



# *Getting Started:* An fMRI-CPCA Tutorial

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#### Definition of fMRI-CPCA Data

Constrained Principal Component Analysis (CPCA) is a general method for multivariate data analysis that is in no way specific to neuroimaging. CPCA combines regression analysis and principal component analysis into a unified framework. fMRI-CPCA uses CPCA to analyze fMRI data, and derives images of functional neural networks that are constrained to be relevant to timing of presented stimuli from cognitive experimentation. fMRI-CPCA used with a finite impulse response (FIR) model can be thought of as deconvolution PCA.

FMRI-CPCA with a FIR model allows:

- **1.** Determination of multiple functional neural networks responding to stimulus presentation.
- 2. Estimation of the pattern of BOLD changes associated with each functional network over peristimulus time points.
- 3. A statistical test of the reliability of the neural networks.
- 4. A statistical test of the degree to which experimental manipulations affect each functional network.
- 5. An estimate of the percentage of variance in BOLD signal accounted for by each neural network.

#### Purpose of this tutorial

The purpose of this tutorial is to guide you through your first simple fMRI-CPCA analysis on example data. It does not demonstrate all of fMRI-CPCA's features or provide a detailed explanation of how to interpret CPCA results. For a more extensive demonstrations and explanations refer to the *fMRI-CPCA Manual* available for download at http://www.nitrc.org/frs/?group\_id=203.

This tutorial will focus on the first two steps of fMRI-CPCA analysis (listed and bolded above): You will extract two neural networks involved in a working memory task. For each neural network you will be able to view a plot of the estimated HRF associated with this network and view how the HRF changes for each task condition.

# Prerequisites

This tutorial assumes you have the following installed on your computer:

▷ I MATLAB (ideally version 2008b or newer) <u>http://www.mathworks.com/</u>

MRIcroN http://www.mccauslandcenter.sc.edu/mricro/mricron/index.html

**■** ≪ **D** Windows, Mac or Linux

# **Used Terms Glossary**

Blood oxygen level dependant (BOLD) signal	The "activation" measure imaged with fMRI. Changes in BOLD signal are correlated with increases and decreases in blood flow relative to increases and decreases in oxygen consumption.
Component	In fMRI data, the extracted component(s) represent a network (or networks) of functionally interconnected voxels. The networks are imaged by superimposing component loadings on a brain template.
Component loadings	Can be interpreted as correlation coefficients between the component scores and the BOLD signal that is predictable from the imposed constraints (e.g., stimulus timing).
fMRI scan	A whole brain image (TR, or repetition time) of BOLD signal values for one point in time (usually 2-3 seconds per TR). When taken sequentially over time, these images can provide information about which brain regions show increases and decreases in activation in response to a particular perceptual or cognitive task.
G matrix	Design matrix. G contains a model of the predicted BOLD signal changes (columns) caused by stimulus presentation timing over all fMRI scans (rows). In traditional univariate analysis a hemodynamic response model (HRF) is used, but with fMRI-CPCA a finite impulse (FIR) response model is typically used to allow deconvolution.
Hemodynamic response (HDR)	The change in brain blood flow that is assumed to be associated with neural activity.
Mask	An image file specifying the regions of each fMRI scan that are brain, and not other materials like bone or air. The brain voxels to be included in the analysis are determined by the mask.
Predictor weights	Indicate the contribution of each aspect of the G matrix model to how each component changes over the series of fMRI scans. Can also be interpreted (somewhat loosely) as the correlations between the component scores and the columns of G.
Run	One run is a complete sequence of fMRI scans associated with one completed run of an experiment. A full experiment usually consists of anywhere from 1 to 8 runs, and each run usually consists of approximately 200 to 300 full brain scans.
Time windows (time bins)	Segment of time of interest, for instance, peristimulus time. When using a FIR model it is usually of interest to attempt to map the entire hemodynamic response ( $\pm$ 20 seconds). Therefore time window is usually between 16 and 24 seconds.
TR	Repetition time. Time to collect one full-brain fMRI scan (2-3 seconds).
Z matrix	Z contains the time series of fMRI scans for all subjects (rows) for all voxels of interest in the brain (columns). For fMRI data, Z can be referred to as the Activation matrix.

# Step 1: Install fMRI-CPCA

➢ Go to the fmri-cpca website: <u>www.nitrc.org/projects/fmricpca</u>.

Home	Tools & Resources	Community	Support	About NITRC
	e source for neuroimaging Is and resources		SEARCH Search within this tool/	resource 💌 🛛 🤇 🕻
		omputational Environment is now a n Amazon Marketplace. Check it ou		Select Language Powered by Coogle Tran
	fMRI-CPCA			Reviews & Ratings
Summary	principal component	pal Component Analysis (CPCA) combine nt analysis into a unified framework. This	method derives images of	User Reviews (3)
Reviews/Ratings Support	and allows derivati	etworks from singular-value decompositio on of images when the analyzed BOLD sig mulus time, using all other scans as base	gnal is constrained to the scans	OVERALL: No Votes INSTALLATION: No Votes DOCUMENTATION: No Votes
Advanced Search Docs	pattern of BOLD changes associated	on of multiple functional networks involve with each functional network over peristin	nulus time points, (3)	
Downloads		tion between these multiple functional ne nanipulations affect each functional netwo		Participate!
Mailing Lists	fMRI CPCA provides all results in mat components, rotated and unrotated.	lab.mat file format, as well as writing imag	es in analyze format for all	Report issues Add a review
Tracker	Download Now MRI_CPCA GUI: cpca_1.1.0.05	_June_05_2013.zip (1519K)	OR See All Files »	Join the team Monitor a file release Subscribe to RSS feed
Members Admin			-	Bookmark this page
Admin	Specifications			Home Page

Figure 1 : NITRIC fMRI-CPCA web page

- > Ensure that **fMRI\_CPCA GUI** has been selected in the **Download Now** box.
- > Click on **Download Now** to download the latest fMRI\_CPCA GUI.zip file.
- > Extract the files into a folder of your choosing. The extraction will create a cpca folder

in the selected directory, of the format cpca\_{version}\_{month}year}, such as **fMRI\_CPCA\_GUI: cpca\_1.1.0.05\_June\_05\_2013**.

