Analyzing 3T Trio fMRI data with SPM2: the Ultimate Guide

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(Matlab 6.1 or 6.5 or 6.51on Linux workstations)

Version 2.7 by EM, last revised 7/6/04

OVERVIEW:

1. Linux commands primer
2. Pre- pre-processing: copying on to system from CD, converting from DICOM to Analyze format, and sorting data by run
3. Pre-processing
    1. **Slice timing correction**: shifts signal as if slices were acquired at same time
    2. **Realignment**: aligns all functional volumes to each other
    3. **Co-registration**: pulls T1 into 3D space of functionals
    - ❋ **Segmentation** ***New in version 2.7***
    4. **Normalization**: warps images into 3D space of template brain
        i. Determine parameters
        ii. Write normalized - apply parameters from (i) to T1 and functionals
    5. **Smoothing**: smoothes functionals with Gaussian kernel
4. Create **design matrix** - specify event onsets, regressors, and images
5. Make **contrasts** and view your results - look at activations, small volume correction, print to .ps file and printers

Inter-subject analysis:

1. Create mean image
2. Fixed effects
3. Conjunction
3. Random effects
4. Simple Regression ***New in version 2.7***
5. One-way Anova and Masking ***New in version 2.7***

Miscellaneous Bonus Material:

1. Mosaic images: Overlay contrast on a volume and show multiple slices on one page.
2. Displaying blobs from multiple contrasts on one anatomical image
3. MarsBar ROI (Region of Interest) analysis in spm99: get t and p statistics for predicted region
4. Optimized VBM (Voxel-Based Morphometry) ***New in version 2.7***
    - also setting up symmetric templates, comparing right vs. left hemispheric atrophy

NOTES:

- ✓ In this guide, words in **bold** are actual commands to type or buttons to click. Words in *italics* represent filenames or directory names that you may pick yourself. The guide assumes some elementary knowledge of Linux commands (see below) and how to use the spm_get window (see page 3).
- ✓ You can always click on the green **HELP** button to get information on the various SPM2 functions or go to http://www.fil.ion.ucl.ac.uk/spm/spm2.html

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LINUX commands primer (most can be used in Matlab terminal also)

Directory and file locations are specified by a path name, e.g. */data/mistral1/Subject01/run1* . Each "/" denotes a different level in the tree structure. Thus / by itself is the root directory, or the top of the tree. */data* contains all the harddrive partitions, */data/mistral1* contains all the files on the disk mistral1, and so on. You can move between levels in the tree by using the **cd *pathname*** command. *pathname* can be the full path (e.g. **cd */data/mistral1/Subject01/run1***) or a partial path (e.g. if you are already within the "Subject01" directory and want to move to a subdirectory, "run1", you could type **cd *run1***.

If a command doesn't work in the Matlab terminal window, try the same command with a **!** in front of it, i.e. **!mv *file1 file2***

cd *pathname*	change directory (see above)
cd ..	go up one level in directory tree
ls	list files and directories in your present working directory
pwd	present working directory (i.e. where you are)
cp *filenames location_to_be_copied_to*	copy
mv *filenames location_to_be_moved_to*	move (use to rename files also)
chmod 774 *filenames*	change permissions to give owner and group read/write/ execute permissions and others only read permission

-R Do the command recursively (descend into subdirectories and repeat command). For example, **cp -R Subject01 /data/mistral1** will copy all files, subdirectories, and subfiles in the directory "Subject01" to its new location in /data/mistral1

***** Wildcard. It stands for any character. For example, **cp R* Subject01** will copy all files beginning with "R" to the directory "Subject01". **cp * Subject01** will copy <u>all</u> files in your present working directory to subject01.

PRE- PRE- PROCESSING

COPYING

1. Decide what hard drive you are going to put your data on. The processes will run faster if you can store the data on the same machine that you are going to be working on because then the information will not have to travel over the network. Thus, put your data on one of the mistral partitions (mistral1, mistral2, mistral3) if you will primarily be using mistral to run Matlab and SPM2.
2. Create directory for your subject (**mkdir *directory_name***) on that hard drive and then create another directory called *RAW* within your subject directory.
3. Copy data from CD into *RAW* on to system and check that all files are copied. For instance, from a terminal window type **cd /mnt/cdrom** so that you are in the CD. When you type **ls**, you will see "dicomdir" and a string of numbers (*numberstring*) that is the name of the directory containing the raw data. Type **cd *numberstring*** to get

into that directory. When you type **ls** you should see a bunch of files named with number strings.
To copy the files from the CD to the hardrive, type
cp * ***/data/mistral1/directory_name/RAW*** or whatever the pathname to your RAW directory is. This might take several minutes.

4. You will have to change the permissions of the files you just copied to ensure that you (the user) has the appropriate permission to read and write the files.
   E.g. **chmod 774** ***filenames*** will give user and group read/write/execute permission and others will just get read permission.
5. **cd** into the *RAW* directory if you are not already there
6. Open Matlab. Either click the Matlab icon on the desktop OR if you are at:
   - Chinook, Gust, or Zephyr: type **lmatlab651 -nodesktop** (so that matlab opens in current terminal window) OR type just **lmatlab651** (to create new Matlab command window)
   - Mistral: type **matlab65 -nodesktop** OR **matlab65**
7. **spm2** to open SPM2. Warning - make sure you don't accidentally open spm2b, they look the same!

