Chapter 1

Auditory block fMRI

This data set comprises whole brain BOLD/EPI images acquired on a modified 2T Siemens MAGNETOM Vision system. Each acquisition consisted of 64 contiguous slices (64x64x64 3mm x 3mm x 3mm voxels). Acquisition took 6.05s, with the scan to scan repeat time (TR) set arbitrarily to 7s.

96 acquisitions were made (TR=7s) from a single subject, in blocks of 6, giving 16 42s blocks. The condition for successive blocks alternated between rest and auditory stimulation, starting with rest. Auditory stimulation was bi-syllabic words presented binaurally at a rate of 60 per minute. The functional data starts at acquisiton 4, image `fM00223_004`. Due to T1 effects it is advisable to discard the first few scans (there were no "dummy" lead-in scans). A structural image was also acquired: `sM00223_002`. These images are stored in Analyse format and are available from the SPM site http://www.fil.ion.ucl.ac.uk/spm/data. This data set was the first ever collected and analysed in the Functional Imaging Laboratory (FIL) and is known locally as the mother of all experiments (MoAE).

To analyse the data, first create a new directory DIR `eg. c:\home\wpenny\fmri_analysis\auditory`, in which to place the results of your analysis. Then create 3 subdirectories (i) `jobs`, (ii) `designs`, (iii) `classical` and (iv) `bayesian`. As the analysis proceeds these directories will be filled with job-specification files, design matrices and models estimated using classical or Bayesian methods.

Start up matlab, enter your jobs directory and type *spm fmri* at the matlab prompt. SPM will then open in fMRI mode with three windows (1) the top-left or 'command' window, (2) the bottom-left or 'interactive' window and (3) the right-hand or 'graphics' window. Analysis then takes place in three major stages (i) spatial pre-processing, (ii) model specification, review and estimation and (iii) inference. These stages organise the buttons in SPM's command window.

1.1 Spatial pre-processing

1.1.1 Realignment

Under the spatial pre-processing section of the SPM command window select 'Realign' from the 'Realign' pulldown menu. This will call up a realignment job specification in the graphics window.

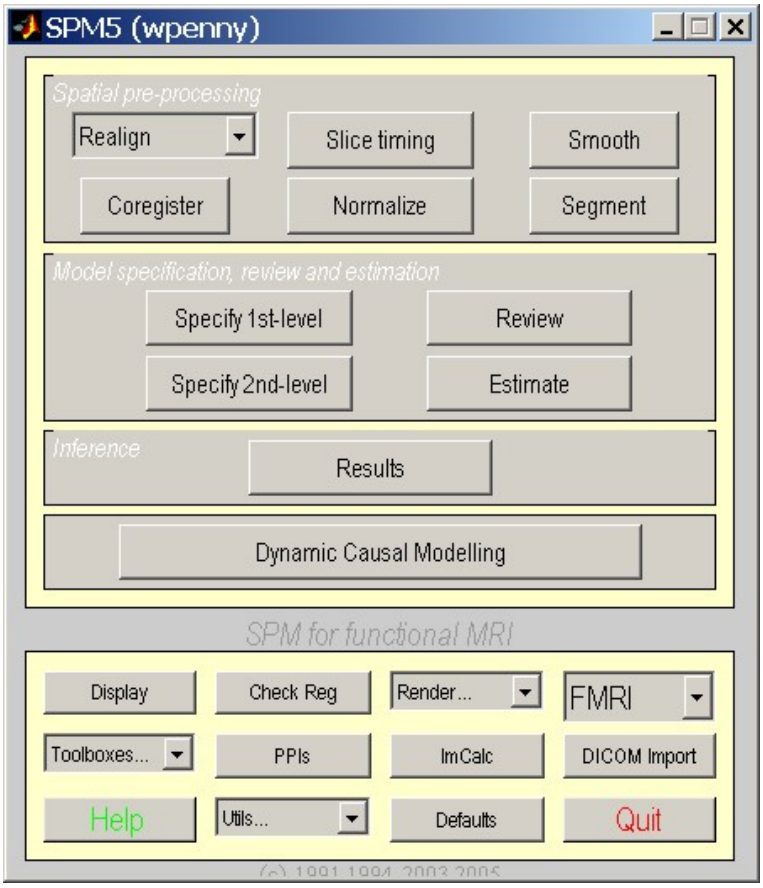


Figure 1.1: *The SPM command window comprises three sections i) spatial pre-processing, (ii) model specification, review and estimation and (iii) inference.*

Select data and then use the SPM file selector to choose all of your functional images eg. 'fM000*.img'. Then save the job file as eg. DIR/jobs/realign.mat.

Then press the RUN button in the graphics window. This will run the realign job which will write realigned images into the directory where the functional images are. These new images will be prefixed with the letter 'r'. SPM will then plot the estimated time series of translations and rotations shown in Figure 1.2. These data are also saved to a file eg. `rp_fM00223_004.txt`, so that these variables can be used as regressors when fitting GLMs. This allows movements effects to be discounted when looking for brain activations.

SPM will also create a mean image eg. `meanfM00223_004.img` which will be used in the next step of spatial processing - coregistration.

1.1.2 Coregistration

Press the 'Coreg' button. This will call up the specification of a coregistration job in the graphics window.

- Select New "Coreg:Estimate"
- Double-click on the newly created Coreg:Estimate
- Highlight 'Reference Image' and then select the mean fMRI scan from realignment eg. `meanfM00223_004.img`
- Highlight 'Source Image' and then select the structural image eg. `sM00223_002.img`.
- Press the Save button and save the job as coreg.job
- Then press RUN

SPM will then implement a coregistration between the structural and functional data that maximises the mutual information. The image in figure 1.3 should then appear in the graphics window. SPM will have changed the header of the source file which in this case is the structural image `sM00223_002.hdr`.

The 'Check Reg' facility is useful here, to check the results of coregistration. Press the 'Check Reg' button in the lower section of the command window and then the select the Reference and Source Images specified above ie `meanfM00223_004.img` and `sM00223_002.img`. SPM will then produce an image like that shown in Figure 1.4 in the graphics window. You can then use your mouse to navigate these images to confirm that there is an anatomical correspondence.

1.1.3 Segmentation

Press the 'Segment' button. This will call up the specification of a segmentation job in the graphics window. Highlight the Data field and then select the subjects registered anatomical image eg. `sM00223_002.img`. Save the job file as segment.mat and then press RUN. SPM will segment the structural image using the default tissue probability maps as priors.

