

Single Subject Demo Data Instructions

- 1) click "New" and answer "No" to the "spatially preprocess" question.
- 2) in "Basic" enter "1" subject, "6" seconds TR, and "1" session
- 3) in "Functional" go to the functional folder and select all of the files in there (you should see two axial images, and read "62 files" in the left plots)
- 4) in "Structural" go to the structural folder and select the "ws*.img"
- 5) in "Rois" go to the structural folder, then first select the "Grey matter" ROI in the ROIs list (at the left) and select the "mwc1*.img" file in the files list (at the right), then select the "White matter" and the "mwc2*.img" file, and then select the "CSF" and the "mwc3*.img" file. The rest of the ROIs are already defined by default (to point to the conn/rois folder) so you can leave those unchanged.
- 6) in "Conditions", enter "rest" as condition name, then enter "0" as onset, and "inf" as duration
- 7) in "covariates->first-level" enter "motion" as covariate name, and then select the "rp_*.txt" file in the realign folder
- 8) press "done" (and enter a new name for the project if you have not already done so), this will take a couple of minutes or so to complete...
- 9) in the "Preprocessing" tab, enter the "band-pass" filter "0.008 0.09", and change the "White matter" and "CSF" confounds to "3" dimensions (it still has the old default of "5"), then press "Done" (this should take only a few seconds to process)
- 10) in the "Analyses" tab, remove all of the BA* rois in the "sources" list (this is just to speed up the computations), to perform the analyses

using our Fox sources as seeds. Then press "Done" (this should take again only a few seconds to process)

11) in the "results" tab, you can select the different fox "sources" to display the connectivity results for this subject (you can also use the "ROI view" button, but not the "Whole brain" button which is only in 2nd level stats)

Overview

The toolbox provides seed-to-voxel and ROI-to-ROI functional connectivity estimation and inference.

The toolbox implements a *CompCor* strategy for physiological (and other) noise source reduction, first-level General Linear Model for correlation and regression connectivity estimation removing typical confounding effects, and second-level random-effect analyses.

The toolbox is designed to work with both resting state scans and block designs where rest is another block amongst other conditions.

Installing the toolbox:

download conn.zip, unzip the file.

add the resulting ./conn/ directory to the matlab path

Requirements:

SPM5 or above

Matlab (no additional toolboxes required)

To start the toolbox:

On the matlab prompt, type : conn

(make sure your matlab path include the path to the connectivity toolbox)

Usage



In order to perform connectivity analyses using this toolbox you will need:

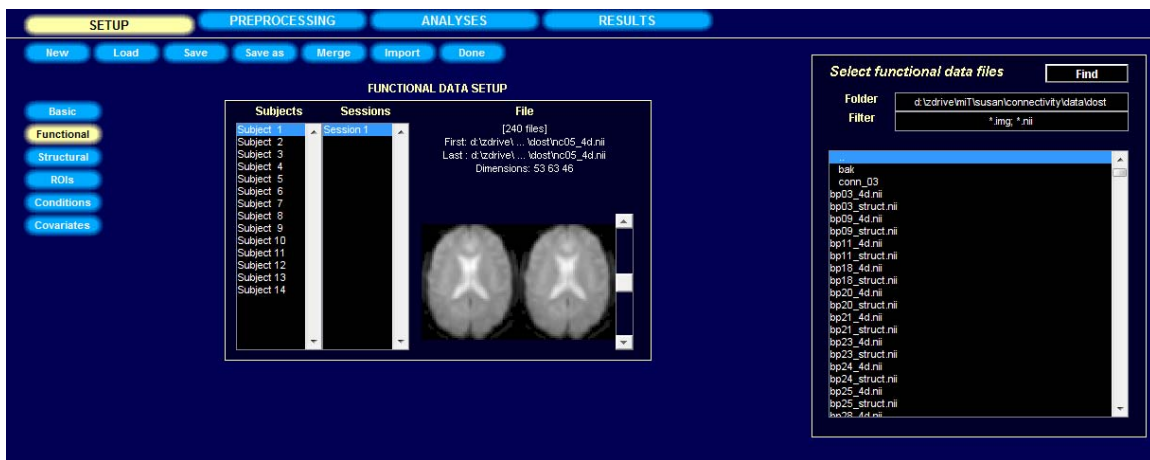
- a) Functional data. Either resting-state or block designs can be analyzed.
- b) Structural data. One anatomical volume for each subject
- c) ROI definitions. A series of files defining seeds of interest. ROIs can be defined from mask images, text files defining a list of talairach positions, or multiple-label images. The toolbox is also provided with a series of pre-defined regions of interest that will be loaded automatically (these include a series of seed areas useful for investigating default network connectivity –FOX_*.img-, as well as a complete list of gyrus-level areas obtained from the Talairach Daemon atlas –TD.img-; see the *utils/otherrois/* folder for additional roi files).

