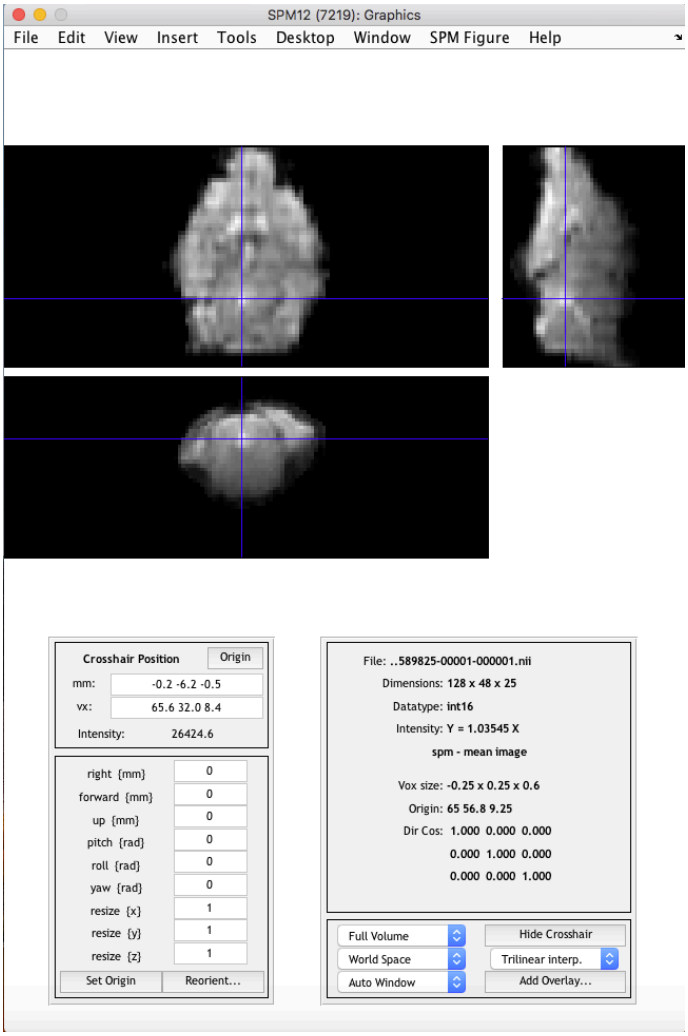
8.Once preserved only the brain should remain. The next step is to save the newly masked image as a NifTi. Under file select “save as NifTi.”



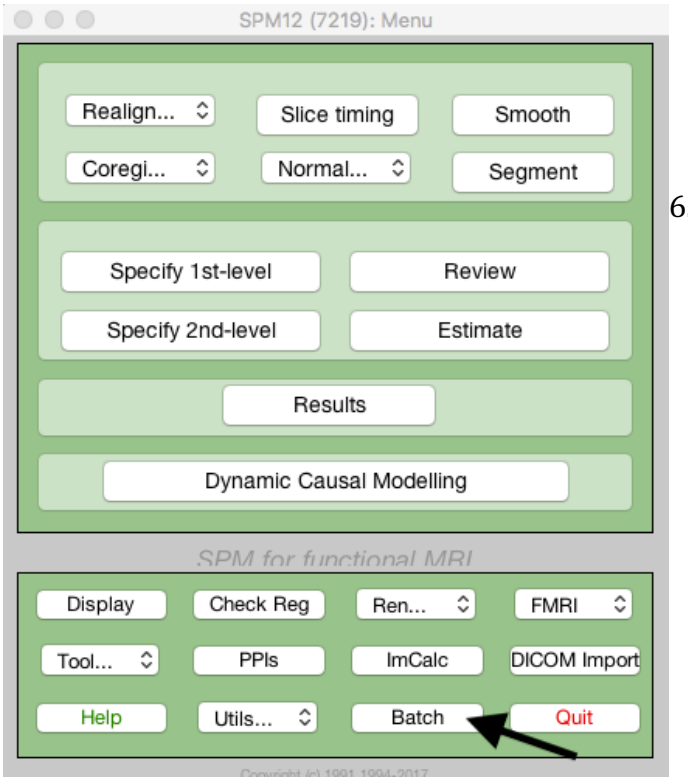
9. The file will be exported as a compressed file. Once unzipped the “.nii” extension will have to be manually added.

Step 4: Image Rotation

1. Use the Display option to open the maskedmean image
2. Move the crosshairs so that they lie over the 4th ventricle

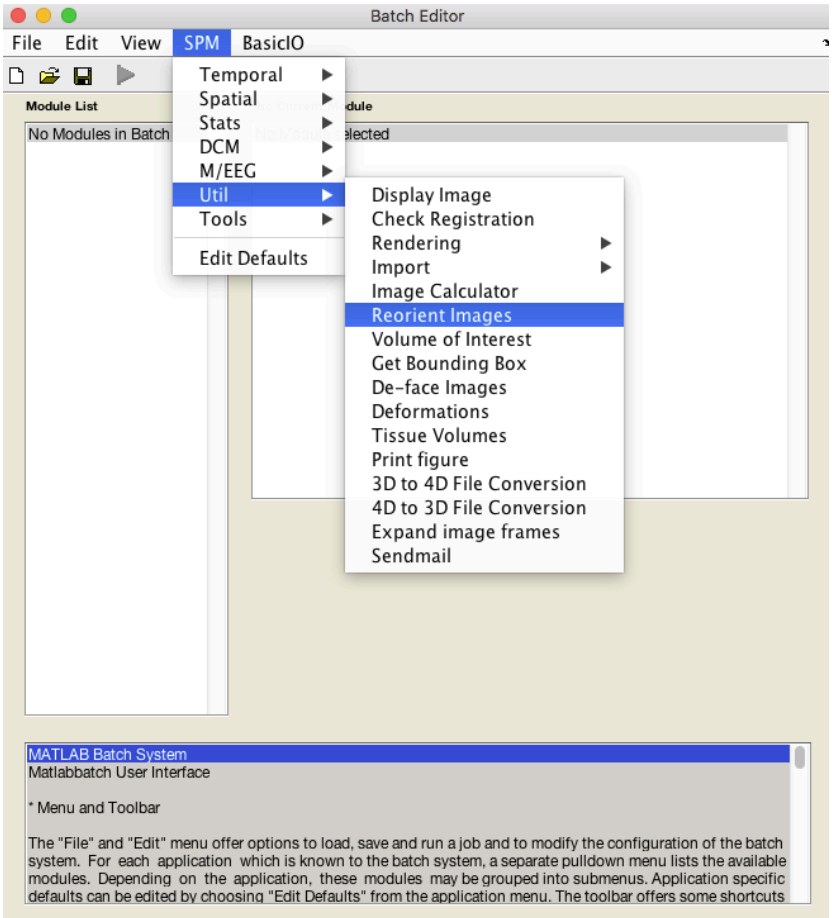


3. In the MatLab Command window type "x=[x; y; z; 1]", where "x", "y", and "z" are representative of the three mm values of the crosshairs. Then hit the "return" key.
4. Use the script "findrotmat" for images that were taken with the mouse in the prone position. Use the script "findrotmatsupine" if the scan was done with the mouse in the supine position.
5. The rotation Matrix will be give with the last column representing the displacement values needed to match the scan to the mouse atlas



