

[www.conn-toolbox.org](http://www.conn-toolbox.org)

[www.nitrc.org/projects/conn](http://www.nitrc.org/projects/conn)

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## Overview

CONN is a Matlab-based cross-platform software for the computation, display, and analysis of functional connectivity in fMRI (fcMRI). Connectivity measures include seed-to-voxel connectivity maps, ROI-to-ROI connectivity matrices, graph properties of connectivity networks, generalized psychophysiological interaction models (gPPI), intrinsic connectivity, local correlation, global correlation, inter-hemispheric correlation, independent component analyses (ICA), functional connectivity multivariate pattern analyses (fc-MVPA), and dynamic component analyses (dyn-ICA).

CONN is available for resting state data (rsfMRI) as well as task-related designs. It covers the entire pipeline from raw fMRI data to hypothesis testing, including spatial coregistration, ART-based scrubbing, a *CompCor* strategy for control of physiological and movement confounds, first-level connectivity estimation, and second-level random-effect analyses and hypothesis testing.

### Installing the toolbox:

download and unzip the latest conn\*.zip file from NITRC ([www.nitrc.org/projects/conn](http://www.nitrc.org/projects/conn)), and add the resulting ./conn/ directory to the matlab path (in Matlab's *File-Set path*)

see <https://web.conn-toolbox.org/resources/installation> for additional details

### Requirements:

SPM12 or above

Matlab R2013b or above (base Matlab only, no toolboxes required)

### To start the toolbox:

On Matlab command window, type : conn

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

### Updating to latest version:

On the CONN gui click on *Help->Update*

Note: a standalone version of CONN for linux64 systems (precompiled including both SPM and CONN) that does not require Matlab installed or a Matlab license can be downloaded from [www.nitrc.projects/conn](http://www.nitrc.projects/conn).

*OS-specific notes:* On Mac OS/X use “ctrl-click” instead of “right-click” to bring contextual menus in the CONN gui; If the default GUI display fonts are too small click on *Tools->GUI settings* to change the GUI font size (your choice of font sizes will be kept across CONN sessions and across toolbox updates); When using VNC to connect remotely to Linux machines type in Matlab’s command window *opengl software* right after starting Matlab if you experience VNC crashes when displaying 3d renderings or when printing;

## General

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

**Functional data.** Either resting-state or task designs can be analyzed.

**Structural data.** At least one anatomical volume for each subject (this is used mostly for plotting purposes but also to derive the gray/white/CSF masks used in the aCompCor confound removal method)

**ROI definitions.** A series of files defining seeds of interest. ROIs can be defined from mask images, text files defining a list of MNI positions, or multiple-label images. The toolbox also provides a series of default pre-defined regions of interest that will be loaded automatically. These include a series of seed areas useful for investigating resting state connectivity –regions characterizing DMN, dorsal attention network, executive control network, etc., as well as a complete brain parcellation including 91 cortical areas and 15 subcortical areas from the FSL Harvard-Oxford Atlas as well as 26 cerebellar areas from the AAL atlas. See the *conn/utils/otherrois/* folder for additional/optional ROI files, including Brodmann areas, large-voxel parcellations, etc.

**tip:** To try out the toolbox in the absence of any data, select *Help. Sample Data* in the CONN gui in order to automatically download and process the NYU test-retest dataset

The toolbox operation is divided in four sequential steps:

**Setup:** Defines basic experiment information, data locations, regions of interest (seeds), temporal covariates, and second-level models. Optionally, perform functional and anatomical preprocessing steps if necessary, including realignment, slice-timing correction, coregistration/normalization, segmentation, outlier identification, and smoothing.

**Denoising:** Define, explore, and remove possible confounds in the BOLD signal, including motion, physiological and other noise sources.

**Analyses:** Perform first-level analyses. Define the seeds of interest and explore the functional connectivity of different sources separately for each subject. Define ICA, voxel-to-voxel analyses, dynamic analyses, etc.

**Results:** Perform second-level analyses. Define group analyses and perform population-level inferences from the resulting connectivity measures of each first-level analysis.

Each of these steps can be defined interactively using the toolbox GUI or programmatically using scripts and *conn\_batch* functionality. In addition, and both when using the GUI or batch processes, all of the analyses can be performed locally on a single computer, or they can be distributed among multiple computers in a cluster environment. Currently CONN supports Sun Grid Engine (SGE), Open Grid Scheduler (OGS/GE) and other Grid Engine implementations, PBS/Torque, LSF, and Slurm batch queuing systems. The following sections describe the experiment definition and analysis steps when using the GUI on a local computer in more detail. See *Help.Documentation.Info:batch processing* for additional information on batch scripts, or see *Help.Documentation.Info:grid computing* as well as *Tools.Grid settings* for additional information on cluster environments.

### General help resources

In the CONN GUI select **Help.Search** to easily search through a database of user questions/answers, select **Help.Updates** to check and update CONN to the latest available release, select **Help.Documentation** for general documentation, and **Help.Web** for additional resources.