The toolbox operation is divided in four sequential steps:

1. **Setup:** Defines basic experiment information, data locations, regions of interest (seeds), temporal covariates, and second-level models.
2. **Preprocessing:** Define, explore, and remove possible confounds in the BOLD signal
3. **Analyses:** Perform first-level analyses. Define the seeds of interest and explore the functional connectivity of different sources separately for each subject
4. **Results:** Perform second-level analyses. Define and explore within- and between-subject contrasts of interest

Each of these steps can be defined interactively using the toolbox gui or by scripting using the *conn_batch* functionality. The following sections describe the steps when using the gui in more detail (see the *conn_batch.m* help and *conn_batchexample*.m* files for additional information and examples regarding batch mode processing).

Step one: Setup (Defines experiment information, file sources for functional data, structural data, regions of interest, and other covariates)



Click on the **SETUP** tab

Click on the **New** button to start a new project. If your data is not already spatially preprocessed (realignment, coregistration, normalization, and smoothing) a wizard will launch that will allow you to specify the locations of your raw functional and anatomical volumes and it will perform using SPM these initial preprocessing steps at this time. This wizard will also initialize some of the basic setup information (you can skip the **Basic**, **Functional** and **Structural** steps below).

Click on the **Basic** button on the left side, enter experiment information (Number of subjects, TR, and number of sessions per subject)

Click on **Functional** button on the left side, from the right side panel, select the functional images (*.img, or *.nii, or 4d nii). This will take a second to load, check the middle panel (Functional data setup) to make sure the correct volumes are loaded. The brain display in the “Functional data setup” window shows the first (left) and last (right) scan for the selected subject/session (as in the figure above). The functional images are expected to be already pre-processed (typically realignment, coregistration, normalization, and smoothing).

GUI tip 1: The “Find” in the “Select functional data files” window can be used to search for all files within the target folder recursively. Change the “Filter” window to narrow the search.

GUI tip 2: If multiple subjects are selected in the “Subjects” list, and the number of files selected in the “Select functional data files” window matches the number of subjects, each subject is assigned one single file from the list (this is useful when one has 4-dimensional .nii files –one file per session- in order to enter all of the functional files for each session simultaneously)

Click on the **Structural** button on the left side to load the structural images. Click sequentially on each subject and select the associated normalized anatomical image. These images should be normalized and coregistered to the functional data for each subject.

Click on the **ROIs** button on the left side to load ROI mask files (.img or .nii volumes), Talairach coordinate files (.tal text files), or atlas files (.nii files with multiple labels). ROI files can be assigned separately to each subject (for subject-specific ROIs) or commonly across all subjects (normalized-space ROIs).

By default all files in the *rois* toolbox folder (*./conn/rois*) will be imported as initial regions of interest. To import new ROIs, click below the last ROI listed and enter the appropriate information. To remove an existing ROI from this list right-click on an ROI and select “*remove*”. When importing subject-specific ROIs click sequentially on each subject and select the corresponding ROI file. When importing subject-independent ROI files, select all subjects simultaneously and then select the corresponding ROI file.

Load the grey matter, white matter and CSF mask for each subject if they already exist (if left blank, the toolbox will generate these masks by performing segmentation on the structural image for each subject)

ROI files should be coregistered to the provided normalized structural of this subject (they could be defined in normalized space, or they could be defined in subject-space and then transformed to normalized space in SPM).