CONVERSION

1. Click on **Toolboxes** -> **DICOM** to convert from DICOM to Analyze format.
   An spm_get window will pop up asking you for your DICOM Working Directory. This is the place where your raw data (DICOM) is stored, i.e. your *RAW* directory. See below for maneuvering in the window. When you are inside your *RAW* directory, click the single black "**.**" to select it, then hit **Done.**

   Using the spm_get window: The red names on the left are the subdirectories inside your present location. Click on a subdirectory name to go into that subdirectory. Click on the red ".." to move up one level in the file tree hierarchy. In the center in black are the files inside your present location. Click on one of them to select it. Click on the single black "." to select the directory you are in. At the top there is a drop-down menu where you can quickly go to previously accessed directories. Click "pwd" in the upper right corner to go to your present working directory.

2. To select your DICOM files, go into your *RAW* directory (so that you can see all your raw files listed below) and hit **all**. All of the raw files should now be selected. Click **Done**.
3. If there is a warning about variable slice spacing, just ignore it.
4. This spm_dicom function will convert the files and rename. Each raw functional file = 1 volume and gives 3 Analyze files:
   1) Image .img
   2) Header .hdr - info about that image
   3) 4x4 matrix .mat - says how image is oriented in 3D space

   This function will append an "f" to the name of your functional files, which begin with the patient ID entered at the time of scanning. If your patient ID was fmri, the functional files will be renamed as

"*ffmri-series#-image#-acquisition#"*
From now on in this guide, this will be abbreviated as "ffmri"

5. All structural raw files are compiled into 1 volume (each T1 file = 1 slice). An "s" will appended to the name so T1 files will start with "*sfmri...*"

SORTING VIA DR. GITELMAN'S SORT_FILES PROGRAM

NOTE: This program may not work properly if you start Matlab by typing "matlab" in a terminal window and/or if you are using the Matlab desktop (i.e. if a separate Matlab command window pops up). It should work fine if you open Matlab using the icon on the desktop. This process may also be done manually if the program doesn't work: create directories for each of your runs (including T1) using **mkdir *directory_name*** and then move (**mv**) the appropriate series into the run folder. For instance, if series #2 is *run1*, make a directory in your subject folder called *run1* , go back into your *RAW* directory, and then type **mv ffmri-0002* ../run1** (i.e. move files starting with that string up one level in the hierarchy and into the directory called "run1").

**** If you do this sorting procedure by hand, you must remember to manually DELETE THE DUMMY FILES (both the .img and .hdr files)!! ****

1. **Toolboxes** -> **CBMG tools**
2. Click on **sort files**
3. Select the base directory. This is the directory where you want your files sorted. For example, if your raw data is in /data/gust4/congruency/F100/RAW, you would select the directory /data/gust4/congruency/F100. Click **done.**
4. Type in the # of runs, including the T1
5. Then for each run, it will ask you the series number (get from Trio run sheet), the # of dummy volumes, and the name of the directory you want to put it in. For the T1, # of dummies = 0!
6. Then it will ask you to select all the images. Select both the functionals and the T1.
7. The program will then sort the files. It takes about 15 seconds to sort ~1000 files.

PRE-PROCESSING

SLICE TIMING CORRECTION

At the 3T Trio magnet, slices are acquired from bottom up in interleaved fashion throughout TR. If there is an even number of slices, it will acquire slice numbers 2, 4, 6... then 1, 3, 5... If there are an odd number of slices then it acquires 1, 3, 5,... 2, 4, 6...SPM considers slice 1 to be at the bottom. The purpose of the interleaved order is to minimize "cross-talk" between slice selection pulses - e.g. slice 2 is partially excited when acquiring slice 1 so you don't want to measure slice 2 right away. Thus, slice timing corrections shift signal so that it is as if all the slices were acquired at the same time (at 1/2 TR).

If you have a block design, you may not want to do this step because several scans are averaged together when analyzing a block design. Thus any gain in "accuracy" from interpolating the data from slices within one scan might be lost in the process of averaging across scans. ??Because it involves interpolating the signal to a time other than when it was acquired and measured, this step can "deteriorate" the data.??

1) **Slice-timing**
2) # of subjects or sessions = # functional runs
3) Select each session's scans separately (that is, press **done** after selecting the "ffmri" .img files for each session). Another spm_get window will pop up to get the next session's scans. The order of sessions does not matter (but in generally it's good to keep a consistent alphabetical/numerical order, i.e. going from top to bottom as the directories are showed on the spm_get window).
4) Sequence type - **user specified**
5) Order of slices (slice 1 = bottom)
   a) Slice timing order is completely sequence dependent. The slice order given here are for the sequences on the Northwestern CAMRI machines, and may not apply elsewhere. We acquire slices in an interleaved order, skipping every other slice.
   b) For an ODD number of slices (max# = N), the order is **1:2:N 2:2:N-1**
      For an EVEN number of slices (max# = N), the order is **2:2:N 1:2:N-1**
      This says that for an odd slice number, start at slice 1 and count up by 2 until you get to slice N, then go back to 2 and count up by 2 until you reach slice N-1. This is because at the Trio, we acquire data from foot to head in an interleaved fashion.
6) Reference slice = **N** (i.e. the slice taken at 1/2 TR)
   For an odd # of slices, slice N is acquired at 1/2 TR. For an even # of slices, slice N and slice 1 are equally in the middle so either one can be chosen, but it's easier to remember to choose N regardless of slice number.
7) Interscan interval = your TR
8) Acquisition time (TA) = how long it actually takes to acquire your number of slices TA = TR - TR/N. This time is from the start of the first slice to the start of the last slice. It figures it out for you so just accept whatever value it puts up.
   a) NOTE: This is not true for sparsely sampled TR's or sequences wherein the slices are bunched together in order to leave some quiet time during the TR. In that case you must find out from the sequence programmer the duration of slice acquisition relative to the TR.
9) Creates "affmri..." files – the "a" is for acquisition corrected. For each volume there will be .img, .hdr, and .mat files.

