ANALYSIS OF FUNCTIONAL MAGNETIC RESONANCE IMAGING DATA USING SPM99:

MODELLING AND RESULTS ASSESSMENT

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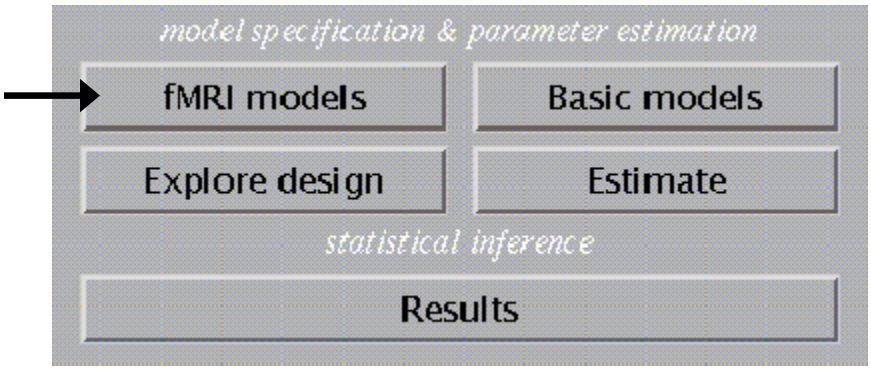
FIRST LEVEL SPM MODEL ESTIMATION (FIXED EFFECTS)

17. Timing files for efMRI

- Before estimating your models, create files listing event onsets in Excel (save in a tab delimited format), Notepad or using the "cat" function in a UNIX terminal
- Create a timing file for each condition for each subject. Place these in the each subject's directory
- List all event onsets in one column
- These onsets should not be in seconds, but in scans (i.e., volume acquisition; so if the TR is 2 seconds, every 2 seconds represents the onset of a new scan).
- Your first few scans may have already been dropped from you set of scans before you started preprocessing (to allow for scanner equilibrium). If this is the case, you should adjust your timing files accordingly (e.g., if you dropped 3 scans what was your fourth scan was preprocessed as your first scan) scan. You can check if they have been dropped by comparing the number of snaf*.img files you have in each subject directory to the number of TRs you collected during scanning.

18. Analysis of an individual subject

- Files needed: snaf*.img; timing files
- Make sure your working directory is the subject's directory (result files will be written here). If it is, change your working directory selecting **Utilities** (from the main menu), **CD**
- Select **fMRI models** from the main menu



- What would you like to do? Select **specify and estimate a model**
- Number of sessions: ***1*** *(individual analysis consisting of one run; if more than one run, enter number of runs, e.g., 3, then sessions 1-3 will represent runs 1-3)*
- Select scans for session 1: select all **snaf*.img** files for Subject 1 (if more than one run, it will prompt for each "session")
- Interscan Interval: Enter TR in secs, e.g., ***2***
- Are the conditions replicated? **Y**
  (Note: This question will only come up if you have more than one run for the subject you are analyzing. Select N if for this subject there are different conditions across runs)

Session 1/1

- You will be prompted to entered information about Session 1/1 (if you entered a subject with only one run); or Session 1/3 (i.e., the first run if you entered one subject with 3 runs)
- number of conditions or trials: ***4***
- name for condition/trial 1?: ***amsp*** *(i.e., specific AMs)*
  name for condition/trial 2?: ***amgen*** *(i.e., general AMs)*
  name for condition/trial 3?: ***sent*** *(i.e., sentence completion)*
  name for condirion/trial4?: ***size*** *(i.e., size discrimination)*

Trial specification

- SOA (stimulus onset asynchrony): Fixed / Variable?: ***V***
  (if onset times differ across scans or you have more than 3 conditions, otherwise enter F)
- Vector of onsets (scans) amsp**:** Type in onsets for amsp (condition 1), or if you have a timing file, right click on area outside of the entry box, select "**load text file**" from menu, and select the **timing textfile**
- Variable durations?: ***N***
  (enter Y unless different durations, e.g., if you wish to consider the reaction times of each event as the duration of the event)
- Vector of onsets (scans) amgen**:** load timefile for amgen (condition 2)
- Variable durations?: ***N***
- Vector of onsets (scans) sent**:** load timefile for sent (condition 3)
- Variable durations?: ***N***
- Vector of onsets (scans) amgen**:** load timefile for size (condition 4)
- Variable durations?: ***N***
- Parametric modulation: none / time / other? Select **none**
  (unless performing a parametric modulation; see section 18)
- Are these trials events / epochs / mixed?: Select ***Events***
  (*If blocked design, select epoch, or if some conditions are blocks and some events, select mixed)*

For event-related designs:

- Select basis set: **hrf (alone)** *(for efMRI)*
- Interactions among trials (Volterra): **No**
- User specified regressors: **Select 1**, then load **realign_param.txt** file
  Allows you to add motion parameters into the model as regressors; if not select 0

For each condition in blocked designs:

- Select type of response: Select **Box-car**
- Convolve with hrf?: Select **Yes**
- Add temporal derivatives: Select **No**
- Epoch length (scans) for amsp: Enter block duration for amsp (condition 1)

For both event-related and blocked deisgns:

- Remove Global effects: Select **None**
  If you select 'scale' the value in each voxels for a given snaf.img will be divided by the global brain mean value for this snaf*.img.. Global scaling can beneficial, since it reduces intersubject variability and improves the sensitivity at the group level of analysis. However, it relies on the assumption that the global brain mean does not correlate with the task. If this assumption is violated, applying global scaling can cause large areas of the brain to appear as activated, in some case almost entire slices. Global scaling should be applied only with extreme caution.*

- Temporal autocorrelation options: High-pass filter? Select **Specify** and enter the default values given for Session cutoff period
  This filters out the low-frequency components of your signal. High-pass filtering is usually beneficial, since the low-frequency components in the fMRI signal contain much more noise than the high-frequency components.

- Temporal autocorrelation options: Low-pass filter (None, Gaussian, hrf)?: **Gaussian**
- Specify**: *4***
  Note that if Gaussian or hrf low-pass filter is selected, this will filter out the high-frequency components in the signal by smoothing the time-series; also known as temporal smoothing. It accounts for autocorrelations in data. It can make the statistical inference overly conservative.
- Model intrinsic correlations?: **None**
  Modelling intrinsic correlations has the same theoretical goal as temporal smoothing, but also makes statistical inferences overly conservative. Only choose if you haven't specified a low-pass filter.
- Set up trial-specific F contrasts: **Yes**
- Estimate?: **Now**
- **Outputs**: **SPM_fMRIDesMtx.mat** (contains design parameters – onsets, number of conditions, names of conditions, etc; if this can be applied to another subject, select to only estimate a specified model and use this file); **Y.mad** (file containing the raw data, directly entered from the snaf*.img files. It contains the values for only those voxels that survived the upper F threshold during the analysis**;** **xCon.mat** (file containing information you will enter (next) about the contrasts, their names, values, etc)

FIRST LEVEL SPM MODEL ESTIMATION (FIXED EFFECTS)

19. Fixed-effects group analysis

Note this is basically the same as the Individual Subject analysis protocol, except you have to enter information for more than one subject (and all their runs if they have more than one run)