The default dimensions (number of PCA components to be extracted) for each ROI can be changed here. In the following steps (preprocessing, analyses), you can later select the number of components among the extracted ones you wish to use in the connectivity analyses. If one dimension is chosen for a ROI the time-series of interest is defined as the average BOLD activation within the ROI voxels. If more than one dimension is chosen for a ROI the time-series of interest are defined as the principal eigenvariates of the time-series within the ROI voxels (note: PCA decomposition is performed after removal of task-effects and first-level covariates).

Select “*mask with grey matter*” on selected ROIs to further restrict these ROIs’ voxels to those voxels within the estimated grey matter mask for each subject.

Select “*multiple ROIs*” if the ROI file contains multiple labels (e.g. atlas file TD.img provided in *roi* folder), or multiple disconnected sets (e.g. functionally-defined ROI composed of multiple clusters, and you wish to use each cluster as a separate seed).

Click on the **Conditions** button on the left side to enter onsets and durations (in seconds) of each experimental condition. If resting state (no experimental conditions) enter a single condition with onset 0 seconds and duration the complete duration of each session.

Click on the **Covariates:First-level** button to define first level covariates such as realignment parameters to be used in the first-level BOLD model. For each covariate select a .txt or .mat file (for each subject and session) containing the covariate time series (e.g. select the realignment parameters file *rp_*.txt* to include the movement parameters as covariates).

When importing *.txt* files they should contain as many rows of number as scans for any given subject/session, and an arbitrary number of columns (columns are separated by spaces). When importing *.mat* files they should contain a single variable (arbitrarily named) consisting of a matrix with as many rows as scans for any given subject/session and arbitrary number of columns.

Click on the **Covariates:Second-level** button to define groups and subject-level regressors (e.g. behavioral measures). Use 1/0 to define subject groups, or continuous values to perform between-subject regression models). Click on the empty space below “All” to add a variable.

e.g. patients: 1 1 1 1 0 0 0 0
 controls: 0 0 0 0 1 1 1 1
 performance : 1 2 3 2 3 4 5 6

Note: Second-level covariates can be defined at any time in the analyses (changes to any of other “Setup” options requires rerunning all the analyses steps, while changes to the second-level covariates do not as they only affect the second-level analyses in the “Results” window)

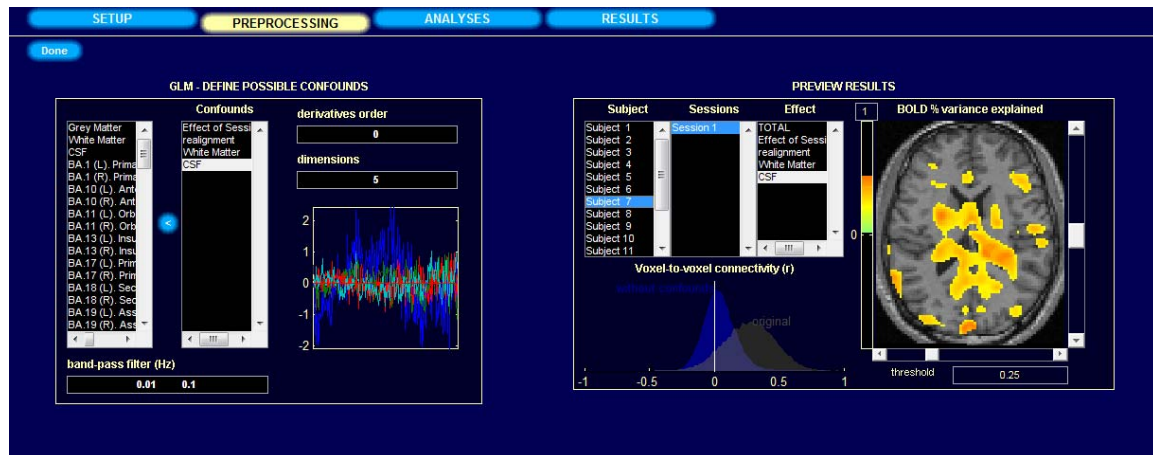
When finished defining the experiment data press **Done**. This will import the functional data. It will also perform segmentation of the structural data in order to define gray matter/ white matter/ CSF regions of interest if necessary. Last it will extract the ROIs time-series (performing PCA on

the within-ROI activations when appropriate). After this process is finished come back to Setup to inspect the resulting ROIs for possible inconsistencies. A .mat file and a folder of the same name will be created for the project.

