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.

	SPM8 (dals	op): Menu					
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	SPM for fun	ctional MF	?/				
Display	Check Reg	Ren	≎ FMRI ≎				
Tool 🗘	PPIs	ImCalc	DICOM Im				
Help	Utils ≎	Batch	Quit				
	Copyright (c) 19	991,1994-2013					

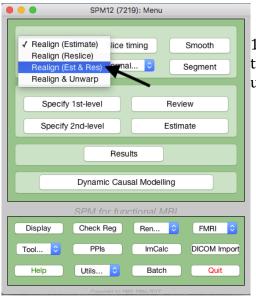
It will ask you to select the files to convert. You have to select all the files by using the shift click option. For fmri there are many files (>30000) so this can take 10-30 minutes depending on the size.

	SPM8 (dals	op): Menu	
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	Dynamic Cau	sal Modelling	
	SPM for fun	ctional MRI	
Display	Check Reg	Ren \$	FMRI 🗧
Tool 🗘	PPIs	ImCalc	DICOM Im
Help	Utils ¢	Batch	Quit
	Copyright (c) 15		

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

Step 2: Realignment

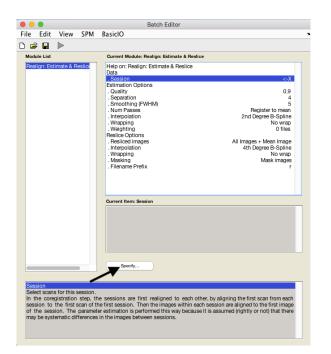
Images will need to be realigned and resliced to create a mean image. This is done under the "realign" drop down menu.



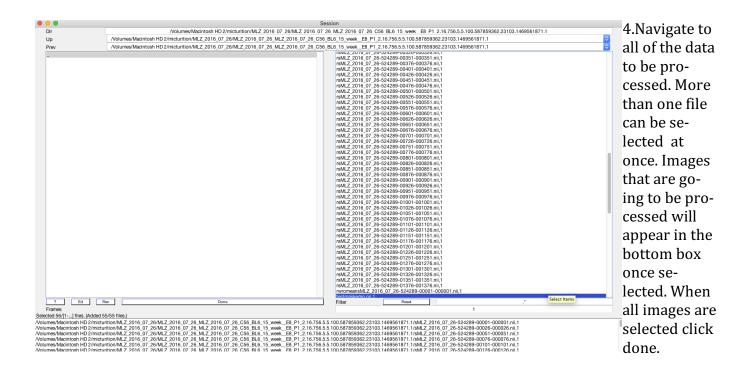
1.click the realign tab and select "Realign (Est & Res)" to open the batch editor. The batch editor will allow you to run modules on multiple sets of data at the same time.

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

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Real	ign: Est	imate & R	eslice	Help on: Realign: Estimate & Reslice	
Heal	IGT. ESI	inate & H		Prep Or Pearing the Submark & Reside Data Session Session County	<-X 0.9 4 5 Register to mean 2nd Degree B-Spline No wrap 0 files All Images + Meen Image 4th Degree B-Spline No wrap Mask images r
				Current Item: Data New: Session Replicate: Session (1) Delete: Session (1)	
Data		ssions for	this sub	Specify	1
Add new sessions for this subject. In the coregistration step, the sessions are first realigned to each other, by aligning the first scan from each session to the first scan of the first session. Then the images within each session are aligned to the first image of the session. The parameter estimation is performed this way because it is assumed (rightly or not) that there may be systematic differences in the images between sessions. 1 or more options must be selected from: 5 Session					



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



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.

	•			Batch Editor		
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				Current Item: Resliced images All Images (1n) Images 2n "All images + Mean Image Mean Image Only		
				Specify		
S A O	Recliced images Specify the images to reslice. All images (1n): This reslices all the images - including the first image selected - which will remain in its original position. Images 2n : Reslices images 2n only. Useful for if you wish to reslice (for example) a PET image to fit a structural MRI, without creating a second identical MRI volume.					