Faster, though perhaps less optimal results can be obtained by eg. reducing the number of Gaussians per class from [2 2 2 4] to eg. [1 1 1 4], increasing the sampling distance from eg. 3 to 4mm. These options can be edited under

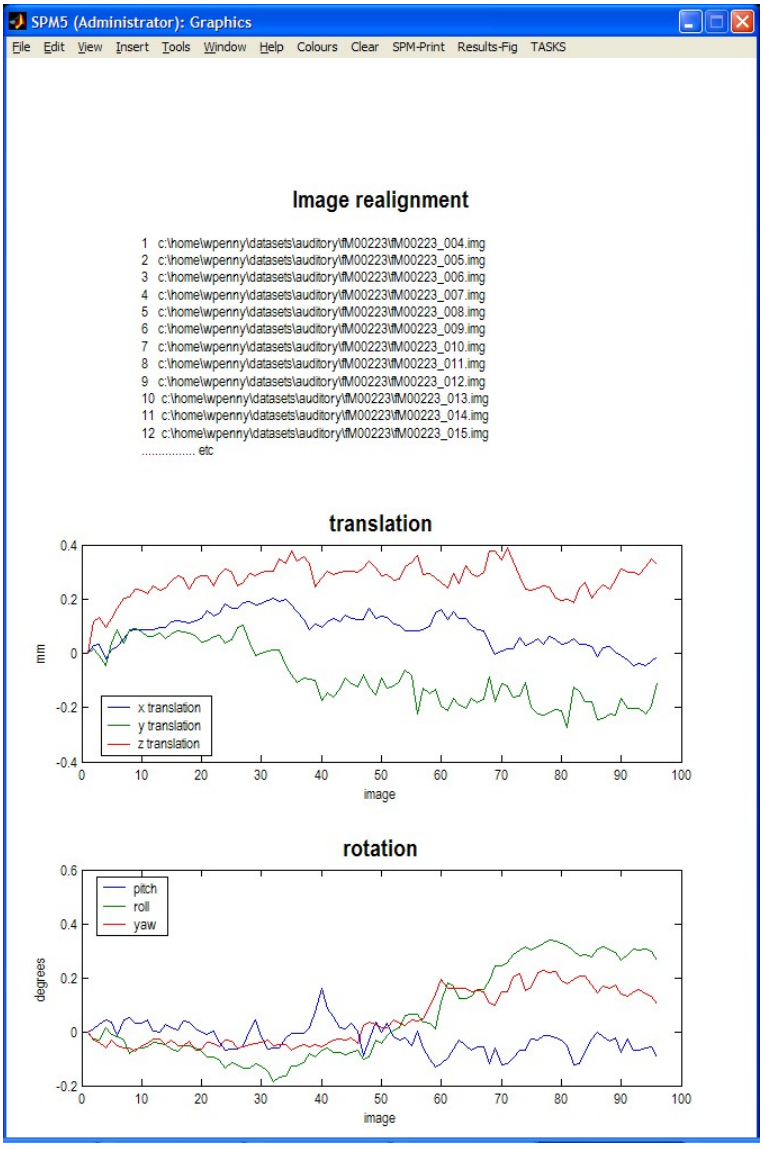


Figure 1.2: *Realignment of auditory data.*

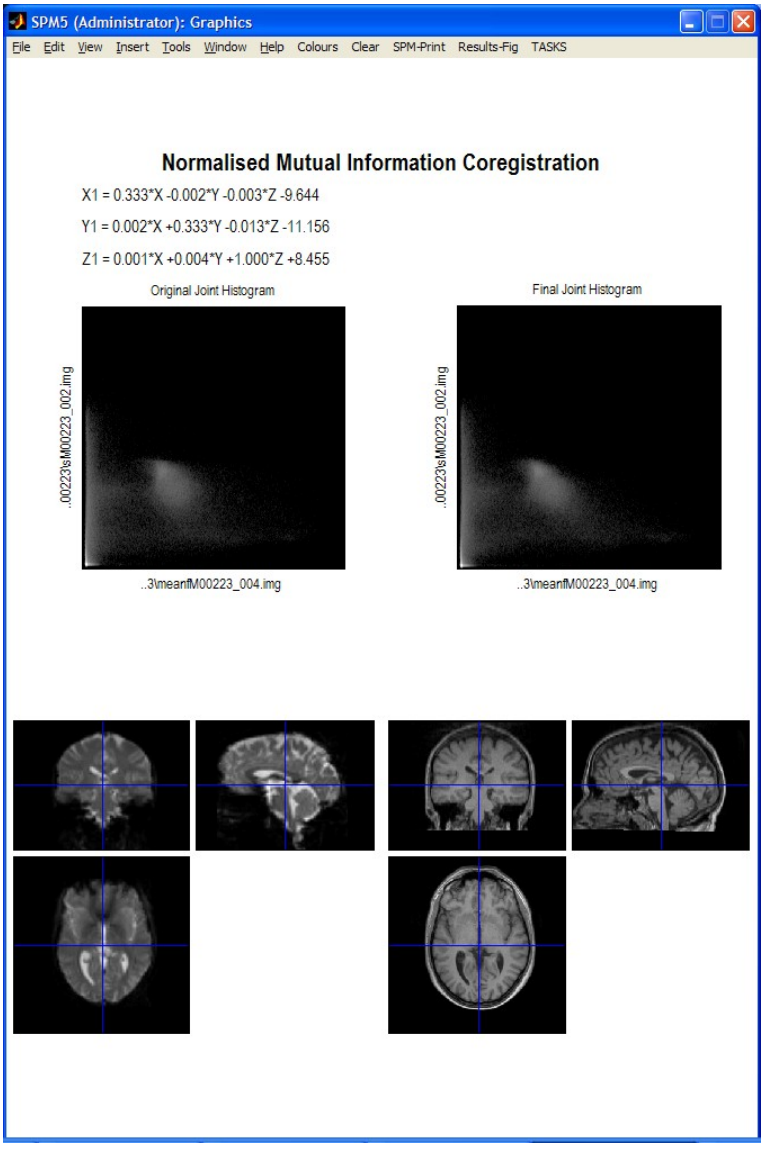


Figure 1.3: *Mutual Information Coeregistration of Auditory data.*

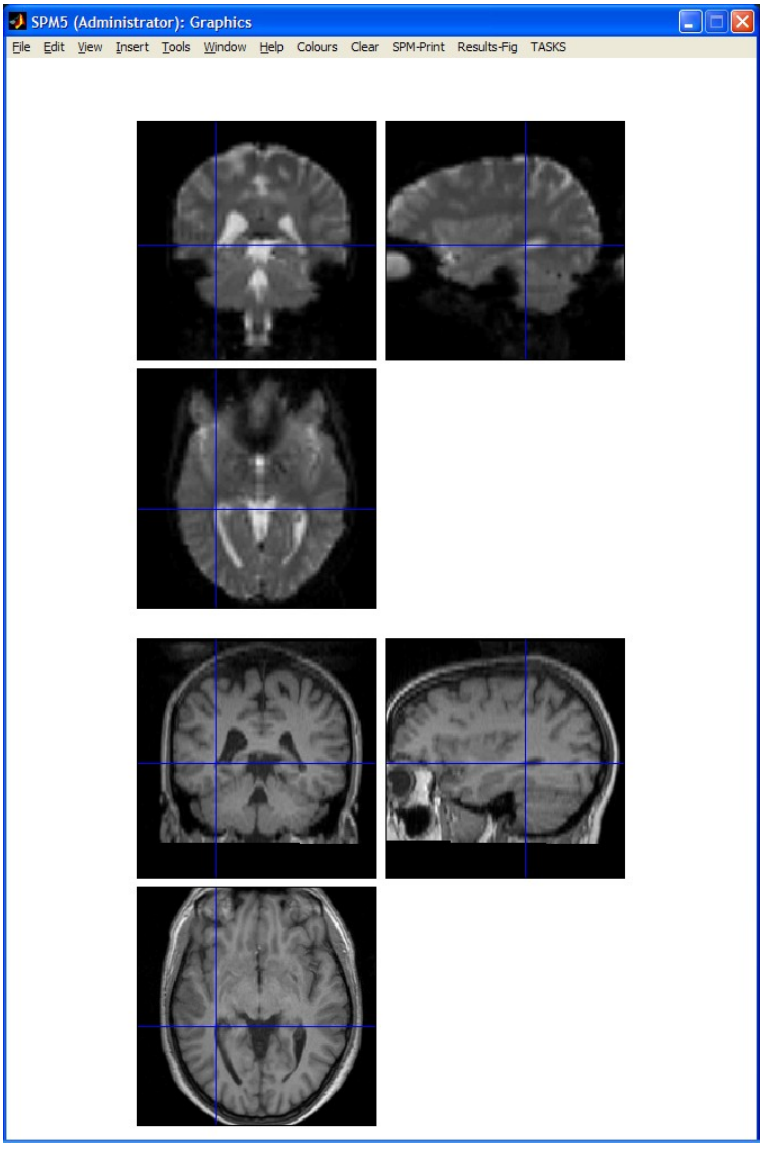


Figure 1.4: *Checking registration of functional and 'registered' structural data.*

the 'Custom' sub-menu and saved before the job is run. The results obtained in figure 1.5 were obtained using the default values.

SPM will create, by default, gray and white matter images and bias-field corrected structral image. These can be viewed using the CheckReg facility as described in the previous section (press segement and select . Figure 1.5 shows the gray matter image, `c1sM0023_002.img` along with the original structural.

SPM will also write a spatial normalisation eg. `sM00223_0020_seg_sn.mat` and inverse spatial normalisation parameters `sM00223_0020_seg_sn.mat` to files in the original structural directory. These can be used to normalise the functional data.

1.1.4 Normalize

Press the 'Normalize' button. This will call up the specification of a normalise job in the graphics window.

- Select a New "Normalise:Write" field. This allows previously estimated warps to be applied to a series of images.
- Highlight 'Data', select New "Subject"