Save / Save as button will save the setup configurations in a .mat file, which can be loaded later (**Load** button). The .mat file will be updated each time the “Done” button is pressed

Note: If the data has initially been defined in SPM you can skip many of the above steps by clicking the **Import** button (right after entering the number of subjects in the **Basic** setup) and specify one SPM.mat file for each subject. The program will extract the location of the functional data, the number of conditions per subject, the onset/length of the conditions of interest, and any specified first-level covariates from these SPM.mat files.

Step two: Preprocess (Define, explore, and remove possible confounds)



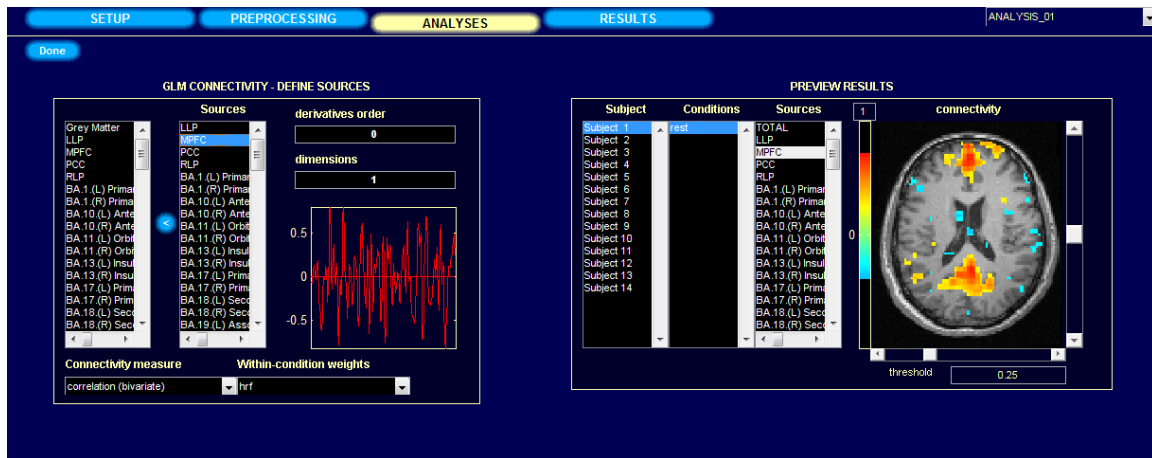
Click on the **PREPROCESSING** tab. By default the system will start with three different sources of possible confounders: 1) BOLD signal from the white matter and CSF masks (5 dimensions each); 2) any previously-defined within-subject covariate (realignment parameters); and 3) the *main* condition effects (condition blocks convolved with *hrf*). For each of the selected possible confounds you can change the number of dimensions (specifying how many temporal components are being used), and the derivatives order (specifying how many successive orders of temporal derivatives are included in the model). For example, the *realignment* confound (derived from the estimated subject motion parameters) is defined by default by 6 dimensions. You can change the *derivative order* to 1 indicating that in addition the first-order temporal derivative of the motion parameters should also be used as covariates. Similarly, the *White Matter* confound is defined by default by 5 dimensions and 0 derivative order (indicating that 5 PCA temporal components are being used, with not additional temporal derivative terms).

The “**Preview results**” window in the right panel shows the total variance explained (r-square) by each of the possible confounding sources (for the selected subject/session data). The histogram plot at the bottom displays the voxel-to-voxel connectivity (r values) before, and after confound removal. Typically confounds introduce a positive bias in connectivity measures so the histogram of original connectivity values can appear “shifted” to the right. After confound removal the distribution of connectivity values appears approximately centered. The dimensions of each confound can be changed up to the values entered in the “Setup” stage, to explore its effect on the total variance explained and on the histogram of voxel-to-voxel connectivity values. Images are displayed in neurological format. Click on the image to see the voxel locations and Talairach areas.

Enter the “**band-pass filter**” information in the bottom-left box (two numbers, in Hz, defining the band-pass frequency window of interest).

When finished, press the **Done** button. This will filter the functional data and remove the effect of the defined confounds on all brain voxels and selected regions of interest.

