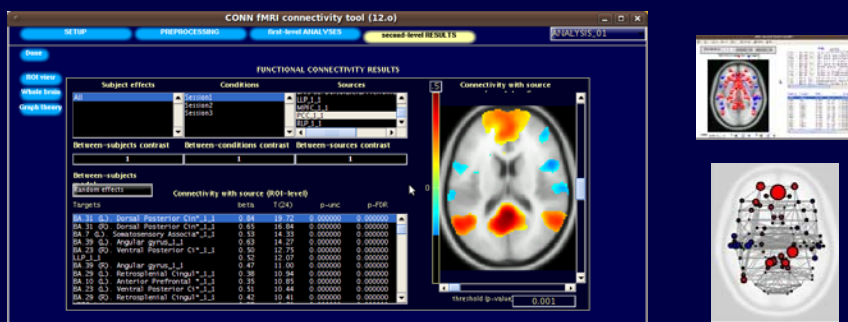


# Conn



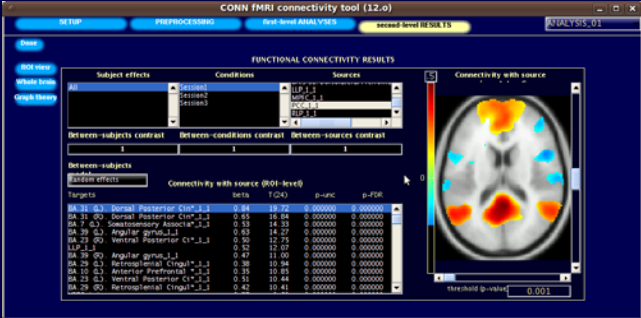
*Susan Whitfield-Gabrieli & Alfonso Nieto-Castanon*  
<http://www.nitrc.org/projects/conn>

## Functional Connectivity Demo



Conn toolbox v.13

# Conn



**FUNCTIONAL CONNECTIVITY RESULTS**

Targets	beta	T(24)	p-unc	p-FDR
BA_31 (L) Dorsal Posterior Cingulate	0.34	10.72	0.000000	0.000000
BA_31 (R) Dorsal Posterior Cingulate	0.33	10.54	0.000000	0.000000
BA_7 (L) Somatosensory Association	0.33	14.33	0.000000	0.000000
BA_7 (R) Somatosensory Association	0.33	14.27	0.000000	0.000000
BA_21 (L) Ventral Posterior Cingulate	0.30	12.75	0.000000	0.000000
BA_21 (R) Ventral Posterior Cingulate	0.30	12.07	0.000000	0.000000
BA_39 (L) Angular Gyrus	0.47	11.00	0.000000	0.000000
BA_39 (R) Angular Gyrus	0.38	10.34	0.000000	0.000000
BA_10 (L) Anterior Prefrontal Cortex	0.35	10.85	0.000000	0.000000
BA_10 (R) Anterior Prefrontal Cortex	0.31	10.44	0.000000	0.000000
BA_29 (L) Retrospatial Cingulate	0.42	10.41	0.000000	0.000000

Threshold p-value: 0.001

**Susan Whitfield-Gabrieli & Alfonso Nieto-Castanon**  
<http://www.nitrc.org/projects/conn>

## fMRI connectivity tool (beta)

**Function:** Perform functional connectivity analyses  
 (seeded voxel correlations)

- The toolbox implements a *CompCor* strategy for physiological (and other) noise source reduction, first-level General Linear Model for correlation and regression connectivity estimation, 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.

The following slides illustrate the operation of the toolbox

## Steps

### Step 1: Setup

Step 2: Preprocess and explore confounds

Step 3: Analyze and view 1<sup>st</sup> level results

Step 4: Define contrasts and view 2<sup>nd</sup> level results

fMRI connectivity tool (beta)

### SETUP

Defines experiment information, file sources for functional data, structural data, regions of interest, and other covariates.

Setup

Preprocessing

first-level Analyses

second-level Results

Help

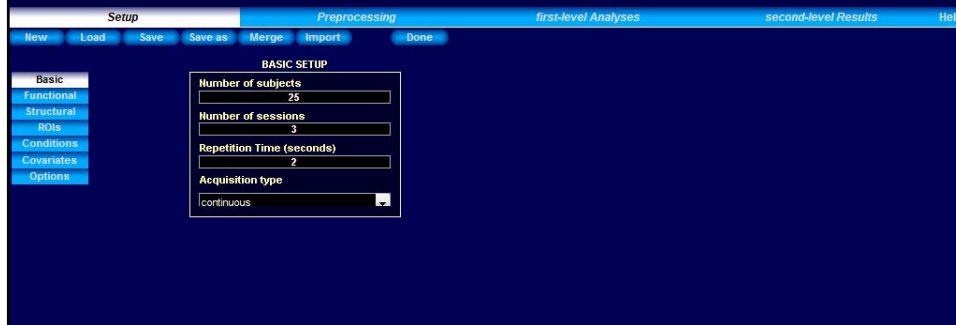
fMRI connectivity tool (beta)

SETUP

**Basic** : Defines basic experimental information

*In this example:*    25 subjects  
                                  TR = 2 seconds  
                                  3 scanning sessions per subject

The "Import" button will load predefined SPM.mat file.