- Start MATLAB.
- > In the *File* dropdown menu, select *Set Path*, then choose the *Add with subfolders* option and select the CPCA directory you created.
- > Save your new path (it doesn't matter where CPCA appears in your path).

Warning: Do not install CPCA over an existing version. When updating to a newer version of the software you must first delete (or remove from the MATLAB path) all file folders containing all older versions.

# Step 2: Install the tutorial data set

➢ Go to the fmri-cpca website: <u>www.nitrc.org/projects/fmricpca</u>.

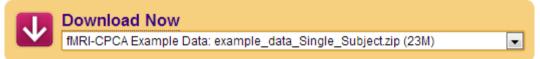


Figure 2: CPCA website Download Now box

- > In the **Download Now** box select **example\_data\_Single\_Subject.zip**
- > Click on **Download Now** (this is a large dataset, so download may take a few minutes).
- Extract the Zip file to a folder of your choosing. The extraction will create a folder in the selected directory, such as 'C:\cpca\_fMRI\example\_data\_Single\_Subject.'

Example\_data\_Single\_Subject.zip needs three matrices needed to carry out the most basic CPCA analysis: a Z matrix, a G matrix and a mask image.

- Z matrix (file Z.mat): Contains the series of fMRI scans for all subjects, stacked vertically. For this tutorial, the scans in the Z matrix have undergone typical preprocessing steps (realignment, spatial normalization, smoothing). It is not directly given in this dataset so it must be created.
- Mask (files mask.img, mask.hdr): One mask.img must be prepared that fits all subjects simultaneously. This requires all subject data to be normalized to a common template. The brain voxels to be included in the analysis are determined by this mask.
- G matrix (file G.mat): Contains the model of the BOLD signal changes associated with the cognitive task timing over all scans. For this example, a finite impulse (FIR) response model was used. This matrix needs to be created as well.

Example\_data\_Single\_Subject.zip also contains a file called '**Shapes.mat'**, which is not needed for this tutorial but which may be required at more advanced stages of analysis (i.e. rotation methods).

# Step 3: Start CPCA

To start the fMRI-CPCA graphical user interface (GUI), type '**cpca**' at the MATLAB prompt in the command window. The following window should appear:

Cache: <na> Utilities/Tools Fix FS</na>	put] Avail Memory: 3.99 GB Drive Space: 252.3 GB	Estimated N/A N/A	Max Partition Mem (Mb) Estimated Time:	500	Create List CD
Z Select Info	Location: Subjects: Runs: Partitions		Total scans Voxels:	Mn:	Mx:
Mask Select ROI Create Verify Model G Select Create	Dimensions:			0	Time Bins: 0 Edit Scree Stats
Z Create Z Regression Covariates Linear Quadratic Head Movement User Defined Covariate	G Regress Extract Rotate Settings				

Figure 3: CPCA GUI initial window

# Step 4: Create and select an output directory

You need to select the directory where all output and temporary files will be written. It will help to organize your work if you create and select a new directory rather than selecting the default directory.

To create and select an output directory:

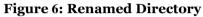
1. Click the **'CD'** button.

View Log	Options		Avail	Estimated	Max Partition Mem (Mb) 500	Create List CD
Cache: <na></na>		Memory:	3.99 GB	N/A	Estimated Time:	
Utilities/Tools	Fix FS	Drive Space:	249.8 GB	N/A		Unload RUN

Figure 4 : Location of CD button

Pick a different drive or directory Pick a different drive or directory	
Folder:     New Folder     Particular     Particular	•
Make New Folder OK Cancel	ncel

Figure 5: Default directory name



- 2. Click on the 'Make New Folder' button or icon
- 3. Rename the created folder/directory with a name of your choice. In Windows this is done by double clicking on the name or right clicking on the folder and selecting 'Rename.' In this example the directory has been renamed 'Output.'
- 4. Click **'OK.'**

# Step 5: Creating the Z Matrix

The Z matrix contains preprocessed BOLD signal from 214 fMRI scans (1 subject x 1 run x 214 scans per subject) collected during a working memory task. The Z matrix must is typically normalized to ensure that all voxels for all subjects have standardized variance. In order to create and normalize a new Z matrix, a File List containing information on the location of scan data must be created first.

The **'Subject Scan Files List'** directs the application to the fMRI scans to be analyzed. It is created in a structure that allows the scans associated with each group, subject and run to be differentiated. The following steps are involved in the creation of this scan list which will later be used to create the Z matrix.

 Click the 'Create List' button, located on the *System Information* panel to open the 'Scan File List Creation' window.