Step three: Analyze (Define and initially view the functional connectivity of different sources in single subject level)



Click on the **ANALYSES** tab to define source of interest (seed ROIs).

Select from the left-most list the **sources** (seeds) of interest and click the right arrow to perform connectivity analyses using the selected sources as seeds.

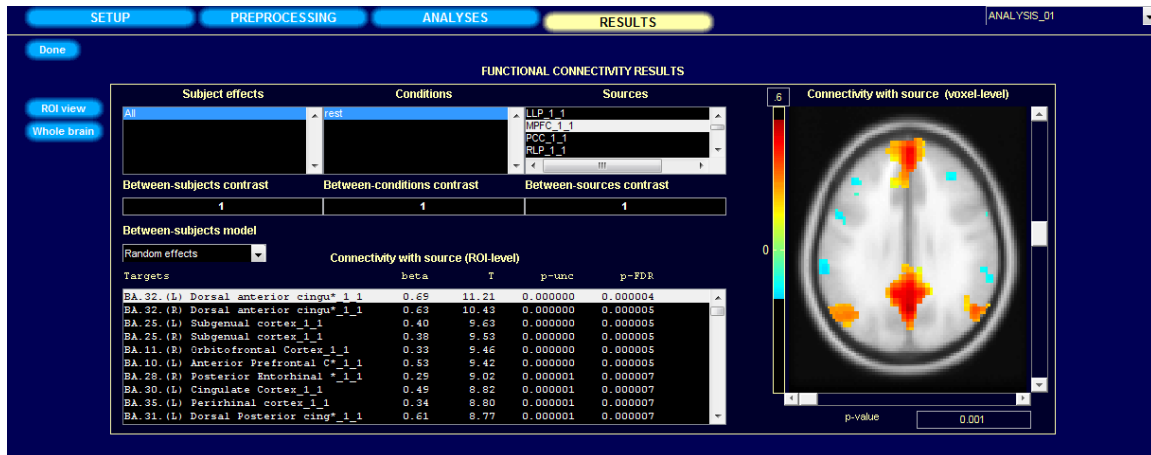
The right panel (“**Preview results**”) displays the connectivity measures for each subject/condition/source. Analyses here are performed in real-time (any changes in the “Define sources” definitions affect directly the results displayed in the “Preview” window). The measures displayed in the “Preview results” brain image correspond to the *connectivity measure* selected (r if correlation is selected, beta if regression is selected). The threshold value is also defined in the same units (in the figure above, voxels with correlation coefficients above 0.3 are shown/colored). Images are displayed in neurological format. Click on the image to see the voxel locations and Talairach areas.

Click on “**Connectivity Measures**” to change the method of analysis (correlation or regression, semipartial for multiple sources).

Click on “**With-in condition weights**” to change the relative weighting of the scans within each condition. When estimating connectivity from rest blocks in a more complex design (with other task blocks) between-condition differences in activation can affect the activation at the beginning or end of the rest block. These effects are partially controlled by entering the “condition” regressors as possible confounds. Hrf and hanning within-condition weights attempt to further reduce these effects by weighting down the initial scans within a block (hrf weights) or both the beginning and end scans within a block (hanning weights).

When finished defining/exploring the connectivity analyses press **Done**. This will perform the defined analyses for all subjects, constructing seed-to-voxel connectivity maps for each selected source (as well as complete ROI-to-ROI connectivity matrices for these sources) for each subject and for each condition. First-level results (beta maps and correlation maps when appropriate) are also exported as .nii volumes (one per Subject/Condition/Source combination) in the *results/firstlevel* folder.

Step four: Results (Define and explore contrasts of interest and second-level results)



At this point, second level analysis can be defined in the **RESULTS** window.

A second-level model is defined by selecting one or multiple elements in the “**Subject effects**” list and specifying the desired “**between-subjects contrast**”. For example, if we have two groups defined (in the “covariates:second level” setup step) : patients, and controls, simply select both of them in the “Subject effects” list, and enter 1 -1 in the “Between-subject Contrast” window to compare the connectivity between the two groups, or enter 1 0 or 0 1 in the “Between-subject Contrast” window to look at connectivity within each of the groups separately.