- Open 'Subject', highlight 'Parameter File' and select the `sM00223_0020_seg_sn.mat` file that you created in the previous section
- Highlight images to write and select all of the realigned functional images 'rfM000*.img'. Note: This can be done efficiently by changing the filter in the SPM file selector to `^r.*`. SPM will then only list those files beginning with the letter *r* ie. those that have been realigned. You can then right click over the listed files, choose 'Select all' and press 'Done'.
- Open 'Writing Options', and change 'Voxel sizes' from [2,2,2] to [3,3,3].[1]
- Press 'Save', save the job as normalise.mat and then press 'Run'.

SPM will then write spatially normalised files to the functional data directory. These files have the prefix 'w'.

If you wish to superimpose a subject's functional activations on their own anatomy[2] you will also need to apply the spatial normalisation parameters to their (bias-corrected) anatomical image. To do this

- Press 'Normalise', select New "Normalise:Write"
- Open 'Normalise: Write', highlight 'Data', select New "Subject"
- Open 'Subject'
- Highlight 'Parameter File', select the `sM00223_0020_seg_sn.mat` file that you created in the previous section, press 'Done'.

[1] This step is not strictly necessary. It will write images out at a resolution closer to that at which they were acquired. This will speed up subsequent analysis and is necessary, for example, to make Bayesian fMRI analysis computationally efficient.

[2] Beginners may wish to skip this step, and instead just superimpose functional activations on an 'average structural image'.