🙏 cpca 1.1.0(05) — System Inform		tput]				n same Aut	August		_   □   >
View Log			Avail	Estimated	1	Max Partition Mem (Mb) Estimated Time:	500	Create List	CD
Cache: <n:< th=""><th>&gt;</th><th>Memory:</th><th>3.99 GB</th><th>N/A</th><th></th><th>Estimated time.</th><th></th><th></th><th></th></n:<>	>	Memory:	3.99 GB	N/A		Estimated time.			
Utilities/Too	s Fix FS	Drive Space:	252.3 GB	N/A				Unload	RUN

Figure 7: Create List button

- Press the directory 'Select' button, located at the top right of the 'Scan File List Creation' window to navigate to and select the root directory that contains the fMRI scans: example\_data\_Single\_Subject.' Press 'OK.'
- 3. In the **Use File Specification box**, edit the wildcard name so that '**fsn\*.img**' is displayed. In this example, a common root for image names is 'fsn\*.img.'

Scan File List Creation			X
C:\CPCAManual\example_data_Sing	le_Subject	Select	
\$01	*		•
V Subje			
Sample File Specification	Use File Specification fsn* ing Output File	Verify	
		lect File Location	File Name files.txt
-	Create File List		Cancel

Figure 8: Scan File List Creation window

- 4. Click the '**Verify**' button to the right of the '**Use File Specification**' text box to display '**File Specification Results**'. All the scan files contained in a directory that match the File Specification will be displayed. An asterisk (\*) beside a file name indicates a possible error.
- 5. Click the **'Create File List'** button to save the file list. Output will be written as **files.txt** (this can be edited in the File Name box).

6. In the **'Subjects'** panel, click **'Select'** and navigate to the correct directory to select either your files.txt if creating a new Z or ZInfo.mat to use a previously created Z. For this example, select the **'files.txt'** file.

— Sy	/stem Informatio	n ———	-			-				
	View Log	Options		Avail	Estimated		Max Partition Mem (Mb)	500		Create List
(	Cache: <na></na>		Memory:	2.86 GB	N/A		Estimated Time:			
	Utilities/Tools	Fix FS	Drive Space:	284.1 GB	N/A					Unload
Su	ubjects									
z	Select	Info	Location:							
		Verify		Runs:	Multiple Hz: no		Total scans	Mn:	M×:	
			Partitions		Ranges:		Voxels:			
Mas	sk Select	ROI								
	Create	Verify	Dimensions:							

Figure 9: Subjects panel, Select button

# **Step 6: Load the Mask**

The mask must be loaded to indicate how the computed voxel information should be written to a brain image.

> Click on the Mask '**Select'** button located on the Subjects panel.

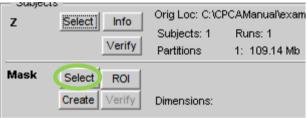


Figure 10: Mask Select button location

- Navigate to the directory containing the tutorial data set.
- Select and open the 'mask.img' file.

Once your mask is selected, the *Subjects* information panel will update with the **location** of the mask image and the image slice **dimensions** (x, y and z), with the voxel size in brackets.

Mask	Select ROI	C:\CPCAManual\example_data_Single_Subject\mask.img	
	Create Verify	Dimensions: 40 x 48 x 34 (4x4x4)	

Figure 11: Mask image information

# Step 7: Standardize the Z Matrix

1. Select the **'Normalize'** button and this will make the button green. The **'Run'** option will be available as well.

2. Ensure that 'Create Z', 'Regression Covariates' ('Linear' and'Quadratic') are all selected

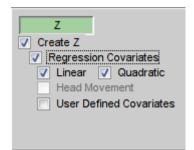


Figure 12: Z normalization options

 Click the 'RUN' button located in the top, right-hand corner of the 'System Information' panel. A 'CPCA Process' window will open to inform you of the status of the processing. Do not close the progress window yourself. Once progress has gone to completion, the window will close by itself.

This process will output the following files to your output directory:

- A '**Z{n}.mat**' for the subject (n being the subject number for the subject) located in the newly created '**Z**' folder.
- A 'ZInfo.mat' which holds the header information for the subject and the calculated Total Sum of Squares. If you would like to repeat this analysis at any time, the outputted 'ZInfo.mat' can be selected in place of the original 'Z{n}.mat', and the Z processing steps outlined above can be bypassed.

# **Step 8: Create the G Matrix**

The G matrix contains the predictor model that will be applied to the Z matrix (subject data). The G matrix is used to constrain the variance in Z to that related to stimulus presentation.

• Click on the **'Create'** button next to **G** on the *Model* panel.

¢	Model Selec	Create			0	Time Bins:	0	Edit	Scree	Stats

Figure 13: G Select button location

1. To define the conditions that are applied to subject runs, click the **'Runs and Conditions'** button

🛃 G Matrix Cre	eation			x
Subjects	Runs		Conditions	
s01	*			*
	-	-		Ŧ
─ Timing Ve Bins 0	ctor Units TR 1	<ul> <li>Scar</li> </ul>	is 🔘 Secon	ds
Model Type				
I FIR	O HRF			
		Runs and	Conditions	
Okay	Cancel			

Figure 14: Runs and Conditions button

In the box located at the top right add type in the condition names (2 letters, 4 letters, 6 letters, 8 letters) and press 'Enter' or click 'Add' after each entry

Conditions and	l End	oding		A COLUMN		
Subjects s01	•	Runs	*	Conditions 2 Letters 4 Letters 6 Letters 8 Letters	*	Add Load Apply To All Subjects Apply To All Runs Create Timing Onsets Template
		Select All		Select All		
Okay	/	Cancel		🗆 Ea	ich	Run Encoding At Least 1