Step 3:Mask image

Applications list

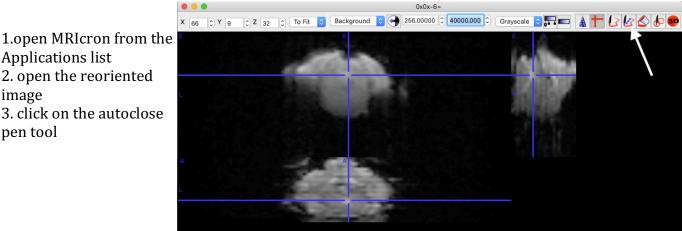
image

pen tool

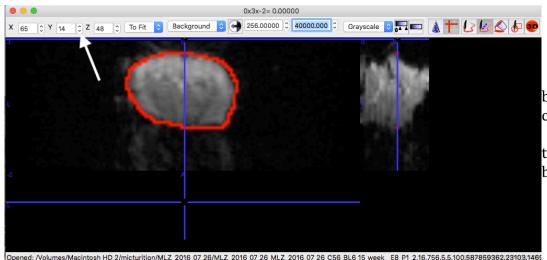
2. open the reoriented

3. click on the autoclose

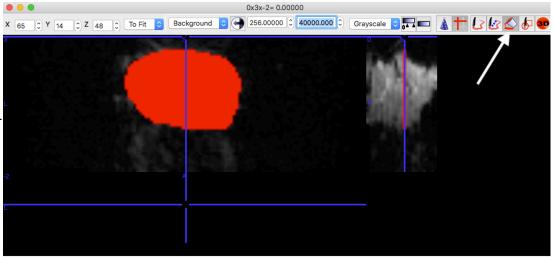
Before normalizing the images it is helpful to mask the scans to help the program's algorithms run smoothly. Masking is done in the MRIcron program.



Opened: /Volumes/Macintosh HD 2/micturition/MLZ_2016_07_26/MLZ_2016_07_26_MLZ_2016_07_26_C56_BL6_15_week_E8_P1_2.16.756.5.5.100.587859362.23103.1466



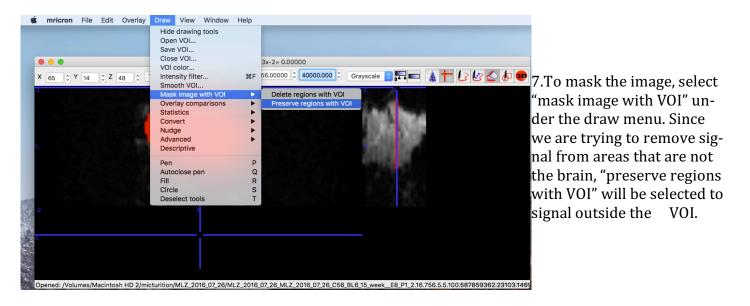
4.draw around the brain sections for each coronal slice. Filter through the Y-axis until all regions of the brain are outlined.

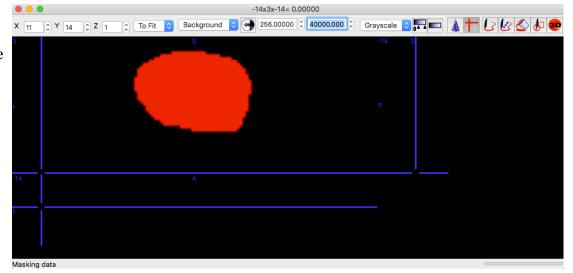


5.click on the fill tool and fill in the ROIs for each slice but clicking within the outlined region of the brain slice.