- Create a directory for this analysis (e.g., Fixed_FX)
- Change directory so this is your PWD (Utilities, CD)

- Select **fMRI models** from main menu
- What would you like to do? Select **specify and estimate a model**
- Number of sessions: ***28***
  (this refers to 28 individuals with one run each, or 14 individuals with 2 runs each etc)
- Select scans for session 1: select all **snaf*.img** files for Subject 1
- Select scans for session 2: select all **snaf*.img** files for Subject 2 (or for run 2 of Subject 1)
- Interscan Interval: Enter TR in secs, e.g., ***2***
- Are the conditions replicated? **Y**
  (Note: Select N if different subjects have different conditions, e.g., in a subsequent memory paradigm where not every subject has events in every post-hoc condition, or there are different conditions across runs)

Session 1/28

- number of conditions or trials: ***4***
- name for condition/trial 1?: ***amsp*** *(i.e., specific AMs)*
  name for condition/trial 2?: ***amgen*** *(i.e., general AMs)*
  name for condition/trial 3?: ***sent*** *(i.e., sentence completion)*
  name for condirion/trial4?: ***size*** *(i.e., size discrimination)*
- SOA (stimulus onset asynchrony): Fixed / Variable?: ***V***
  (if onset times differ across scans or you have more than 3 conditions, otherwise enter F)
- Vector of onsets (scans) amsp**:** Type in onsets for amsp (condition 1), or if you have a timing file, right click on area outside of the entry box, select "**load text file**" from menu, and select the **timing textfile**
- Variable durations?: ***N***
  (enter Y unless different durations, e.g., if you wish to consider the reaction times of each event as the duration of the event)
- Vector of onsets (scans) amgen**:** load timefile for amgen (condition 2)
- Variable durations?: ***N***
- Vector of onsets (scans) sent**:** load timefile for sent (condition 3)
- Variable durations?: ***N***
- Vector of onsets (scans) amgen**:** load timefile for size (condition 4)
- Variable durations?: ***N***
- Parametric modulation: none / time / other? Select **none**
  (unless performing a parametric modulation; see section 18)
- Are these trials events / epochs / mixed?: Select ***Events***
  (*If block design, select epoch, or if some conditions are blocks and some events, select mixed)*

For event-related designs:

- Select basis set: **hrf (alone)** *(for efMRI; note this is only asked for the first session)*
- Interactions among trials (Volterra): **No**
- User specified regressors: **Select 1**, then load **realign_param.txt** file for Subject 1
  Allows you to add motion parameters into the model as regressors; if not select 0

Session 2/28

- As for session 1/28, except entering timefiles and realignment parameters for Subject 2

For each condition in blocked designs:

- Select type of response: Select **Box-car**
- Convolve with hrf?: Select **Yes**
- Add temporal derivatives: Select **No**
- Epoch length (scans) for amsp: Enter block duration for amsp (condition 1)

For both event-related and blocked deisgns:

- Remove Global effects: Select **None**
  (see individual analysis for explanation)
- Temporal autocorrelation options: High-pass filter? Select **Specify** and enter the default values given for Session cutoff period
  (see individual analysis for explanation)
- Temporal autocorrelation options: Low-pass filter (None, Gaussian, hrf)?: **Gaussian**
- Specify**: *4***
  (see individual analysis for explanation)

  Model intrinsic correlations?: **None**
  (see individual analysis for explanation)
- Set up trial-specific F contrasts: **Yes**
- Estimate?: **Now**
- **Outputs**: creates **SPM_fMRIDesMtx.mat**, **Y.mad**, **xCon.mat** files

FIRST LEVEL SPM MODEL ESTIMATION (FIXED EFFECTS)

20. Parametric Modulation Analysis

Note that this is basically the same as the Group analysis protocol, except for the parametric modulation stage. In this example, we are modeling specific AMs and entering detail as a covariate of interest in our parametric modulation, and also recency as a nuisance covariate that we want to take into account.

- For each covariate, create a textfile for each subject in each condition that contains a column of the parameters for each event in that condition (e.g., ***detail.txt, recency.txt***). Save in the subject's directory.
- Create a directory for this analysis (e.g., ***PM_Detail_Recency***)
- Change directory so this is your PWD (**Utilities, CD**)

- Select **fMRI models** from main menu
- What would you like to do? Select **specify and estimate a model**
- Number of sessions: ***6***
  (this refers to 28 individuals with one run each, or 14 individuals with 2 runs each etc)
- Select scans for session 1: select all **snaf*.img** files for Subject 1
- Select scans for session 2: select all **snaf*.img** files for Subject 2 (or for run 2 of Subject 1)
- Interscan Interval: Enter TR in secs, e.g., ***2***
- Are the conditions replicated? **Y**

Session 1/6

- number of conditions or trials: ***1***
- name for condition/trial 1?: ***amsp*** *(i.e., specific AMs)*
- SOA (stimulus onset asynchrony): Fixed / Variable?: ***V***
  (if onset times differ across scans or you have more than 3 conditions, otherwise enter F)
- Vector of onsets (scans) amsp**:** Type in onsets for amsp (condition 1), or if you have a timing file, right click on area outside of the entry box, select "**load text file**" from menu, and select the **timing textfile**
- Variable durations?: ***N***

Parametric modulation step:

- Parametric modulation: Select **other**
- Expansion: linear / exponen / polynom: Select the function you wish to test, e.g., **Linear**
- Name of parameter: ***Detail_recency***
- Which trials? 1: Enter ***1 1***
  (By entering trial one twice, you will be prompted to enter a set of parameters for condition 1 twice. If you have modeled 2 conditions and have 3 parameters, enter 1 1 1 2 2 2)
- [10] Parameters for trial 1: Using right click, **Load text file,** load textfile for first parameter
  (The "10" is prompting you to enter a number of parameters same as number of events in that condition)
- [10] Parameters for trial 1: Using right click, **Load text file,** load textfile for second parameter

- Are these trials events / epochs / mixed?: Select ***Events***
- Select basis set: **hrf (alone)** *(for efMRI; note this is only asked for the first session)*

- Interactions among trials (Volterra): **No**
- User specified regressors: **Select 1**, then load **realign_param.txt** file for Subject 1
  Allows you to add motion parameters into the model as regressors; if not select 0

Session 2/6

- As for session 1/6, except entering timefiles, parametric modulation files, and realignment parameters for Subject 2

- Remove Global effects: Select **None**
  (see individual analysis for explanation)
- Temporal autocorrelation options: High-pass filter? Select **Specify** and enter the default values given for Session cutoff period
  (see individual analysis for explanation)
- Temporal autocorrelation options: Low-pass filter (None, Gaussian, hrf)?: **Gaussian**
- Specify**: *4***
  (see individual analysis for explanation)

  Model intrinsic correlations?: **None**
  (see individual analysis for explanation)
- Set up trial-specific F contrasts: **Yes**
- Estimate?: **Now**
- **Outputs**: creates **SPM_fMRIDesMtx.mat**, **Y.mad**, **xCon.mat** files