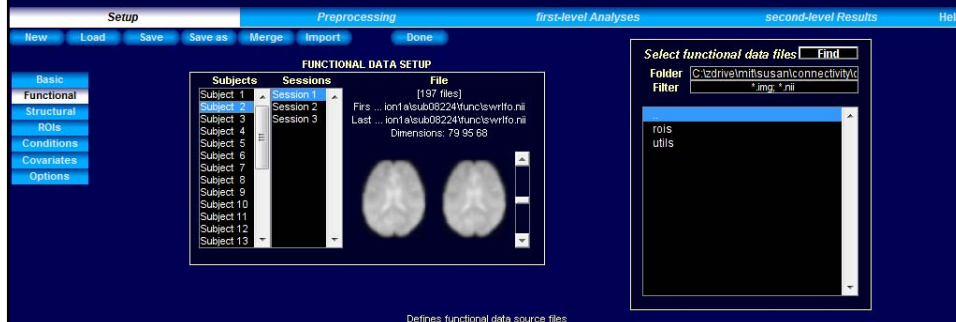


fMRI connectivity tool (beta)

SETUP

**Functional** : Defines functional data source files

(assumes realigned, smoothed)



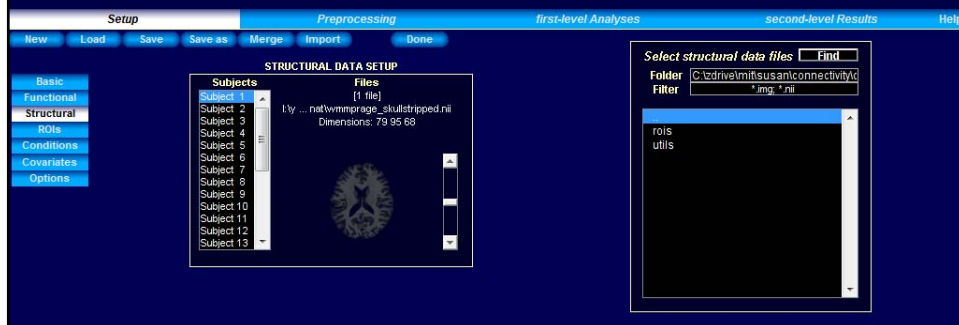
Defines functional data source files

## fMRI connectivity tool (beta)

SETUP

**Structural** : Defines structural data source files

Assumes coregistered to functional volumes –i.e. same orientation; use spm checkreg to check orientation)