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

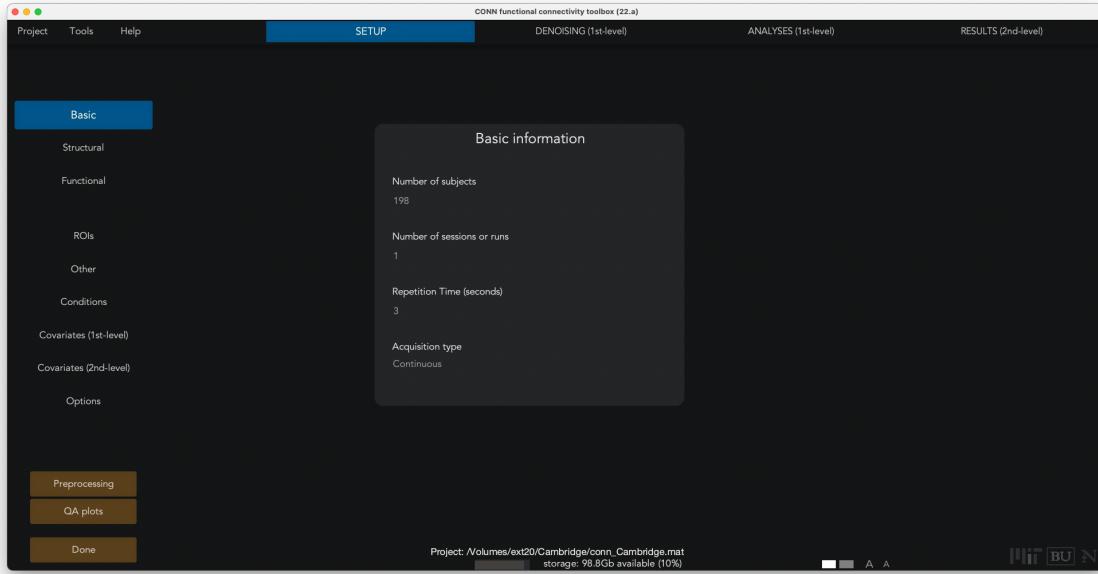


Click on **Project.Load** to load an existing CONN

Or click on the **Project.New (blank)** button to start a new project

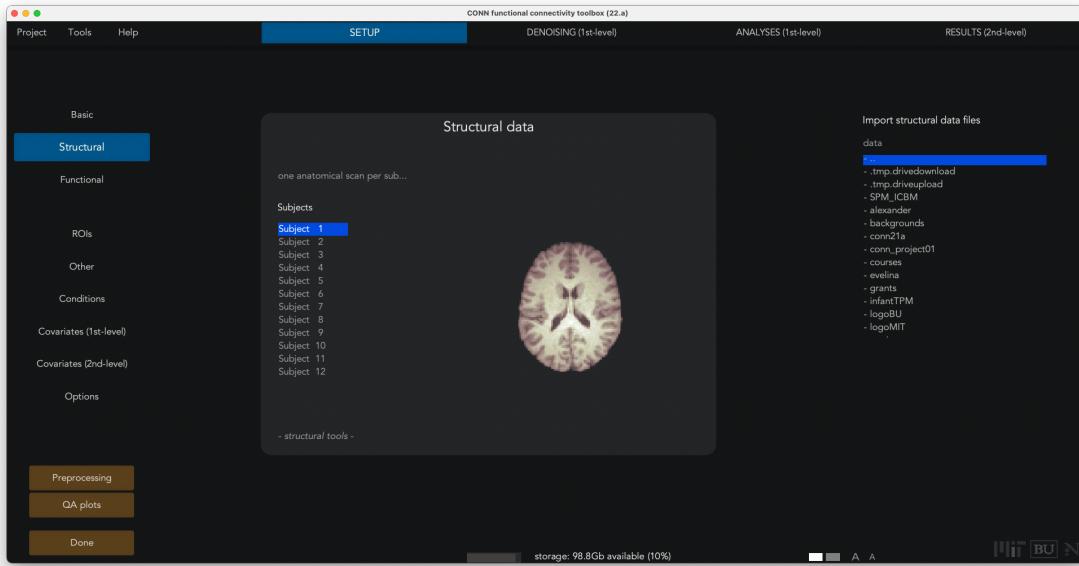
Note: If the data has initially been defined/analyzed in SPM you can skip several of the steps explained below to import your data by using instead the automatic **Import** functionality. If you want to use this functionality select the option **Project.New (import)** instead of **Project.New (blank)** when creating your project, and then choose the source and format of your input data (DICOM files, a set of SPM first-level analyses, a BIDS dataset, or a fMRIPrep preprocessed dataset) and CONN will automatically import several relevant data files automatically from those sources (and you can then proceed to manually import any additional files or data if necessary).

## Basic experimental info Setup



Click on the **Basic** button on the left side, enter experiment information (Number of subjects, TR, number of sessions per subject, and acquisition type). If the same number of sessions was acquired for every subject enter a single number in *Number of sessions*, otherwise enter the subject-specific values (one value per subject). If your data was acquired continuously select '*continuous*' in *Acquisition type*, and if you used sparse sampling select '*sparse*' (this will skip hrf-convolution when computing task effects). If your data was acquired using different TR values, enter *NaN* in the TR field and CONN will read this information from the *RepetitionTime* field of a sidecar .json file associated with each functional run NIFTI .nii file.

## Structural files Setup



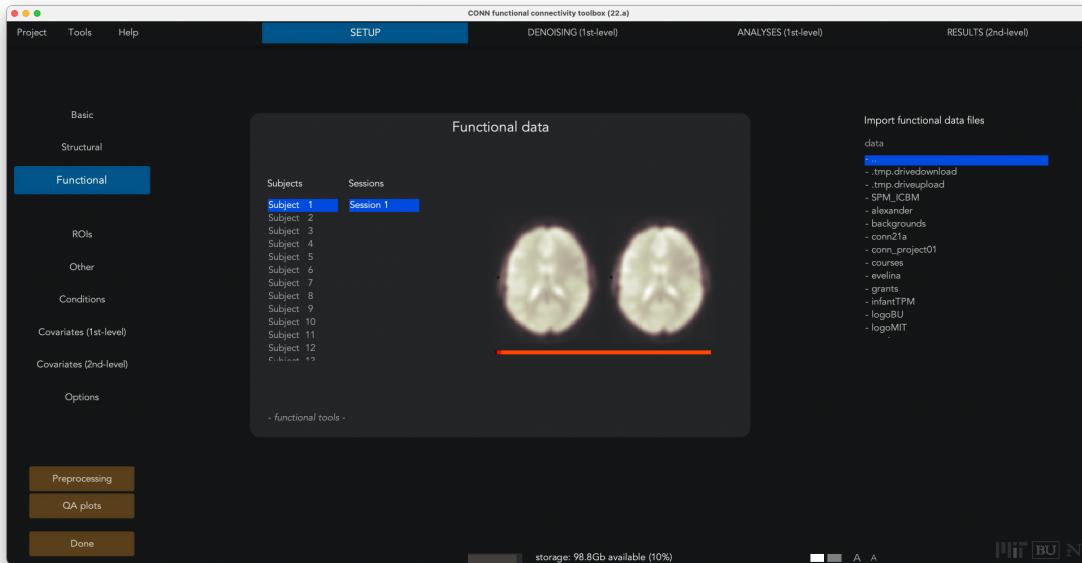
Click on the **Structural** button on the left side to load the structural images. Click sequentially on each subject and select the associated anatomical image (one anatomical volume per subject). If you have multiple anatomical volumes per subject (e.g. one per session/scan), select '*Multiple anatomical scans per subject*' and then enter the corresponding session-specific anatomical volumes. Anatomical volumes should typically be coregistered to the functional and ROI volumes for each subject (e.g. if using MNI-space normalized functional volumes you should enter here the normalized anatomical volume). Alternatively you may enter the raw (subject-space) anatomical volumes and transform them to your desired target space (e.g. MNI) using '*structural tools: individual preprocessing step*' or '*Preprocessing*' (see *Notes on Structural, Functional, and ROI files coregistration* section below).