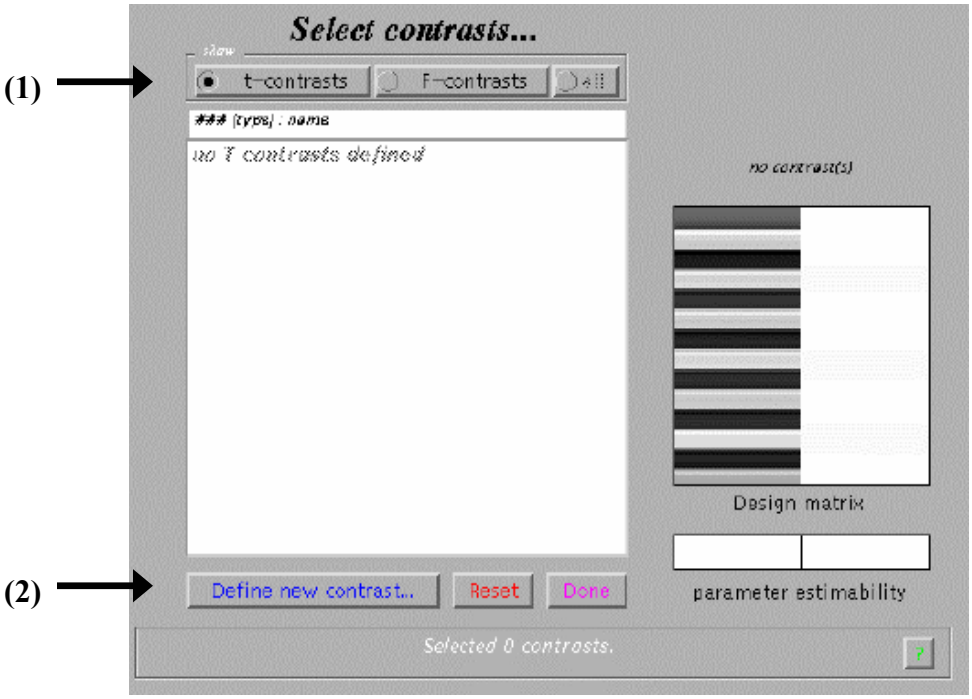
Note: Specifying contrasts in this instance is different; please read Section 22

RESULTS: DEFINING CONTRASTS

21. Results: Defining contrasts for fixed effects models

- Select **Results** from main menu

- Select **SPM.mat** file (for an individual subject, or from the group analysis)



- In SPM contrast manager: Select **t-contrasts (1)**
  Select **Define new contrast (2)**
- Type in name of contrast, then the contrast values, e.g. ***1 1 -1 -1 0 0 0 0 0 0 0 1 1 -1 -1 0 0 0 0 0 0***
- *Determining your contrasts: note that each column represents a condition for each subject (in the order you specified them in the model), followed by the next condition for that subject, followed by the six motion parameters*
  e.g., S1cond1, S1cond2, S1cond3, S1cond4, S1motion1, S1motion2, S1motion3, S1motion4 S1motion5, S1motion6, S2cond1, S2cond2, S2cond3, S2cond4, S2motion1, S2motion2, S2motion3, S2motion4 S2motion5, S2motion6 …etc
  Thus, to contrast cond1 + cond2 against cond3 + cond 4, enter the following:
  1 1 -1 -1 0 0 0 0 0 0 …. for all subjects (i.e., 1 1 -1 -1 0 0 0 0 0 0 0 1 1 -1 -1 0 0 0 0 0 0 0 etc)
  Note, zeros are added for motion parameters; they are considered in the model in that other variables are othoganilized to these.
- Click **Submit**
- Check the design matrix on the right, to see if the contrast is what you want. If so, click **OK**
- The contrast will appear in the contrast manager window.

- Select the contrast you want to display, and click **Done**.
  (if more than one contrast is selected, the conjunction between them will be computed and displayed)
- Mask with other contrast(s): **No**
  Note, if you select Yes, you will prompted to select another contrast from the same SPM.mat file for inclusive masking (displays everything in contrast A plus voxels activated by contrast B and not activated by contrast A) or exclusive masking (displays voxels activated by contrast A that are also activated by contrast B).
- Corrected height threshold: **Yes** (corrects for multiple comparisons) or **No** (uncorrected)
- Threshold {T or p value}: Enter desired p-value
- Extent threshold {voxels}: Enter the min number of voxels that a cluster should have in order to be displayed. For the least conservative estimate, enter 0.
- **Outputs**: **con*.img** (i.e., an image for the contrast) and **spmT*.img** (i.e., an image coding every voxel with its t-value) is created for each contrast. The con*.img can be entered in a random effects analysis.

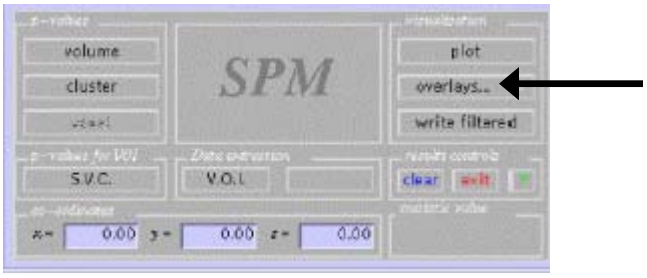
22. Results: Defining contrasts for parametric modulation models

- Select **Results** from main menu
- Select **SPM.mat** file (for an individual subject, or from the group analysis)
- In SPM contrast manager: Select **t-contrasts**
- Select **Define new contrast**
- Type in name of contrast, then the contrast values, e.g. ***1 1 -1 -1 0 0 0 0 0 0 1 1 -1 -1 0 0 0 0 0 0***
- *Determining your contrasts: note that in this instance, the first column for each subject represents the effect of the modeled condition, followed by the parametric effect of parameter 1 (e.g., detail), parameter 2 (e.g., recency), and the six motion parameters*
  e.g., S1cond1, S1detail, S1recency, S1motion1, S1motion2, S1motion3, S1motion4 S1motion5, S1motion6, S2cond1, S2detail, S2recency, S2motion1, S2motion2, S2motion3, S2motion4 S2motion5, S2motion6 …etc
  Thus, to look at the positive parametric effect of parameter 1 (orthogonal to parameter 2), enter the following:
  0 1 0 0 0 0 0 0 0 …. for all subjects.
  To look at the negative parametric effect of parameter 1, enter: 0 -1 0 0 0 0 0 0 etc
  To look at the positive parametric effect of parameter 2, enter: 0 0 1 0 0 0 0 0 0 etc
- Click **Submit**
- Check the design matrix on the right, to see if the contrast is what you want. If so, click **OK**
- The contrast will appear in the contrast manager window.
- Select the contrast you want to display, and click **Done**.
- Mask with other contrast(s): **No**
- Corrected height threshold: **Yes** (corrects for multiple comparisons) or **No** (uncorrected)
- Threshold {T or p value}: Enter desired p-value
- Extent threshold {voxels}: Enter the min number of voxels that a cluster should have in order to be displayed. For the least conservative estimate, enter 0.
- **Outputs**: **con*.img** (i.e., an image for the contrast) and **spmT*.img** (i.e., an image coding every voxel with its t-value) is created for each contrast. The con*.img can be entered in a random effects analysis.