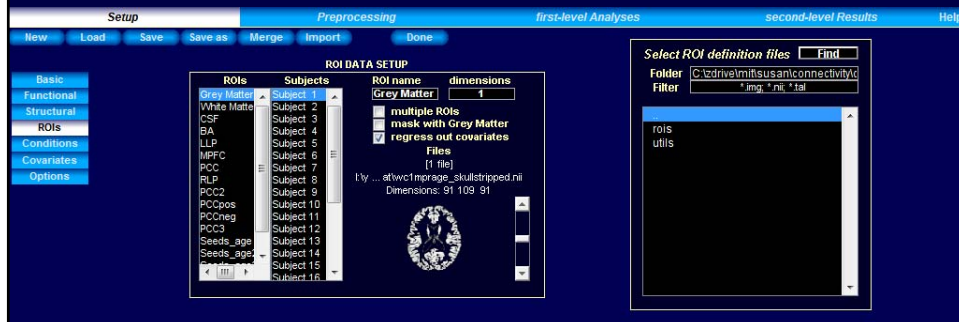


## fMRI connectivity tool (beta)

SETUP

**ROIs** : Define ROI masks (mask files or Talairach coordinate files).

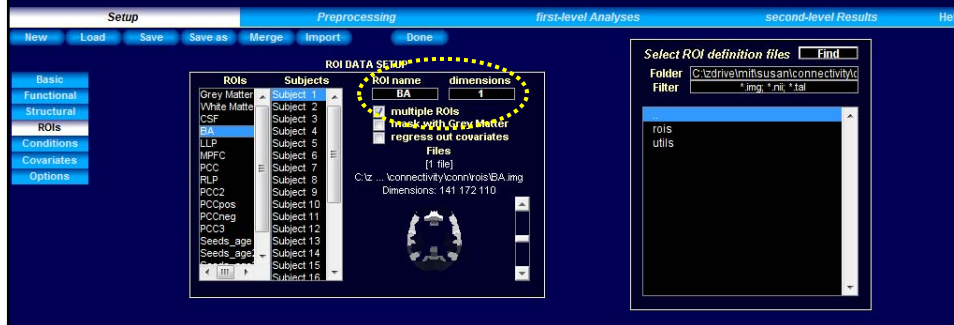
- By default all files in the *rois* toolbox folder (./conn/rois) will be imported as initial regions of interest. To import new ROIs, click below the last ROI listed.
- The special ROIs corresponding to grey matter, white matter, and CSF can be imported here (if they have already been created) or they will be automatically created from each subject structural data.
- Talairach coordinates are defined in mm



SETUP

**ROIs :**

For each ROI a number of functional time-series (*dimensions*) can be extracted: the first time-series is the average BOLD activation within the ROI; the following time-series are the ones associated with each sequential eigenvariate (from a principal component decomposition of the BOLD activation among all voxels within the ROI).

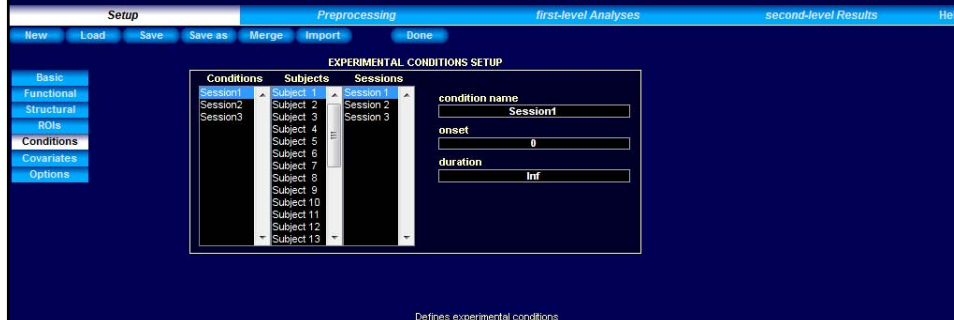


SETUP

**Conditions :** Defines experimental conditions.

(assumes block design; conditions are defined by *onset* and *duration* of each block)

- *Onsets* and *Durations* are in seconds.



## fMRI connectivity tool (beta)

SETUP

**Covariates – first level:** Defines within-subject covariates (e.g. realignment parameters)

(one *.txt* or *.mat* file per subject/session; files should contain as many rows as scans)

Defines 1st level (within subject) covariates

## fMRI connectivity tool (beta)

SETUP

**Covariates – second level:** Defines between-subject covariates (e.g. subject groups)

(each covariate is defined by a vector with as many values as subjects; use 1/0 to define subject groups, or continuous values to perform between-subject regression models)

Defines 2nd level (between subject) covariates

fMRI connectivity tool (beta)

SETUP

**Options:** Defines additional analysis options

Planned analyses: ROI-to-ROI, **Seed-to-Voxel**, Voxel-to-Voxel

Spatial resolution: voxel size for analyses (e.g. 2mm isotropic)

Analysis mask: brainmask.nii or implicit mask (SPM subject-specific 'analysis' mask)

Optional output files

fMRI connectivity tool (beta)

SETUP

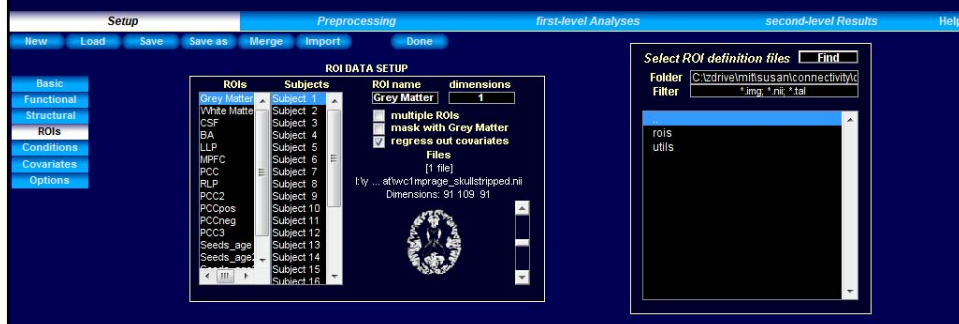
- When finished defining the experiment data press **Done**
  - This will import the functional data, it will also perform normalization & segmentation of the structural data in order to define gray matter/ white matter/ CSF regions of interest if these have not been already defined. Last it will extract the ROIs time-series (performing PCA on the within-ROI activations when appropriate).
  - This process could take between 5-10 minutes per subject.
  - After this process is finished come back to **Setup** to inspect the resulting ROIs for possible inconsistencies.
- a *conn\_\*.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