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

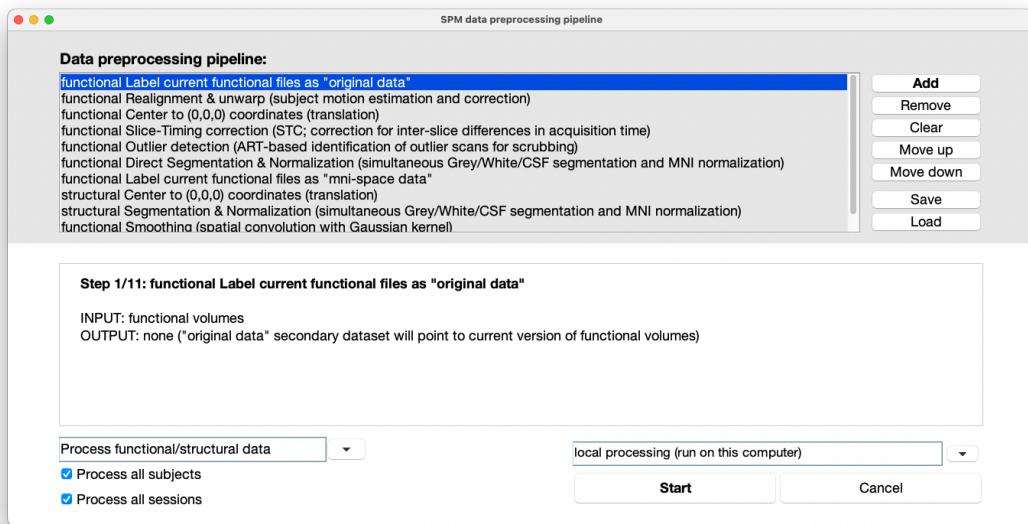
If your structural volumes have been preprocessed using Freesurfer (<https://surfer.nmr.mgh.harvard.edu/>), you may enter here the T1 or brainmask volumes in the subject-specific *mri* folder generated by Freesurfer. The toolbox will identify the associated *surf* folder containing the estimated cortical surfaces and it will display at this point a 3d-rendering of the pial surface (and this will allow you to obtain connectivity measures on the cortical surface; see *Options Setup* section below).

## Functional files Setup



Click on **Functional** button on the left side, from the right side panel, select the functional images (4D NIFTI .nii files, or multiple 3D NIFTI .nii or ANALYZE .img files). 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 (realigned and smoothed), as well as coregistered with the structural and ROI volumes. If they are not, you may select ‘functional tools: individual preprocessing step’ or ‘Preprocessing’ to perform the appropriate preprocessing steps (see *Notes on Structural, Functional, and ROI files coregistration* section below). Clicking on ‘functional tools: check registration’ displays the coregistration between the functional and structural volumes for the selected subjects/sessions.

## Preprocessing functional & structural data



After defining your raw Functional and Structural data, using *Setup.Preprocessing* you may run any combination of preprocessing steps (e.g. realignment, normalization, segmentation, outlier detection, etc.). Select individual spatial preprocessing steps or select one of the provided default pipelines (defaultMNI for analyses in MNI-space, and defaultSS for analyses in subject-space or surface-based analyses), add, remove, or resort individual steps if you wish to better tailor it to your data or planned analyses, and then click ‘Ok’ to run the entire sequence of spatial preprocessing steps on all of the defined subjects or on a subset of subjects. For reference, CONN’s *defaultMNI* pipeline consists of the following steps: functional realignment and unwarping, slice-timing correction, outlier detection, structural segmentation&normalization, functional normalization, and smoothing. If your functional and structural data has been already preprocessed using SPM or other software you may simply skip all or any unnecessary preprocessing steps here. See [www.conn-toolbox.org/fMRI-methods/preprocessing-pipeline](http://www.conn-toolbox.org/fMRI-methods/preprocessing-pipeline) and “*help conn\_batch*” for details about all of the different preprocessing steps available in CONN

### Notes:

Typically, after selecting all structural, functional, and ROI files in the Setup step, and further applying any additional spatial preprocessing if necessary, all of the structural, functional, and ROI files should be coregistered to each other (in the same space; e.g. MNI-space)<sup>1</sup>. This will happen, for example, when using CONN’s initial spatial preprocessing pipeline (*defaultMNI*), which will result in both functional and structural files being in MNI-space (note that all of the ROIs provided in the toolbox conn/rois folder are also defined in MNI-space).

In some cases you might want to relax this condition and use structural/functional/ROI files that might not be all in the same space (e.g. using subject-specific ROI files, surface-based analyses, etc.). The toolbox uses separate ROI-level and voxel-level pipelines, and the tables below show typical configurations of Structural/Functional/ROI files that one would select depending on the desired analysis option within each of these pipelines. For example, if you want to perform volume-based analyses on MNI-space for voxel-level analyses, but use subject-specific ROIs for ROI-level analyses, you will only need to have the functional files coregistered to the structural files (both normalized in MNI-space), and separately the functional files (ROI extraction option) coregistered to the ROI files (typically in subject-space).

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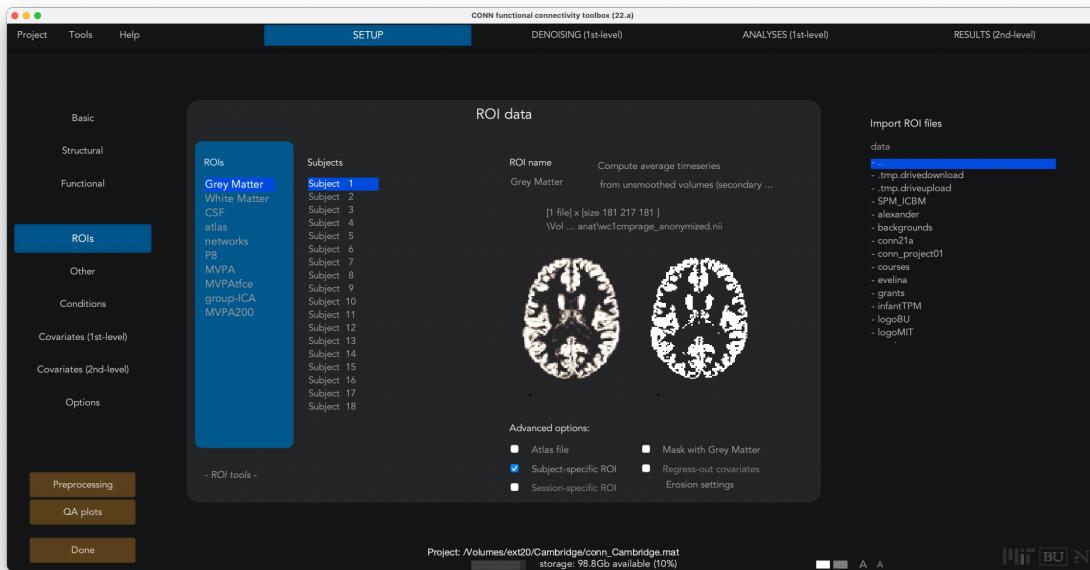
<sup>1</sup> although not necessarily having the same dimensions or resolution (i.e. coregistered, but not necessarily resliced to the same reference space)