Opened: /Volumes/Macintosh HD 2/micturition/MLZ 2016 07 26/MLZ 2016 07 26 MLZ 2016 07 26 C56 BL6 15 week E8 P1 2.16.756.5.5.100.587859362:23103.146

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



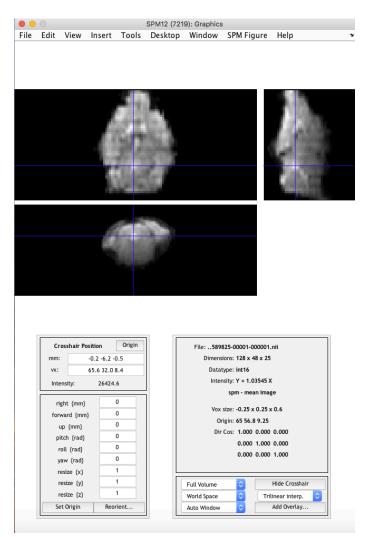


9. The file will be exported as a compressed file. Once unzipped the ".nii" extension will have to be manually added.

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."

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

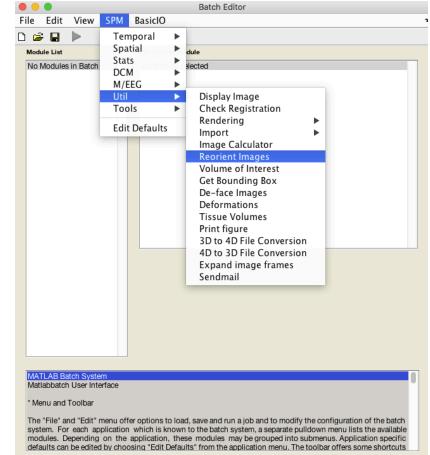


- 3. In the MatLab Command window type "x=[x; y; z; 1]", where "x", "y", and "z" are representative of the three mm values of the crosshairs. Then hit the "return" key.
- 4. Use the script "findrotmat" for images that were taken with the mouse in the prone position. Use the script "findrotmatsupine" 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

	SPM12 (72	19): Menu		
Realign	Slice t	iming	Smooth	
Coregi	Norma	I ¢	Segment	
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	Resu	its		
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	SPM for fund	_		
Display	Check Reg	Ren	S FMRI S	
Tool ≎	PPIs	ImCalc	DICOM Impo	ort
Help	Utils ≎	Batch	Quit	

In SPM Open the batch editor

7. Under the SMP tab navigate to "Reorient Images"

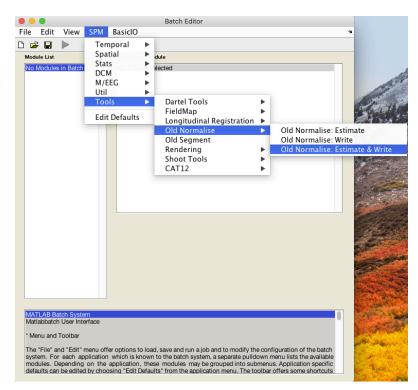


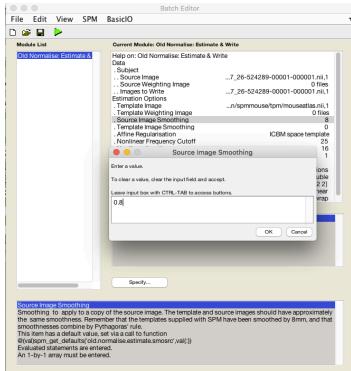
- 8. Once loaded click "specify" to select the mean images that you would like to process.
- 9. Once scans are selected, the reorientation matrix will need to be edited. click on "reorientation Matrix" and then "specify" to edit this.
- 10. The reorientation Matrix is a 4 x 4 matrix which will determine 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".

						Batch Editor	
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	1	Modu	le List			Current Module: Reorient Images	
		Reor	ient Im	ages	<-X	Help on: Reorient Images Images to reorient NsMLZ_2016_07_26-524289-00001-000001.nii,1 Reorient by . Reorientation Matrix <-X Filename Prefix	
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ł						lue. I value, clear the input field and accept. ut box with CTRL-TAB to access buttons.	
						0; 0 0 1 0; 0 1 0 0; 0 0 0 1]	
					-	OK Cancel	
						Current Item: Reorientation Matrix	
"						Specify	
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12. Click the run button. New images will be saved with the "ro" prefix.