6. In SPM Open the batch editor

7. Under the SMP tab navigate to “Re-orient Images”

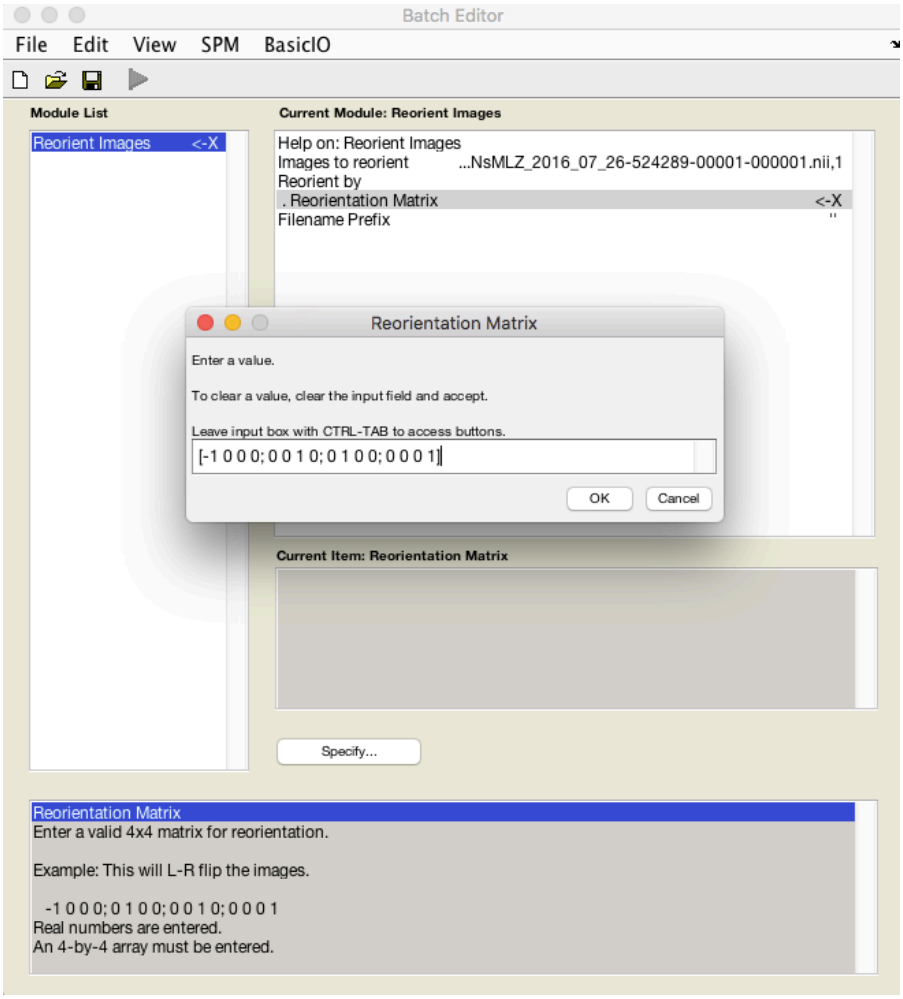


8. Once loaded click “specify” to select the mean images that you would like to process.

9. Once scans are selected, the reorientation matrix will need to be edited. click on “reorientation Matrix” and then “specify” to edit this.

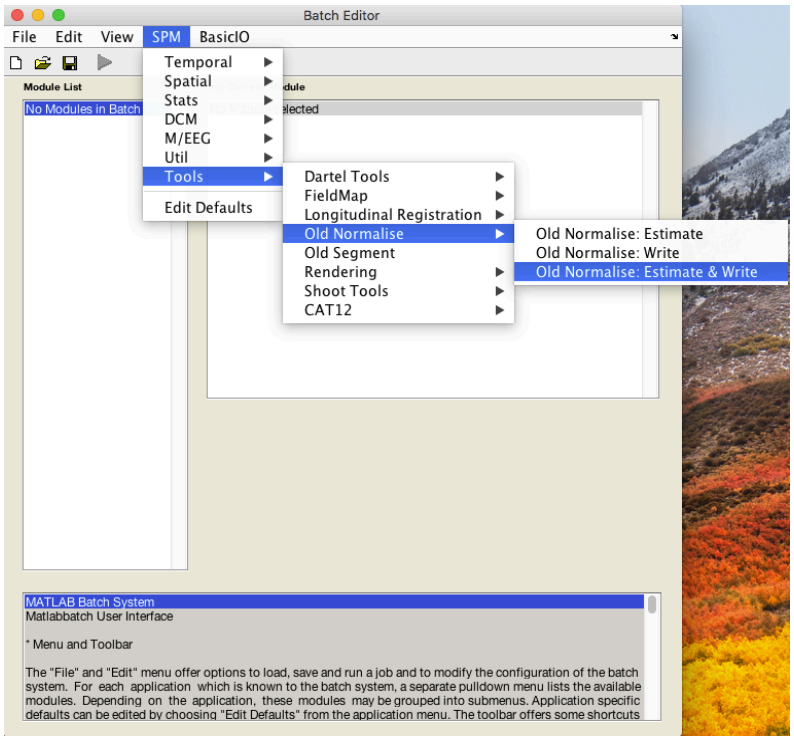
10. The reorientation Matrix is a 4 x 4 matrix which will determine how the mean image is rotated. In order to match the mouse atlas reference image enter “ [-1 0 0 0; 0 0 1 0; 0 1 0 0; 0 0 0 1]”

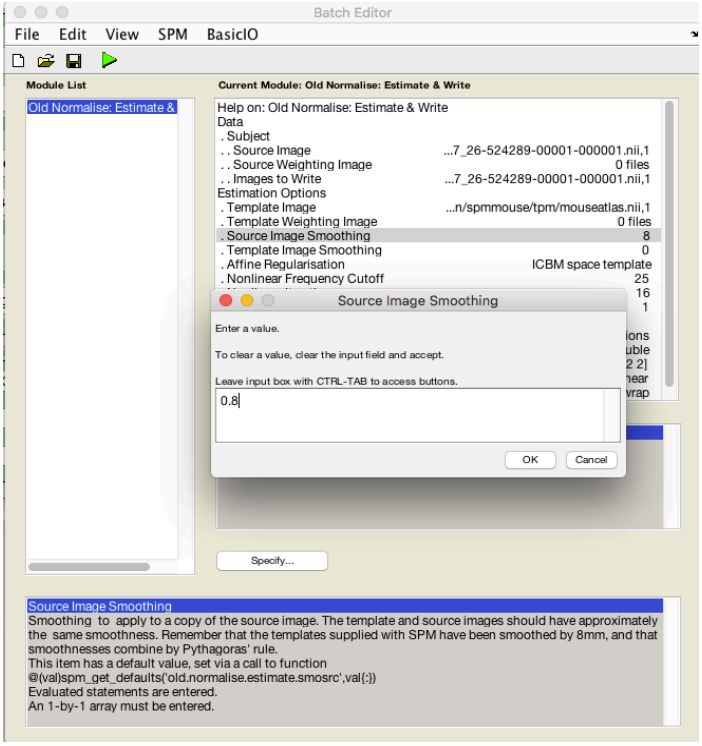
11. Click “OK”, and then change the file prefix to “ro”.



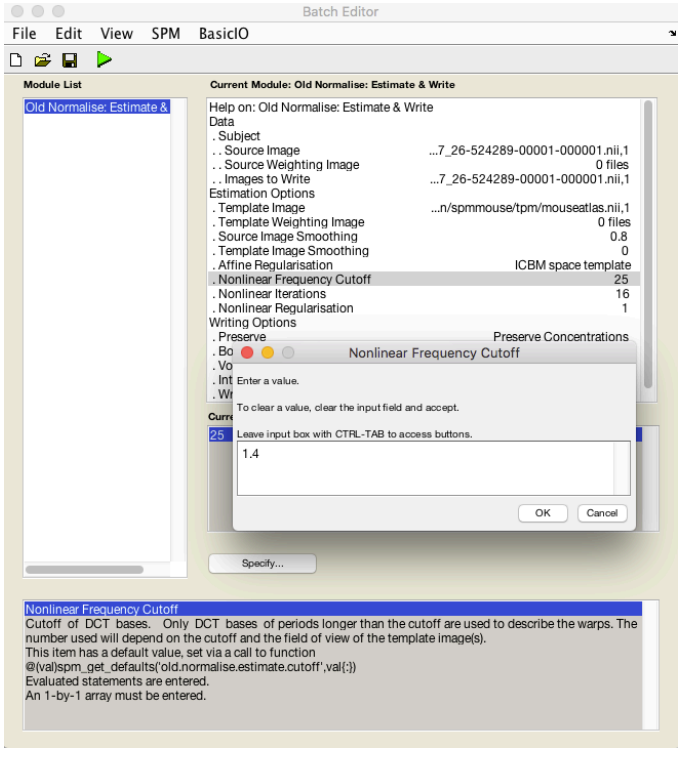
12. Click the run button. New images will be saved with the “ro” prefix.