10) NOTES ON VISION DATA: Data on the vision was acquired top to bottom. The slice timing order was always from the top slice down. It does not matter if the top slice is even or odd. So for 32 slices the order is 32:-2:2, 31:-2:1.

<u>REALIGNMENT</u>

The realignment procedure figures out how to move each image volume so that it lines up spatially with other image volumes. This acts to minimize the effects of a subject's head movements. Note that if head movements are > 1 mm between scans within a run. or if head movements are correlated with the task (e.g., more movements occur when the

subjects responds) this introduces additional complications in the preprocessing data analysis. The realignment algorithm assumes that the movement is taking place between image volumes. This is not quite correct since subjects can also move between slices, but it is a reasonable approximation. The realignment procedure is used within modalities (e.g. across functional volumes, NOT between functional and structural). Instructions in step 4 are for realigning to the functional volume taken closest to the T1. If you don't care what volume the functionals are realigned to, skip step 4. According to DRG, it is currently just easier and better to realign to the mean image of all the realigned volumes rather than worrying about which scan was taken closest to the T1.

1. **Realign**
2. # subjects = **1**
3. # sessions = # functional runs
4. TO REALIGN TO VOLUME CLOSEST TO T1 IMAGE:
   a) It will ask you for the scans to realign. *The first volume you choose* will be the reference volume to which the other functionals will be realigned. Thus we want to first choose the volume taken closest to the T1 anatomical image. Go to the functional session immediately preceding the T1 session. If the T1 was the first series, go to the functional session immediately following it.
   b) We want to select the "affmri" .img files. Click on the white number next to the "affmri" .img files - this will list out each volume separately.
   c) If this is the session *before* the T1 scroll to the bottom and select the last volume in the series. If this is the session *after* the T1, select the first volume in the series. In other words, choose the volume acquired closest in time to the T1. This is what all other functionals in this session and in the remaining sessions will be aligned to.
   d) Then click **all** to select the rest of the volumes in the series.
   e) Click **done** because you have selected all the volumes for the first series.
   f) Now for each of the remaining sessions, go to that directory, choose the "affmri" .img files, and hit **done**. For these remaining sessions, you can select all of the .img files within one session and the order of sessions should not matter.
   g) Skip to Step 6.

5. IF YOU DON'T CARE WHAT VOLUME YOU REALIGN TO:
   a) Go into a functional run directory (It doesn't matter which one, though it is nice to be consistent and start at the top of the alphabetical/numerical list in the spm_get window. This way you'll know later on which volume you realigned to in case you want to know).
   b) Click on the "affmri" .img files to select them all, then press **done**. All the functionals will be realigned to the first volume in this session.
   c) For each of the remaining sessions, go to the appropriate directory, select the "affmri" files, and click done. Again, the order in which you select the directories does not matter.
6. Which option - **coregister and reslice**
7. What do you want to create? **Mean image only**

A mean image of all functional volumes will be created and used to co-register with the T1. Only after coregistration and the determination of the normalization parameters will a new set of .IMAGE files be created for the functionals

8. Background information about reslice interpolation method (this is just for your edification, this is not necessary to do the analysis) : through the defaults, one can choose between nearest-neighbor, trilinear, various B-splines and fourier. DRG says: Never choose nearest neighbor unless you don't care about the results. Only choose fourier if the image volume have been acquired with isotropic voxels sizes (i.e., 2x2x2 or 3x3x3). Trilinear can be used if the volumes won't be normalized and resliced again. The best choice is 4^{th} degree B-spline.
9. What is happening: First it realigns each volume WITHIN the session to the first file selected in each session. To do this it figures out how each volume should be moved and puts that in the *.mat file for each volume. Similarly the algorithm can figure out how each session should be moved to line up with the preceeding session.. In the end, the .mat files of each volume will be adjusted so that all functionals are in the same 3D space. The procedure will also create an "rp…txt" file, which contains the information used for realignment in a text format.

COREGISTRATION

Line up the functional and T1 volumes by pulling T1 into the space of the functionals (easier to adjust one T1 volume than 2000+ functional volumes). It can work by segmenting the volumes into gray and white matter and then matching up the segments and figuring out the parameters necessary to rotate/translate/squeeze/pull those segments into alignment. However, SPM2 is currently set to use a mutual information algorithm rather than the segmentation algorithm. The end result for the user is the same.

1. **Coregister**
2. # subjects = **1**
3. **Coregister only**
   - if you are only looking at a single-subject and will not be normalizing, you can choose **coregister and reslice**; this will create an "rsfmri" anatomical image that is resliced into the space of the functionals. This should not be normalized.
4. Select target image = what you want to shoot towards
   - choose the "mean..." .img image file just created in realignment (probably in your first functional run directory).
5. Source image = what you want to move into target space
   - select the T1 .img file ("sfmri")
6. It will ask for other images - don't select anything - click **done**.
7. The coregistration procedure will alter the T1 .mat file

❋SEGMENTATION

This is an optional step. If you have problems normalizing your brains with the T1.mnc template, you may want to first segment your T1, then determine normalization parameters by matching your gray matter segment to the apriori gray matter template, and then applying those parameters to your functional and T1 data. We have found in Spring

2004 that this procedure worked better. To follow this procedure, make the appropriate adjustments (as noted by the ❋ symbol in the following pre-processing steps).

Segmentation is also a step to take if you want to do voxel-based morphometry (VBM).