Step 5: Normalization1.Open the batch editor2. From the SPM menu select "Old Normalise" (estimate & write)

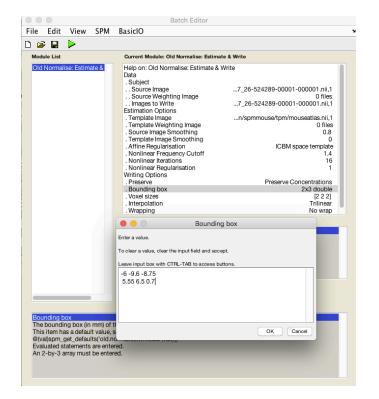




6. Change nonlinear cutoff frequency to 1.4

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

Batch Editor File Edit View SPM BasiclO D 🛩 🖬 🕨 Module List Current Module: Old Normalise: Estimate & Write Old Normalise: Estimate & Help on: Old Normalise: Estimate & Write Data . Subject ...7_26-524289-00001-000001.nii,1 . . Source Image . . Source Weighting Image 0 files ...7_26-524289-00001-000001.nii,1 Images to Write Estimation Options Template Image ...n/spmmouse/tpm/mouseatlas.nii,1 . Template Weighting Image . Source Image Smoothing 0 files 0.8 Template Image Smoothing 0 Affine Regularisation Nonlinear Frequency Cutoff ICBM space template 25 Nonlinear Iterations Nonlinear Regularisation 16 Nonlinear Regu Writing Options Preserve Bo Vo Int Enter a value. Preserve Concentrations Nonlinear Frequency Cutoff To clear a value, clear the input field and accept Leave input box with CTRL-TAB to access button: 1.4 ок Cancel Nonlinear Frequency Cutoff Cutoff of DCT bases. Only DCT bases of periods longer than the cutoff are used to describe the warps. The number used will depend on the cutoff and the field of view of the template image(s). This item has a default value, set via a call to function @[valspm.get_defaults[cold.normalise.estimate.cutoff',val{:}] Evaluated statements are entered. An 1-by-1 array must be entered.

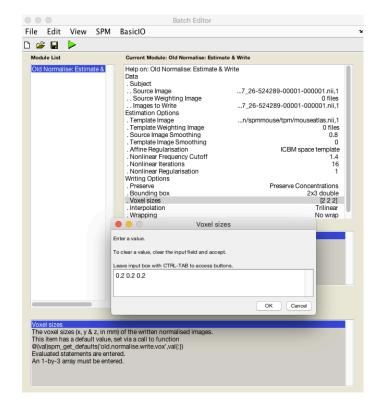


8. Change voxel size to 0.2 0.2 0.2

9.Click the run batch button

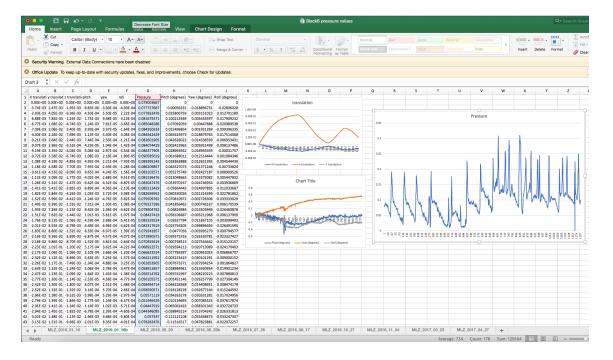
10. The normalized file will be saved with the prefix "w". A normalization report will also be exported as a .ps file.