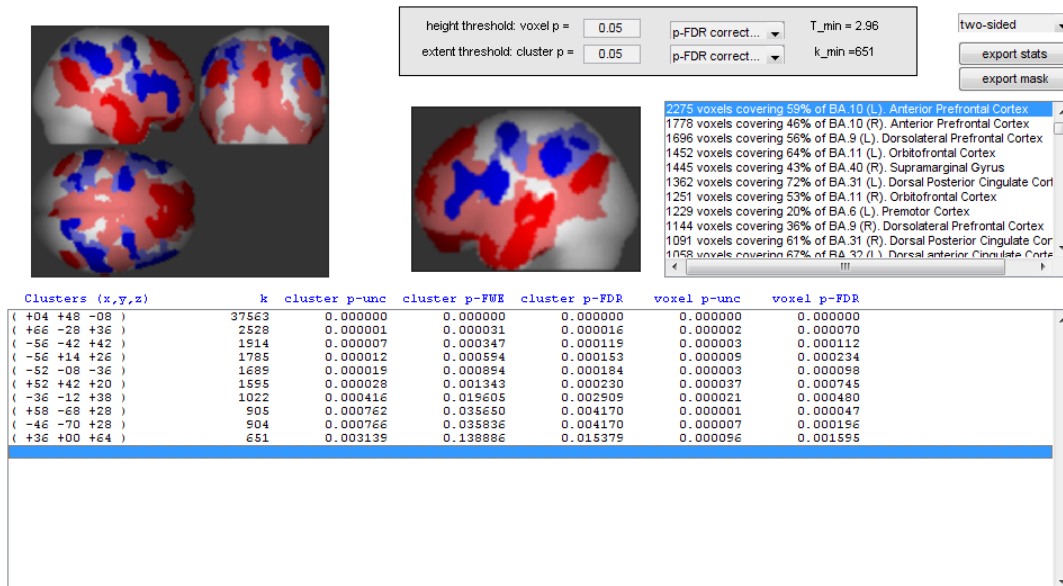
Multiple conditions can be selected simultaneously in order to analyze connectivity results across several conditions by specifying the contrast in “**Between conditions contrast**” (e.g. use 1 -1 to compare connectivity during rest vs. task).

Similarly multiple ROIs/sources can be selected simultaneously in order to analyze connectivity results across several ROIs by specifying the contrast in “**Between source contrast**” (e.g. select both “PCC” and “Angular Gyrus” sources and enter a [1 -1] contrast to compare the connectivity between these two seeds).

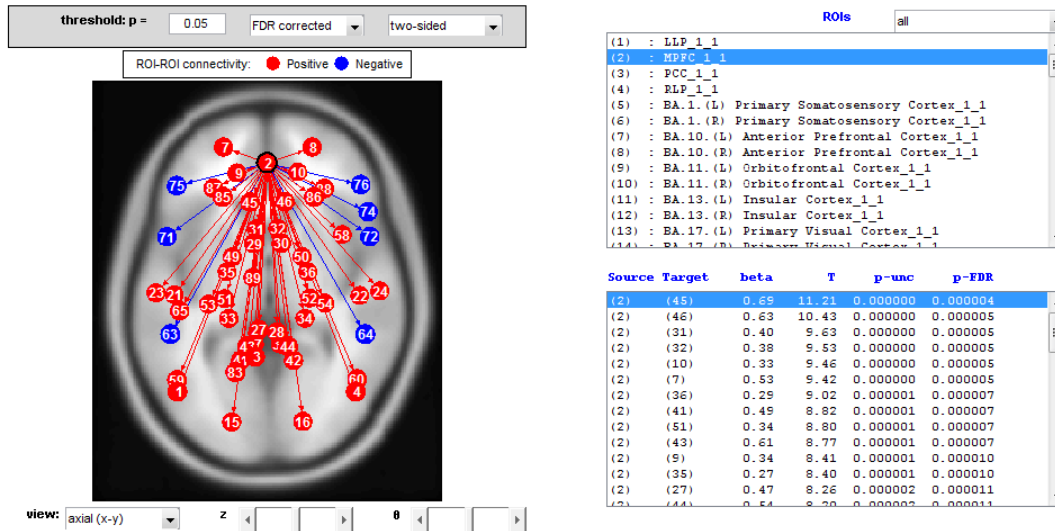
Both between-condition and between-source contrasts represent within-subject contrasts and the second-level analyses will correspond to repeated-measures analyses of the selected effects. Specifically the second-level model will be a general linear model that includes as regressors the selected terms in the “*Subject effects*” list. The outcome variable will be the within-subjects linear combination of effects specified by the “*between-conditions*” and “*between-sources*” contrasts, applied to the first-level connectivity-measure volumes (for the seed-to-voxel analyses) or to the first-level connectivity-measure matrix (for the ROI-to-ROI analyses).