To segment the T1 into gray matter, white matter, and CSF:
1. click **Segment**
2. Select the T1 sf . . .img file.
3. Subj 2? click **done**.
4. Next you get two questions: click "no" and then select the "*T1. . ." option

NORMALIZATION

Warp functionals/T1 into the space of template brain. First it minimizes differences via translation, rotation, zooming (affine movement). Then it performs 3D cosine transformations to match curves (e.g. sulcal differences). Thus, it is a mathematical algorithm and is not based on landmarks. Three steps you have to perform:

1. Determine parameters needed to warp your (co-registered) T1 to the template
2. Apply those parameters to warp your functionals
3. Apply those parameters to warp your T1 (note that in step 1, T1 not actually resliced). Steps 2 and 3 can be done in any order

Step 1 - determine parameters

1. **Normalize** -> **Determine parameters only**
2. Target image = T1.mnc in /usr/contrib/spm/templates (should go here automatically)
   ❋ go up one level to the "apriori" folder and select "gray.mnc"
3. Source image = your "sfmri" T1 image
   ❋ sfmri . . . _seg1.img
4. Source image 2 = none. Just click **done**

Step 2 - normalize functionals

5. Before applying those parameters to functionals, you should check that the voxel size used is 3 x 3 x 3. The default value as of this writing is 3 x 3 x 3 so if you're confident this hasn't changed, you can skip this changing defaults procedure when normalizing the functionals (but you MUST change the voxel size to 1 x 1 x 1 when normalizing the T1!!) SPM99 used to ask for this number within the procedure itself, but for some reason this has been moved to the defaults in SPM2. Don't forget to make sure your images are resliced at the correct resolution.
6. Changing Defaults Procedure:
   Defaults -> **Spatial normalization** -> **Writing normalized**
   Click through options (selecting those with the *) until you reach voxel size. Be sure that 3 x 3 x 3 is selected (should be starred as default already). Continue click though options and selecting the * until it is done.
   ❋ This isn't necessary for segmentation, but one can change the bounding box to the larger: "-90:90 -126:90 -72:108 (Template)".
7. **Normalize** -> **Write normalized only**

8. Parameters, subject 1 = what the program just figured out, the parameters needed to move T1 to template space. Select the ..._sn.mat file in T1 directory
    - ❋ Select the . . . _seg1_sn.mat file in the T1 directory.
9. Images to write - select "affmri" .img image files for each session ALL TOGETHER (order does not matter). Then press **done**.
10. It will ask for subject 2, just select **done**.
11. This will created "waffmri.." files = w for warped

Step 3 - normalize T1

12. Since we are now normalizing the T1, the voxel size must be 1 x 1 x 1.
13. Go through the Changing Defaults Procedure section again (# 6 in Step 2 above), but select voxel size = 1 x 1 x 1
14. Repeat the **Normalize** -> **Write normalized only** instructions but select the T1.img file as the image to write.

SMOOTHING

FWHM of a Gaussian distribution = the distance from the mean to the point where the probability density function drops from 1.0 to 0.5 (half of the maximum value). The wider the kernel the greater the smoothing and the larger impact neighboring voxels have on each other. Smoothing increases sensitivity by averaging out uncorrelated noise across voxels, but reduces spatial resolution by blurring small areas of activity across the area over which it was smoothed. A positive side effect of the blurring is that cross-subject averaging is less affected by inter-subject anatomical variability.

We want to smooth the functionals, NOT the T1 anatomical.

1. **Smooth**
2. 10 mm FWHM kernel is usually used, especially for an older population where there will be a lot of anatomical variation and if you are looking for larger cortical activations. For a younger population and/or if you are looking for smaller activations in areas like the hippocampus or amygdala, a 7mm kernel may be appropriate.
3. Select "waffmri" .img functional images from each session ALL TOGETHER, then click **done**
4. Creates "swaffmri..." images - s for smoothed

* After pre-processing, an spm2.ps file will be created that graphical illustrations of each of the preprocessing steps. For the realignment procedure the amount of movement will be displayed. For the coregistration and normalization procedures an example of the results will be displayed. This can be viewed with Ghostview (see section on viewing .ps files on page 14).

DESIGN MATRIX

Create *anal* folder in subject directory and cd into it. Place necessary vector files into *anal* folder. Start Matlab and spm2.

Specify the event or block onsets

Event and block onsets are specified as vectors containing the numerical value (in

scans or seconds) corresponding to the start of the event or block. If you have multiple conditions within your study, you will need multiple vectors (e.g. for 2 conditions: A and B, you need two vectors). These vectors can be specified in two ways:

(1) as two variables within one Matlab m-file (.m) document. For instance, A = *[Atime1 Atime2 Atime3...]* B = [*Btime1 Btime2 Btime3...*].

(2) as two separate text (.txt) files, each containing a column of numbers specifying the onsets. The two files should have unique names (e.g. *Aonsents*, *Bonsets*).

Variables or filenames must start with a letter! Matlab won't accept those starting with a number.

1. Load necessary vectors into Matlab command window
   - if vectors saved as variables inside *filename*.m, type ***filename***
   - if vectors saved as column of numbers inside regular text file named *filename,* type **load *filename*** . The column of numbers will be saved as a variable named *filename.*
   - type **who** to double-check that vectors are properly loaded. There should be a variable for each unique condition.
2. **fmri** -> **design**
3. Interscan interval = TR
4. Scans per session = # volumes per functional run (don't forget that you have taken out the dummies!). You need to enter a vector whose length equals the number of sessions. For instance, 3 sessions of 100 scans each would be entered as *100 100 100*
5. How to specify - select scans or seconds (must match the units of your vectors)
6. Are sessions replications - **no** (i.e. sessions are different)
7. Select basis set - **hrf with time derivative** (the time derivative will account for extra variance in case the onsets are off by a little). For a study with regressors (e.g. cue benefit), might select **hrf**
8. Model interactions - **no**
9. # conditions
10. name of condition
11. vector of onset - type in name of vector variable
12. Duration
    - for event-related design, duration = **0**
    - for block design, = block duration in seconds or block duration/TR in scans
13. Parametric modulation
    - **none** if not regressing with another variable
    - **other** if regressing (e.g. cue benefit)
      a) # parameters
      b) type in regressor name and vector
      c) polynomial order = **1** (i.e. linear)
14. Other regressors - **0**
15. Repeat steps 9-13 for each condition
16. Repeat steps 8-15 for each session