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

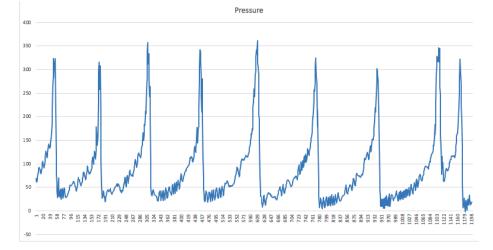


Step 6: Analyzing CMGs and movement

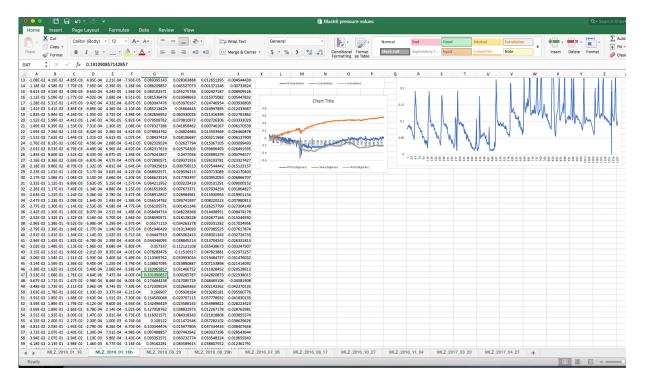
1. The data for the pressure curves are saved in their own text document. Copy and paste this file into excel and create a graph based on the values. The values from the motion files should also be imported to gauge whether either portions or entire subjects should be omitted from the analysis. portions 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.

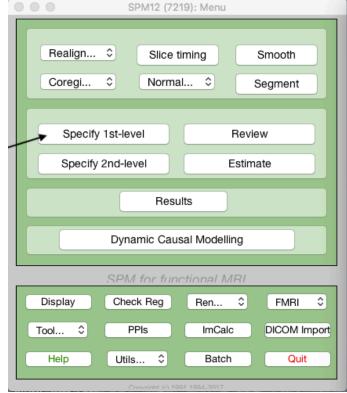


3.Find the peak of each CMG on the pressure graph in excel. If you hover the cursor over each peak in the pressure graph, you will be given a rough idea of the scan at which the peak occurs. Find the scan number in the pressure column and mark it.



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.



• •	•			Batch Editor	
File	Edit	View	SPM	BasicIO	3
D 🖻	۹				
Mode	ule List			Current Module: fMRI model specification	
Dire	ctory	specificat		Help on: fMRI model specification <-X	

Step 8: Estimate

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

2. Change the parameters to the following values:

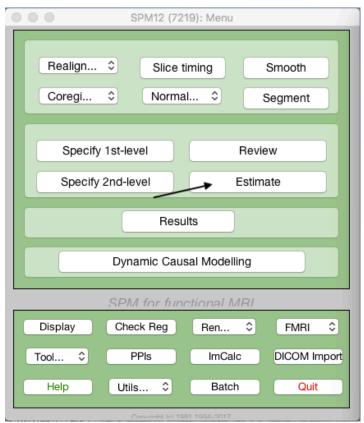
-Units for design "Scans"

-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.



•	•	•			Batch Editor
F	ile	Edit	View	SPM	BasiclO
D	Ē				
	Modu	le List			Current Module: Model estimation
	Sele	et sPM	.mat	<x ile that or ing this f</x 	Heijon: Select SPM.mat With residuals No Outrant Item: Select SPM.mat Specify Specify ontains the design specification. ile is known as the input directory.