Step 5: Normalization
1.Open the batch editor
2. From the SPM menu select “Old Normalise” (estimate & write)

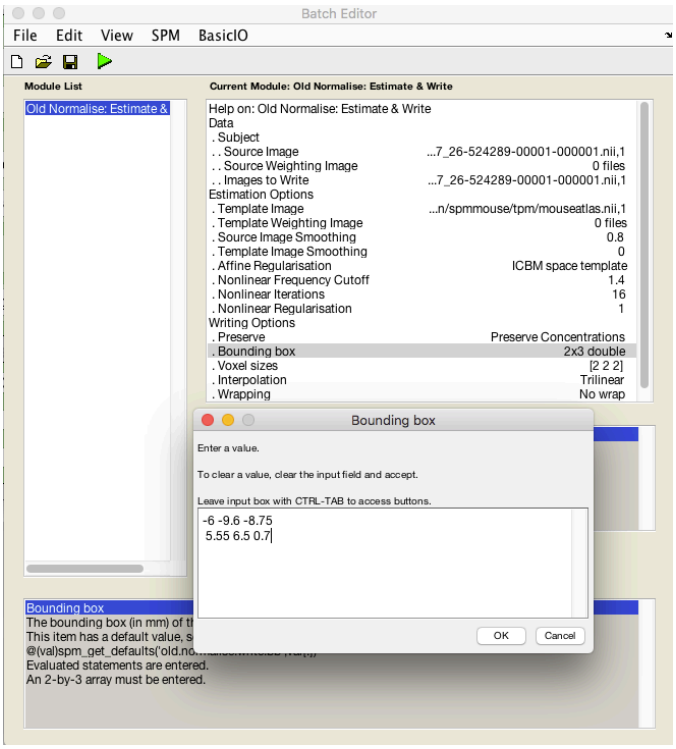




3.Click on data and hit specify
4.Specify a source image (images you want normalized), images to write (images you want normalized), and a template image (mouse atlas).
5. Change smoothing to 0.8



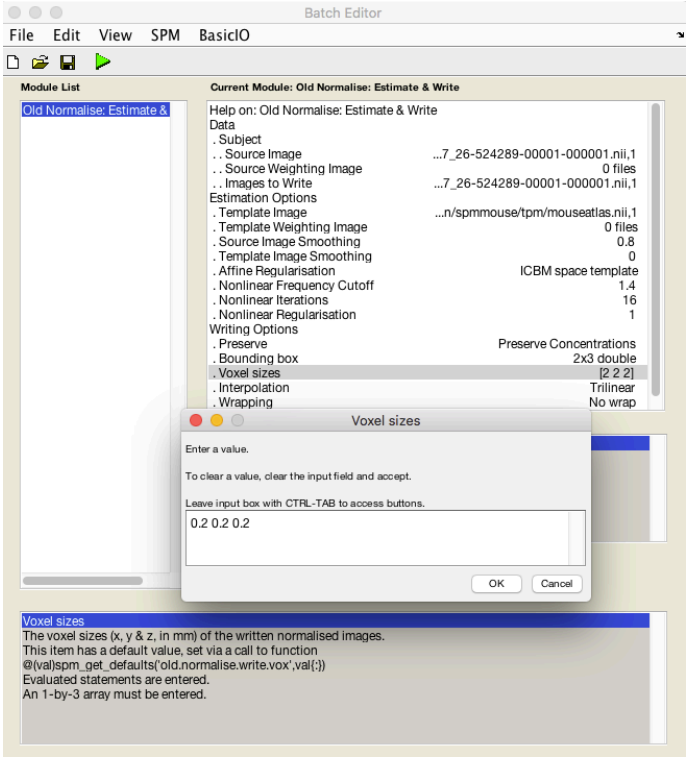
6. Change nonlinear cutoff frequency to 1.4



7. change the bounding box to : -6 -9.6 -8.75
5.55 6.5 0.7

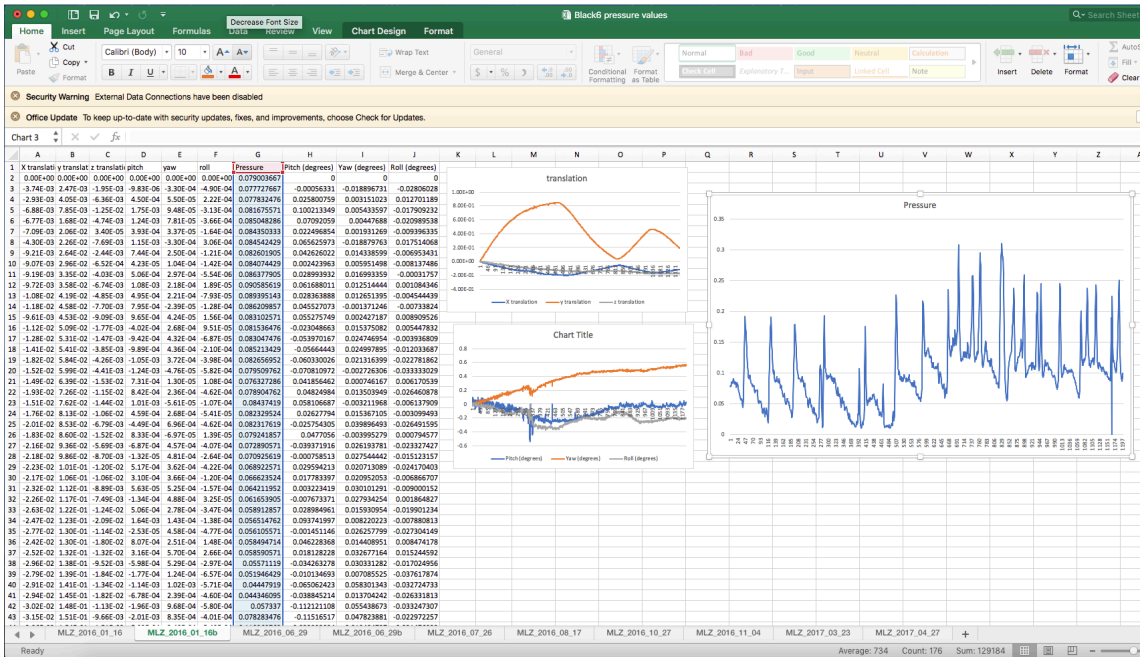
8. Change voxel size to 0.2 0.2 0.2

9.Click the run batch button
10. The normalized file will be saved with the prefix “w”. A normalization report will also be exported as a .ps file.