17. Creates SPM.mat file saved in your *anal* directory

Output graphs:

- can switch between conditions using square SPM2 window on left
- lower left = the hrf with time derivative basis set
- upper left = signal vs. time. A Fourier transform gives you... ->

Important for high-pass filter

- upper right = signal (power, spectral density) vs. frequency, i.e. how strong the response is at different frequencies. This should look about the same for subjects within the same study. The default vertical line is at 128 seconds, any signal within the gray area will be cut off by high-pass filter. You should use this graph to determine what filter you want to use (don't want to lose too much signal power). The high-pass filter is necessary to get rid of low frequency noise.
- For most event-related designs, 128 seconds should be fine.
- For block designs, it could be higher (e.g. 256 seconds).

Specify the data

1. **fmri** -> **data**
2. Select the SPM.mat file just created
3. Select the "swaf" files for each session separately (i.e. click **done** after selecting each session's files).
4. Remove global effects - **scale**. This normalizes the intensity of the signal so that comparisons across subjects can be made.
5. High pass filter - **specify**
    - Use the high pass filter frequency you've determined for your study based on the graph output after specifying the fMRI design (see above)
    - You need to specify a vector that contains one number for each of the sessions: e.g. if you wanted to use a 256 second filter, type **repmat(256,1,x)** where x is the number of sessions or type in 256 x times (with a space in between each number). Repmat means "repeat matrix" so repmat ([x],y,z) means to repeat matrix x for y rows and z columns.

6. Unlike spm99, there is no low pass filter option. That was included to deal with autocorrelations in the data related to the time series but now this is taken care of with AR(1) modeling (see next step).
7. AR(1) serial correlations (corrects for the fact that each scan in the time series is correlated by lowering degrees of freedom).
    - Select **yes** for repeated measures analyses or non-random effect analyses (e.g. single subject)
    - Select **no** if you will be doing random effects level analyses. RE collapses away from the time domain and is across subjects. You cannot report single subject results without the AR(1) model because the significance will not have been estimated correctly since autocorrelations will be ignored.

8. This step updates and replaces the previously made SPM.mat file.

Output
Each condition will have two columns, the first for the hrf and the second for the hrf time derivative. Rows indicate the scans. Vector onsets should roughly show which scan is matched up to each condition in each session.

Estimate the matix you have designed
1. Click on **Estimate** in the SPM2 window
2. Select the SPM.mat file, then click **done**.
3. This process could take some time (several hours if it's a big matrix with a lot of sessions and scans).
4. SPM will solve the general linear model for each voxel ($y = \beta x + e$, solving for b), therefore will get beta images
5. There are 46 planes because normalized data has 46 slices
6. Main effect analysis asks: is β different than 0 for a condition x?
7. Subtractions: are two β (different conditions) different from each other?
8. F contrasts automatically done: They show any activation that comes from a specified column but we usually don't care about that, so we want to look at T contrasts

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CONTRASTS

It is often convenient to create a text document that lists all contrasts for a study. It is then easy to cut and paste these contrasts into the contrast manager window, thereby reducing the error of having to type them in each time. A contrast is built by "weighting" each condition.

- If you built your design matrix with the "hrf with time derivative" basis function, each condition will have two columns. The first represents the hrf, the second represents the time derivative. In our analyses we want to look at the hrf. The numbers in the contrast should sum to 0 (e.g. If your study has conditions A, B, and C in that order, the contrast A - (B+C) would translate into 2 0 -1 0 -1 0. Note that the time derivative column for column is assigned a 0.
- If you built your design matrix with the "hrf " basis set and included a regressor, the first column of each condition will represent the hrf alone and the second column will represent the regressor. Since you probably want to look at the activations correlated with the regression if you built the matrix this way, put a 0 for the first column and your numerical value for the second. For example, for A - (B+C), the contrast is 0 2 0 -1 0 -1.
- When defining a contast, each column of every session in the matrix must be specified. Thus, the pattern of numbers for one session described above (e.g. 1 0 1 0 -2 0) must be repeated for each session. You can use the "repmat" function to accurately build a contrast for a matrix that has multiple sessions. For instance, if your study has 4 sessions, each of which have the same 3 conditions, and you used the "hrf with time derivative" basis set, an example

contrast A-(B+C) might look like 2 0 -1 0 -1 0 for one session. To define the contrast, you would type **repmat([2 0 -1 0 -1 0],1,4)**. In other words, repeat that matrix in one row and 4 columns.

If you plan on doing random effects, you should make a spreadsheet where you write down the contrast number for each contrast for each subject. The contrast number appears in the contrast manager window next to the name of each contrast you build.