Figure 15: Runs and Conditions window

- 3. Highlight all **'Conditions'** by clicking the **'Select All'** buttons located at the bottom of each box.
- 4. Click **'Apply to All Subjects.'** Ensure the **'Each Run Encoding At Least 1'** box is checked.
- 5. Click the **'Create Timing Onsets'** button. An editor will appear displaying the timing onsets template text file.
- 6. In the **'Runs and Conditions'** window, click **'OK'** to return to the **'G Matrix Creation'** window
- 7. In the **G Matrix Creation** window make sure that the number of **Bins** is 8, the timing rate **TR** is 3, the timing units are **Scans**, and the **Model Type** is **FIR**.
- 8. Click on **'Import Onsets List'**, navigate to the directory where the text file is saved (for this example, a previously created **'timing\_onsets.txt'** file is included in the

example data you downloaded), and open the file. An editor will appear showing the previously created template with the imported data inserted into it

Once the G matrix has been loaded, it will be partitioned into separate G matrix files for each subject and run and written to the folder named **Gsegs** in your current output directory.

The *Model* panel will be updated as follows:

Model				
G Clear Select Create 214 x 32	[53.50 Kb]	4 0 Time	e Bins: 8 0 Edit	Scree Stats

Figure 16: G matrix information

If the G matrix is of the proper size to be applied to the data, a small faded check mark will appear beside the "Select" button. The size of the G matrix will also be displayed, in this case 214 X 32.

# Step 9: Run the analytic processing

Now that the G matrix has been loaded you are ready to set the parameters for your analysis.

Z Create Z Regression Covariates Linear Quadratic	G Regress Resume Extract 2
<ul> <li>Head Movement</li> <li>User Defined Covariates</li> </ul>	Rotate Settings Clr

The processing panel will now look as follows:

Figure 17: fMRI-CPCA Processing panel

The **'G'** button will be green indicating all necessary data has been loaded in order to regress G onto Z.

Under the 'G' button ensure that the box labeled 'Regress' is checked.

- You will apply **'Extract'** at a later stage.
- 'Rotate' (rotation) is more advanced and is discussed in the comprehensive manual.

You are now ready to run your first fMRI-CPCA analysis.

- > Press the '**RUN**' button located in the upper right hand corner of the GUI.
- A 'CPCA Process' window will appear indicating the status of the analytic process. When the application has completed its calculations the text "CPCA Processing Completed" will appear on the MATLAB console screen.

View Log	Options		Avail	Estimated	Max Partition Mem (Mb) 500 Create List	CD
Cache: <na></na>		Memory:	3.99 GB	7.29 Mb	Estimated Time:	
Utilities/Tools	Fix FS	Drive Space:	249.7 GB	0.3 GB	00 Minute 48 Seconds Unload	RUN



```
214 x 29151 ] Runs: 1
Subjects:
      1 [
Estimated Completion Time: 00 Minute 48 Seconds
- Subject s01 1 of 1 Run: 1 [========] [00:11]
 Process
             start
                    end
                          Duration
_____
             10:47:59 10:48:11 O Minute 12 Seconds
 Normalization
  _____
            10:47:58 10:48:11
     Elapsed
                            O Minute 12 Seconds
Subjects: 1 [ 214 x 29151 ] Runs: 1
            214 x 32 ] C:\.cpca\Output\Gheader.mat
        [
    G:
         [ 32 x 29151 ]
    GZ:
  -Subject: 1 Run: 1
  - Creating C*C
            10:51:20 10:51:22 O Minute 2 Seconds
     Apply G
*******************< Computation of SS GC >***********************
SS Z
             = 6238314.00
SS GC
             = 1516432.70
SS percent of Z
             = 24.31
 Process
             start end Duration
_____
                                     _____
     Apply G
             10:51:20 10:51:22
                            O Minute 2 Seconds
_____
     Elapsed
             10:51:20
                     10:51:23 O Minute 2 Seconds
>>
```



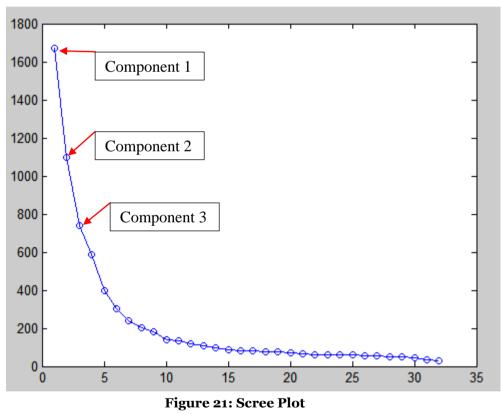
# **Step 10: Determine the number of components to extract**

\*\*\* For this tutorial, you will only be extracting **two** components, in order to keep the analysis succinct. However, the method used to determine the number of components to extract will be introduced and briefly explored. For further explanation, please refer to the comprehensive user manual.

Model G Clear Select Create 214 × 32 [53.50 Kb]	4 0 Time Bins: 8 0 Edit Scree tats

Figure 20: 'Scree' button

Press the 'Scree' button located on the Model Panel (see Fig. 21 above). You will see a graph similar to Fig. 22 below.



Each point on the scree plot represents a component that can be extracted. The higher the value on the y-axis, the more variance it accounts for. When points correspond to a low y-value, they should not be included as they could represent noise etc., and potentially compromise the data. To interpret a scree plot, work your way from right (error) to left (increasingly strong signal), and when a subjectively important "jump" occurs, you may consider keeping that component and all components larger than it. In the scree plot shown above, a four-

component solution appears most appropriate, due to the subjectively important jump from the 5<sup>th</sup> to the 4<sup>rd</sup> component. Following this, significance testing is necessary, but this will be covered in the comprehensive user's manual. Interpretation of scree plots is more an art form than a science, but has a long history in the area of component analysis (starting with Cattell, 1966).