<i>Voxel-level analyses</i>	<i>Volume-based analyses in MNI-space</i>	<i>Surface-based analyses</i>
<i>Functional files</i>	Normalized and smoothed volumes (e.g. <i>swauFunct.nii</i> )	Subject-space non-smoothed volumes (e.g. <i>auFunct.nii</i> )
<i>Structural files</i>	Normalized structural (e.g. <i>wTl.nii</i> )	Subject-space structural (e.g. <i>mri/Tl.mgh</i> )

<i>ROI-level analyses</i>	<i>MNI-space ROIs</i>	<i>Subject-specific ROIs</i>
<i>Other files (dataset #1)</i>	Normalized non-smoothed volumes (e.g. <i>wauFunct.nii</i> )	Subject-space non-smoothed volumes (e.g. <i>auFunct.nii</i> )
<i>ROI files</i>	Normalized-space (e.g. <i>wc1_Tl.img</i> )	Subject-space ROIs (e.g. <i>c1_Tl.img</i> )

If your structural or functional files are not in the desired space or they have not been yet spatially preprocessed, you may (during the Setup step) apply any combination of structural segmentation, structural segmentation&normalization, functional removal of intial scans, functional realignment, functional realignment&unwarp, functional realignment&unwarp&B0correction, functional slice timing correction, functional coregistration to structural volumes, functional normalization, functional outlier detection (ART-based scrubbing), and functional smoothing steps to your structural/functional files using the *tools: individual preprocessing step* menu on each of the *Structural* and *Functional* tabs, or from *Setup.Preprocessing:*

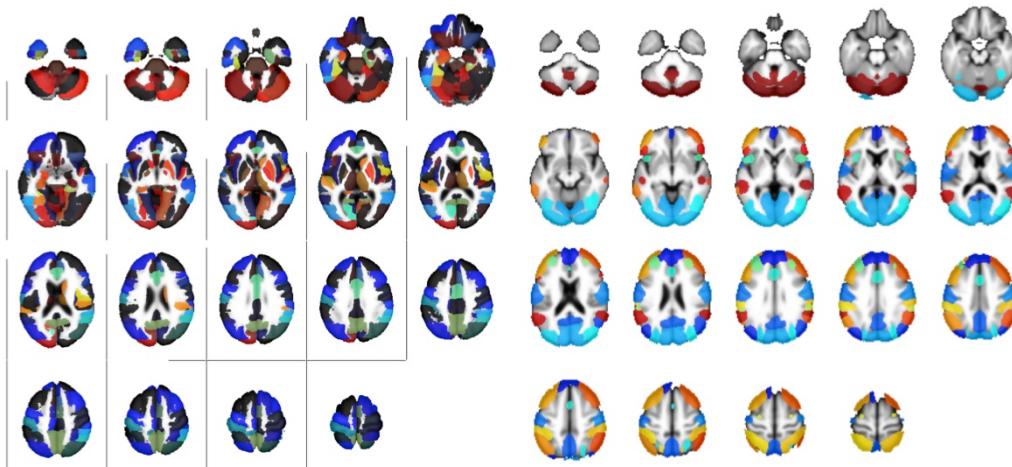
## ROI files Setup



Click on the **ROIs** button on the left side to load ROI mask files (.img or .nii volumes), MNI coordinate files (.tal text files), or atlas files (.nii files with multiple labels, or 4d files with multiple masks). ROI files can be assigned separately to each subject (for subject-specific ROIs) or commonly across all subjects (normalized-space ROIs). From each ROI, CONN may extract an average BOLD signal timeseries within the ROI voxels (*extract average timeseries* option), multiple timeseries from a Principal Component decomposition of the BOLD signal within ROI voxels (*extract PCA decomposition* option), or a weighted sum of the BOLD signal within ROI voxels (weighting each voxel timeseries by a different factor; *extract weighted sum timeseries* option). Also you may indicate which dataset, among potentially multiple alternative functional datasets, each ROI BOLD signal is to be computed from ('*from functional dataset #*' field; see ***Other files Setup*** section below).

Running the structural or functional segmentation *Preprocessing* step will automatically fill-in the first three ROIs in this tab (Gray/White/CSF) with the corresponding subject-specific masks. By default all new projects in CONN are set up to extract a Principal Component decomposition from both the White and CSF regions which can later be used during the *Denoising* step to implement aCompCor (removal of White/CSF noise components).

When starting a new project all files in the *rois* toolbox folder (./conn/rois) will be imported as initial regions of interest. This folder includes: 1) **atlas.nii/.txt/.info**, an atlas of cortical and subcortical areas from the FSL Harvard-Oxford Atlas, as well as cerebellar areas from the AAL atlas; and 2) **networks.nii/.txt/.info**, an atlas of a few commonly used networks and areas (e.g. Default Mode MPFC/PCC/RLP/LLP areas)



Default ROIs. atlas.nii (left, 132 ROIs) and networks.nii (right, 31 ROIs across 8 networks).

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 or simply uncheck the *Subject-specific ROI* option before selecting a single ROI file.

Select “*Subject-specific ROI*” and/or “*Session-specific ROI*” to specify whether you would like to enter different ROI files for each subject and/or each session.

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)