2. Specify the SPM file and then and then run the batch. SPM will ask if it is okay to overwrite the SPM file in the current directory. Click 'Ok". The estimation step can take anywhere 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

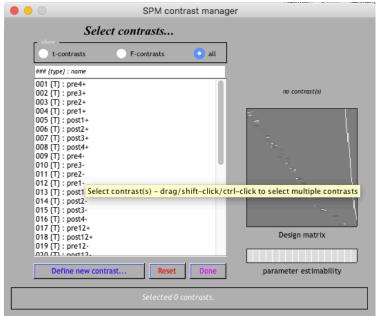
1. Once the estimation is finished you can view the results by clicking on "Results"

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

	SPM12 (721	oj. Menu			
Realign	Slice ti	ming	Smooth		
Coregi	Norma	l 🌣	Segment		
Specify	1st-level	R	eview		
Specify	2nd-level	Es	timate		
Results					
	Dynamic Caus	al Modelling	J		
	SPM for fund	tional MR	1		
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Tool ≎	PPIs	ImCalc	DICOM Impor		
Help	Utils ¢	Batch	Quit		
	Convisit Is) 100	1 100/ 2017			

SPM contrast manag	jer
define contrast name type t-contrast rootrast within the second secon	ro contrast(s)

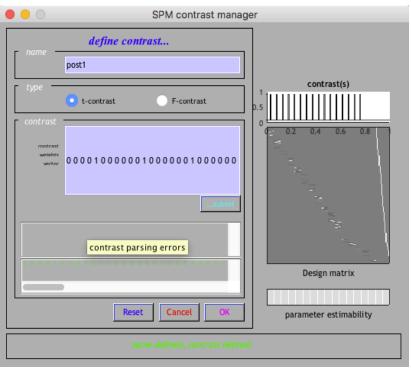
3. The Contrast manager will be brought up. To add a new contrast click on "Define New Contrast"



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"



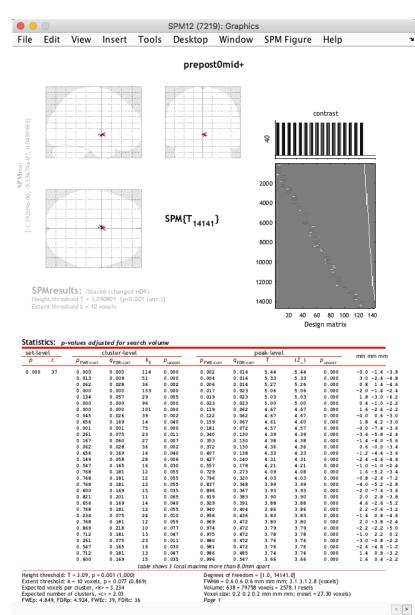
• •	SPM cor	ntrast manag	ger
Se	elect contrasts		
t-contrasts	F-contrasts	💽 all	
### {type} : name			contrast(s)
021 [T] : all+ 022 [T] : all- 023 [T] : pre34+ 024 [T] : pre34- 025 [T] : post34+ 026 [T] : post34- 027 [T] : mid4+ 028 [T] : mid4+ 030 [T] : post+ 031 [T] : mid- 032 [T] : pre- 033 [T] : post- 034 [T] : post- 035 [T] : mid+ 036 [T] : pre-post-			21
037 {T} : pre+pos 038 {T} : mid+pre 039 {T} : mid-pos	P Select contrast(s) - c	drag/shift-clio	ck/ctrl-click to select multiple contrasts
Define new (contrast Reset	Done	parameter estimability
	Selected 1 contrast, pro	ess "Done" whe	n finished.

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".

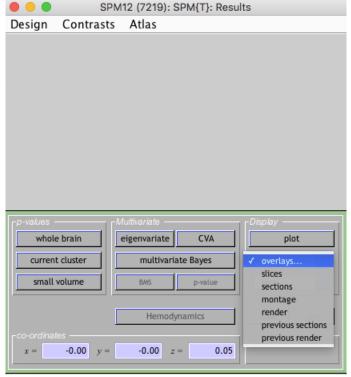
• • •	SPM12 (7219): S	tats: Results
	apply masking	none contrast image atlas