SPM RESULTS: OVERLAYS

23. Results: Overlaying on anatomical scans

- Once you select a contrast to display, the results will appear on a glass brain.
- To overlay these activations on a anatomical scan: Select **Overlays, Sections**

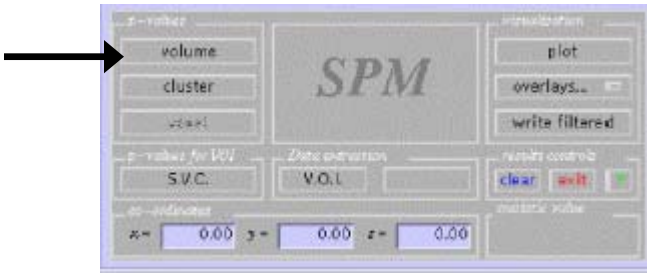


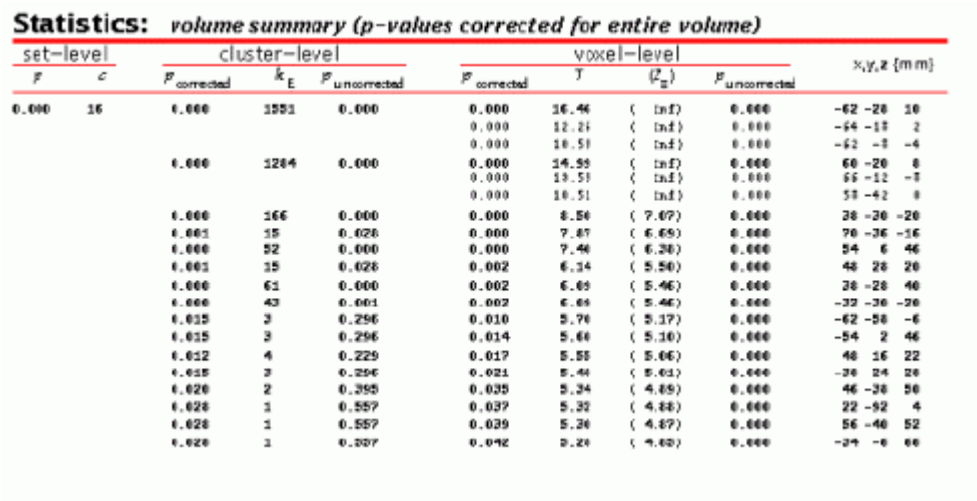
- Select the anatomical image, e.g., **single_subj_T1.img** (located users/crawley/spm99/canonical directory)
- Image will be displayed
- To remove crosshairs: in the matlab window, type the following command line: ***spm_orthviews xhairs off*** and ***spm_orthviews xhairs on*** to reinstate them
- To save the images: either use the Print Screen function on your computer and paste it into a picture-editing programme; or before selecting the contrast, change the defaults for Printing (select Defaults from main menu; defaults for printing; print to file; other format; tif)
- Additionally, you can render the image on a 3D brain by selecting: **Overlays, Render**

SPM RESULTS: CO-ORDINATES

24. Results: Extracting co-ordinates to a textfile

- To display a table of local maxima below the glass brain, select **Volume** from the main menu





Statistics: *volume summary (p-values corrected for entire volume)*

set-level		cluster-level			voxel-level				x,y,z {mm}
p	c	$p_{corrected}$	k_E	$p_{uncorrected}$	$p_{corrected}$	T	(Z_E)	$p_{uncorrected}$	
0.000	16	0.000	1551	0.000	0.000	16.46	(Inf)	0.000	-62 -28 10
					0.000	12.21	(Inf)	0.000	-64 -18 2
					0.000	10.51	(Inf)	0.000	-62 -8 -4
		0.000	1284	0.000	0.000	14.55	(Inf)	0.000	60 -20 8
					0.000	13.51	(Inf)	0.000	66 -12 -8
					0.000	10.51	(Inf)	0.000	58 -42 8
		0.000	166	0.000	0.000	8.56	(7.67)	0.000	38 -26 -20
		0.001	15	0.028	0.000	7.87	(6.65)	0.000	70 -36 -16
		0.000	52	0.000	0.000	7.46	(6.30)	0.000	54 6 46
		0.001	15	0.028	0.002	6.34	(5.50)	0.000	48 28 20
		0.000	61	0.000	0.002	6.05	(5.46)	0.000	38 -28 40
		0.000	43	0.001	0.002	6.05	(5.46)	0.000	-32 -36 -20
		0.015	3	0.296	0.010	5.70	(5.17)	0.000	-62 -58 -6
		0.015	3	0.296	0.014	5.60	(5.10)	0.000	-54 2 46
		0.012	4	0.229	0.017	5.55	(5.06)	0.000	48 16 22
		0.015	3	0.296	0.021	5.44	(5.01)	0.000	-38 24 28
		0.020	2	0.395	0.035	5.34	(4.85)	0.000	46 -38 50
		0.028	1	0.557	0.037	5.32	(4.84)	0.000	22 -92 4
		0.028	1	0.557	0.039	5.26	(4.87)	0.000	56 -40 52
		0.028	1	0.557	0.042	5.20	(4.80)	0.000	-24 -8 66

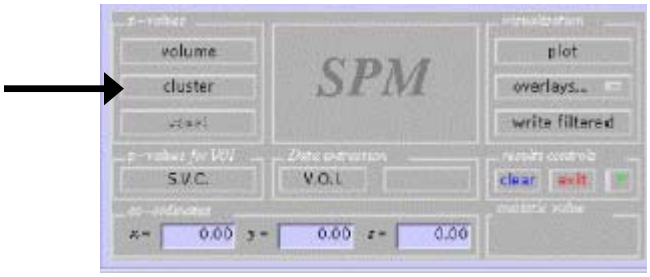
- By clicking on co-ordinate in the table, the pointer on the glass brain will move to coordinate
- By default, the local maxima listed in the table are 8mm apart. To change this, and the number of local maxima listed for each cluster, type the following command line in matlab:
  d=5 *(d being distance between each local maxima)*
  m=6 *(m being the number of maxima listed for each cluster)*
  title='Extended list of local maxima'
  tabData=spm_list('list', SPM, VOL, d, m, title, hReg)
- To save table of co-ordinates and z-scores etc: print into matlab (right click on table, select **print**).
- Once in matlab, you can copy this to Notepad (if working in windows) or to a terminal window where you have begun a "cat" process (to create a txt file).
- Note co-ordinates are MNI and must be converted to TAL before localization.
- First, open your txt file in Excel, copy the list of co-ordinates to another Excel spreadsheet and make sure everything looks okay.