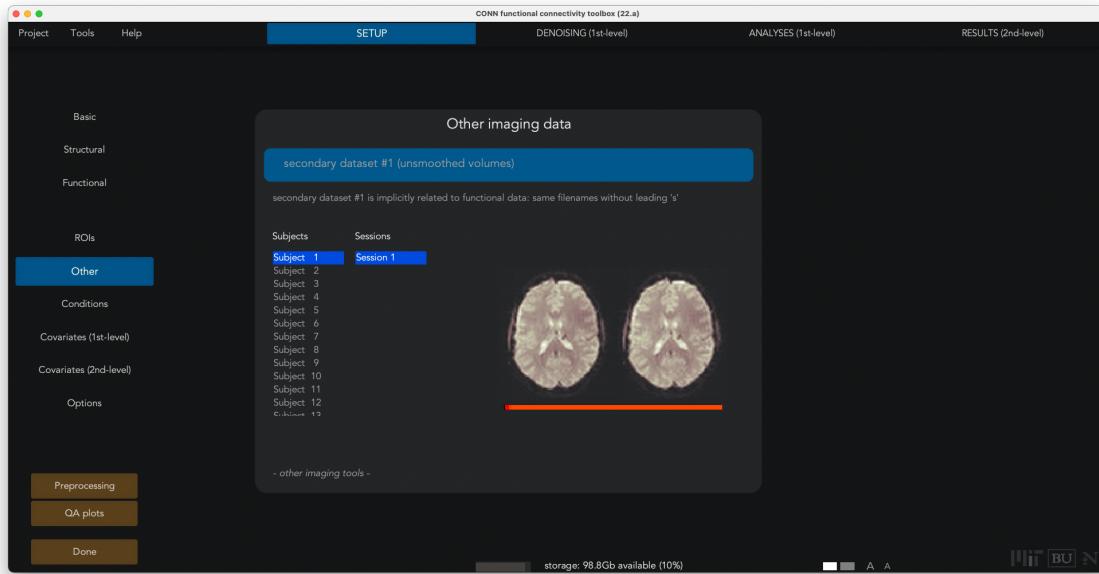
ROI files should be coregistered to the provided structural and functional volumes of this subject (they could be defined in normalized space, or they could be defined in subject-space). Click on *ROI tools: check registration* to display the coregistration between the selected ROI(s) and the structural or functional volumes (see *Notes on Structural, Functional, and ROI files coregistration* section below for additional options).

The default dimensions (number of PCA components to be extracted) for each ROI can be changed when selecting the *extract PCA decomposition* option. In the following steps (denoising, 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 by 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 when selecting the *regress-out covariates* option).

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).

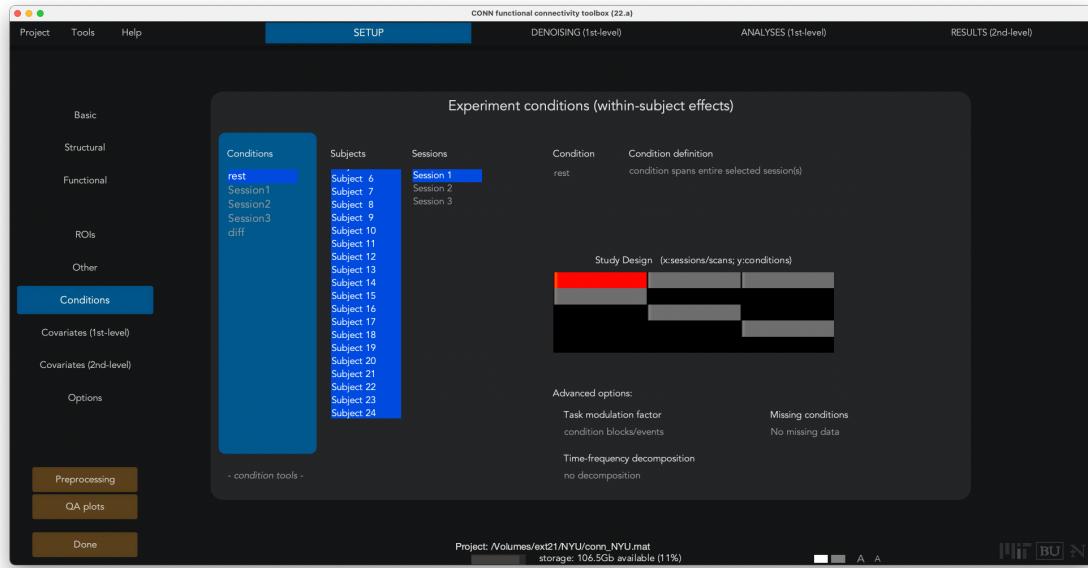
## Other files Setup



Click on **Other** button on the left side, from the middle panel select an existing secondary dataset that you would like to import or select '*New dataset*' to define a new secondary dataset. Secondary datasets are where any additional subject- and session- specific image files that may be useful or relevant to your study are defined. For example, if you wish to define an alternative set of functional volumes that you would like to be used as sources when extracting ROI-level BOLD signal estimates for some particular ROI, that dataset can be defined in this tab, assigned a label, and then in the Setup.ROIs tab you would only have to specify which particular ROI(s) you would like to extract data from this particular dataset.

By default the toolbox will automatically define a dataset named '**unsmoothed volumes**' pointing to the unsmoothed functional volumes (using the SPM-convention for unsmoothed volumes, same filenames without the initial 's' in the file name) and this dataset will be the one used unless specified otherwise when extracting ROI-level BOLD signal timeseries for all ROIs, in order to avoid "spillage" from nearby regions (see ***ROI files Setup*** section above). You may also create a new secondary dataset named '**VDM**' to define subject and session-specific .vdm files for susceptibility distortion correction which can later be used by the realign&unwarp preprocessing step. A secondary dataset named '**FMAP**' can also be created to enter fieldmap files for each subject and session, which can later be used by the 'creation of VDM files' preprocessing step to automatically compute the above .vdm files. A secondary dataset named '**TPM**' can also be used to enter subject-specific TPM (Tissue Probability Maps) which can later be used by the functional/structural normalization preprocessing steps. Other secondary datasets may be arbitrarily created to store different versions of the functional data. For example, the default preprocessing pipeline in CONN will create a secondary dataset named '**original data**' pointing to the original functional data (before running any preprocessing steps), and a dataset named '**MNI-space data**' pointing to the data in MNI-space (before smoothing). If at some point you need to revert your functional data to a previous state (e.g. original data) simply select the option '*move datasets*' and transfer the '**original data**' dataset to the main functional dataset.

## Rest or task conditions Setup



Click on the **Conditions** button on the left side to define your experimental conditions. CONN will typically compute separate connectivity measures for each individual condition that you define in this tab. Here you specify how each individual condition is defined (is it associated with BOLD data from one or multiple runs? Does it cover the entire run or only some blocks or events during the run? etc.)