- > Return to the *Processing* panel.
- > Press the **'G'** button in the *Processing* panel.
- Check the box labelled 'Extract' and enter '2' in the box to the right of it. This is the number of components that will be extracted in this tutorial.
  - In a full analysis, you would determine the number of components to enter based on your decision after viewing the scree plot. For a full analysis, you would enter '3' in the box to the right of the 'Extract' box. You also have the option of extracting a multiple number of components in a single run by entering all the numbers you wish to extract in the box to the right of the 'Extract' box, for example you could enter '3 4 5'. As aforementioned, for simplicity reasons, we will only extract two components in the tutorial. Please refer to the comprehensive user manual for further explanation.

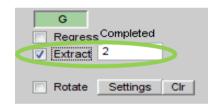


Figure 22: Extract 2 components

Again, Press the 'RUN' button located in the upper right hand corner of the GUI. A 'CPCA Process' window will appear indicating the status of the analytic process. When the application has completed its calculations the text "CPCA Processing Completed" will appear on the MATLAB console screen.

### **Step 11: Plot the estimated HDRs**

Predictor weights are computed and saved for each component extracted. For each component, these mean predictor weights can be plotted to reveal the estimated deconvolved HDR for each condition over time. These values can be plotted using any program such as Excel or SPSS, or via the fMRI-CPCA GUI interface.

Press the 'Stats' button that will have appeared in the *Model* panel upon completion of the analysis. This will open the 'Multivariate Statistics' window.



#### Figure 23: Location of the Stats button

To view the statistics for the first component, select '1' in the component selection pull down menu.

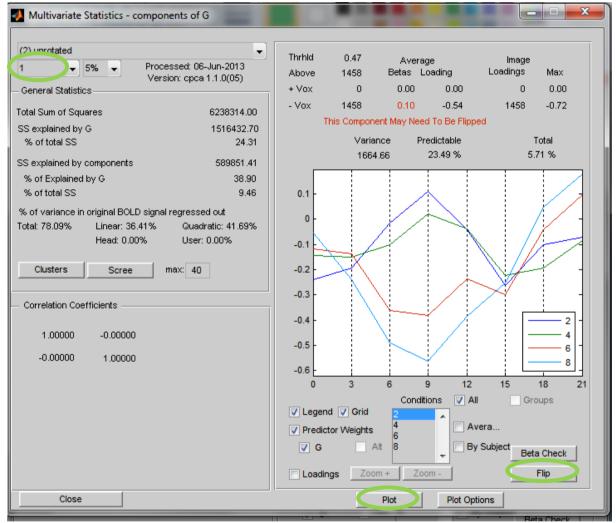


Figure 24: Component 1 Statistics

In this example, component 1 needs to be flipped. *See the fMRI-CPCA Manual for instructions on how to determine if a component needs to be flipped prior to plotting.* 

- > To create and view a plot of the estimated HDR for the two extracted components:
- > To flip component 1:
  - > Ensure '1' is selected in the component selection pull down menu.
  - Press the 'Flip Component(s)' button (see Fig. 25 above). The 'flipping data' window will appear throughout the process.
  - Note: On some platforms, clicking the 'Flip' button will cause Matlab to crash. To flip data in this case, open up Matlab command window and type in edit MvsfMRI.m. Run the 'Flip' using debug mode by searching for the word 'flip' and clicking on these corresponding lines: 1271, 1286, and 1289.

On some platforms, the graph may not update properly; so to refresh the flipped predictor weights, select the **'1'** again.

- Select '1' in the component selection pull down menu.
- > Press the 'Plot Component(s)' button, as shown in Figure 25.
- > The plot image will appear in a new window, as shown below.
- > To save an image of your plot, In the *File* dropdown menu, select *Save As...*
- Close the plot window when finished.

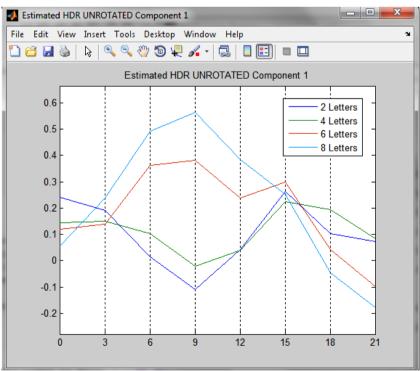


Figure 25: Component 1 HDR plot window

- Similar to the procedure outlined above, return to the 'Multivariate Statistics' window, and select '2' in the component selection pull down menu.
- Press Flip and Plot Component(s)' buttons and save the resulting plot for component 2 if desired.

# Step 12: View the neural networks

The neural networks are whole brain images representing systems of functionally interconnected brain voxels. To view a neural network the loadings contained in the image file are overlaid onto a brain image template. A given neural network may contain only positive loadings (activations), only negative loadings (deactivations), or both positive and negative loadings.

The neural network images will have been written to a folder in your output directory located in **'Output\G\2\_components\unrotated**. Each network image is composed of an .img and an .hdr file. In this example only the unrotated solution was extracted, but it is possible to extract multiple rotations in one analysis (see the comprehensive manual). For each rotated solution, a separate neural network is created for each component. In this unrotated solution example, one neural network was created for each of the two components extracted:

unrotated\_Component\_1.img/.hdr
unrotated\_Component\_2.img/.hdr

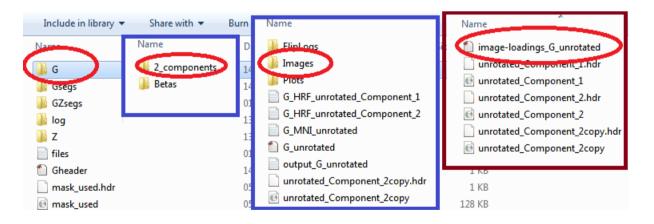


Figure 26: CPCA output folders

#### View the neural network for component 1

#### Step 12A: Determine the display values

In the Statistics window:

- > Ensure that '(2) unrotated' is selected in the top left hand corner.
- > Select '1' in the component selection pull down menu. The statistics for the selected component will be displayed in the upper right hand section of the statistics window.