25. Results: Converting co-ordinates from MNI to TAL space

- Copy just the list of co-ordinates to another spreadsheet. Save as a **tab-delimited** textfile, e.g., ***contrastA-B_MNI.txt***.
- Open textfile of MNI co-ordinates in a terminal window (i.e. view file using "more" command)
- To convert co-ordinates, a programme called **mni2tal** is used
  Download from: http://www.mrc-cbu.cam.ac.uk/Imaging/mnispace.html
- In the Matlab window type,
  mni2tal([x y z
  x y z
  x y z])
  Note, x y z represents the MNI co-ordinates you should copy and paste over from the textfile open in your terminal window.
- Save output (TAL co-ordinates) as a textfile by pasting into the terminal window where you have started a "cat" process (to create a textfile)
- To make this file compatible for programmes such as **Talairach Daemon** *(see Section 33)*, open the textfile in Excel, make sure negative signs are in appropriate columns (sometimes they are attached to the end of the value in front); reduce to **no decimal places**; save as a **tab-delimited** textfile

26. Results: Listing local maxima for a cluster

- To display a table of local maxima for a specified cluster, place pointer on cluster on glass brain.
- Select **Cluster** from the main menu



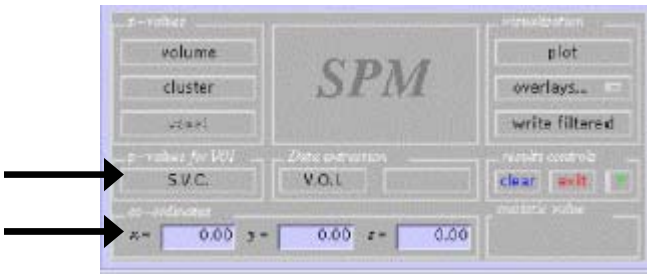
- Follow guidelines above to print table to a textfile.

SPM RESULTS: SMALL VOLUME CORRECTIONS

27. Results: Small Volume Corrections

Corrects statistics for an ROI rather than the whole brain volume.

- In order to list a table of statistics where p-values are corrected for a small volume rather than the whole brain, place pointer on glass brain in area of interest
- Place pointer at appropriate voxel on glass brain (or enter co-ordinates)
- Select S.V.C.

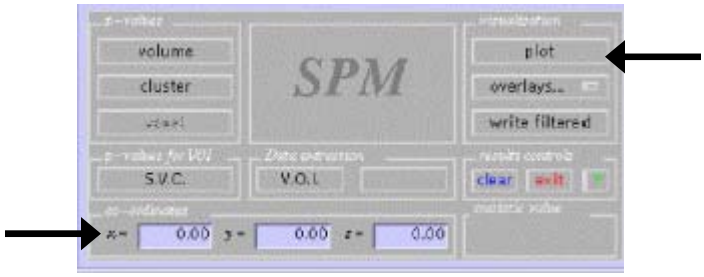


- Search volume: Sphere *(centered on selected voxel)*, box *(at selected voxel)*, nearest cluster, image
- If you selected **Sphere**, radius of spherical ROI (mm): Enter ***radius*** in mm
- If you selected **Box**, box dimensions [k l m] (mm): Enter ***box dimensions***
- If you selected **nearest cluster**, this will be computed
- If you selected **image**, choose a mask image (e.g., created in MARINA): **mask*.img**
- Table will be printed, listing local maxima at 4mm apart.
- To save as a textfile, follow guidelines in Section 23.

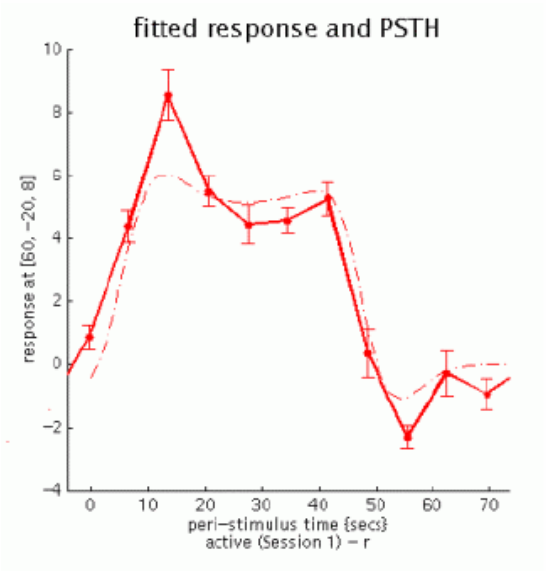
SPM RESULTS: PLOTS

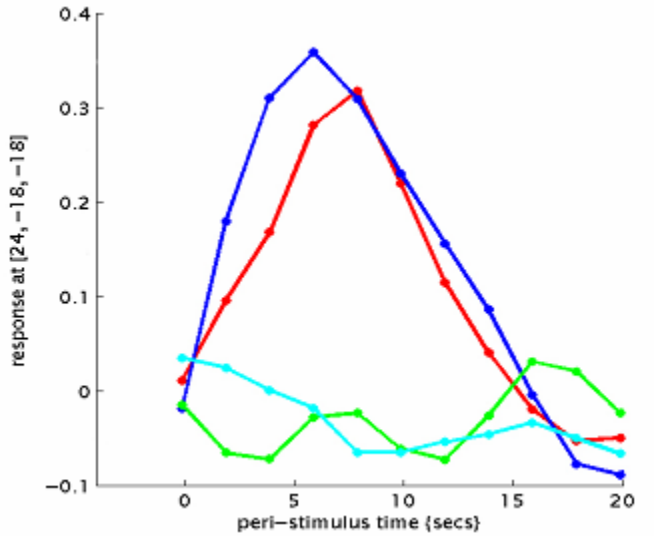
28. Results: Plotting Timecourses for Peak Voxel

- Place pointer at appropriate voxel on glass brain (or enter co-ordinates)
- To plot the mean signal over the timecourse of the event (PSTH), select **plot**



- Select **Event/epoch-related responses**
- Average over sessions? ***Y*** *(to include all subjects; to select certain sessions, enter* ***N)***
- Which trial or conditions?: ***1 2 3 4*** Specify the conditions you wish to include
- Plot in terms of: Select **Fitted response and PSTH**
- You will get the graph below on the left (but for as many conditions you specified to plot)
- By altering the program, **spm_graph.m** so it only plots PTSH (and not the fitted response), you can get the graph seen below on the left to look like the one on the right (see appendix for code changes)

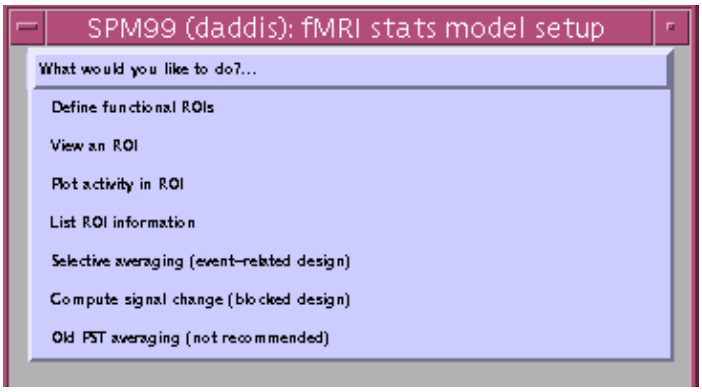




- Note, if you want to change the x-axis (i.e., the amount of time it covers), use the attributes button, and enter the limits for the x-axis (e.g., ***-4 20***)