## fMRI connectivity tool (beta)

SETUP (revisited)

**ROIs** : If these had not been defined previously gray matter, white matter, and CSF masks will have been created now.

(check results; problems may occur when structural data is not reasonably reoriented)



## Steps

Step 1: Setup

Step 2: Preprocess and explore confounds

Step 3: Analyze and view 1<sup>st</sup> level results

Step 4: Define contrasts and view 2<sup>nd</sup> level results

## fMRI connectivity tool (beta)

PREPROCESSING

Define, explore, and remove possible **confounds**.

Any global signal that simultaneously affects otherwise unrelated areas (e.g. physiological noise, subject movement) can act as a confound in functional connectivity analyses.

Setup Preprocessing first-level Analyses second-level Results Help

## fMRI connectivity tool (beta)

PREPROCESSING

Define possible confounds:

By default the system will utilize white matter and CSF BOLD time-series (5 dimensions each), as well as any previously-defined within-subject covariate (realignment parameters) together with their first-order derivatives, and the *main* condition effects (blocks convolved with *hrf*) as possible confounds.

Setup Preprocessing first-level Analyses second-level Results Help

Bone

GLM - DEFINE POSSIBLE CONFOUNDS

Confound: White Matter

derivatives order: 0

dimensions: 5

band-pass filter (Hz): 0.01 0.1

PREVIEW RESULTS

Subject: Subject 1, Session 1

Effect: White Matter

Voxel-to-voxel connectivity (r) (dof=172)

BOLD % variance explained

threshold: 0.5

Define/Edit preprocessing options

## fMRI connectivity tool (beta)

PREPROCESSING

## Define possible confounds:

- User can define at this step these or other possible confounds, and inspect, for each subject and session, the contribution of each confound to the BOLD response (displayed as percentage BOLD variance explained)
- Threshold in the preview window represents r-square values

Setup Preprocessing first-level Analyses second-level Results Help

Done

GLM - DEFINE POSSIBLE CONFOUNDS

Confound: White Matter  
 derivatives order: 0  
 dimensions: 5

band-pass filter (Hz): 0.01 0.1

PREVIEW RESULTS

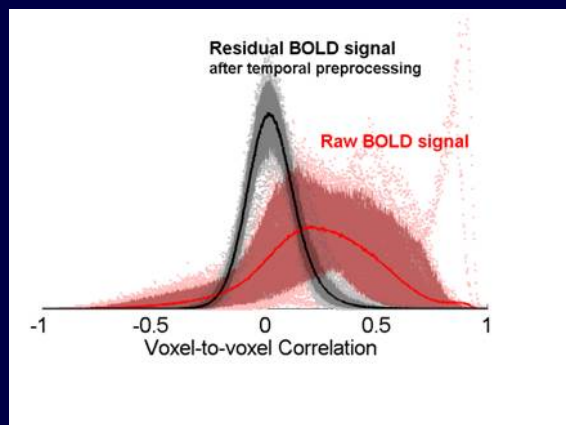
Subject	Sessions	Effect	BOLD % variance explained
Subject 1	Session 1	TOTAL	0.5
Subject 2	Session 2	White Matter	0.1
Subject 3	Session 3	CSF	0.1
Subject 4		Effect of Se	0.1
Subject 5		Effect of Se	0.1
Subject 6		Effect of Se	0.1
Subject 7		Effect of Se	0.1
Subject 8		Effect of Se	0.1

Voxel-to-voxel connectivity (r) (dof=172)

threshold: 0.5

Define/Edit preprocessing options

## Effects of noise reduction on correlation distribution

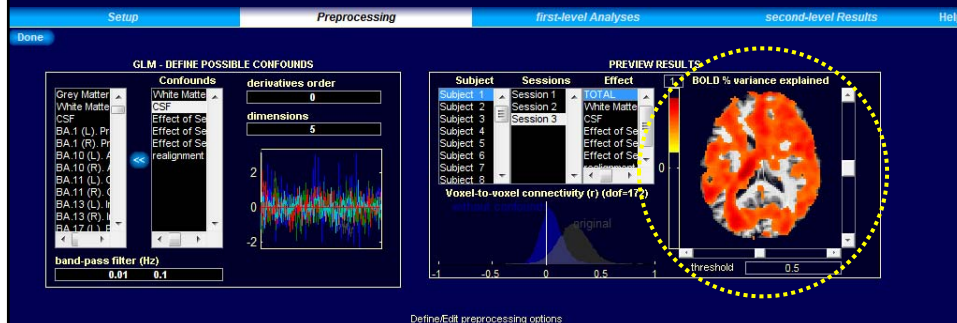


## fMRI connectivity tool (beta)

PREPROCESSING

## Define possible confounds:

... as well the *total* variance explained by all confounds (usually a large percentage of widespread effects, highlighting the importance of this preliminary step)