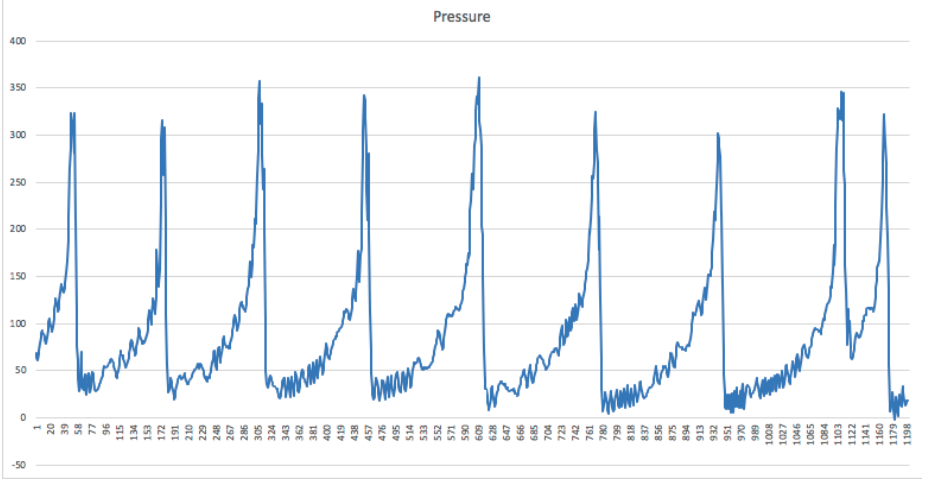


Step 6:Analyzing CMGs and movement

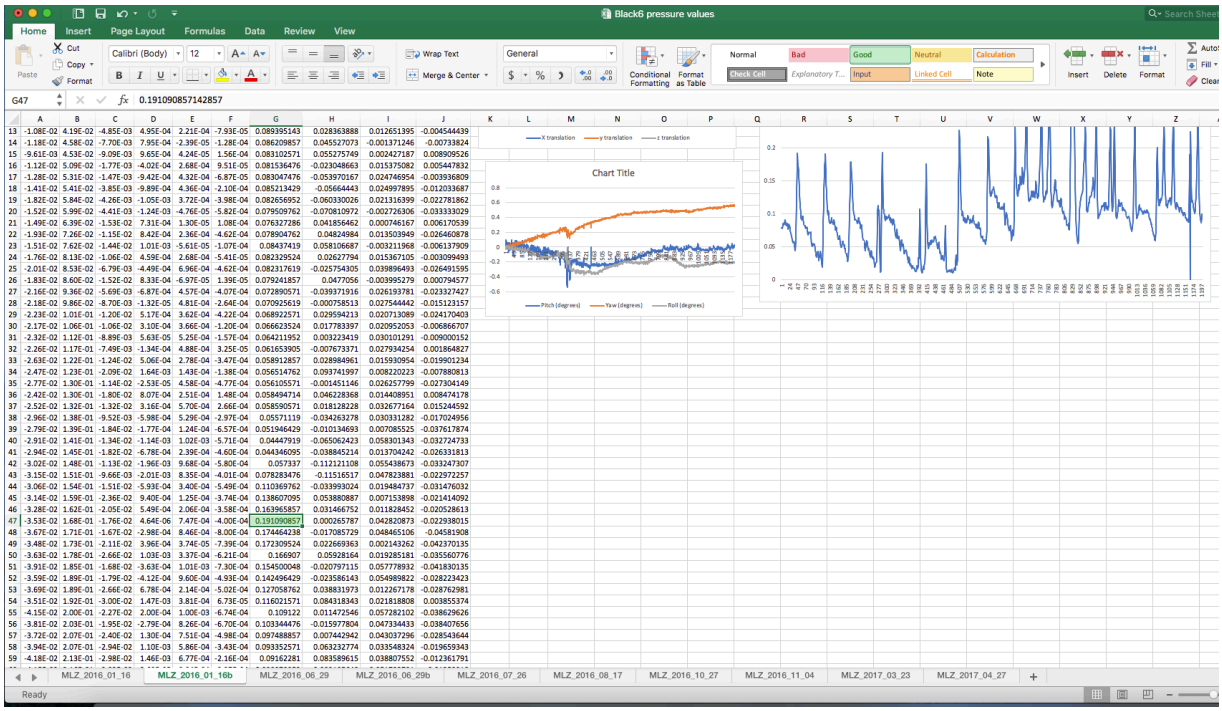
1.The data for the pressure curves are saved in their own text document. Copy and paste this file into excel and create a graph based on the values. The values from the motion files should also be imported to gauge whether either portions or entire subjects should be omitted from the analysis. portions of the scans which have over 1 degrees or 1 mm deviations should be excluded. Along with these scans, mice with poor CMGs should also be removed from the analysis.



2. A good CMG will have well defined and evenly spaced peaks. You should see a general trend of a slow buildup of pressure and then a rapid drop after voiding. Many animals will not have ideal CMGs, for those subjects it is best to omit certain portions of the scans.

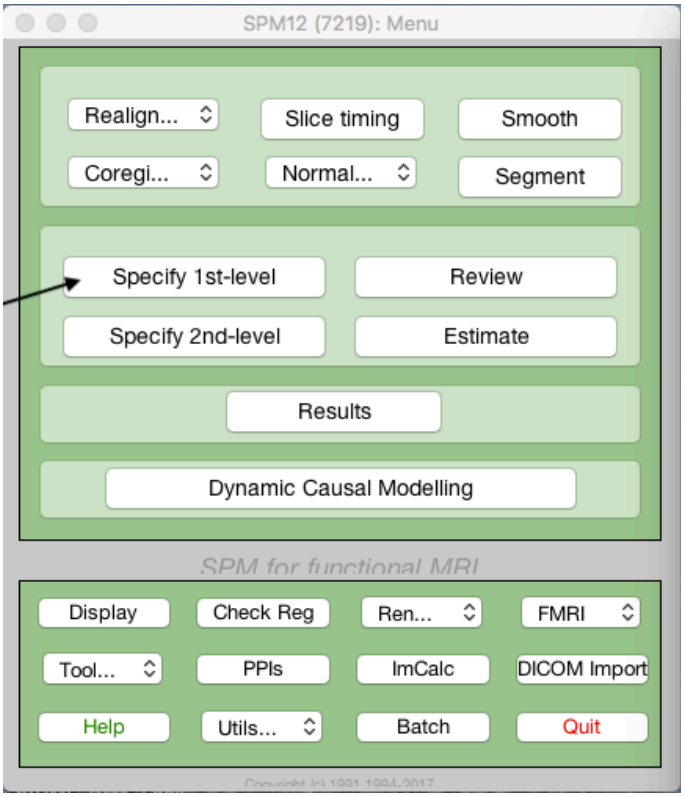


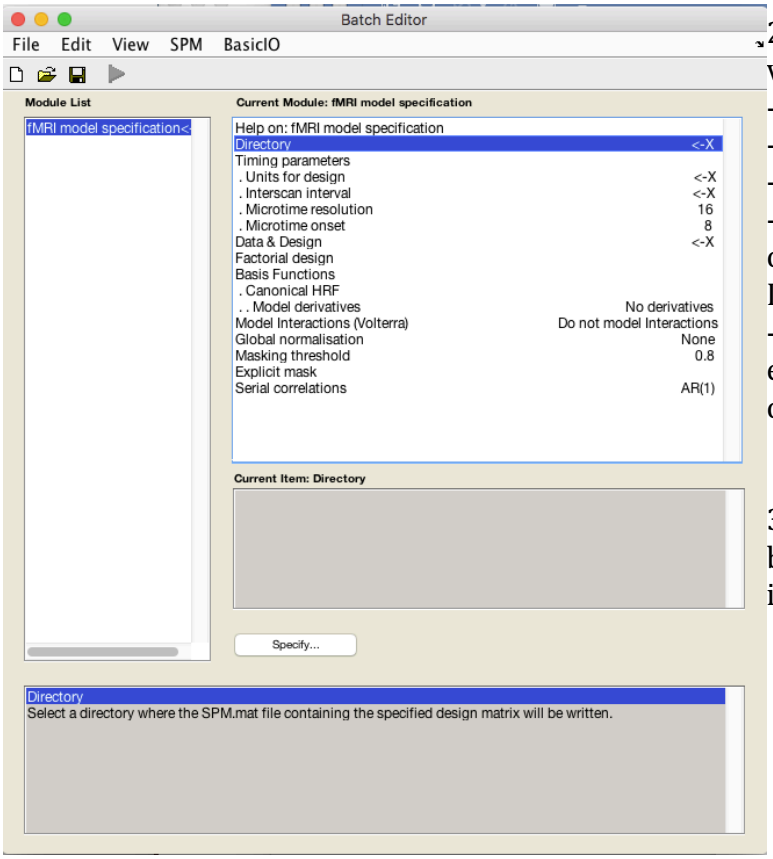
3.Find the peak of each CMG on the pressure graph in excel. If you hover the cursor over each peak in the pressure graph, you will be given a rough idea of the scan at which the peak occurs. Find the scan number in the pressure column and mark it.



Step 7: 1st Level Analysis

1. Navigate to the directory where you will want the SPM file saved. It needs to be in a unique folder with no other SPM files. If you try to save it in a path with another SPM file the older file will be overwritten.