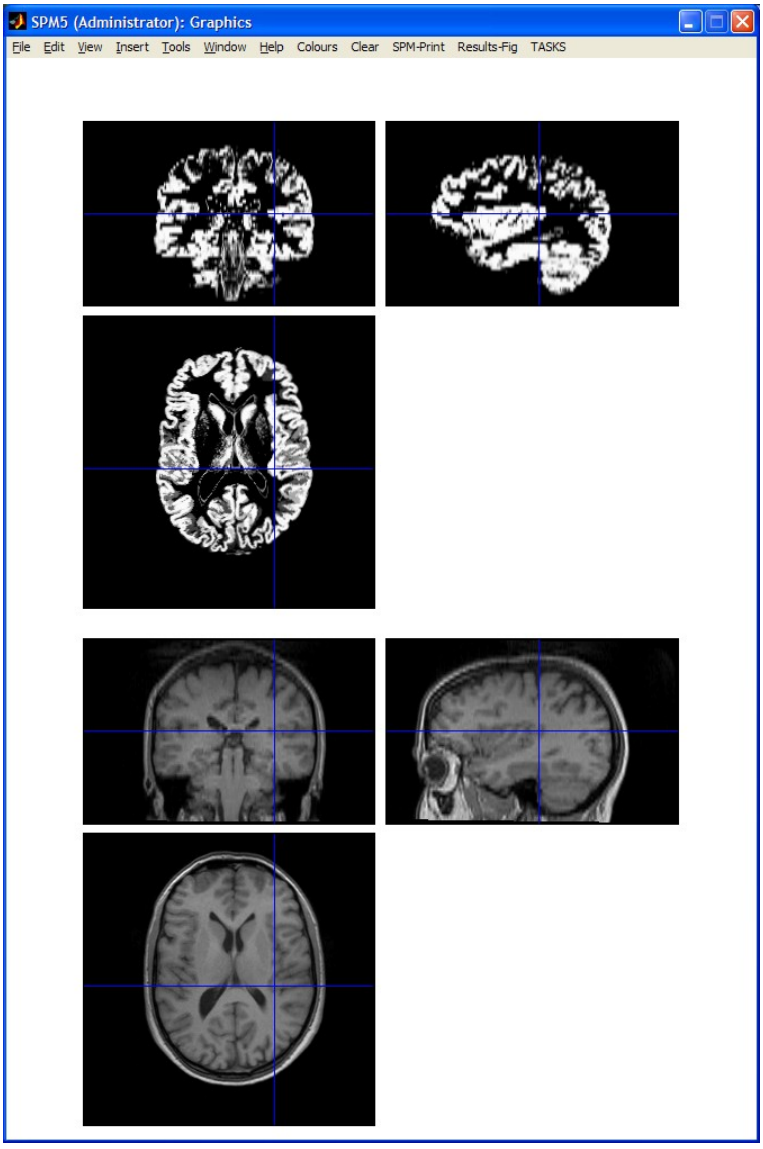


Figure 1.5: *Gray matter image and 'registered' structural image.*

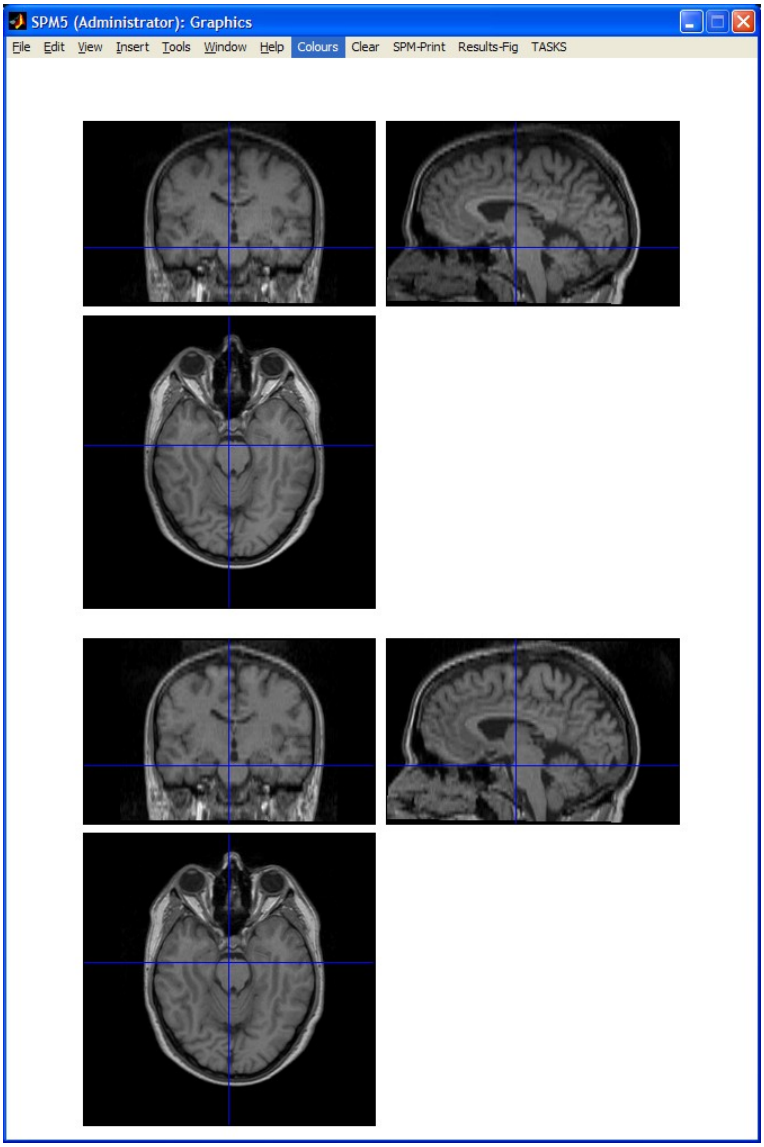


Figure 1.6: *Stuctural image (top) and bias-corrected structural image (bottom). Notice that the original structural is darker at the top than at the bottom. This non-uniformity has been removed in the bias-corrected image.*

- Highlight 'Images to Write', select the bias-corrected structural eg. `msM00223_002.img`, press 'Done'.
- Open 'Writing Options', select voxel sizes and change the default [2 2 2] to [1 1 3] which corresponds to the original resolution of the images.
- Save the job as `norm_struct.mat` and press 'Run'.

1.1.5 Smoothing

Press the 'Smooth' button[3]. This will call up the specification of a smooth job in the graphics window.

- Open 'Smooth', select 'Images to Smooth' and then select the spatially normalised files created in the last section eg. wrfM000*.img.
- Highlight, 'FWHM' and change [8 8 8] to [6 6 6]. This will smooth the data by 6mm in each direction.
- Save the job as smooth.mat and press 'Run'.

1.2 Model specification, review and estimation

To avoid T1 effects in the initial scans of an fMRI time series we recommend discarding the first few scans. To make this example simple, we'll discard the first complete cycle (12 scans, 04-15), leaving 84 scans, image files 16-99. This is best done by moving these files to a different directory.

Press the 'Specify 1st-level' button. This will call up the specification of an fMRI specification job in the graphics window. Then

- Open the fMRI model specification option
- Open the 'Timing paramaters' option
- Highlight 'Units for design' and select 'Scans'
- Highlight 'Interscan interval' and enter 7
- Highlight 'Data and Design' and select 'New Subject/Session'. Then open the newly created 'Subject/Session' option.
- Highlight 'Scans' and use SPM's file selector to choose the 84 smoothed, normalised functional images ie `swrfM00223_016.img - *_099.img`. These can be selected easily using the `^s.*'` filter, and select all (provided you have moved the scans 4 to 15 into a different directory). Then press 'Done'.
- Highlight 'Condition' and select 'New condition'
- Open the newly created 'Condition' option. Highlight 'Name' and enter 'active'. Highlight 'Onsets' and enter '6:12:84'. Highlight 'Durations' and enter '6'.

[3]The smoothing step is unnecessary if you are only interested in Bayesian analysis of your functional data.

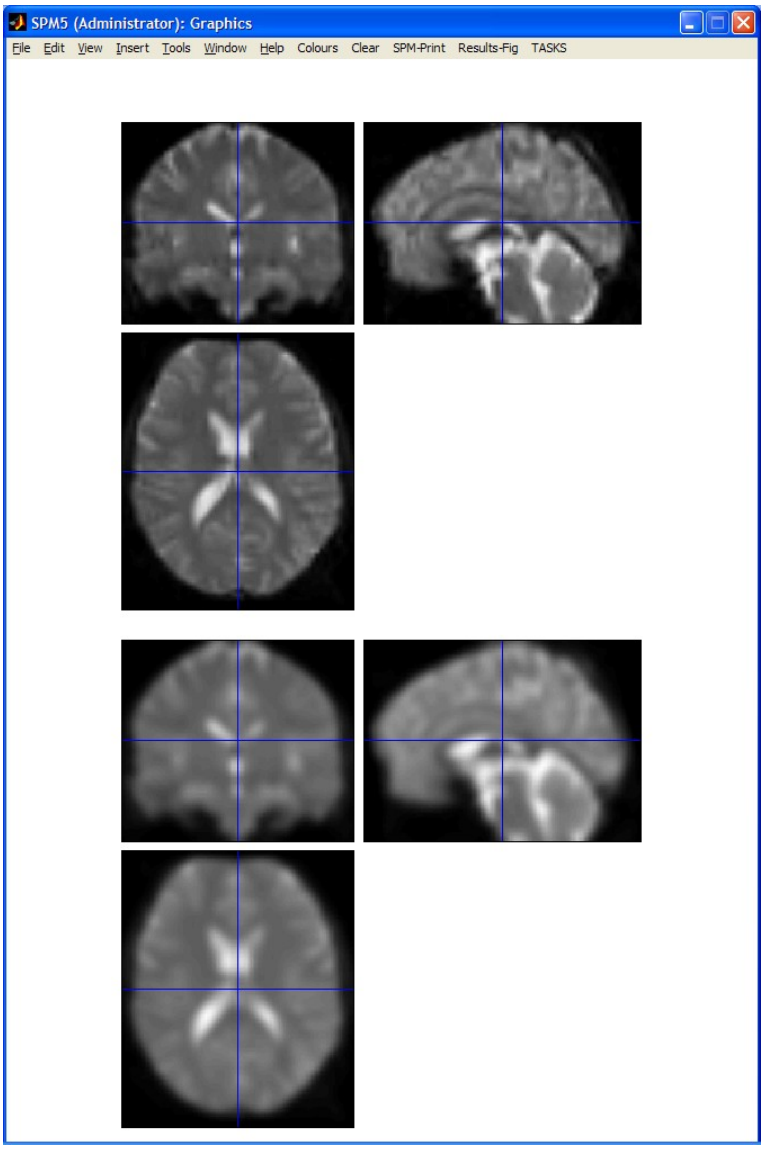


Figure 1.7: *Functional image (top) and 6mm-smoothed functional image (bottom). These images were obtained using SPM's 'CheckReg' facility.*