29. Results: Plotting Timecourses for an ROI

- Install ROI toolbox (download from http://sourceforge.net/projects/spm-toolbox and save in your spm99/toolbox directory; note it must be unzipped and un-tarred)
- To plot the mean signal over the timecourse of the event averaged across an ROI, select **Toolboxes, ROI** from the main menu



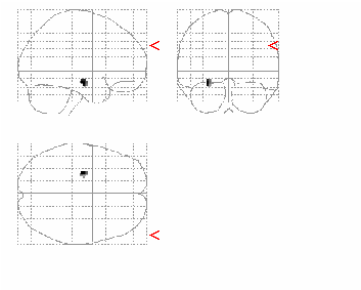
- Select **Define functional ROIs**
- Enter the ***radius*** of ROIs in mm *(i.e., radius of a sphere centred on each peak voxel across the brain)*
- Select **SPM.mat**
- Select the relevant contrast, threshold etc. In the Matlab window, ROIs will be calculated
- Outputs: **con* clust#.img, con* clust#.hdr, con* clust#.tal**; *(Note * is the number assigned to this contrast by SPM when you originally defined this contrast; # is the number of that particular cluster; files will be created for each cluster in the brain)* **con* roi.mat**

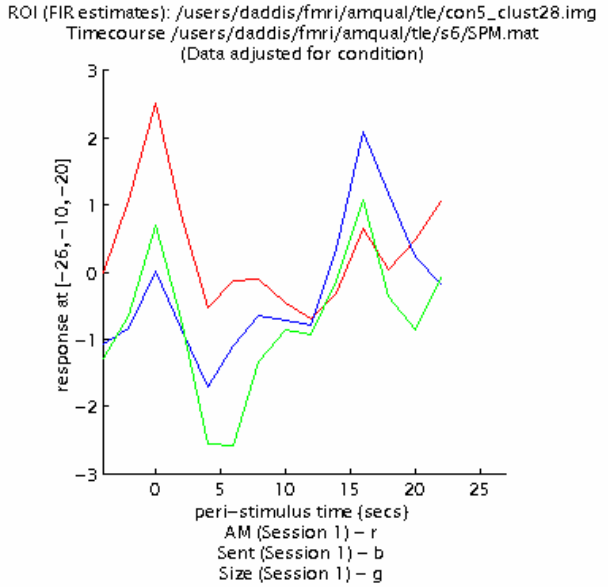
- Select **Toolboxes, ROI** from the main menu
- Select **View an ROI**
- Select anatomical image over which to superimpose cluster, e.g., **a.img**
- Select **con* roi.mat**
- All clusters are listed according to their number and peak voxel in the SPM graphics window. Note down the number of each cluster you are interested in (this denotes the relevant con*_clust#.img)

To plot an ROI for one subject:

- Select **Toolboxes, ROI** from the main menu
- Select **Selective averaging**
- Select cluster image **con* clust#.img** *(e.g., con5_clust28.img)*
- Select **SPM.mat**
- Adjustment: **by condition**

- **Select subject (sessions) and trials**
- y values for each voxel of the cluster are computed in matlab
- Displays the cluster on a glass brain, and a timecourse:





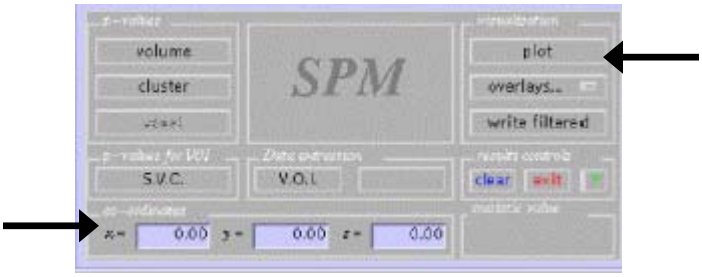
**To plot an ROI averaged across multiple subjects:

- Select **Toolboxes, ROI** from the main menu
- Select **Plot activity**
- Select cluster image **con*_clust#.img** *(e.g., con5_clust28.img)*
- Select **SPM.mat**
- Average across subjects? **Yes**
- y values for each voxel of the cluster are computed in matlab

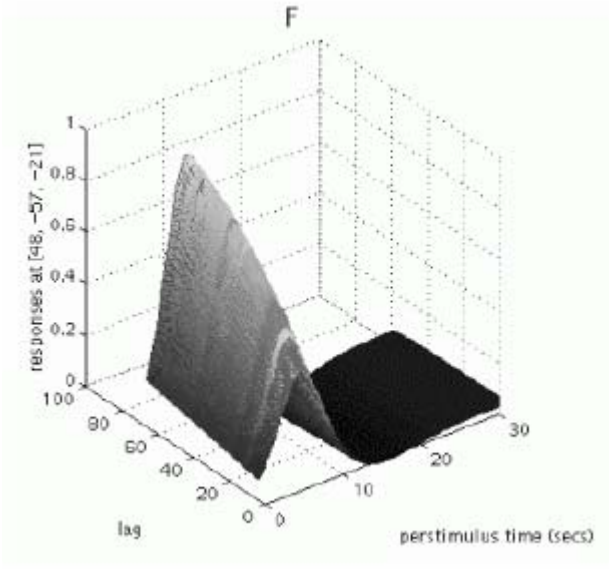
Results: 3D plots of parametric effects

Note: this option is appropriate if you have one parameter in your parametric modulation; when you have more than one, both effects will be plotted on the same 3D plot. Additionally, this feature can only plot the parametric effect of one subject per plot (i.e., you can't plot the average effect; see Section 28).

- Place pointer at appropriate voxel on glass brain (or enter co-ordinates)
- To plot the parametric effect from an individual subject, select **plot**



- Select **Plots of Parametric Responses**
- Which session?: Enter ***subject number***
- Which effect?: Select desired ***effect*** from choices given



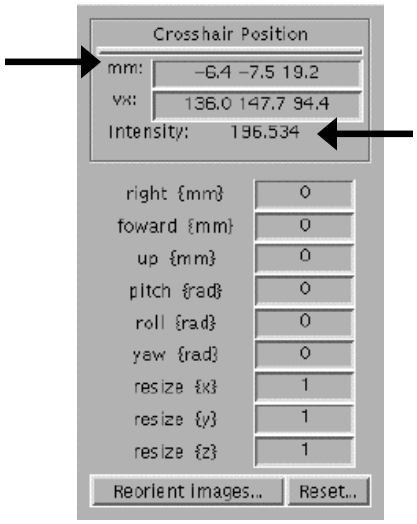
30. Results: Plots of group parametric effects

This requires use of a script written by A.P. Crawley (email donnad@psych.utoronto.ca for code) and allows you to plot the group parametric effect of the covariate of interest, orthogonal to nuisance covariates. First, however, it requires you define an additional contrast for the effect of the modelled condition.