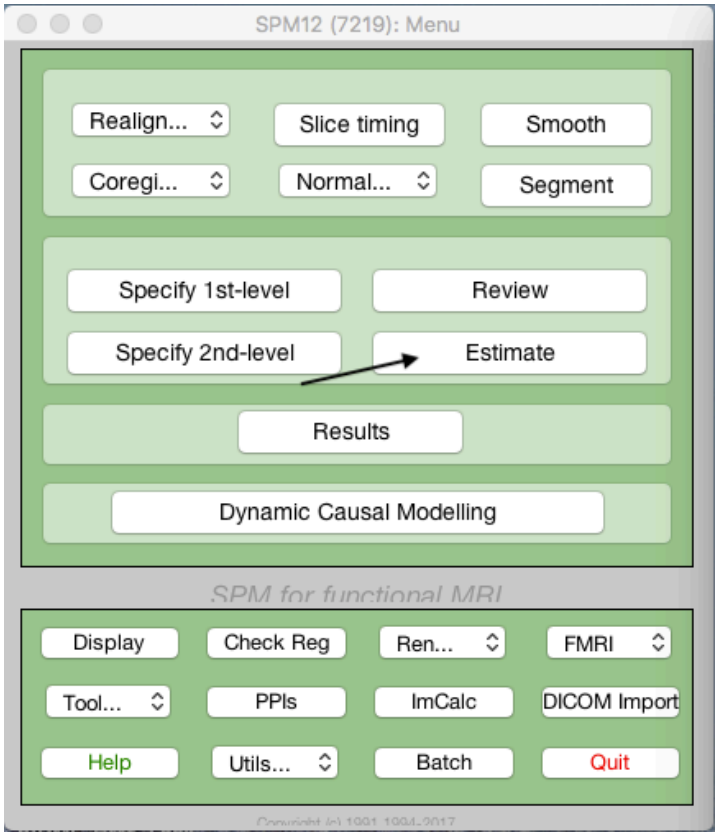


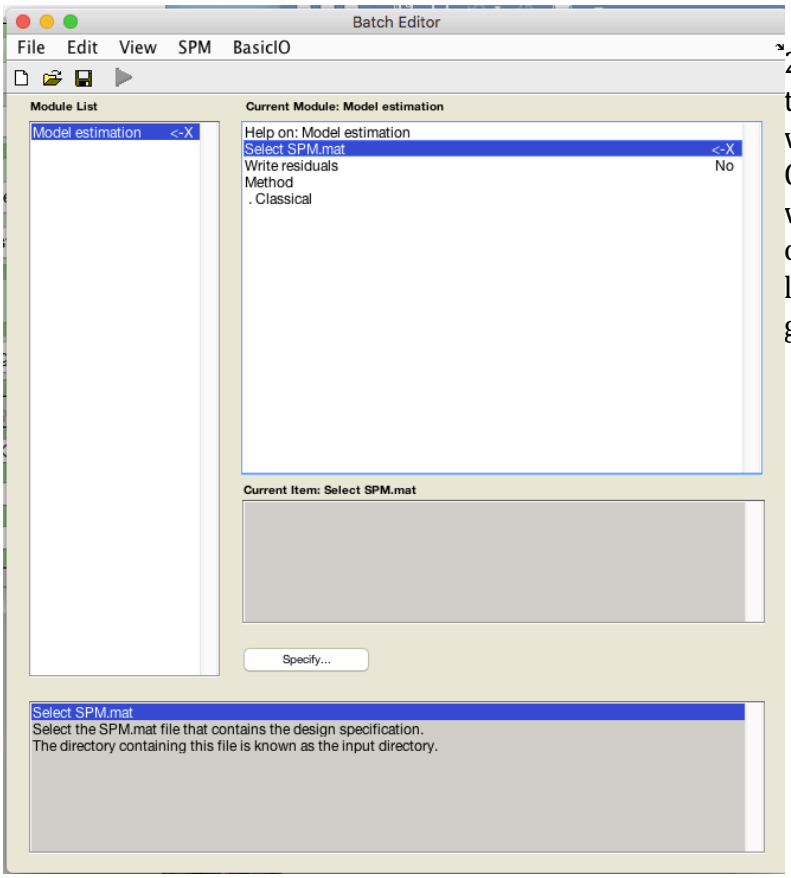


2. Change the parameters to the following values:
-Units for design “Scans”
-Scan Interval=2
-Set 8 conditions for each subject
-After finding the scans that contain the peak of each CMG, enter them into the Onsets box. Data will be analyzed around these scans.
-Set duration of the onsets to 1. This will make each condition measure the scan right after the onset.

3. When all of the settings are correct run the batch. This can take a little bit of time depending on how many subjects are analyzed.

Step 8: Estimate
1. Once the SPM file is generated it will need to be estimated. Click on Estimate



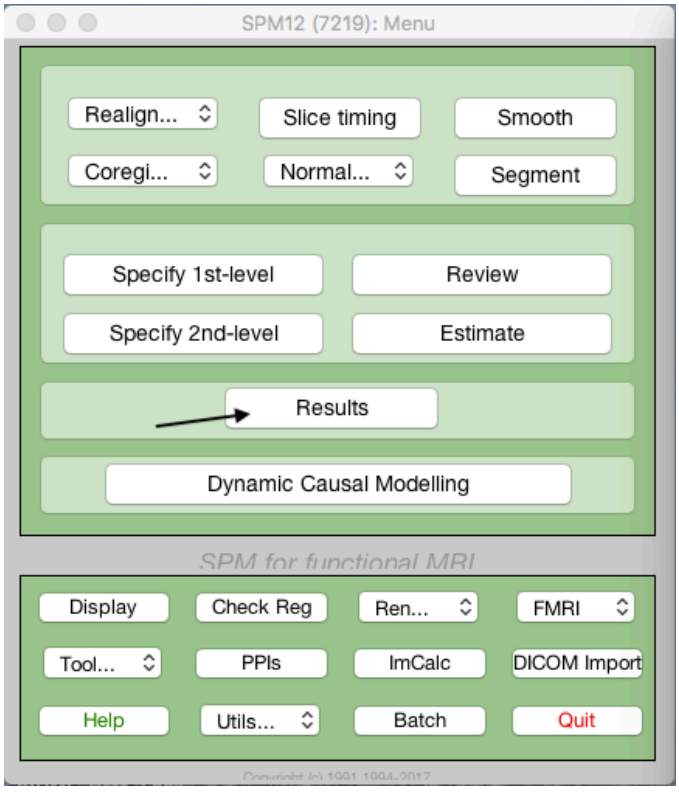


2. Specify the SPM file and then and then run the batch. SPM will ask if it is okay to over-write the SPM file in the current directory. Click 'Ok". The estimation step can take any-where from a few minutes to hours depending on how many subjects were in analysis. If a large number of subjects are involved it is a good idea to run the estimation overnight.

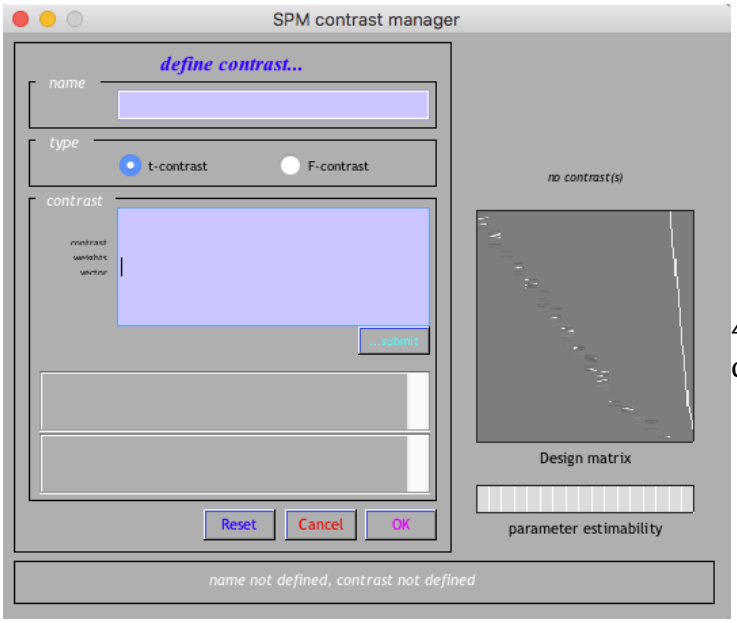
Step 9: Reviewing Results

1. Once the estimation is finished you can view the results by clicking on "Results"

2. Navigate to the estimated SPM file in the data browser



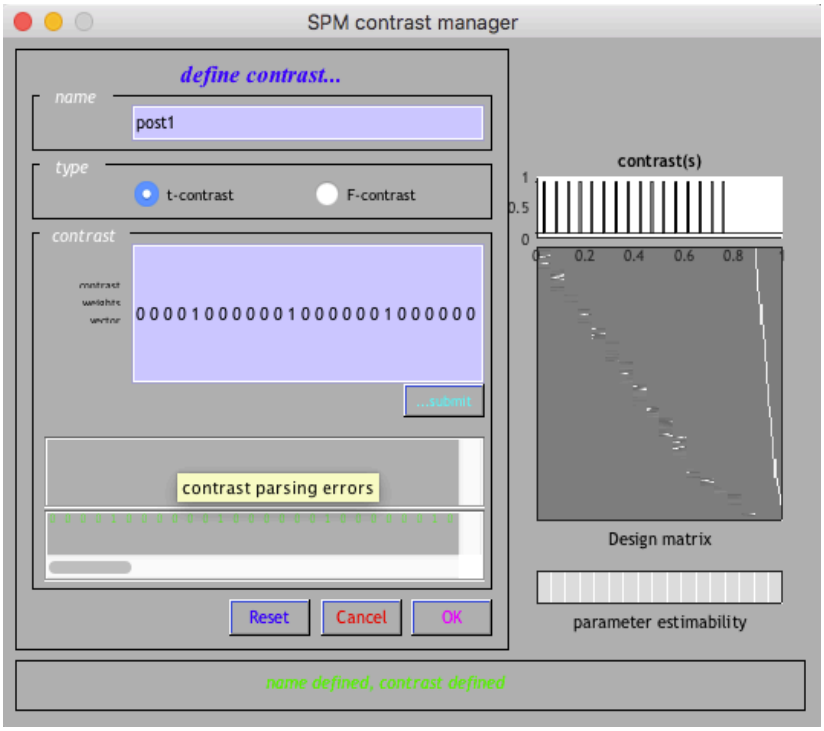
3. The Contrast manager will be brought up. To add a new contrast click on “Define New Contrast”