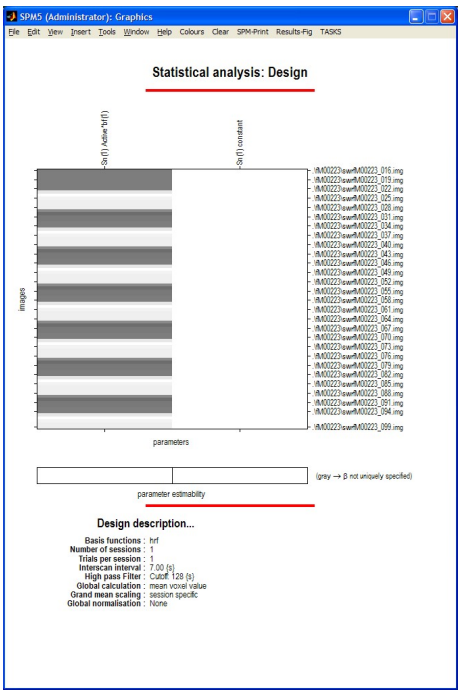


Figure 1.8: *Design matrix.*

- Highlight 'Directory' and select the `DIR/design` directory you created earlier.
- Save the job as `specify.mat` and press 'RUN'

SPM will then write an `SPM.mat` file to the `DIR/design` directory. It will also plot the design matrix, as shown in Figure 1.8.

At this stage it is advisable to check your model specification using SPM's review facility which is accessed via the 'Review' button. This brings up a 'design' tab on the interactive window clicking on which produces a pulldown menu. If you select the first item 'Design Matrix' SPM will produce the image shown in Figure 1.8. If you select 'Explore' then 'Session 1' then 'active', SPM will produce the plots shown in Figure 1.9.

1.2.1 Estimate

Press the 'Estimate' button. This will call up the specification of an fMRI estimation job in the graphics window. Then

- Open the 'fMRI model estimation' option
- Highlight the 'Select SPM.mat' option and then choose the SPM.mat file saved in the design subdirectory
- Highlight the 'Directory' option and then choose the `DIR\classical` directory

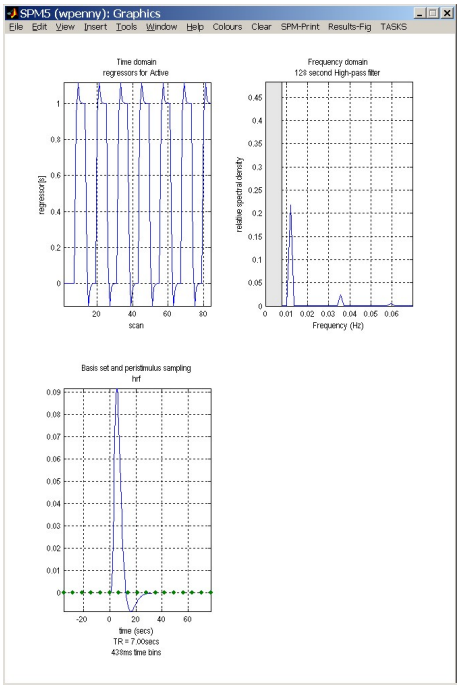


Figure 1.9: *Exploring the design matrix in Figure 1.8. This shows the time series of the 'active' regressor (top left), a frequency domain plot of the active regressor (top right) and the basis function used to convert assumed neuronal activity into hemodynamic activity. In this model we used the default option - the canonical basis function. The frequency domain plot shows that the frequency content of the 'active' regressor is above the set frequencies that are removed by the High Pass Filter (HPF) (these are shown in gray - in this model we accepted the default HPF cut-off of 128s or 0.008Hz).*

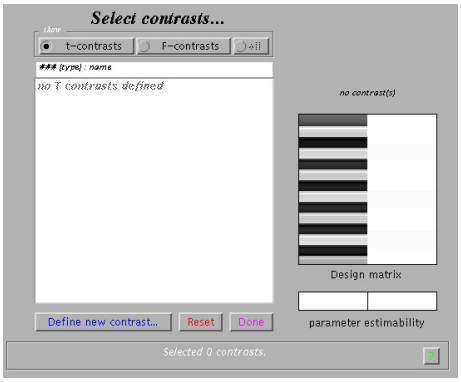


Figure 1.10: *The contrast manager*

- Save the job as `estimate.job` and press Run

SPM will write a number of files into the selected directory including an `SPM.mat` file.

1.3 Inference

After estimation:

- Press 'Results'
- Select the `SPM.mat` file created in the last section

This will invoke the contrast manager.

1.3.1 Contrast manager

The contrast manager displays the design matrix (surfable) in the right panel and lists specified contrasts in the left panel. Either 't-contrast' or 'F-contrast' can be selected. To examine statistical results for condition effects

- Select 'Define new contrast'

One sided main effects for the active condition (i.e., a one-sided t-test) can be specified (in this example) as '1' (active > rest) and '-1' (rest > active). SPM will accept correct contrasts only. Accepted contrasts are displayed at the bottom of the contrast manager window in green, incorrect ones are displayed in red. To view a contrast

- Select the contrast name e.g., 'active > rest'
- Press 'Done'

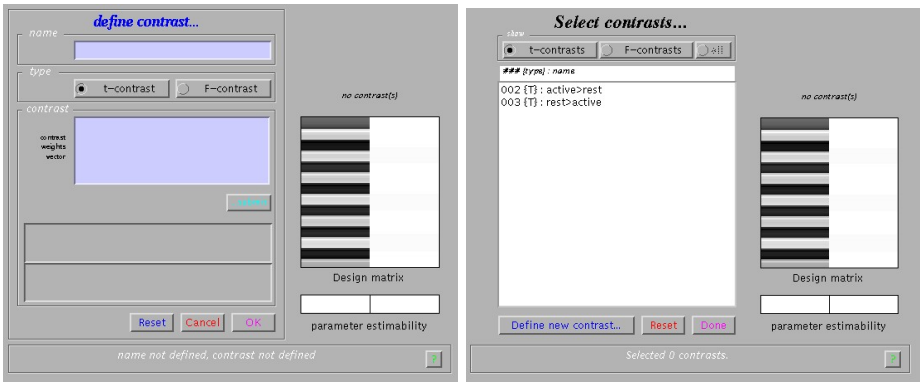


Figure 1.11: *Left: A contrast is entered by specifying the numeric values in the lower window and the name in the upper window. Right: After contrasts have been specified they can be selected.*

1.3.2 Masking

You will then be prompted with

- *Mask with other contrast ? [Yes/No]*
- Specify No.