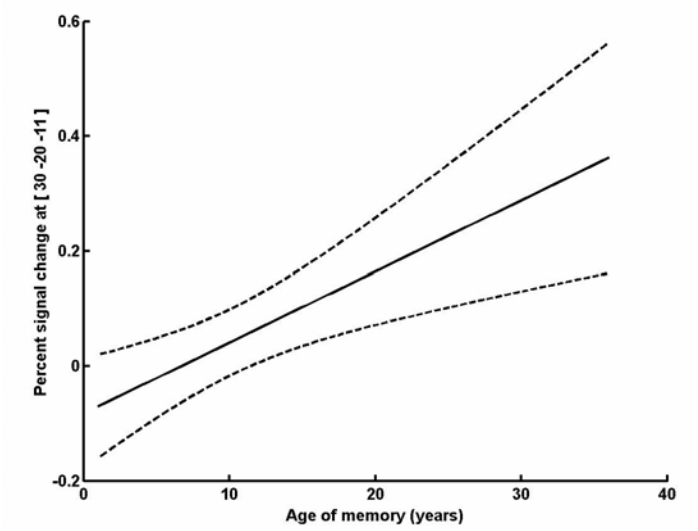
- Select **Results** from main menu
- Select **SPM.mat** file (e.g., from the group parametric modulation analysis)
- In SPM contrast manager: Select **t-contrasts**
- Select **Define new contrast**
- Type in name of contrast, then the contrast values, e.g. ***1 0 0 0 0 0 0 0 0***
- *Determining your contrasts: Remember, the first column for each subject represents the effect of the modeled condition, followed by the parametric effect of parameter 1 (e.g., detail), parameter 2 (e.g., recency), and the six motion parameters*
  e.g., S1cond1, S1detail, S1recency, S1motion1, S1motion2, S1motion3, S1motion4 S1motion5, S1motion6, S2cond1, S2detail, S2recency, S2motion1, S2motion2, S2motion3, S2motion4 S2motion5, S2motion6 …etc
  Thus, to create a contrast for the modeled condition, enter the following:
  1 0 0 0 0 0 0 0 0 …. for all subjects.
- Click **Submit**
- Check the design matrix on the right, to see if the contrast is what you want. If so, click **OK**
- The contrast will appear in the contrast manager window.
- Select the contrast you want to display, and click **Done**.
- Mask with other contrast(s): **No**
- Corrected height threshold: **Yes** (corrects for multiple comparisons) or **No** (uncorrected)
- Threshold {T or p value}: Enter desired ***p-value*** *(not overly important in this case)*
- Extent threshold {voxels}: Enter the min number of voxels that a cluster should have in order to be displayed. For the least conservative estimate, enter ***0***.

Obtaining values for pm_plot script

- Select **Display** from main menu
- Select the **con*.img** for the effect of the modeled condition (the contrast you just specified)
- Enter co-ordinates of voxel you wish to plot
- Note down the intensity value



- Repeat for the **con*.img** for the parameter of interest, and for the corresponding **spmT*.img**
- In a terminal window, type (name of script**)
- Enter these values into the script.



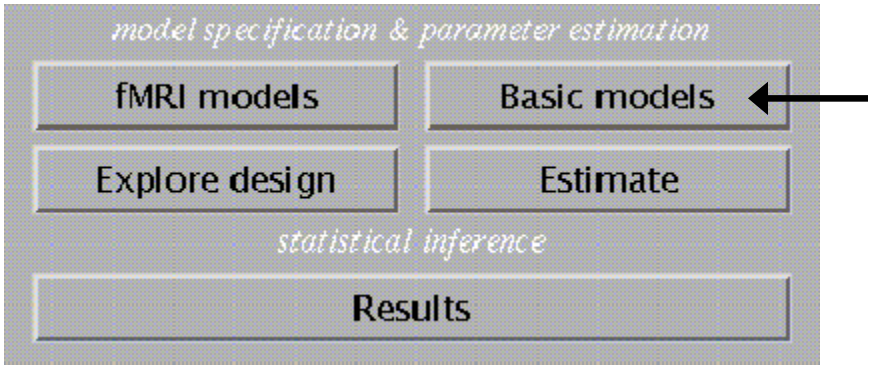
SECOND LEVEL SPM MODEL ESTIMATION (RANDOM EFFECTS)

31. Random effects model: One group

This analysis is second level as it takes the contrast images (con.img) created in your first analysis on each individual subject and runs a t-test on these.*

- Create a new results directory (e.g., ***RFX_am-con***)
- Decide which contrast **con*.img** files you need from each subject. Each is numbered according to the number that contrast was assigned by the contrast manager. If you're not sure, click Results, select the SPM.mat for that subject, and see which number each contrast has been assigned. If you entered all your contrasts in the same order for each subject when running your individuals analyses and there were no other con*.img files in their directories, then they should all have the same number.
- E.g., con009.img for each subject (e.g., am-con, the contrast between AM tasks and control tasks)

- Select **Basic Models** from the main menu



- Select design type: **One-sample t-test**
- Select images: Select the appropriate **con*.img** from each subject's directory
- Grand mean scaling?: **No**
- Explicitly mask images?: **No**
- Global Calculation?: **Omit**
- Estimate? **Now**

The estimation of this model is fairly fast, as you have inputted contrast images rather than actual data (i.e., snaf.img)*

32. Random effects model: Two groups (e.g., patients and controls)

- Select **Basic Models** from the main menu
- Select design type: **Two-sample t-test**
- Select images: Select the appropriate **con*.img** from directory of each Gp. 1 subject, then each Gp. 2 subject
- Groups: e.g., ***1 1 1 2 2 2***
  (Enter number for each subject in the order you selected the con.img files, denoting their group membership)*
- Threshold masking?: **None**
- Grand mean scaling?: **No**
- Explicitly mask images?: **No**
- Global Calculation?: **Omit**
- Estimate? **Now**

SPM RESULTS: RANDOM EFFECTS MODELS

33. Random effects model: One group

- Select **Results** from main menu
- Select **SPM.mat** file (for an individual subject, or from the group analysis)
- In SPM contrast manager: Select **t-contrasts**
- Select **Define new contrast**
- Type in name of contrast, then the contrast values, e.g. ***1***
- *Determining your contrasts: Note in this instance, you are testing whether the effect in the con*.img is different from zero. To test if it is greater than 0, simply enter 1 (or -1 to see if it is less than zero)*
- Click **Submit**
- Check the design matrix on the right, to see if the contrast is what you want. If so, click **OK**
- The contrast will appear in the contrast manager window.
- Select the contrast you want to display, and click **Done**.
- Mask with other contrast(s): **No**
- Corrected height threshold: **Yes** (corrects for multiple comparisons) or **No** (uncorrected)
- Threshold {T or p value}: Enter desired ***p-value***
- Extent threshold {voxels}: Enter the min number of voxels that a cluster should have in order to be displayed. For the least conservative estimate, enter ***0***.
- **Outputs**: **con*.img** (i.e., an image for the contrast) and **spmT*.img** (i.e., an image coding every voxel with its t-value) is created for each contrast. The con*.img can be entered in a random effects analysis.