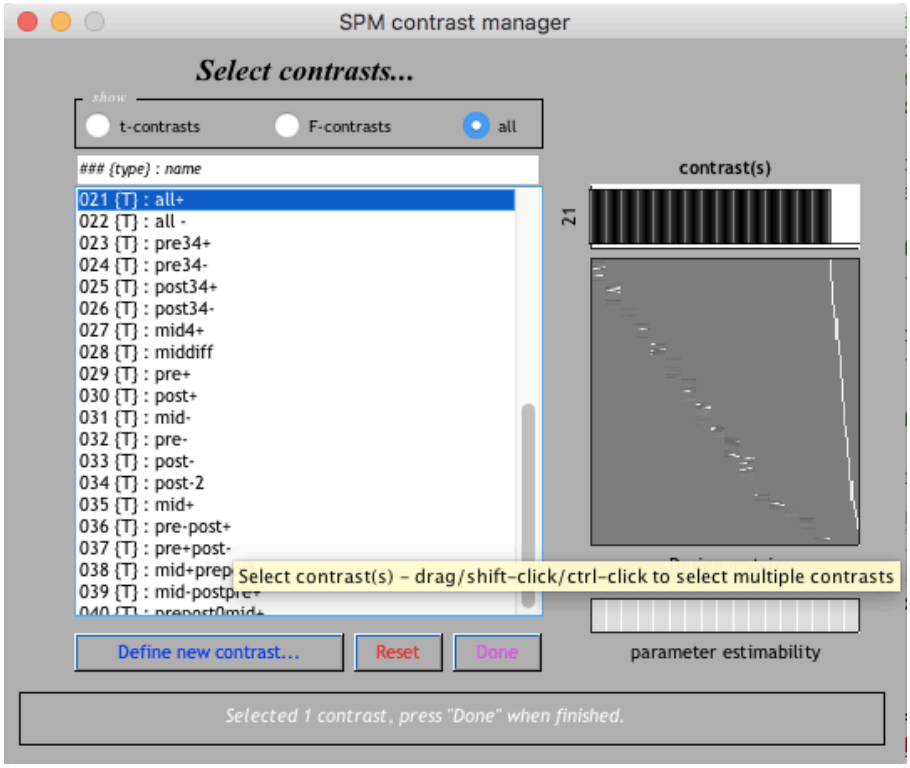


4. Select t-contrast and create a name for the new contrast.

5. You will have to enter the weighting for all conditions. Use “0” for unweighted conditions, “1” for positive weighting, and “-1” for conditions that you want weighted negatively. You will need to enter eight values per subject.

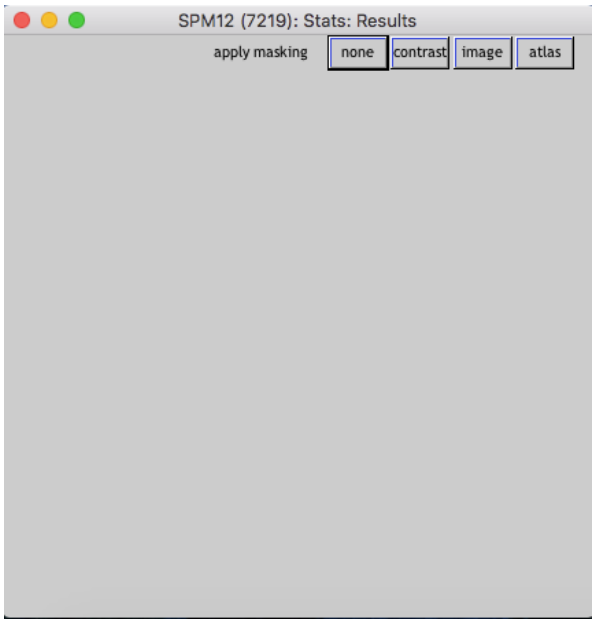
Click “submit” to check if the contrast are entered as desired. A preview will show up in the contrast(s) box above the design matrix. If the contrast is entered correctly click “OK”

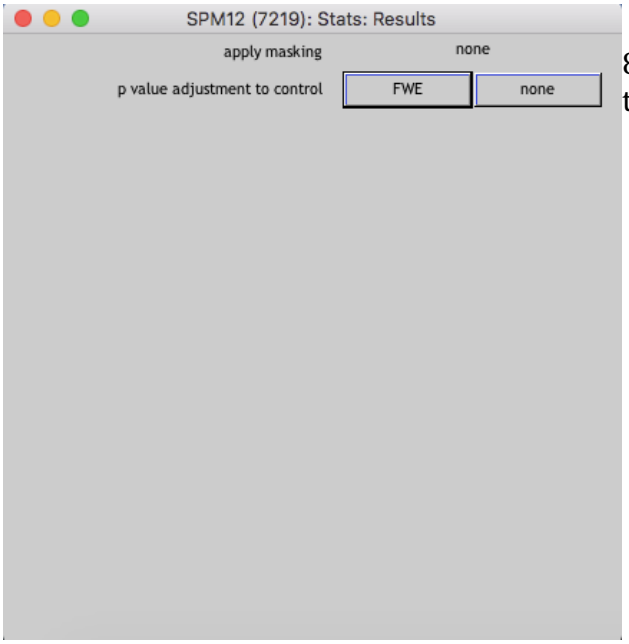




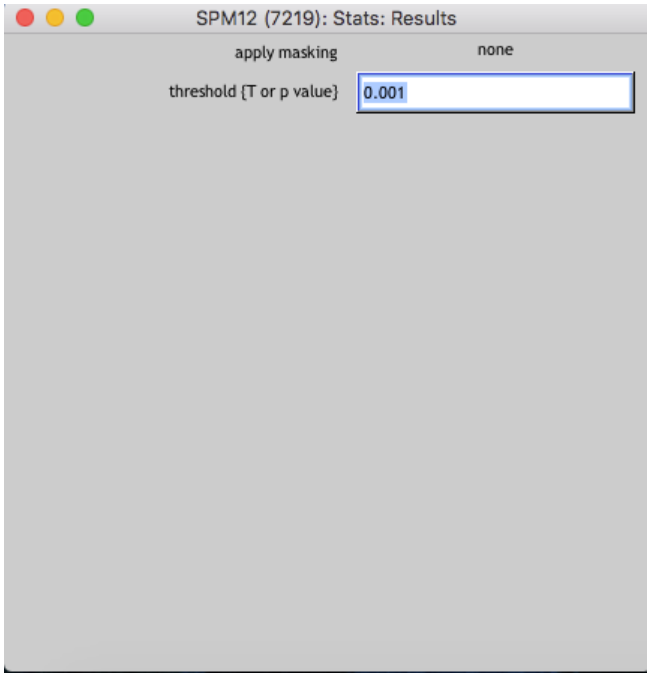
6. You will be brought back to the contrast selection. All contrasts that have been entered will appear here. Click on the contrast you would like to view and then click "Done".