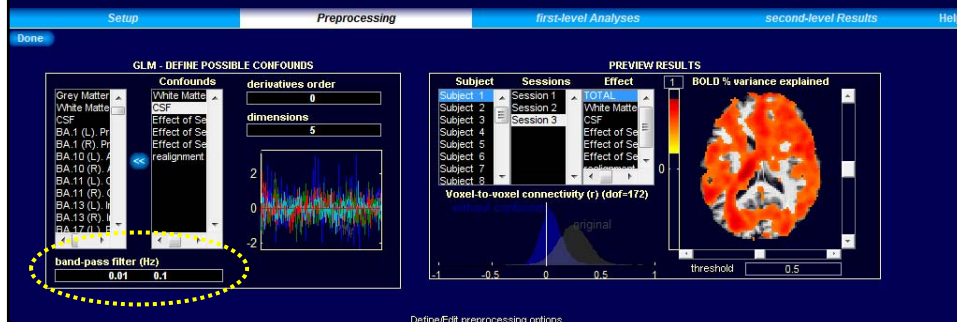


## fMRI connectivity tool (beta)

PREPROCESSING

## Define possible confounds:

- Last the user can also define a band-pass filter at this stage to further reduce the effect of possible confounds or to limit the subsequent connectivity analyses to a given frequency window of interest
- To see a description of an input field, point cursor to the field.



fMRI connectivity tool (beta)PREPROCESSING

When finished defining/exploring the effect of confounds press **Done**. This will remove the effects of the defined confounds on all brain voxels and regions of interest.

This process could take ½ minute per subject.

After this process is finished go to the **Analyses** section

## Steps

Step 1: Setup

Step 2: Preprocess and explore confounds

**Step 3: Analyze and view 1<sup>st</sup> level results**

Step 4: Define contrasts and view 2<sup>nd</sup> level results

fMRI connectivity tool (beta)

ANALYSES

Define and initially explore the functional connectivity of different **sources**.

Setup    Preprocessing    **first-level Analyses**    second-level Results    Help

fMRI connectivity tool (beta)

ANALYSES

**Define sources of interest:**

In this step the user defines the sources (ROIs) for the functional connectivity analyses. Each source can be defined by a single time-series, or it can include several time-series (several dimensions from a single ROI, or first- or higher-order derivatives of the above).

Setup    Preprocessing    **first-level Analyses**    second-level Results    Help

ROI-to-ROI  
Seed-to-Voxel  
Voxel-to-Voxel

GLM CONNECTIVITY - DEFINE SOURCES

Sources: BA 6 (L), Pr; BA 6 (R), Pr; BA 7 (L), S; BA 7 (R), S; BA 8 (L), D; BA 8 (R), D; BA 9 (L), D; BA 9 (R), D; LLP; MFC; PCC; RLP; PCCpos; PCCneg; PCC3

derivatives order: 0  
dimensions: 1

Measure: correlation (R)    Weights: trf    Analyses: all

PREVIEW RESULTS

Subject	Conditions	Sources
Subject 1	Session1	BA 6 (L), Pr
Subject 2	Session2	BA 6 (R), Pr
Subject 3	Session3	BA 7 (L), S
Subject 4		BA 7 (R), S
Subject 5		BA 8 (L), D
Subject 6		BA 8 (R), D
Subject 7		BA 9 (L), D
Subject 8		BA 9 (R), D
Subject 9		LLP
Subject 10		MFC
Subject 11		PCC
Subject 12		RLP
Subject 13		

connectivity

threshold: 0.25

## fMRI connectivity tool (beta)

## ANALYSES

**Define sources of interest:** Functional connectivity analyses can be performed based on *regression* (beta values) or *correlation* (Fisher transformed) measures. In addition when multiple sources are used one can define whether the analyses should focus on *bivariate* or *semipartial* correlation measures (or *bivariate* or *multivariate* regression)

## Linear measures of functional connectivity

**Bivariate regression**  $b = (\mathbf{x}^t \cdot \mathbf{x})^{-1} \cdot (\mathbf{x}^t \cdot \mathbf{y})$

**Bivariate correlation**  $r = (\mathbf{x}^t \cdot \mathbf{x})^{1/2} \cdot b \cdot (\mathbf{y}^t \cdot \mathbf{y})^{-1/2}$