Add any new condition to the ‘conditions’ list by clicking on the ‘+’ button there. Then enter the name of your condition in the ‘Condition’ field, and then select a combination of subjects and sessions in the ‘Subjects’ and ‘Sessions’ lists and specify how this condition is defined for these subjects/sessions:

Select ‘***condition spans entire selected session(s)***’ if the condition spans the entire duration of these session(s), e.g. during a resting state run

Select ‘***condition is not present in selected session(s)***’ if the condition is not present at any time during these session(s), e.g. in a pre- post- design, the ‘pre’ condition is not present in the second functional session, and the ‘post’ condition is not present in the first functional session

Select ‘***task designs: condition present at blocks/events during these session(s)***’ for task designs. In this case you will need to then specify the onsets and durations of the blocks or events associated with this condition during these session(s). See the table below for a few examples of condition onset/duration specification. For complex designs, condition information can also be imported using the ‘functional tools. Import condition info from text file(s)’ menu from BIDS \_events.tsv files, SPM .mat files, or custom .csv or .txt files (see *help conn\_importcondition* for additional details)

Select ‘***hierarchical designs: condition defined as a function of other conditions***’ to define second- or higher- order conditions in a multi-level design. In this case you will need to specify how this (output) condition is defined as a function of other (input) conditions. Available options are: average across input conditions; standard deviation across input conditions; minimum across input conditions; maximum across input conditions; regressor in linear model fitting input conditions; and custom function across input conditions. These options are particularly useful in the context of non-linear relationships between the input and output conditions. For linear relationships it is simpler to define the specific between-conditions contrast later in the second-level results tab, without the need to manually define those contrasts at this point.

Additional options: Right-click on the *conditions* list to delete or replicate a condition; select *condition tools* to enter all condition information simultaneously from a text file, or to copy or move a condition to the first-

level covariates list (e.g. when you are not interested in obtaining task-specific measures of functional connectivity, but want instead to use Fair et al. method for estimating resting-state connectivity from block- or event-related designs). Optionally, you may also define a temporal modulation factor (in *condition-specific temporal modulation*; this defaults to a timeseries defined by hrf-convolution of the condition blocks/events) in order to perform gPPI analyses for task-related designs, or analyze potential temporal-modulation in fcMRI measures. You may also enter for each condition a condition-specific band-pass filter (in *condition-specific filter*) as a way to explore potential frequency- modulation of fcMRI measures<sup>2</sup>.

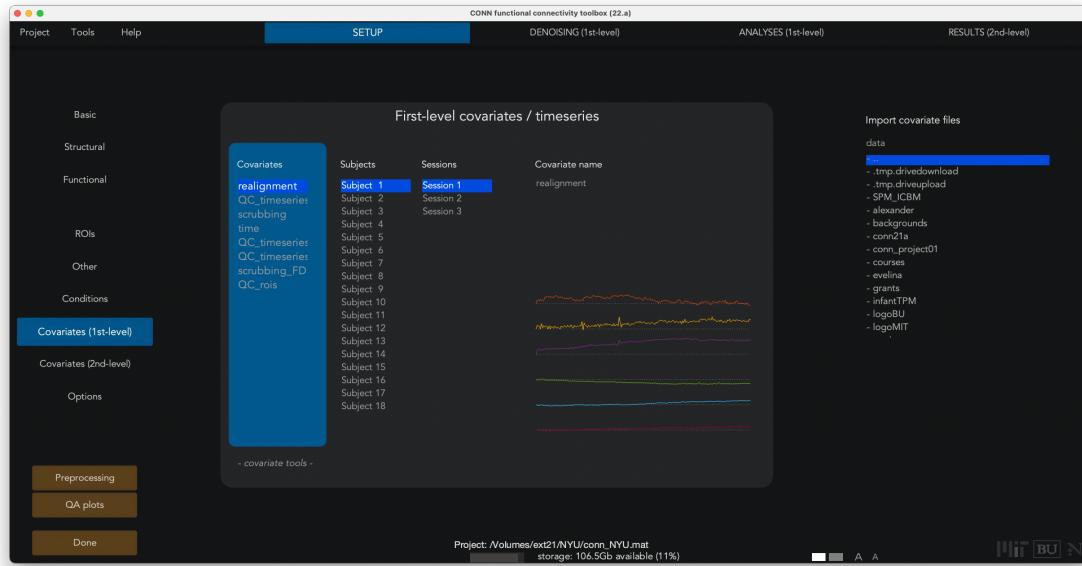
	Condition name	Session 1		Session 2	
		Onset	Duration	Onset	Duration
<i>Resting state, single condition</i>	rest	0	inf	0	inf
<i>Resting state, pre-post- design</i>	pre	0	inf	[ ]	[ ]
	post	[ ]	[ ]	0	inf
<i>Block design (30s blocks, 10s silence)</i>	taskA	0 80	30	40 120	30
	taskB	40 120	30	0 80	30
	rest	30 70 110 150	10	30 70 110 150	10

Condition design examples

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<sup>2</sup> If you want to explore frequency-dependent variations in fcMRI measures, but do not wish to specify a series of frequency filters manually, you may select *filter-bank* in the *Temporal filter* field, and enter there the desired number of frequency filters. This will partition the frequency band defined in the *Denoising* step into n equally-sized frequency regions, and it will automatically create one condition associated with each of these frequency bands. This allows you to use between-condition effects/contrasts as a way to analyzing potential frequency-dependent differences in any of your fcMRI measures.

## First-level (within-subjects) covariates Setup



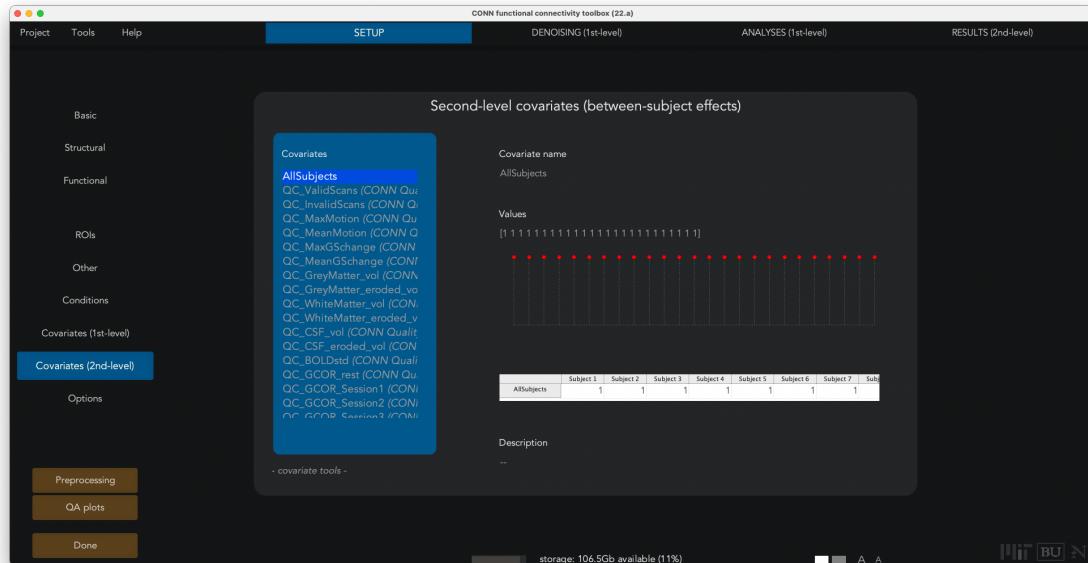
Click on the **Covariates:First-level** button to define first level covariates such as realignment parameters to be used in the first-level BOLD model. For each covariate select a .txt or .mat file (for each subject and session) containing the covariate time series (e.g. select the realignment parameters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

If you want to obtain aggregated subject-level measures of some of your first-level covariates (e.g. to compute the maximum amount of movement for each subject, or the number of outlier scans for each subject) you may do so by selecting each first-level measure in the *covariates* list and clicking on *covariate tools: compute summary measures*.