Masking implies selecting voxels specified by other contrasts. If 'yes', SPM will prompt for (one or more) masking contrasts, the significance level of the mask (default p = 0.05 uncorrected), and will ask whether an inclusive or exclusive mask should be used. Exclusive will remove all voxels which reach the default level of significance in the masking contrast, inclusive will remove all voxels which do not reach the default level of significance in the masking contrast. Masking does not affect p-values of the 'target' contrast, it only includes or excludes voxels.

1.3.3 Thresholds

You will then be prompted with

- *Title for comparison ?*
- Enter eg. 'active > rest'
- *Corrected height threshold ? [Yes/No]*
- Enter Yes.
- *p value adjustment to control: [FWE/FDR/none]*
- Select FWE
- *p value(family-wise error)*
- Accept the default value, 0.05

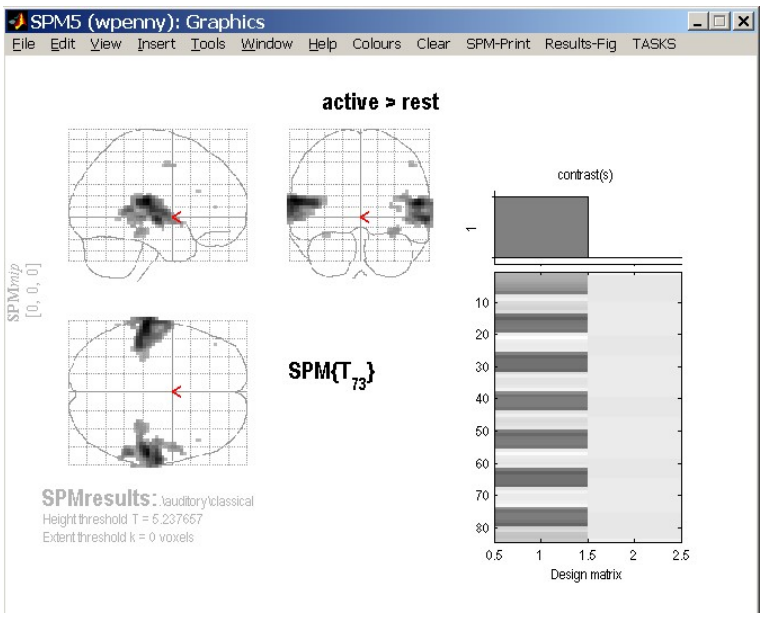


Figure 1.12: *SPM showing bilateral activation of auditory cortex.*

A Family Wise Error (FWE) is a false positive anywhere in the SPM. Now, imagine repeating your experiment many times and producing SPMs. The proportion of SPMs containing FWEs is the FWE rate. A value of 0.05 implies that 1 in 20 SPMs contains a false positive somewhere in the image.

If you choose the 'none' option above this corresponds to making statistical inferences at the 'voxel level'. These use 'uncorrected' p values, whereas FWE thresholds are said to use 'corrected' p values. SPM's default uncorrected p value is p=0.001. This means that the probability of a false positive at each voxel is 0.001. So if, you have 50,000 voxels you can expect $50,000 \times 0.001 = 50$ false positives in each SPM.

The final option here is False Discovery Rate (FDR). If you set this at 0.1, this means that of all the discoveries you make (ie. above threshold voxels that appear in the SPM) 10% of them are likely to be false.

You will then be prompted with

- *Extent Threshold {voxels} [0]*
- Accept the default value, 0

Entering a value v here will produce SPMs with clusters containing at least v voxels. SPM will then produce the SPM shown in Figure 1.12.

- Select 'Define new contrast'

1.3.4 Files

A number of files are written to the working directory at this time. Images containing weighted parameter estimates are saved as con-0002.hdr/img, con-

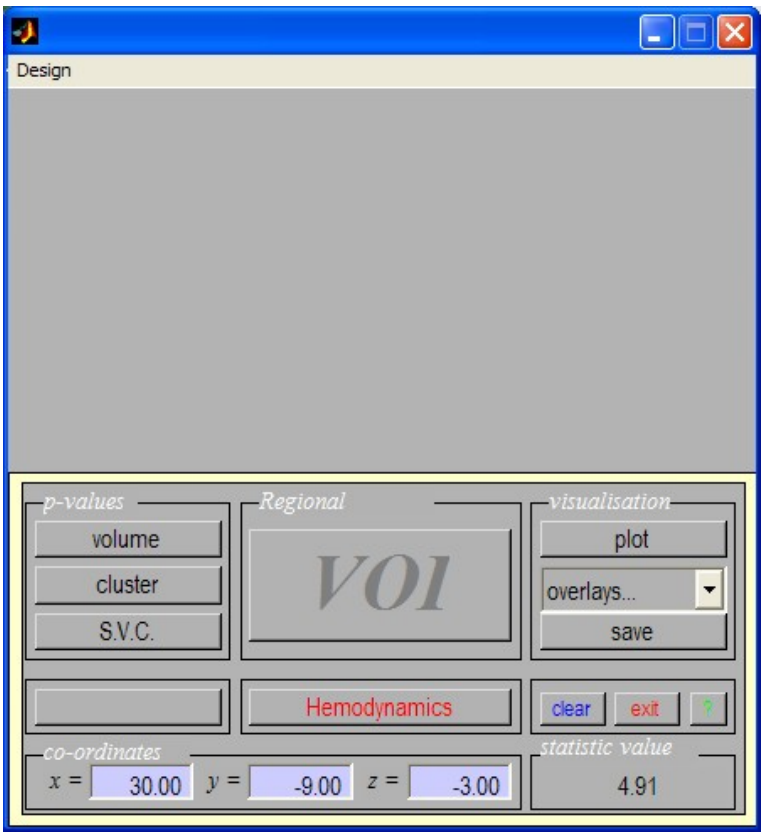


Figure 1.13: *SPM's interactive window during results assessment. The 'p-values' section is used to produce tables of statistical information. The visualisation section is used to plot responses at a voxel or to visual activations overlaid on anatomical images. The 'Regional' section, ie. the VOI button, is used to extract data for subsequent analyses such as assessment of PsychoPhysiological Interactions (PPIs) or Dynamic Causal Models (DCMs).*