From the statistics displayed for component 1, it is evident only positive loadings passed the display threshold (positive loadings as listed in the +Vox row) and the display threshold values (threshold and maximum) can be determined.

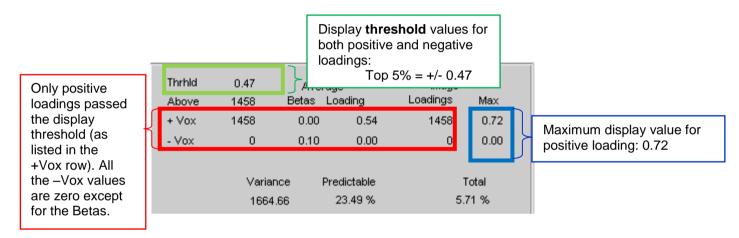


Figure 27: Display values for component 1

Note that the threshold values are the same for both the positive and negative voxels, but the maximum (most extreme positive and negative) values differ. When viewing/processing images in MRIcroN, the thresholds for display must be the same for both the positive and negative loading voxels (changing the threshold value will result in different neural networks being seen). However, the maximum values can optionally be adjusted to control activation brightness.

#### Step 12B: Start MRIcroN

- Start the application *MRIcroN.exe*
- The Statistics window and the MRIcroN window can be viewed side by side to facilitate viewing of the network.



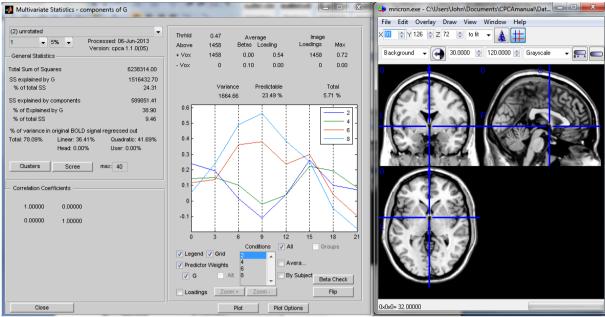
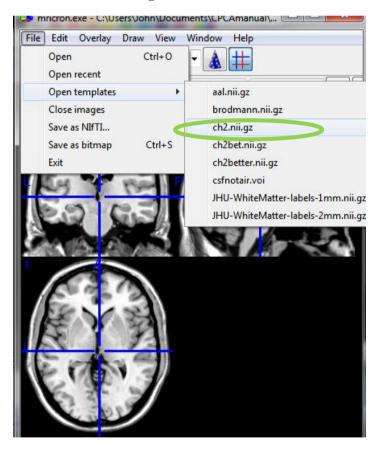


Figure 28: Statistics window viewed next to MRIcroN



- In the MRIcroN 'File' dropdown menu, select 'Open Templates.'
- Select the template named:'ch2.nii.gz.'

Figure 29: Opening Templates in MRIcroN

Step 12C: Load the neural network images

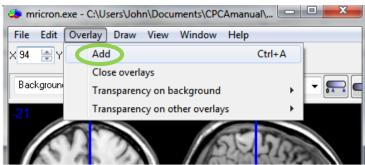


Figure 30: Add an overlay in MRICroN

In the 'Overlay' dropdown menu, select 'Add'.

Look in:	鷆 Images	•	G 🤌 📂 🛄 -	
(Pa)	Name		Date modified	Туре
	unrotated_Component_1.hdr		14/05/2013 12:41	HDR File
Recent Places	university		14/05/2013 12:41	HDR File
	unrotated_Component_2copy.hdr		01/05/2013 3:16 PM	HDR File
Desktop				

Figure 31: Select positive loadings overlay

for component 1: G\_Component\_1\_unrotate d.hdr

Select the .hdr of the image

 $\geq$ 

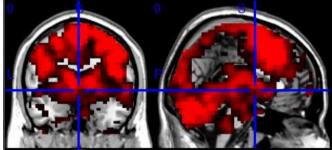


Figure 32: Unthresholded positive loadings

The loadings will appear as a red coloured activation map. (Red is the default colour for the first overlay added and blue is the default for the second.)

#### Step 12D: Set the display values

The MRIcroN menu contains two boxes where the threshold and maximum display values must be entered. The relation between the first number (left-side box) and the second number (right-side box) is always from the lowest to the highest value. Mostly, to display activations or deactivations, the threshold value is entered into the left-side box and the maximum value into the right-side box. *When displaying negative deactivations both the thresholds and maximum values should include a minus sign*. This may cause their values (and therefore the relation between the numbers) to change. You will need to adjust what you enter into the boxes accordingly with the lower value entered in the left-side box and the higher value entered in the right-side box.