Running some of the functional *Preprocessing* steps will automatically create or fill-in several first-level covariates in this tab. In particular, running the outlier scan identification *ART-based scrubbing* step will automatically create a first-level covariate named '**scrubbing**' containing the offending scans for each subject/session (this variable can later be used during the *Denoising* step to perform scrubbing), and running any of the *functional realignment* steps will automatically create a first-level covariate named '**realignment**' containing the 6 rigid-body parameters characterizing the estimated subject motion for each subject/session (this variable can also be later used during the *Denoising* step to perform regression of residual movement-related effects). Also during ART preprocessing step a covariate named '**QC\_timeseries**' is created containing two timeseries (BOLD global signal changes, in z-score units, and framewise displacement, in mm units) that can be used for computing other quality control measures (e.g. using the '*covariate tools. Compute new/derived second-level covariates*' menu) and/or for later changing the choice of outlier thresholds (using the '*covariate tools. Compute new/derived first-level covariates*' menu and recomputing the '*scrubbing*' first-level covariate).

## Second-level (between-subjects) covariates Setup



Click on the **Covariates:Second-level** button to define groups and subject-level regressors (e.g. behavioral measures). Click on the empty space below the last variable in the *Covariates* list to add a new variable. For each variable enter the corresponding subject values in the *values* field (one number per subject). Use 1/0 to define subject groups, or continuous values for between-subject regression models. Missing values may be indicated using the special symbol *Nan* (second-level analyses that contain a variable with missing values will automatically disregard the corresponding subjects' data). Some examples of second-level covariates definitions are below:

Name	Values	comment
AllSubjects	1 1 1 1 1 1 1 1 1 1	Used to identify the entire group of subjects
Patients	1 1 1 1 1 0 0 0 0 0	Used to identify a subset/group of subjects
Controls	0 0 0 0 0 1 1 1 1 1	
Age	23 13 19 25 90 66 12 83 74 72	Used for regression analyses or to control for covariates of no interest
Age*Patients	23 13 19 25 90 0 0 0 0 0	Used for regression analyses on subset/group of subjects
Age*Controls	0 0 0 0 0 66 12 83 74 72	(or covariate-by-group interactions)
Severity	3 5 4 3 1 3 nan 2 1 nan	Nan's are used to indicate missing-values in a covariate
Outliers	0 0 0 0 nan 0 0 nan 0 0	Nan's can also be used to explicitly remove individual subjects from an analysis

## Second-level covariates example

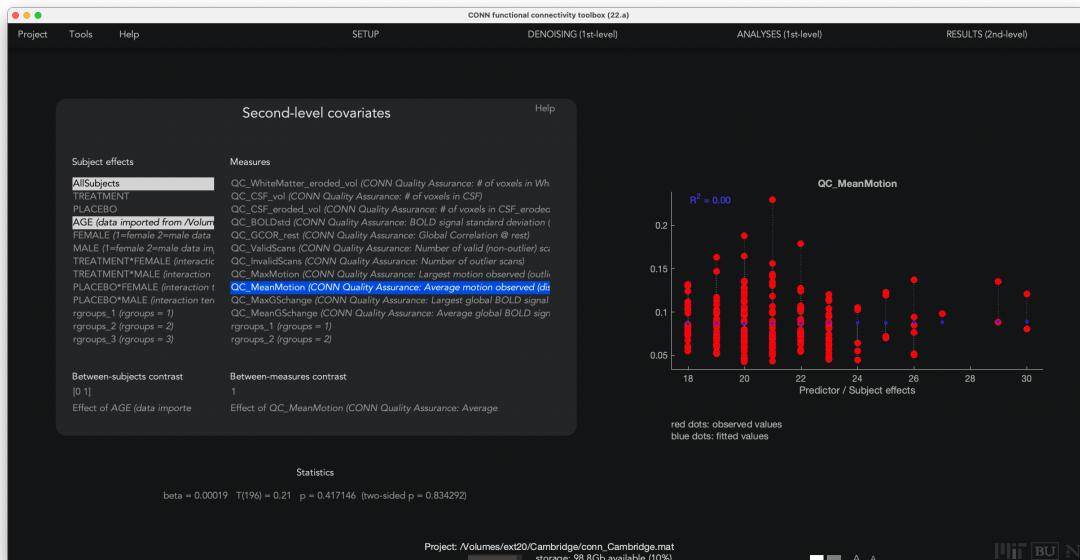
**Tip:** In addition to simple lists of numbers like the examples above, the *values* field will also accept any valid Matlab syntax (e.g. `[ones(1,5) zeros(1,5)]` or `(1:10)<=5` would produce the same values as the *Patients* example above). You may also refer to variables in Matlab workspace by name, or refer to other already-defined second-level covariates by name (e.g. in the example above the covariate *Controls* could also be defined simply entering in the values field *AllSubjects -Patients*).

By default the variable '**AllSubjects**' is always created when starting a new project. In addition, running some of the *Preprocessing* or *Denoising* steps will automatically create or fill-in several second-level covariates in