7. SPM about applying a mask. Click "None".

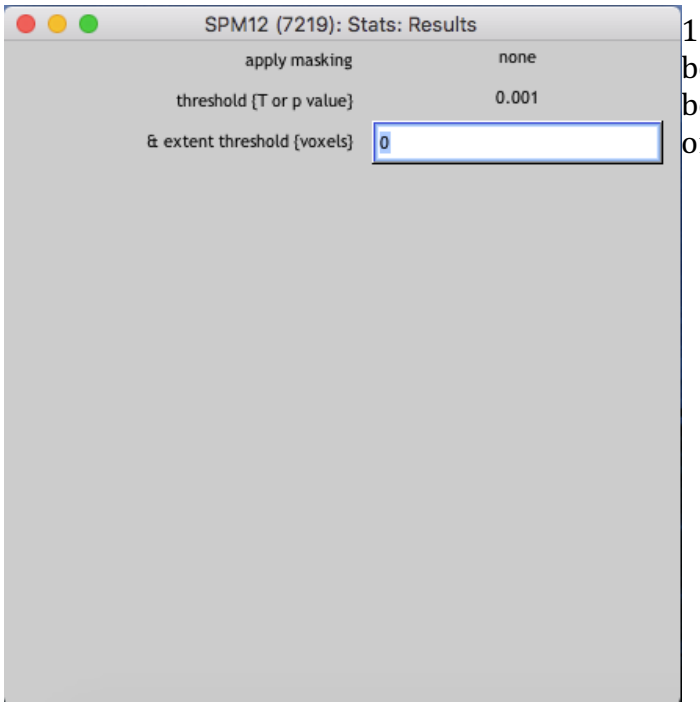




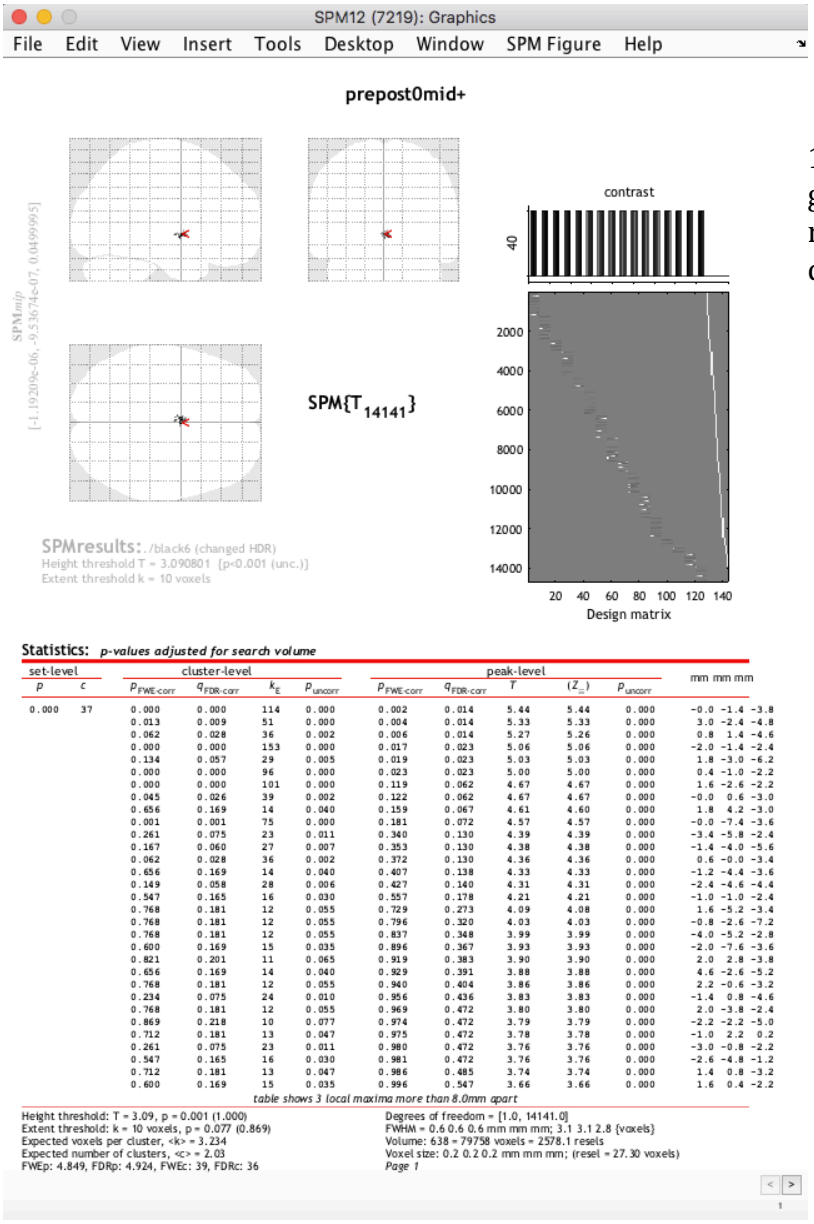
8. Select whether or not you want the p-value adjusted to the control. Hit “none”.



9. Set the threshold for significance. Hit enter after applying the value you would like to use.

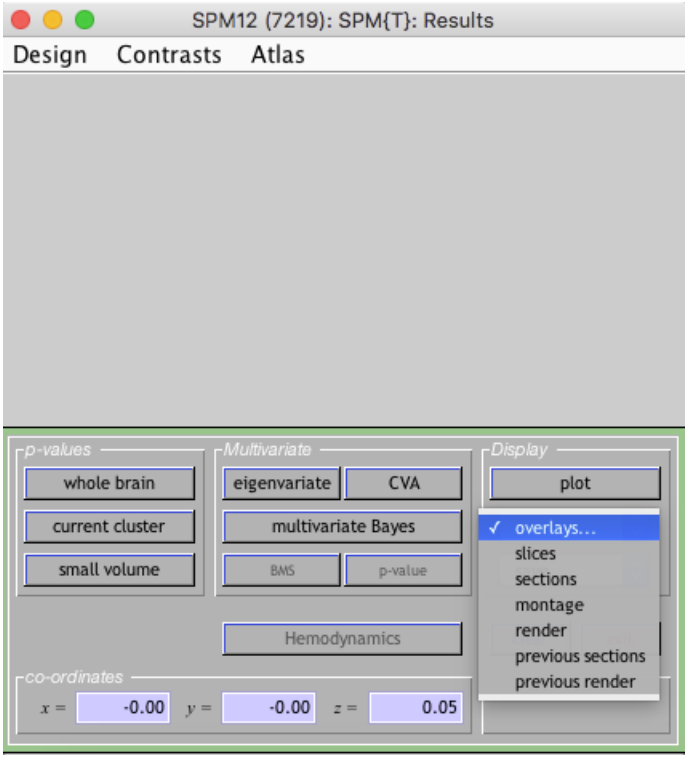


10. The cluster size required for significance will then be set. Hit enter when you have set the desired number of voxels. Only significant clusters of the threshold or greater will be included.

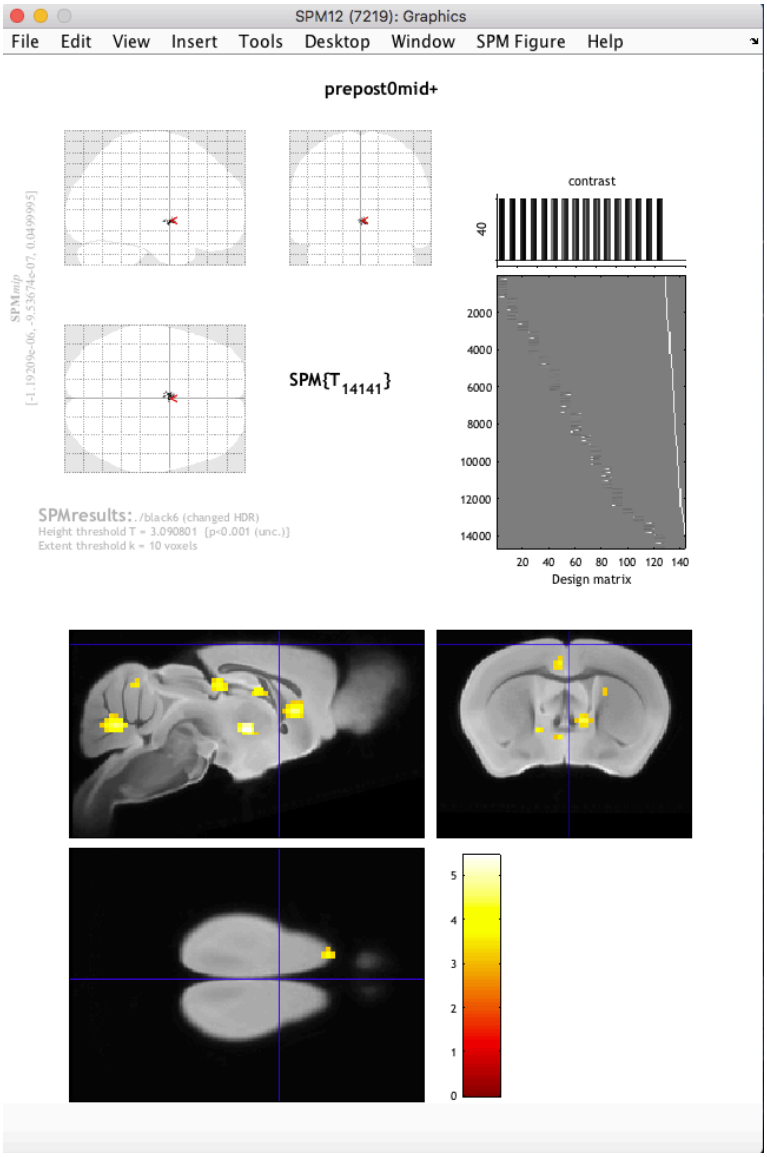


11. Significant clusters will be listed in the graphics window. To view the clusters on a mouse brain you will need to overlay the data on the mouse atlas.