Left-side box: Threshold	Right-side box: Maximum
File Edit Overlay Draw View	Window Help
🗙 91 🚔 Y 126 🚔 Z 72 🚔 to fit	- <b>A H</b>
unrotated_Cor 🗸 💽 0.0023 🛊	0.3067 🔿 Red 🗸 戻 🗨
Figure 22. MRIcro	N display value boxes

Figure 33: MRIcroN display value boxes

- > Match the threshold and the maximum values to those in the statistics window:
- > Set the display values for the positive loadings:
  - Enter 0.47 in the threshold / left-side box (top 5% of loadings in this case).
  - $\circ$  Enter 0.72 in the maximum / right-side box

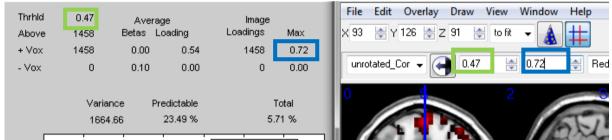


Figure 34: Setting component 1 positive loadings

#### Step 12E: Set the viewing options

The neural networks loaded in MRIcroN can be viewed in several different orientations and slice levels. Any of the selected views can be saved as bitmap images. One viewing option is the multislice option.

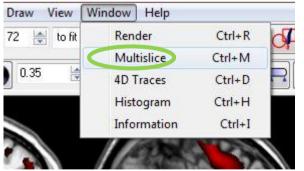
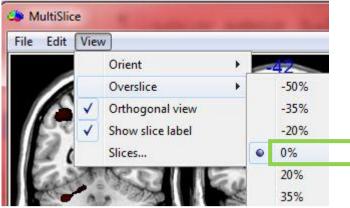


Figure 35: Open Multislice view



Figure 36: Set Multislice view to coronal

- In the 'Window' dropdown menu, select 'Multislice', which will open the MultiSlice window.
- In the MultiSlice View dropdown menu:
- Check the 'Orthogonal View' and the 'Show
   Slice Label' options.
- Select 'Orient', and choose 'Coronal.'
- Select 'Overslice', and select '0%.'





Slice numbers [e.g. 10,16,24]	
68, 87, 123, 136, 162	

Figure 38: Select multislices window

- In the MultiSlice View dropdown menu:
- Select 'Slices'..., and enter Slice Numbers '68,87,123,136,162' (y-coordinate values) into the 'Select multislices' window.
- Press 'OK' to see this:

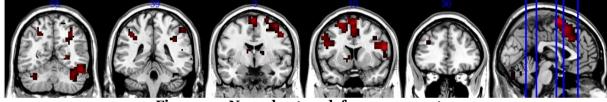
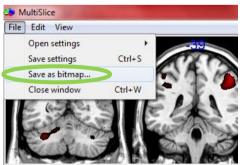


Figure 39: Neural network for component 1

#### Step 12F: Save an image of the neural network



In the MultiSlice 'File' dropdown menu, select 'Save as bitmap...'

Figure 40: Save image as bitmap

# View the neural network for component 2

Now you are ready to view the neural network for component 2, using the same steps used to view the neural network for component 1.

#### Step 12A: Determine the display values

In the Statistics window:

- > Ensure that (2) unrotated is selected in the top left hand corner.
- > Select '2' in the component selection pull down menu.

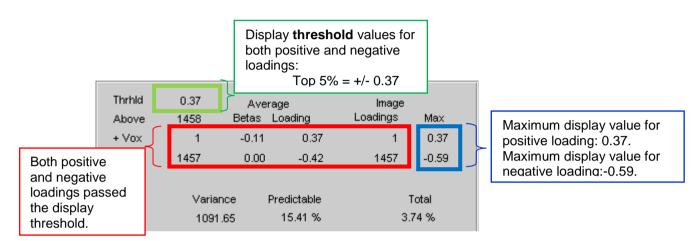


Figure 41: Display values for component 2

From the statistics displayed for component 2, it is evident that both activations/+loadings and deactivation/-loadings pass the threshold display values.

#### Step 12B: Start MRIcroN

If you have not closed and reopened MRIcroN, you can reset the display by closing the overlays associated with component 1, as shown below:

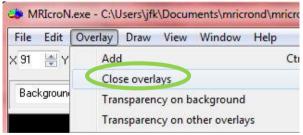


Figure 42: Closing overlays in MRIcroN

In the 'Overlay' dropdown menu, select 'Close overlays.'

#### Step 12C: Load the neural network images

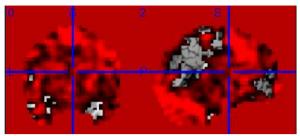


Figure 43: Component 2, 1st overlay added

# Step 12D: Set the display values

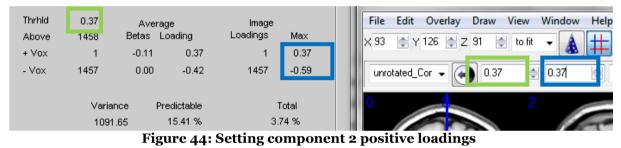
- In the 'Overlay' dropdown menu, select 'Add.'
- Select the .hdr of the image for component 2:

#### G\_Component\_2\_unrotated.hdr

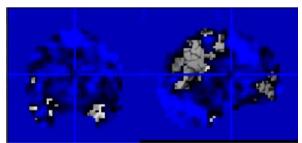
The image should appear as shown at left.

#### > Set the display values for the positive loadings:

- Enter '0.37' in the threshold box (top 5% of loadings in this case).
- Enter '0.37' in the maximum box



In order to also view the negative loading associated with this component, a second copy of the same overlay must be added. *Note: If you are working in Linux it will not be possible to add two copies of the same overlay in MRICron. Thus, Linux users will have to select the second overlay from the folder 'Component\_Images/Duplicates'. The second overlay should* 



have the same name as the first, but appended with '\_copy'.