1. Click **Results**
2. Select SPM.mat file
3. **Define new contrasts** (under T contrasts tab)
4. Type in name of contrast
5. Specify contrast: can type in strings of numbers or use repmat function
    - Remember that each column in the matrix must be assigned a number: SPM will right-pad the contrast with zeroes if necessary
    - You can type in the numbers by hand or cut and paste from your contrasts text file (see above)
    - Don't forget that each condition has two columns. You should use the first column (the hrf) and put a 0 for the second (the time derivative). Exception: if matrix was built with hrf alone and a regressor was included, you should use the second column (the regressor) and put a 0 for the first (the hrf alone).
6. Click **done**
7. If you are making a contrast spreadsheet so that you can do random effects later, be sure to note down the contrast number (number to the left of the contrast name in the contrast manager window).
8. Select the contrast you just built (or that you want to see) and click **done**
9. Mask with other contrasts
    – usually **no**
    – if you say yes, you'll select which contrast you want to mask with and then be asked whether you want inclusive or exclusive masking. For more info on masking, see "One-way Anova and Masking" under "Intersubject analyses" on page 17.
    – A time-saving shortcut for entering in a large number of contrasts and then estimating them at one time: After entering all your contrasts in the contrast manager window, select one of them and click **done**. Select **yes** to mask with other contrasts, and then select the rest of the contrasts that you want to specify. Choose inclusive or exclusive, it doesn't matter. The result you get will be meaningless, but all of the chosen contrasts will be estimated.
10. Title - can usually keep default
11. Program will take some time to calculate...
12. p-value adjustments
    Family-wise error (fwe) = threshold is corrected over # of voxels in volume: very stringent
    None - select this
13. p-value - usually start with **0.001**. You can increase it to 0.005 or 0.01 to be less stringent.

14. extent threshold - **3** = at least 3 voxels must be clumped together. You can decrease this to 0 to be less stringent.

Output notes:

1. Click **Results-Fig** on graphics window to create satellite window.
2. Then click **Volume** in the lower left window to pull up activation peak statistics in that satellite window (can also click this when a satellite window is not open and the stats will come up on the main graphics window).
    - Coordinates are clickable and crosshairs will jump to that location
    - Click on p values to get full value in Matlab window
    - P corrected = family wise error (see above)
    - Cluster level : e.g. what is the significance of these 37 voxels given that there is a z=0.003 maxima within it?
3. **Overlays** -> **Sections**, then select your T1 (or mean) anatomical image on which activation will be laid. The three sections of the anatomical image are clickable.
4. **Overlays** -> **Render** will allow you to pull up 3D images with overlaid activation
5. Can right-click in upper half of graphics window and select "jump to local maxima." The crosshairs will then (you guessed it) jump to the local maxima.
6. Can brighten or darken image via **Effects** in graphics window
7. The crosshairs appear on the anatomical image by default. To turn them on, go to the Matlab command window and type **spm_orthviews('Xhairs', 'off')** . To turn them back on, type **spm_orthviews('Xhairs', 'on')** .
8. Small Volume Correction
    - Center the crosshairs on your peak of interest (jump to local maxima)
    - Click **S.V.C.** on square SPM2 window on lower left
    - Select **sphere at *[coordinates]***
    - Statistics table will show in satellite figure; stats corrected the specified sphere
9. Can save to .ps file via **Print**. These will always be called "spm2.ps" but you can rename them later. If there is already a file with named spm2.ps in your current working directory, output will be appended to it.

Viewing .ps files and Printing

1. View .ps files with Ghostview
2. Click on **Toolboxes** -> **CBMGtools** -> **Ghostview**
3. Select the .ps file you want to view.
4. Print to color printer: press **Print**; print command = **lpr -PLexmarkC720**
5. Print to black and white printer; print command = **lpr**

Inter-subject Analyses

Mean image

Overlay inter-subject analyses on to mean image (averaged T1 of all subjects included in analysis).

- Be sure you are in the directory where you want your mean image saved.

- In SPM99: Click on **Toolboxes** -> **CBMGtools** -> **mean**
- Select normalized T1 image ("wsfmri" .img) for each subject. When all are selected, press **done**
- mean.img and mean.hdr will be created in current working directory
- In SPM2: Click on **ImCalc** [image calculation]
- Select T1 image
- Type in name that you want output file to be called (i.e. *mean*)
- Function: ***(i1+i2+i3...in)/n*** where n = # images. For example, if you want to average 5 subject's T1s, you should type *(i1+i2+i3+i4+i5)/5*

Fixed Effects

Create a new folder and build a design matrix just as you would for an individual subject, but put data from several subjects all together. You may have to adjust your vectors so that each subject's variables have individual names. If all variables are in one text file, one solution is to assign each subject a letter of the alphabet and than to prefix all of that subject's variables with its letter. If vectors consist of a column of numbers within a text file (no variables assigned within the file), give each subject's vector file a unique name. See section on loading vectors into a design matrix (page 10).

This analysis looks at what activation is present when averaging across subjects. It does not account for inter-subject variance (less stringent than random effects). Build contrasts just as you would for an individual subject.

Conjunction

Use the fixed effects matrix but when building contrasts, make the specified contrast for each individual. Then in the contrast manager window, highlight each individual subject's contrast all together (if all are next to each other, click on the blank white space to the right of the topmost individual contrast and with the mouse button pressed, drag down). On the right you should see the contrasts you've selected, one on top of the other. Click **done** and proceed just as you would for a regular contrast. Note that it will not ask you for a voxel threshold. This analysis accounts for inter-subject variance better than fixed effects.

Random Effects

Uses contrast images created for each subject. Shows activation only if present in each subject for that contrast. Similar to conjunction analysis in that it takes into account inter-subject variance. See DRG's 1999 review for a clear and very helful explanation of some of the issues involved in random effects analyses:
http://www.brain.northwestern.edu/cbmg/ranfx.html

1. Make a directory for that particular contrast and cd into it.
2. **Basic Models** -> **1 sample T-test**
3. Select images: choose the con_00XX....img contrast image for each subject. Find contrast number (XX) from your contrast spreadsheet (see page 13).
4. Grand mean scaling - **no**
5. Explicit mask - **no**

6. Global calculation - **omit**
7. Click the **Estimate** button on the main spm2 menu
8. **Results** - Select .mat file. This may take a few minutes to bring up the contrast manager window.
9. **Define new contrast**
   - name it and put **1** into vector area
10. Continue just as with other contrasts...