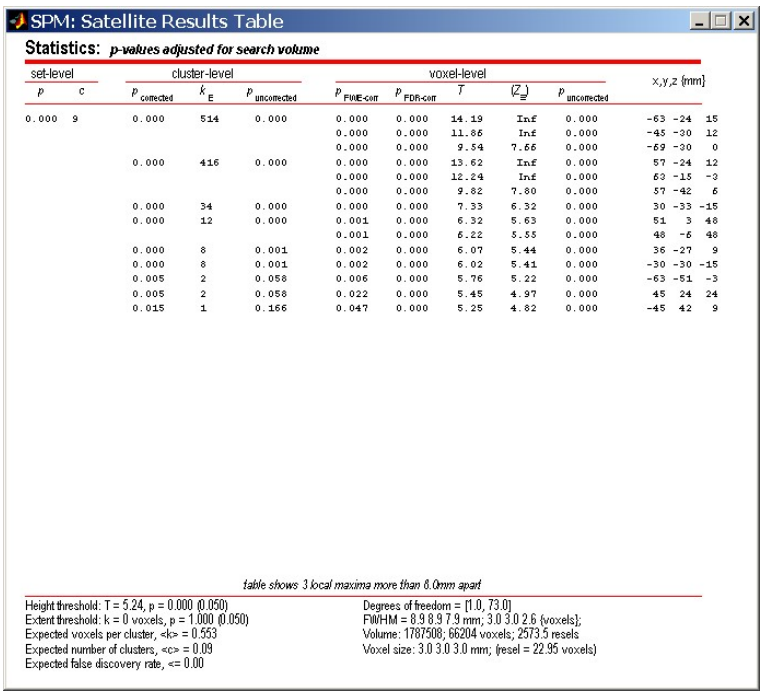
0003.hdr/img, etc. in the working directory. Images of T-statistics are saved as spmT-0002.hdr/img, spmT-0003.hdr/img etc., also in the working directory.

1.3.5 Maximum Intensity Projections

SPM displays a Maximum Intensity Projection (MIP) of the statistical map in the graphics window. The MIP is projected on a glass brain in three orthogonal planes. The MIP is surfable: R-clicking in the MIP will activate a pulldown menu, L-clicking on the red cursor will allow it to be dragged to a new position.

1.3.6 Design matrix

SPM also displays the design matrix with the selected contrast. The design matrix is also surfable: R-clicking will show parameter names, L-clicking will



SPM: Satellite Results Table

Statistics: *p-values adjusted for search volume*

set-level		cluster-level			voxel-level					x,y,z {mm}
p	c	$p_{corrected}$	k_E	$p_{uncorrected}$	$p_{FWE-corr}$	$p_{FDR-corr}$	T	$(Z_{\equiv})$	$p_{uncorrected}$	
0.000	9	0.000	514	0.000	0.000	0.000	14.19	Inf	0.000	-63 -24 15
					0.000	0.000	11.86	Inf	0.000	-45 -30 12
					0.000	0.000	9.54	7.66	0.000	-69 -30 0
		0.000	416	0.000	0.000	0.000	13.62	Inf	0.000	57 -24 12
					0.000	0.000	12.24	Inf	0.000	63 -15 -3
					0.000	0.000	9.82	7.80	0.000	57 -42 6
		0.000	34	0.000	0.000	0.000	7.33	6.32	0.000	30 -33 -15
		0.000	12	0.000	0.001	0.000	6.32	5.63	0.000	51 3 48
					0.001	0.000	6.22	5.55	0.000	48 -6 48
		0.000	8	0.001	0.002	0.000	6.07	5.44	0.000	36 -27 9
		0.000	8	0.001	0.002	0.000	6.02	5.41	0.000	-30 -30 -15
		0.005	2	0.058	0.006	0.000	5.76	5.22	0.000	-63 -51 -3
		0.005	2	0.058	0.022	0.000	5.45	4.97	0.000	45 24 24
		0.015	1	0.166	0.047	0.000	5.25	4.82	0.000	-45 42 9

table shows 3 local maxima more than 8.0mm apart

Height threshold: T = 5.24, p = 0.000 (0.050)
Extent threshold: k = 0 voxels, p = 1.000 (0.050)
Expected voxels per cluster, <k> = 0.553
Expected number of clusters, <c> = 0.09
Expected false discovery rate, <= 0.00

Degrees of freedom = [1.0, 73.0]
FWHM = 8.9 8.9 7.9 mm; 3.0 3.0 2.6 {voxels};
Volume: 1787508; 66204 voxels; 2573.5 resels
Voxel size: 3.0 3.0 3.0 mm; (resel = 22.95 voxels)

Figure 1.14: *Volume table for 'active > rest' effect. This table of values was created by pressing the 'Results-Fig' tab at the top of the graphics window and then pressing the 'Volume' button. This displays the table of results in a separate window.*

show design matrix values for each scan.

In the SPM Interactive window (lower left panel) a button box appears with various options for displaying statistical results (p-values panel) and creating plots/overlays (visualisation panel). Clicking 'Design' (upper left) will activate a pulldown menu as in the 'Explore design' option.

1.3.7 Statistical tables

To get a summary of local maxima, press the 'volume' button in the p-values section of the interactive window. This will list all clusters above the chosen level of significance as well as separate (>8mm apart) maxima within a cluster, with details of significance thresholds and search volume underneath, as shown in Figure 1.14 The columns in volume table show, from right to left:

- x, y, z (mm): coordinates in Talairach space for each maximum
- voxel-level: the chance (p) of finding (under the null hypothesis) a voxel with this or a greater height (T- or Z-statistic), corrected (FWE or FDR)/ uncorrected for search volume.
- cluster-level: the chance (p) of finding a cluster with this many(ke) or a greater number of voxels, corrected / uncorrected for search volume

- set-level: the chance (p) of finding this (c) or a greater number of clusters in the search volume

It is also worth noting that

- The table is surfable: clicking a row of cluster coordinates will move the pointer in the MIP to that cluster, clicking other numbers will display the exact value in the Matlab window (e.g. 0.000 = 6.1971e-07).
- To inspect a specific cluster (e.g., in this example data set, the R auditory cortex), either move the cursor in the MIP (by L-clicking and dragging the cursor, or R-clicking the MIP background which will activate a pulldown menu).
- Alternatively, click the cluster coordinates in the volume table, or type the coordinates in the co-ordinates section of the interactive window.

It is also possible to produce tables of statistical information for a single cluster of interest rather than for the whole volume. Firstly, elect the relevant cluster in the MIP and then press the 'cluster' button in the p-values section of the interactive window. This will show coordinates and voxel-level statistics for local maxima (>4mm apart) in the selected cluster. This table is also surfable.