34. Random effects model: Two groups

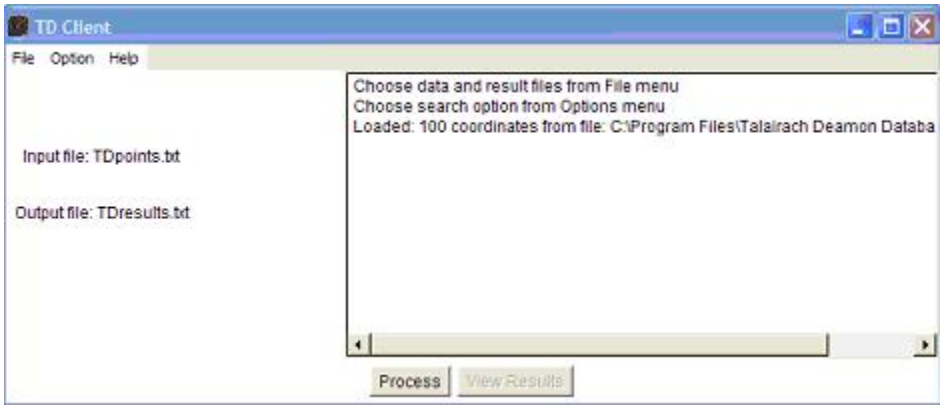
- Select **Results** from main menu
- Select **SPM.mat** file (for an individual subject, or from the group analysis)
- In SPM contrast manager: Select **t-contrasts**
- Select **Define new contrast**
- Type in name of contrast, then the contrast values, e.g. ***1 -1***
- *Determining your contrasts: Note that in this instance, you are testing whether the effect in the con*.img for patients is different from controls. To test if patients are greater than controls, enter 1 -1; to test if controls are greater than patients, enter -1 1*
- Click **Submit**
- Check the design matrix on the right, to see if the contrast is what you want. If so, click **OK**
- The contrast will appear in the contrast manager window.
- Select the contrast you want to display, and click **Done**.
- Mask with other contrast(s): **No**
- Corrected height threshold: **Yes** (corrects for multiple comparisons) or **No** (uncorrected)
- Threshold {T or p value}: Enter desired ***p-value***
- Extent threshold {voxels}: Enter the min number of voxels that a cluster should have in order to be displayed. For the least conservative estimate, enter ***0***.
- **Outputs**: **con*.img** (i.e., an image for the contrast) and **spmT*.img** (i.e., an image coding every voxel with its t-value) is created for each contrast. The con*.img can be entered in a random effects analysis.

LOCALISING MAXIMA: TALAIRACH DAEMON

35. Localising maxima: Talairach daemon

Download from: http://biad02.uthscsa.edu/RIC_WWW.data/Components/talairach/talairachdaemon.html
Note: the co-ordinates used at this stage should be converted to TAL using ***mni2tal***

- Co-ordinates: you should have created a textfile of Talairach co-ordinates, with all co-ordinates reduced to **zero decimal places**. If this file was created in Excel, it must have been saved as a **tab-delimited** text file (not a Unicode textfile)
- Run Talairach daemon database.



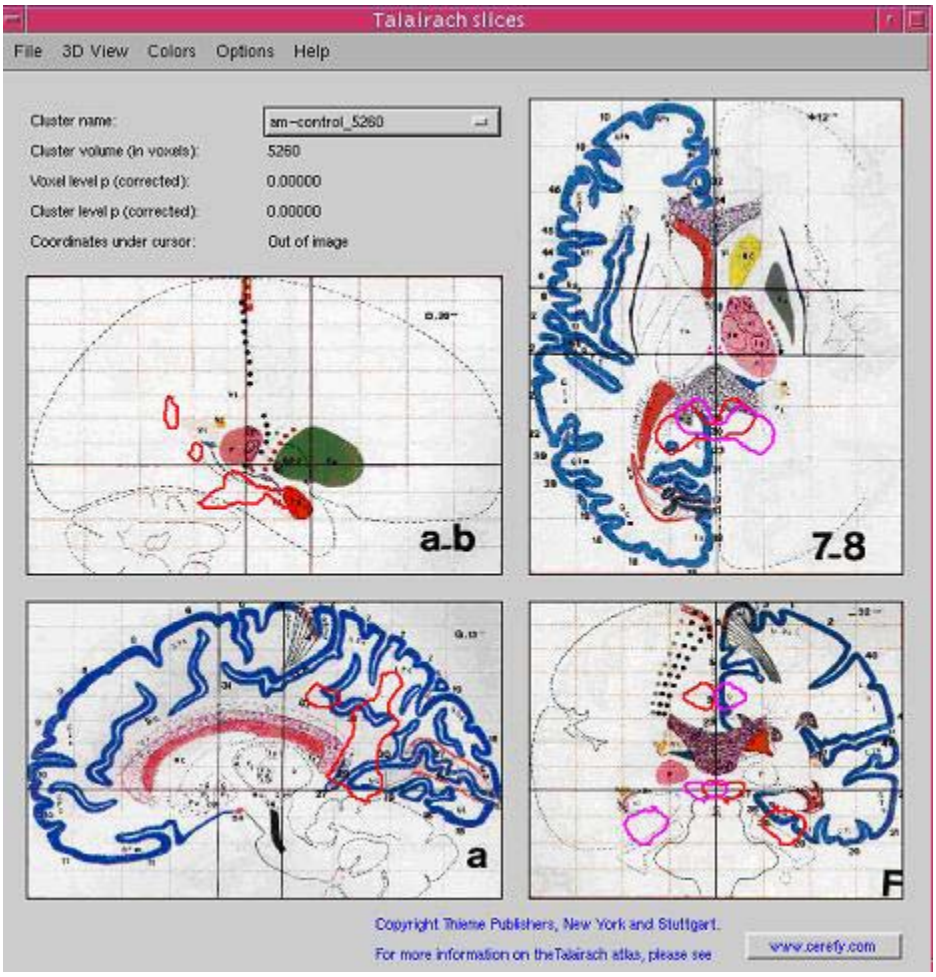
- To define the output you want, select **Option**.
- Choose either "assign a Talairach label" (default) or "search nearest gray matter"
- To load a file of co-ordinates, select **File**, **Choose data file**
- The number of co-ordinates successfully loaded will be printed in the window.
- If the number of co-ordinates it less than 100, you will be able to view the results in a window. If you have more than 100, you must specify an output file. Select **File**, **Choose Result file**
- Click **Process**
- Talairach labels for each co-ordinate will be assigned. Copy and paste into your spreadsheet of statistics outputted from SPM.

LOCALISING MAXIMA: TALAIRACH SPACE UTILITY (TSU)

36. Localising maxima: Talairach space utility

Download from: http://www.ihb.spb.ru/~pet_lab/TSU/TSUMain.html
Note this programme runs in the matlab window and uses some of the SPM windows.

- In matlab, open SPM in the desired working directory
- In the matlab window, type ***TSU***
- You will then be prompted through the SPM window to select the **SPM.mat** and **contrast** you wish to view results for.
- On TSU, all the significant clusters will be loaded, and you can view each overlayed on a Talairach atlas.
- Note, you can identify clusters by their cluster size (this is included in the table of local maxima from the SPM Results section)



APPENDIX: CODE

Changes to spm_graph.m

Purpose: to plot PTSH (timecourses) without fitted response or SE bars

- In section beginning at Line 459, titled ***case 'fitted response and PSTH'***, comment out the following lines:

```
errorbar(PST,PSTH,SEM,[':' COL(u)])
plot(x,Y,['-.' COL(u)])
```