**Multivariate regression**  $\mathbf{B} = (\mathbf{X}^t \cdot \mathbf{X})^{-1} \cdot (\mathbf{X}^t \cdot \mathbf{Y})$

**Semipartial correlation**  $\mathbf{R} = [(\mathbf{X}^t \cdot \mathbf{X})^{-1}]^{-1/2} \cdot \mathbf{B} \cdot [\mathbf{Y}^t \cdot \mathbf{Y}]^{-1/2}$

## Analysis Options in Conn

- **Use Correlation (bivariate)** to study the pairwise connectivity between every voxel of the brain and each source ROI separately (effect sizes represent correlation coefficients)
  - Use Correlation (semipartial) to study the connectivity that is unique to each ROI (that is not mediated by connectivity from the other ROIs)
- Use Regression (bivariate) to study bivariate regression models predicting each voxel BOLD signal in terms of the BOLD signal from each of the ROIs separately (effect sizes represent % changes in BOLD activity at each voxel associated with a 1% change of BOLD activity in the source ROI)
- Use Regression (multivariate) to study multivariate models predicting each voxel BOLD signal in terms of the BOLD signal from all of the ROIs simultaneously

## fMRI connectivity tool (beta)

### ANALYSES

Within-condition weights determines how the different scans within each condition should be weighted when estimating connectivity measures. *None* weights all scans equally, *hrf* weights them with a block-convolved hrf function (incorporating expected hemodynamic delays), and *hanning* weights them using a hanning window (selecting the scans at the center of each block in order to minimize possible border effects)

The screenshot displays the Conn software interface during the 'first-level Analyses' phase. The main window is titled 'GLM CONNECTIVITY - DEFINE SOURCES' and is divided into several sections:

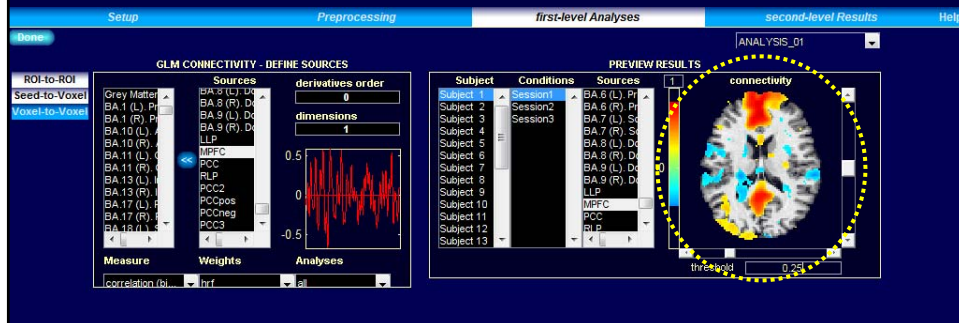
- ROI-to-ROI / Seed-to-Voxel / Voxel-to-Voxel:** A list of brain regions (Sources) including BA 1 (L), BA 1 (R), BA 10 (L), BA 10 (R), BA 11 (L), BA 11 (R), BA 13 (L), BA 13 (R), BA 17 (L), BA 17 (R), BA 18 (L), BA 18 (R), MIPFC, PCC, RLP, PCC2, PCCneg, and PCC3.
- Sources:** A list of brain regions (Sources) including BA 6 (L), BA 6 (R), BA 7 (L), BA 7 (R), BA 8 (L), BA 8 (R), BA 9 (L), BA 9 (R), LLP, MIPFC, PCC, RLP, PCC2, PCCneg, and PCC3.
- derivatives order:** Set to 0.
- dimensions:** Set to 1.
- Measure:** Set to correlation (bivariate).
- Weights:** Set to hrf (highlighted with a yellow dashed circle).
- Analyses:** Set to all.

The 'PREVIEW RESULTS' window shows a connectivity map of the brain with a color scale from 0 to 1 and a threshold of 0.25.

## fMRI connectivity tool (beta)

ANALYSES

- At any time the resulting connectivity maps can be inspected for each subject/condition in the **preview** window (Analyses here are performed in real-time ).
- Threshold represents correlation coefficients, or beta values for regression.



## fMRI connectivity tool (beta)

ANALYSES

When finished defining/exploring the connectivity analyses press **Done**. This will perform the defined analyses for all subjects and allow the user to explore second-level (between subject) results.

First-level results are also exported as .nii volumes (one per Subject/Condition/Source combination) in the *results/firstlevel* folder

This process could take 1 minute per source (depending on number of subjects in the study).