Simple Regression

Use this analysis when you have one number for each subject that you would like to regress against (i.e. age, perceived intensity of a taste). This should be a continuous variable. Note that if you have one number for each TRIAL (i.e. many numbers per subject) you should build a design matrix using a regressor (see parametric modulation on page 10). In this analysis you select one contrast image from each subject and look for activation present in that contrast that is correlated with your variable of interest. You can look for positive or negative correlation.

1. Optional: Load in your variable into the Matlab window if you have it saved as a variable (similar to loading variables in when making a design matrix).
2. **Basic Models** -> **Simple Regression (correlation)**
3. Select the con_*XX*.img files for each subject that you want to include in your analysis, where *XX* is the contrast number of your contrast of interest for each subject. After you have selected all the images, click **Done.**
4. [X] covariate: Here X is the number of images you have selected. Thus you must put in a vector of numbers that is X long, one number for each image. You can type these numbers in by hand or type in the name of the variable you loaded in step 1.
5. covariate name - type in the name of your variable
6. Grand mean scaling - **no**
7. Threshold masking - **none**
8. Explicitly mask images - **no**
9. Global calculation - **omit**
10. An SPM.mat file will be created showing the matrix you just made.
11. Press **Estimate** and select this SPM.mat file, then click **Done**.
12. When it is done estimating, press **Results** and select your SPM.mat file
13. **Define new contrast**
    - name it (e.g. ***Contrast name*** **(+1) with** ***variable,*** or (-1) if you'll be doing a negative correlation
    - In the vector area, type **1** for a positive correlation, **-1** for a negative
14. Continue just as with other contrasts...

One-Way ANOVA and Masking

Use this to compare contrasts to each other (e.g. the main effect of A across subjects – the main effect of B across subjects) and to do inclusive or exclusive masking of contrasts across subjects. A inclusively masked by B will give you only activation from A that was also present in B. A exclusively masked by B will give you only activation from A that

was not present in B. Note that A exclusively masked by B is not equivalent to B exclusively masked by A.

You can also use these results to create nice bar graphs that show group parameter estimates for each of your conditions if you specify each condition main effect as a group.

1. Make a directory for that particular contrast and cd into it.
2. **Basic Models** -> **One-way Anova**
3. #groups - Enter the number of contrasts you want to compare in this analysis (NOT the number of subject images). If you wanted to look at A masked by both B and C, you would have 3 groups.
4. Enter your Group 1 images. Select the con_*XX*.img files for each subject that you want to include in your analysis, where *XX* is the contrast number of your Group 1 contrast for each subject. After you have selected all the images in one group, click **Done.** If you still have remaining groups, you will be prompted to enter more images. Although I don't think the order in which the contrast images are entered has to be identical in each group, it is a good idea to keep them constant.
5. Grand mean scaling - **no**
6. Threshold masking - **none**
7. Explicitly mask images - **no**
8. Global calculation - **omit**
9. non-sphericity correction? - The FIL spm2 website says "Sphericity refers to the assumption of identically and independently distributed errors." This is what I think this means: if, for instance the contrasts you are comparing are drawn from the same set of image data (e.g. contrast A = sugar water and contrast B = (sugar water – baseline), then the errors for the two groups are not independent and you need non-sphericity correction. If your contrasts are completely separate from each other, you can select no. But you should definitely ask someone who actually knows statistics what to put for your particular study.
10. An SPM.mat file will be created showing the matrix you just made. There should be one column for each group.
11. Press **Estimate** and select this SPM.mat file, then click **Done**.
12. When it is done estimating, press **Results** and select your SPM.mat file
13. Define a contrast for each group
    - **Define new contrast**
    - name it (e.g. ***Group 1 contrast name*** **)**
    - in the vector area, if you want to do masking, create the main effects of each group by typing in "1" for the column that represents that group and "0s" for other columns. (e.g. for group 2, type ***0 1***). If you want to do a subtraction (e.g. Group 1 – Group 2), type in the appropriate specification (e.g. ***1 -1***).
14. For subtraction contrasts, proceed as usual.
15. For masking (A masked by B)
    - select your first contrast (A) and hit **done**.
    - mask with other contrasts? – **yes**
    - select the contrast(s) you want to mask by - **done**

- choose exclusive or inclusive masking. See intro to this section for explanations.
- continue as usual…

Miscellaneous Bonus Material

1. Create Mosaic images

Display on page different anatomical slices overlaid with activation. Usually done with group analyses.

1. **Toolboxes** -> **CBMG tools** -> **Mosaic**
2. Select anatomical volume on which to overlay activation - usually mean image
3. Superimpose SPM - **yes**
4. Select the .mat file containing the contrast activation you want to overlay
5. Select the contrast you want to see, proceed as usual
6. Orientation - **axial**
7. Starting coordinate = **-48** or **-51**
8. Rows = **6**
9. Columns = **6**
10. Slice thickness = **3**
11. Mosaic progression - **ascending**
12. Contrast enhance background - **no**
13. Interpolation type - **point**
14. Background color - **black**
15. Draw ROI... - **no**

Then you have to repeat all steps to see top portion of volume because usually all slices won't fit on one page. Use starting coordinate = 57mm or whatever would be next slice

2. Display activation blobs from multiple contrasts on 1 anatomical volume

15. **Display**
16. Select the T1 or mean image on which you want to overlay activation
17. The 3 orientations of the image will come up on the main graphics window.
18. Click **add blobs** in the lower right corner of that window.
19. Number of blobs = the number of contrasts you want to overlay.
20. It will ask you to select the SPM.mat file. Choose the SPM.mat file from the analysis that contains the first contrast you want to look at.
21. The contrast manager window will pop up. Choose the contrast you want to view.
22. Go through the masking/thresholding options as you usually would when viewing a contrast.
23. Finally, it will ask you to select a color to represent activation from that contrast. Pick one.
24. Repeat steps 6-9 for each contrast that you want to view.
25. The activations will then be overlaid on to the anatomical image you selected. You can click around the anatomical image to view any area.