12. To view the results overlaid on the mouse atlas, select “Overlays” and navigate then select “sections” from the drop down menu. Select the mouse atlas from the file browser.



13. The areas that are significant according to your parameters will now be overlaid over the mouse atlas.



Step 10: Identifying significant regions

To identify significant regions the results will need to be cross-referenced with a mouse brain atlas. The coordinates of significant clusters can be found in the statistics window.

Statistics: *p-values adjusted for search volume*

set-level		cluster-level				peak-level					mm mm mm
p	c	$p_{FWE\text{-}corr}$	$q_{FDR\text{-}corr}$	k_E	p_{uncorr}	$p_{FWE\text{-}corr}$	$q_{FDR\text{-}corr}$	T	$(Z_=)$	p_{uncorr}	
0.000	37	0.000	0.000	114	0.000	0.002	0.014	5.44	5.44	0.000	-0.0 -1.4 -3.8
		0.013	0.009	51	0.000	0.004	0.014	5.33	5.33	0.000	3.0 -2.4 -4.8
		0.062	0.028	36	0.002	0.006	0.014	5.27	5.26	0.000	0.8 1.4 -4.6
		0.000	0.000	153	0.000	0.017	0.023	5.06	5.06	0.000	-2.0 -1.4 -2.4
		0.134	0.057	29	0.005	0.019	0.023	5.03	5.03	0.000	1.8 -3.0 -6.2
		0.000	0.000	96	0.000	0.023	0.023	5.00	5.00	0.000	0.4 -1.0 -2.2
		0.000	0.000	101	0.000	0.119	0.062	4.67	4.67	0.000	1.6 -2.6 -2.2
		0.045	0.026	39	0.002	0.122	0.062	4.67	4.67	0.000	-0.0 0.6 -3.0
		0.656	0.169	14	0.040	0.159	0.067	4.61	4.60	0.000	1.8 4.2 -3.0
		0.001	0.001	75	0.000	0.181	0.072	4.57	4.57	0.000	-0.0 -7.4 -3.6
		0.261	0.075	23	0.011	0.340	0.130	4.39	4.39	0.000	-3.4 -5.8 -2.4
		0.167	0.060	27	0.007	0.353	0.130	4.38	4.38	0.000	-1.4 -4.0 -5.6
		0.062	0.028	36	0.002	0.372	0.130	4.36	4.36	0.000	0.6 -0.0 -3.4
		0.656	0.169	14	0.040	0.407	0.138	4.33	4.33	0.000	-1.2 -4.4 -3.6
		0.149	0.058	28	0.006	0.427	0.140	4.31	4.31	0.000	-2.4 -4.6 -4.4
		0.547	0.165	16	0.030	0.557	0.178	4.21	4.21	0.000	-1.0 -1.0 -2.4
		0.768	0.181	12	0.055	0.729	0.273	4.09	4.08	0.000	1.6 -5.2 -3.4
		0.768	0.181	12	0.055	0.796	0.320	4.03	4.03	0.000	-0.8 -2.6 -7.2
		0.768	0.181	12	0.055	0.837	0.348	3.99	3.99	0.000	-4.0 -5.2 -2.8
		0.600	0.169	15	0.035	0.896	0.367	3.93	3.93	0.000	-2.0 -7.6 -3.6
		0.821	0.201	11	0.065	0.919	0.383	3.90	3.90	0.000	2.0 2.8 -3.8
		0.656	0.169	14	0.040	0.929	0.391	3.88	3.88	0.000	4.6 -2.6 -5.2
		0.768	0.181	12	0.055	0.940	0.404	3.86	3.86	0.000	2.2 -0.6 -3.2
		0.234	0.075	24	0.010	0.956	0.436	3.83	3.83	0.000	-1.4 0.8 -4.6
		0.768	0.181	12	0.055	0.969	0.472	3.80	3.80	0.000	2.0 -3.8 -2.4
		0.869	0.218	10	0.077	0.974	0.472	3.79	3.79	0.000	-2.2 -2.2 -5.0
		0.712	0.181	13	0.047	0.975	0.472	3.78	3.78	0.000	-1.0 2.2 0.2
		0.261	0.075	23	0.011	0.980	0.472	3.76	3.76	0.000	-3.0 -0.8 -2.2
		0.547	0.165	16	0.030	0.981	0.472	3.76	3.76	0.000	-2.6 -4.8 -1.2
		0.712	0.181	13	0.047	0.986	0.485	3.74	3.74	0.000	1.4 0.8 -3.2
		0.600	0.169	15	0.035	0.996	0.547	3.66	3.66	0.000	1.6 0.4 -2.2

table shows 3 local maxima more than 8.0mm apart

Height threshold: T = 3.09, p = 0.001 (1.000)
Extent threshold: k = 10 voxels, p = 0.077 (0.869)
Expected voxels per cluster, <k> = 3.234
Expected number of clusters, <c> = 2.03
FWEp: 4.849, FDRp: 4.924, FWEc: 39, FDRc: 36

Degrees of freedom = [1.0, 14141.0]
FWHM = 0.6 0.6 0.6 mm mm mm; 3.1 3.1 2.8 {voxels}
Volume: 638 = 79758 voxels = 2578.1 resels
Voxel size: 0.2 0.2 0.2 mm mm mm; (resel = 27.30 voxels)
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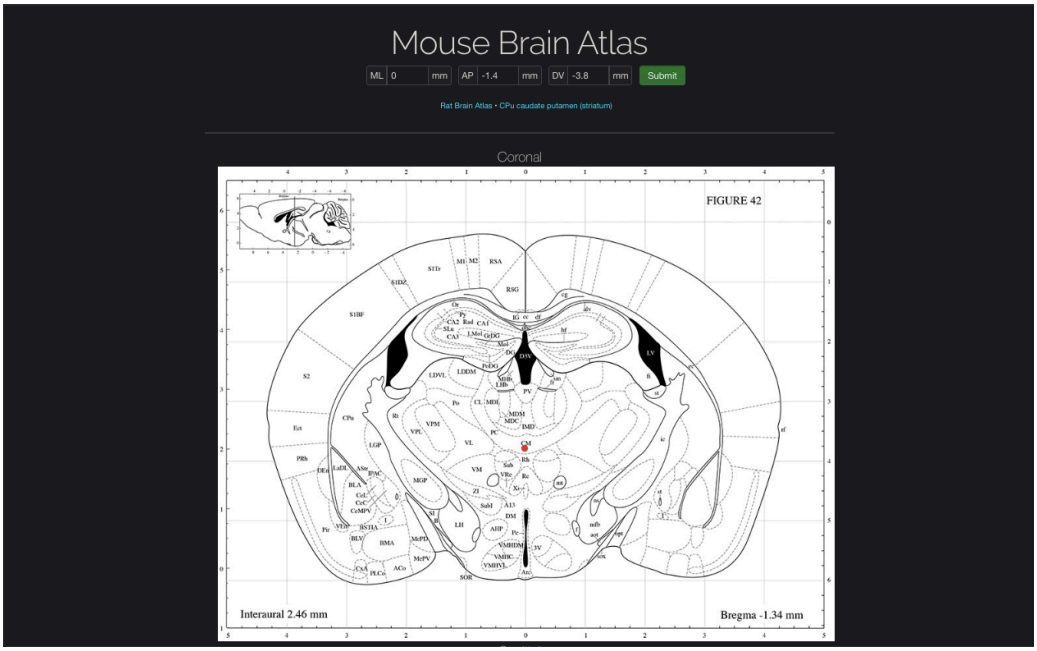

Enter the coordinates in the atlas found at: http://labs.gaidi.ca/mouse-brain-atlas/?ml=2.5&ap=-.4&dv=3.2 will give you brain region of interest. It is also a good idea to cross reference this position with the Allen Mouse Brain Atlas found here:

http://atlas.brain-map.org/atlas?atlas=1&plate=100960520#atlas=1&plate=100960520&resolution=9.10&x=2199.9097741168475&y=2032.044916567595&zoom=-3