## Steps

Step 1: Setup

Step 2: Preprocess and explore confounds

Step 3: Analyze and view 1<sup>st</sup> level results

Step 4: Define contrasts and view 2<sup>nd</sup> level results

fMRI connectivity tool (beta)

### RESULTS

Define and explore contrasts of interest and **second-level results**

Setup

Preprocessing

first-level Analyses

second-level Results

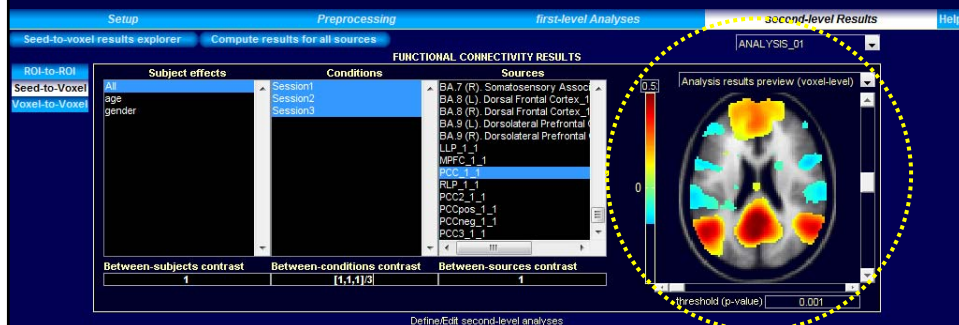
Help

## fMRI connectivity tool (beta)

RESULTS

## Explore second-level results:

Functional connectivity measures are tested at the second-level (between-subject) using random-effect analyses. The results display shows effect sizes (measures defined in the previous step; e.g. bivariate correlation), which can be thresholded using an (uncorrected) false-positive threshold (p-values).



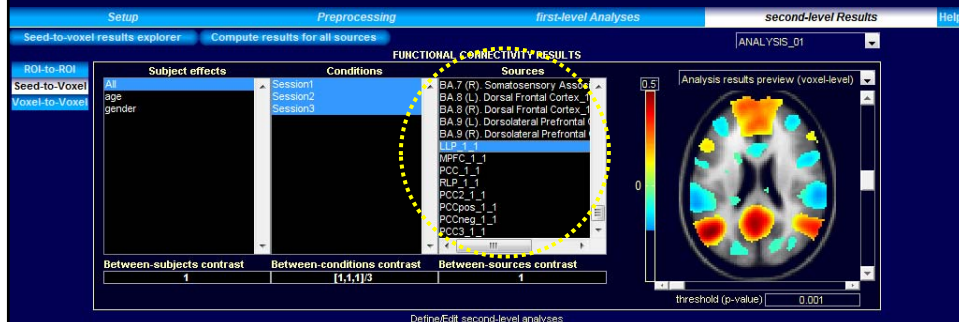
## fMRI connectivity tool (beta)

RESULTS

## Explore second-level results:

Each source connectivity can be tested separately simply selecting the corresponding ROI in the menu

(note: when sources were defined by multiple time-series, source names will follow the convention *ROI\_DimensionNumber\_DerivativeOrder*)

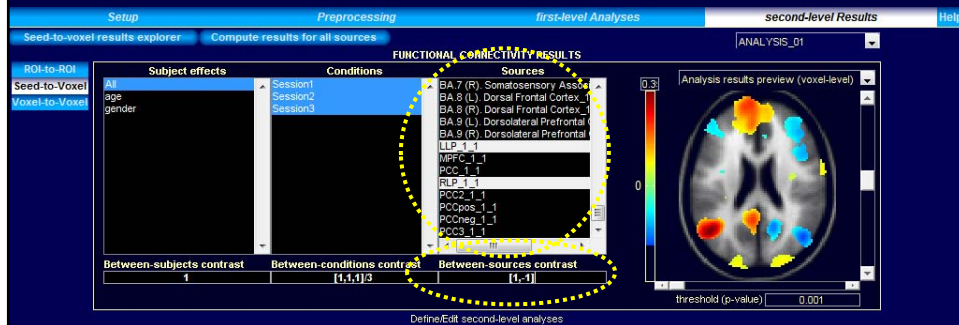


## fMRI connectivity tool (beta)

## RESULTS

## Explore second-level results:

Multiple ROIs/sources can be selected simultaneously in order to aggregate or compare the connectivity results across several ROIs (e.g. to compare the connectivity between LLP & RLP select both sources and enter [1,-1] in the 'between-sources contrast' field)