The brain display at the right allows you to explore the **seed-to-voxel connectivity results** for the specified second-level analysis estimated in real time. These results can be thresholded using a voxel-level uncorrected **p-value** threshold ($p < .001$ uncorrected in the figure above; see below for additional thresholding options). Images are displayed in neurological format. Click on the image to see the voxel locations and Talairach areas. In addition you can click on **Whole-brain** to launch a graphical display showing the results of these second-level analyses using a MIP view of supra-threshold voxels for the entire brain. This display can be thresholded by a combination of height (voxel-level) and extent (cluster-level) thresholds. Clusters are listed below together with their peak-voxel location (in mm), number of voxels (k), uncorrected, FWE-corrected, and FDR-

corrected cluster-level p- values, and uncorrected and FDR-corrected voxel-level p-values of the peak voxel within each cluster. Statistics are one-sided, select “**negative contrast**” in the dropdown menu at the top right to view the results for the reverse directionality of the contrast, or “**two-sided**” to perform two-sided analyses. From this graphical display you can also export the results as a text file containing the supra-threshold clusters and their statistics (“**export stats**”), or as a .nii mask file defining supra-threshold voxels (“**export mask**”). In addition when choosing “*export mask*” the toolbox offers the option to perform ROI analyses on supra-threshold clusters for this or a different between-subjects contrast.



Back in the connectivity toolbox window the list at the bottom allows you to explore the results of the **ROI-to-ROI connectivity results** for the specified second-level analysis estimated in real time. For each target ROI these results display the between-ROIs connectivity contrast effect sizes (between the selected source –or linear combination of sources- and each target), as well as T- values, uncorrected p-values, and FDR-corrected p- values for the specified second-level analysis. In addition by clicking **ROI-view** a graphical display of the ROI-to-ROI connectivity measures (for the selected between-subjects and between-condition contrasts) is shown. The *ROIs* list at the top-right can be used to select a different source, and the bottom-right list will display the ROI-to-ROI connectivity values between this source and all of the other ROIs. The graphical display can be thresholded using either uncorrected or FDR-corrected p- values, and it can be set to display one-sided or two-sided results.



When finished defining/exploring second-level contrasts, press **Done**. This will export the defined second-level model to SPM (second-level SPM.mat, beta and contrast volumes are saved in a folder selected by the user) for all of the sources. The results for each performed second-level analysis will be stored in the *results/secondlevel/* folder (organized by folder names indicative of the between-subjects and between-condition contrasts, as well as the source for each analysis). Each of the results folders will contain a SPM.mat file that can be later used to explore the results without the need to repeat the second-level model estimation. To that end you can:

- Type *conn_display* in the matlab command window, select the SPM.mat file in the results folder of interest, and select the between-subjects contrast that you wish to explore. This will launch again the MIP display for the selected results / contrast of interest.
- You can also explore these same results from SPM. Simply click “Results” in SPM, select the SPM.mat file in the results folder of interest, then select the between-subjects contrast that you wish to explore, and follow the standard steps to explore the corresponding seed-to-voxel second-level connectivity results within SPM (see the *SPM* documentation for further information).
- Type *conn_displayroi* in the matlab command window, select the ROI.mat file in the results folder of interest. This will display the ROI-to-ROI graphical display for the selected analysis.
- Type *rex* in the matlab command window, click “Sources” and select the SPM.mat file in the results folder of interest, then click “ROIs” and select the ROI files of interest, then click “Extract”, and last click “Results” and select the between-subjects contrast that you wish to explore. This will perform ROI-based analyses on the selected ROIs of the seed-to-voxel second-level connectivity results (see the *REX* documentation for further information).