Figure 45: Component 2, 2nd overlay added

- In the 'Overlay' dropdown menu, select 'Add.'
- Again, select the .hdr of the image for component 2:

G\_Component\_2\_of\_2\_unrotated.hdr \*Or in Linux, select: Duplicates/G\_Component\_2\_of\_2\_unrotated\_copy.hdr

The user can switch between the two overlays using the selection box on the left side of the MRIcroN menu.

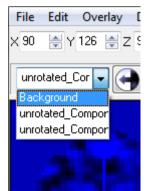


Figure 46: Switching between overlays

- > Set the display values for the **negative** loadings:
  - Note: The relation between the first number (left-side box) and the second number (right-side box) is always from the lowest to the highest value. Therefore, when dealing with negative loadings, we will have to look at the values of the negative maximum and the value of the threshold (remember to make this negative) to determine which number should be entered into which box.
  - Enter -0.59 in the left-side box (maximum in this case).
  - Enter -0.37 in the right-side box (top 5% in this case).

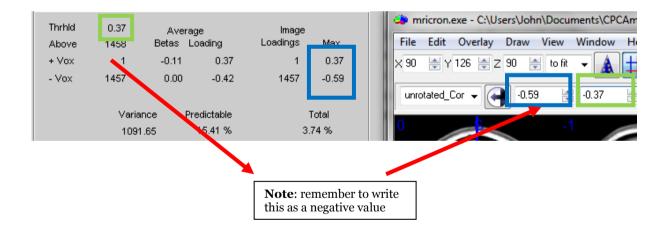


Figure 47: Setting component 2 negative loadings

#### Step 12E: Set the viewing options

- Again, this uses the same steps taken for component 1: In the 'Window' dropdown menu, select 'Multislice', to open the MultiSlice window
- > In the MultiSlice 'View' dropdown menu, select 'Slices...'
- Enter Slices numbers '71,84,113,118,175' into the 'Select multislices' window. Press 'OK':

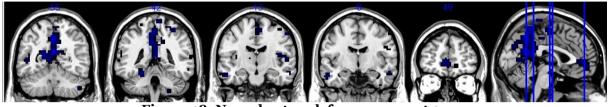
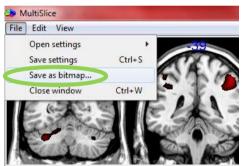


Figure 48: Neural network for component 2

#### Step 12F: Save an image of the neural network



In the MultiSlice 'File' dropdown menu, select 'Save as bitmap...'

Figure 49: Save image as bitmap

# Step 13: Interpret the results

The tutorial data is from a working memory task that required subjects to remember 2, 4, 6 or 8 Letters across a delay period. When fMRI-CPCA was used to extract two components, aspects of the task-positive and task-negative (i.e. default) networks emerged. The following example of result interpretation is not identical to the CPCA results you have just obtained by working through this manual. However, the results are very similar and the following information is included here to provide an example of interpretation.

#### Interpretation of component 1

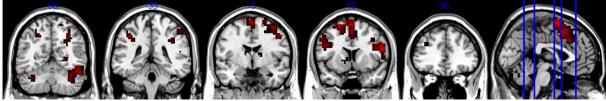


Figure 50: Interpretation of component 1

The network for component 1 reveals the system of functionally connected brain areas that are active during the working memory task. Included in this system are the dorsal anterior cingulate gyrus, dorsolateral prefrontal cortex, bilateral middle and inferior frontal gyrus, bilateral supramarginal gyrus, and insular cortex.

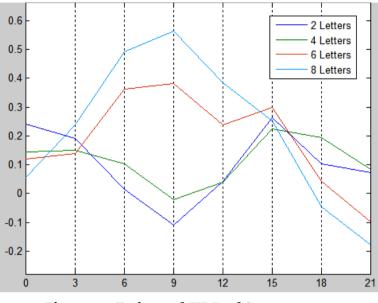


Figure 51: Estimated HDR of Component 1

The plot of the estimated HDR for component 1, reveals that the activation of this neural network is load dependent, with the greatest activation present for the highest memory load condition (8 letters; condition 4) and the lowest level of activation present for the lowest memory load condition (2 letters; condition 1).

#### Interpretation of component 2

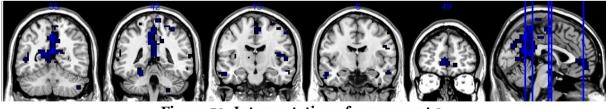


Figure 52: Interpretation of component 2

The Neural network for component 2 contains elements of the task positive network found in component one, but it also contains elements of the task negative (i.e., default) network. The task negative network consists of a system of functionally connected brain areas that are suppressed during the working memory task. Included in this system are the ventral anterior cingulate gyrus, posterior cingulate/precuneus, bilateral inferior parietal lobes, primary auditory cortex, planum temporale and posterior superior temporal gyrus.

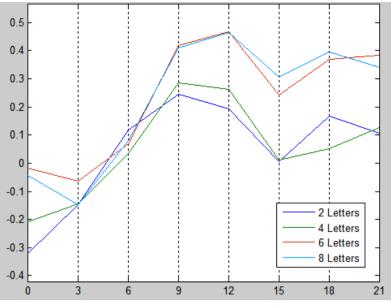


Figure 53: Estimated HDR of component 2

The plot of the estimated HDR for component 2 reveals that the intensity of the activation increases (positive red loadings) and decreases (negative blue loadings) is clearly load dependent, with the greatest intensities present for the highest memory load condition (8 letters; condition 4) and the least for the lowest memory load condition (2 letters; condition 1).



Congratulations! You have completed your first fMRI-CPCA analysis!