SPM12 (7219): Stats: Results	
apply masking none	8. Select whether or not you want the p-value adjusted to
p value adjustment to control FWE none	the control. Hit "none".
	SPM12 (7219): Stats: Results
	apply masking none
	threshold {T or p value}
9. Set the threshold for significance. Hit en	ter after
applying the value you would like t	
applying the value you would like t	
SPM12 (7219): Stats: Results	10. The cluster size required for significance will then
apply masking none	be set. Hit enter when you have set the desired num-
threshold {T or p value} 0.001	ber of voxels. Only significant clusters of the threshold
& extent threshold {voxels}	or greater will be included.
<u>-</u>	

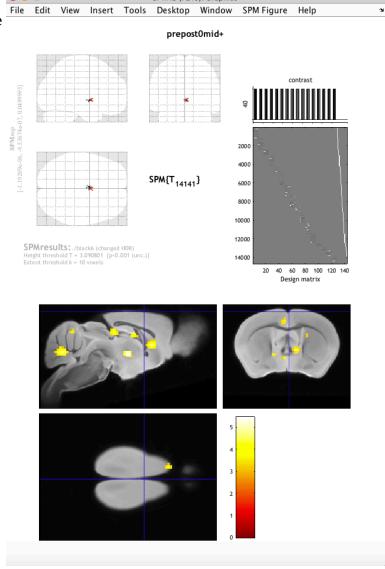


11. Significant clusters will be listed in the graphics window. To view the clusters on a mouse brain you will need to overlay the data on the mouse atlas.

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.



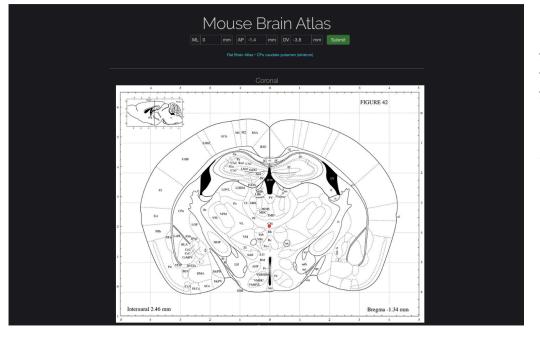
13. The areas that are significant according to your parameters will now be overlaid over the mouse atlas.



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.

set-lev	el	cluster-level				peak-level					
р	c	P _{FWE-corr}	q _{FDR-carr}	k _E	P uncorr	P _{FWE-corr}	q _{FDR+carr}	Т	(Z_)	P uncorr	mm mm mm
0.000	37	0.000	0.000	114	0.000	0.002	0.014	5.44	5.44	0.000	-0.0 -1.4 -3.6
		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 -3.4
		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 -2.0
		0.600	0.169	15	0.035	0.896	0.367	3.93	3.93	0.000	-2.0 -7.6 -3.
		0.821	0.201	11	0.065	0.919	0.383	3.90	3.90	0.000	2.0 2.8 -3.0
		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 sl	hows 3 local i	maxima more t	han 8.0mm a	part			
Extent th	reshold:	T = 3.09, p = 0 k = 10 voxels,	p = 0.077 (0.	869)		FWHM = (of freedom =).6 0.6 0.6 m	m mm mm	; 3.1 3.1 2.		
		er cluster, <k></k>					638 = 79758				
		of clusters, <c< td=""><td></td><td></td><td></td><td></td><td>e: 0.2 0.2 0.2</td><td>mmmn</td><td>mm; (resel</td><td>= 27.30 voxels</td><td>)</td></c<>					e: 0.2 0.2 0.2	mmmn	mm; (resel	= 27.30 voxels)
FWEp: 4.	849, FDR	p: 4.924, FWE	:: 39, FDRc: 1	36		Page 1					



Chatletier.

Enter the coordinates in the atlas found at: <u>http://labs.gaidi.ca/mouse-</u> <u>brain-atlas/?ml=2.5&ap=-</u> <u>.4&dv=3.2</u> 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:

<u>http://atlas.brain-</u> map.org/atlas?atlas=1&plate= 100960520#atlas=1&plate=1 00960520&resolution=9.10& x=2199.9097741168475&y=2 032.044916567595&zoom=-3