3. MarsBar ROI Analysis (in spm99)

Compute statistics over a defined region of interest (instead of over whole volume).
This is available in spm2 but I'm not sure if it is fully installed on CNADC workstations.

1. Make directory for this ROI and cd into it
2. **Toolboxes** -> **marsbar**
3. **ROI definition** -> **Build**
4. Type of ROI

16. **activation cluster** = select the .mat file from which you want to get the cluster
17. **sphere** with x y z center coordinates and specify radius

5. Description and name of ROI
6. ROI will be saved as ROI.mat files
7. Build all ROIs before moving in to estimating (?? True??)
8. **Design** -> **Estimate ROI(s)**

18. select spmcfg.mat from fixed effects or random effects

9. Select appropriate ROI.mat

10. Summary function - **1st eigenvector** (takes variance into account)
11. Creates a marsestimated.mat file (corrected just for this area, not whole brain)
12. **Results** in MarsBar window
    a) Can **import contrasts** already made from appropriate xCon.mat
    b) Or can go straight to **statistics table** to define a new contrast (name it and type **1** in vector area). This will print out t and p statistics in Matlab window.

4. Optimized VBM (Voxel-Based Morphometry)

An optimized VBM button is under the CBMG tools list on the CNADC workstations. Just press the button, select your T1, and have a cup of java. The batch script is from John Ashburner. DRG comments about the script:

I have commented the script so you can see the command for each of the steps. They are largely the same as we discussed, but for spm2 the modulation step is now done within the program called spm_write_sn. In spm99 it was called spm_preserve_quantity.

Also when we were discussing VBM today there was a value called "cutoff" under both segmentation and normalization and I wasn't sure what that meant. It stands for the cutoff in mm of the period of the cosine basis functions. It is selected to be 25 mm as a default. Roughly translated this means the program will only estimate warps on the order of 25 mm or larger. This gives you an idea of how basically coarse the normalization is. Selecting a finer value might work but you might also see distortions. In spm99 this was called the number of non-linear basis functions. I think the current value name is more intuitive. It was harder to know exactly what 7x8x7 basis functions meant.

To compare Right and Left hemispheric atrophy:
Note: Once the symmetric templates have been created once, they can be saved and reused.

1. Create symmetric templates.

You will need to make symmetric templates for normalization and segmentation.

Copy the following files to a directory of your choosing- all directory references are relative to the SPM2 directory which is in /usr/contrib/spm2

templates/T1.mnc
apriori/gray.mnc
apriori/white.mnc
apriori/csf.mnc
apriori/brainmask.mnc

2. Rename all these files by putting an r in front of the name.
   2a. Now start SPM2 and cd to the directory where you just put these images.
3. Click on the display button and choose one of these images.
4. When the display comes up there will be a set of parameters you can change on the left side of the display window.
5. Type -1 in the resize {x} parameter and press the enter key. The image will flip.
6. Click the Reorient images button at the bottom of the graphics window. A dialog will come up that will say "This will flip the images" Click OK. Then choose the image you just displayed. So if you had displayed rT1.mnc then choose rT1.mnc again. This will add a mat file for the image. The mat file contains information about how the image is now flipped.
7. Do this with the rest of the above images.

If you get an error in any of the above it probably means you tried to flip the images in the original spm2 directory which you do not have permission to do. Be careful about choosing the images to flip.

Now you have to average the flipped and unflipped images:

8. Click the IMCALC button (2nd row from bottom, 2nd button from right). You will be asked to select images- choose one of the images you just flipped and its unflipped counterpart which is still in the original spm directory. So for example choose rT1.mnc and T1.mnc. You will then be asked for a name for the output image- call it something like avgT1 (you don't need to put in the 3 letter extension- spm will take care of that).
9. You will then be asked for an equation to calculate- enter the following: (i1 + i2)/2 and hit the return key.
10. Do this for all the images above.
11. You will have to re-threshold the avgbrainmask image you"ve made.
12. Click IMCALC again and choose the avgbrainmask image. Give it a name of threshavgbrainmask and the equation is i1>0. All this is telling the program to do is set every voxel with a value greater than 0 to have a value of 1. This gets rid of all the 0.5 values that were in the averaged image. Do not do this for the other images as they are not mask images.
13. Run the optimizedAVGVBM script- go to the directory the script is in and type optimizedAVGVBM. Just as with the original script you will be asked to choose the T1 images to segment. Don't worry about making copies of the original images as I will make sure the segmented output images are written with a different name. Instead of being named Gfilename I will call them avgGfilename and avgWfilename

14. Once the segmentation is done you will need to create a flipped version of the segmented images. Just worry about the gray matter images. Copy them to a new directory and flip the copied images as above. You will then have 2 sets of gray matter images. All images will have been normalized to a symmetrical template. One set of gray matter images will be unflipped (L on L) and the other set will be flipped (L on R).
15. Now you can take each groups image and compare flipped and unflipped using a paired t-test.