1.3.8 Plotting responses at a voxel

A voxel can be chosen with co-ordinates correspoonding to those in the interactive window. The responses at this voxel can then be plotted using the 'Plot' button in the visualisation section of the interactive window. This will provide you with five further options:

1. Contrast estimates and 90% CI: SPM will prompt for a specific contrast (e.g., active>rest). The plot will show effect size and 90% confidence intervals. See eg. Figure 1.15
2. Fitted responses: Plots adjusted data and fitted response across session/subject. SPM will prompt for a specific contrast and provides the option to choose different ordinates ('an explanatory variable', 'scan or time', or 'user specified'). If 'scan or time', the plot will show adjusted or fitted data with errors added as shown in Figure 1.16
3. Event-related responses: Plots adjusted data and fitted response across peri-stimulus time.
4. Parametric responses
5. Volterra kernels

For plotting event-related responses SPM provides three options

1. Fitted response and PSTH (peri-stimulus time histogram): plots mean regressor(s) (ie. averaged over session) and mean signal +/- SE for each peri-stimulus time bin.
2. Fitted response and 90% CI: plots mean regressor(s) along with a 90% confidence interval.

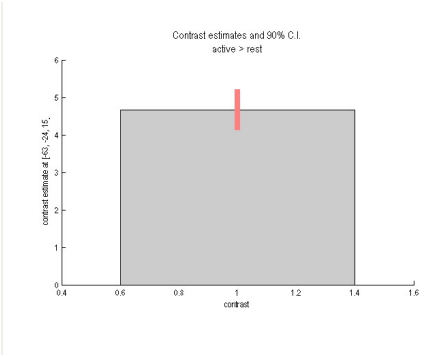


Figure 1.15: *Estimated effect size.*

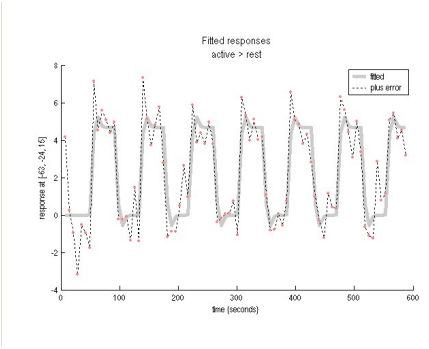


Figure 1.16: *Fitted responses.*

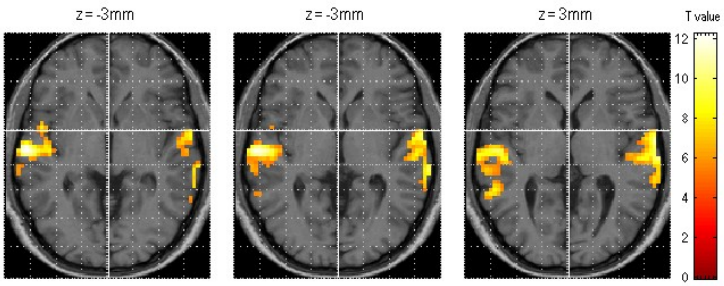


Figure 1.17: *Slices.*

3. Fitted response and adjusted data: plots regressor(s) and individual data (note that in this example the data are shown in columns due to the fixed TR/ISI relationship).

Its worth noting that

- The values for the fitted response across session/subject for the selected plot can be displayed and accessed in the Matlab window by typing 'Y'. Typing 'y' will display the adjusted data.
- 'Adjusted' data = adjusted for confounds (e.g., global flow) and high- and low pass filtering.

1.3.9 Overlays

The visualisation section of the interactive window also provides an overlay facility for anatomical visualisation of clusters of activation. Pressing 'Overlays' will activate a pulldown menu with three options

1. Slices: overlay on three adjacent (2mm) transaxial slices. SPM will prompt for an image for rendering. This could be a canonical image (see spm-template.man) or an individual T1/mean EPI image for single-subject analyses.
2. Sections: overlay on three intersecting (sagittal, coronal, transaxial) slices. These renderings are surfable: clicking the images will move the crosshair.
3. Render: overlay on a volume rendered brain, with options for using a smoothed brain, and old (left) and new (right) style rendering.

Renderings can be saved as filename.img and filename.hdr in the working directory by using the *write filtered* option. In Figures 1.171.181.19 the 'active > rest' activation has been superimposed on the spatially normalised, bias-corrected anatomical image `wmsM00223_002.img` created earlier.

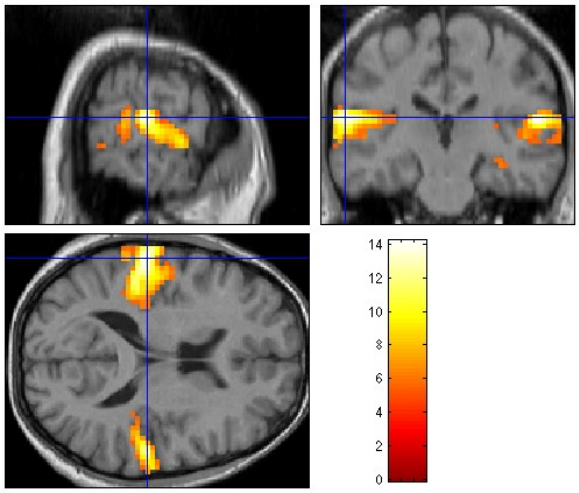


Figure 1.18: *Sections.*

For the 'Render' option we first created a rendering for this subject. This was implemented by . We then selected the gray and white matter images `c1sM00223_002.img` and `c2sM00223_002.img` created earlier.

- Selecting 'Xtract Surface' from the 'Render' pulldown menu
- Selecting the gray and white matter images `c1sM00223_002.img` and `c2sM00223_002.img` created earlier.
- Saving using the default options (Rendering and Surface)

SPM plots the rendered anatomical image in the graphics window and saves it as `render_c1sM00223_002.img` (the surface is saved as `surf_c1sM00223_002.img`).

1.3.10 Miscellaneous

Other options (in the results controls panel):

- clear: clears lower subpanel of Graphics window
- exit: exits the results section
- ? : launches spm-results-ui help

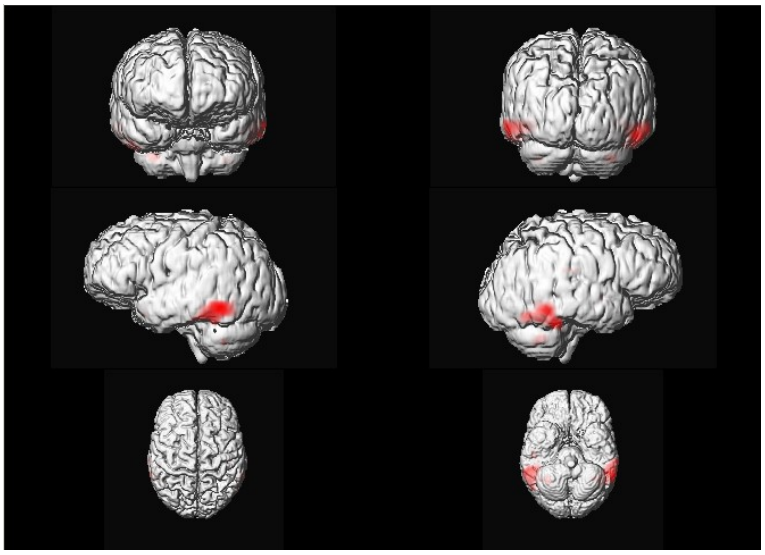


Figure 1.19: *Render.*