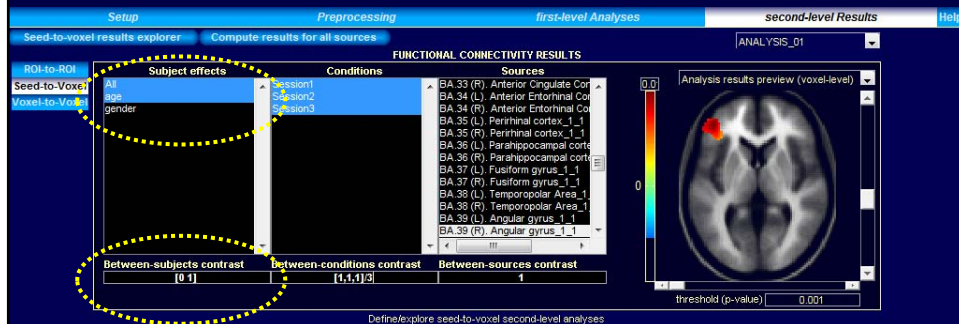


## fMRI connectivity tool (beta)

## RESULTS

## Explore second-level results:

Selecting multiple second-level effects in the *Subject effects* list and defining *Between-subjects contrast* can be used to test more complex second-level models (e.g. regression analyses for age-related connectivity changes)



## fMRI connectivity tool (beta)

### RESULTS

Explore second-level results:

Similarly, selecting multiple conditions in the *Conditions* list and defining *Between-conditions contrast* can be used to test more complex second-level models (e.g. paired t-test for between-condition differences in connectivity)

## fMRI connectivity tool (beta)

### RESULTS

Selecting **Seed-to-voxel results explorer** exports the defined second-level model to SPM (second-level SPM.mat, beta and contrast volumes are saved in the *results/secondlevel/* folder) and it launches a new window that allows you to: explore these results using a combination of **voxel-level thresholds** (based on uncorrected p-values, or FDR-corrected p-values), and **cluster extent thresholds** (based on uncorrected cluster-level p-values, FWE- or FDR-corrected cluster-level p-values); perform one-sided or two-sided tests; export the resulting statistics; create a mask of suprathreshold voxels (e.g. for post hoc analyses); and display the results projected on the brain surface

Coordinates (x, y, z)	N clusters	p-unc	p-FWE	p-FDR	p-FDR	p-FDR	p-FDR
-12 -84 -12	1822	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
-68 -84 -12	742	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
-68 -14 -12	443	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
-12 -76 -48	1242	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
-10 -10 -14	874	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
-12 -84 -18	432	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
-12 -10 -18	78	0.01282	0.01248	0.04383	0.00168	0.00000	0.00000

## Conclusion of 2<sup>nd</sup> level Results

1. Select "All" in the subjects list: *test the connectivity across all subjects.*
2. Select both "GroupA" and "GroupB. Contrast of [1 -1]:  
*This will compare the connectivity between the two groups (t-test / anova)*
3. Select "GroupA" "GroupB" "Behav" with a contrast of [1 -1 0]:  
*This will compare the connectivity between the two groups when controlling for the behavioral measure (ancova)*
4. Select "All" and "Behav" with a contrast of [0 1]:  
*This will test the association between levels of connectivity and the behavioral measure across all subjects (regression)*
5. Select "GroupA" "GroupB" "BehavA" "BehavB" with a contrast of [0 0 1 -1]: *This will compare the behavioral/connectivity association between the subject groups (regression with between-groups interaction term)*

## Homework NYU n=10, 1 session

- *\*Note: Raw Data = 2.9 G,*
- *Data + Analysis Total = 15 G*
- *Script: nyu\_batch.m*
- Optional –add covariates
- `age=[27 21 26 23 22 28 49 23 31 25 27 35 22 49 24 27  
27 22 23 49 32 41 30 25 28];`
- `gender=[2 2 2 2 2 2 1 2 1 1 2 2 1 2 2 1 2 1 1 1 1 2 1 2];`

## RESULTS

If you wish to repeat the same second-level analysis across all of the ROIs/sources select **Compute results for all sources**. This will export the corresponding set of second-level models to SPM (saved in the *results/secondlevel/* folder)

## Specification of 2<sup>nd</sup> level Covariates

“Between-subjects contrast”: in the “Subjects” list will appear all of the defined second-level effects (e.g. subject groups, or subject covariates such as a behavioral measures).

Selecting multiple of these effects will create a second-level GLM that will include only the selected effects.

Between-subjects contrast can then be used to define what aspect of this second-level model one wishes to analyze

## Demos/Tutorials

- Demos: 1. NYU N=25, 3session
- 2. Multi-Group Demo
- 3. 2<sup>nd</sup> Level Correlations

Tutorials 1. Single subject Rest scan  
2. Group Analysis (NYU- n=10)

## THANK YOU!

**Alfonso Nieto Castanon**

Xiaoqian(Jenny)Chai

Tom Zeffiro