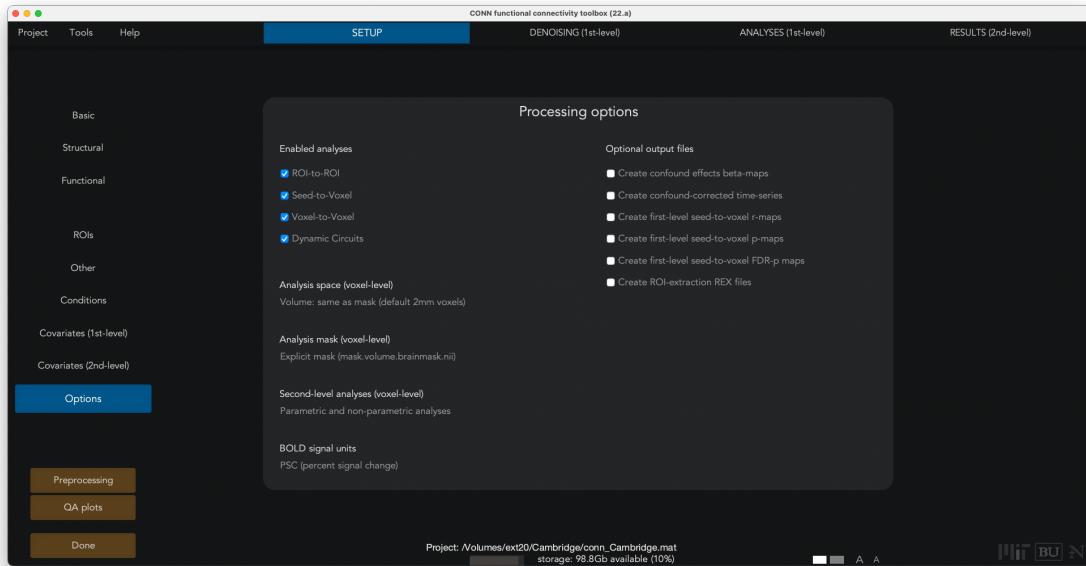
this tab. In particular, running the outlier scan identification *ART-based scrubbing* step will automatically create second-level covariates named '**QC\_MeanMotion**', '**QC\_MaxMotion**', '**QC\_MeanGSCchange**', '**QC\_MaxGSCchange**', '**QC\_ValidScans**', and '**QC\_InvalidScans**' containing the mean (across valid scans only) and maximum inter-scan movement (framewise displacement), the mean (across valid scans only) and maximum global signal change, the number of valid & invalid scans observed for each subject/session. Similarly, running the *Denoising* step will automatically create second-level covariates named '**QC\_BOLDstd**', '**QC\_GCOR**', and '**QC\_dof**', containing the BOLD signal standard deviation (averaged across all voxels), global correlation index (average correlation coefficient between every pair of voxels across the entire brain), and effective degrees of freedom after denoising, for each subject/condition. Any of these variables can later be used during the *Second-level analyses* step for additional control over potential between-subject differences in motion/outliers, or to help identify and remove potential outlier subjects.

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). In addition, using the **covariate tools** menu, you can also import covariates from a file (.txt, .csv, .tsv, or .mat files), discretize an existing covariate into new dichotomous covariates (e.g. from a ‘group’ covariate with three levels, it will create new ‘group1’, ‘group2’, and ‘group3’ covariates), define new covariates characterizing the interaction between other existing covariates (from a ‘age’ and ‘gender’ covariate it will create a new ‘age\*gender’ interaction covariate), or orthogonalize a covariate against one or several of your other covariates (e.g. you may ‘center’ a between-subjects variable by orthogonalizing it against the constant –all subjects- term).

**GUI tip:** Use Tools->Calculator on the main CONN gui to visualize and analyze your current 2<sup>nd</sup>-level covariates (e.g. look at potential between-group differences in subject movement, levels of association between your different subject-level measures, etc.). The way to define 2<sup>nd</sup>-level analyses in the calculator is the same as that of the main CONN gui 2<sup>nd</sup>-level results view (see Step 4 below)



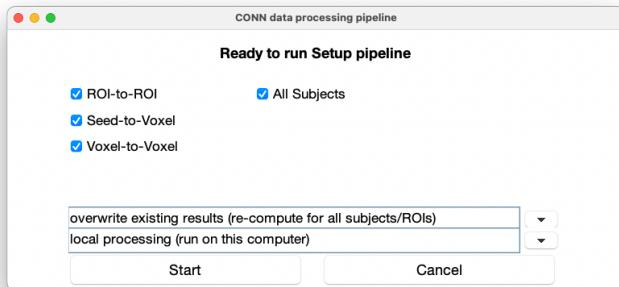
## Options Setup



Click on the **Options** button for additional analysis options. Select the type of analyses that you wish to perform (ROI-to-ROI, seed-to-voxel, and voxel-to-voxel). For voxel-based analyses define the desired analysis space (by default volume-based analyses using isotropic 2mm voxels; you may indicate surface-based analyses only if you have selected Freesurfer generated structural volumes for all of your subjects) as well as the type of analysis mask to be used (by default a gray matter template mask).

Last select if you wish optional/additional output files to be created by the toolbox during the Setup and Denoising steps (these are files that are not necessary for any of the CONN-based analyses, but could be useful for example if you plan to export some of the toolbox intermediate results to other packages; e.g. confound-corrected BOLD timeseries, first-level seed-to-voxel r- or p-maps, etc.).

When finished defining the experiment data in the *Setup* tab press **Done** in order to run the *Setup* processing pipeline.

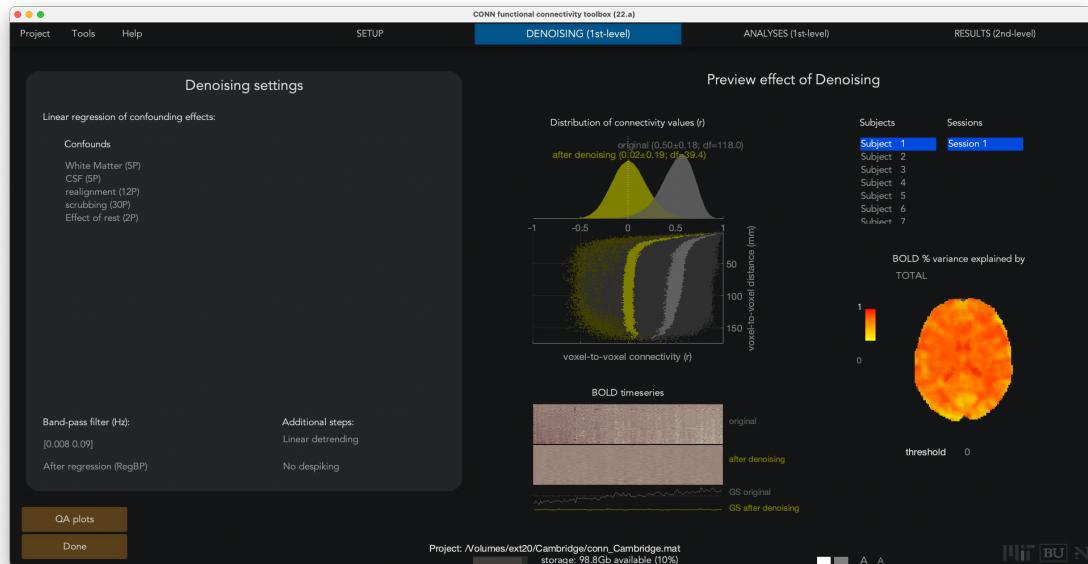


This will import the functional data for each session. If the gray/white/CSF ROIs have not been defined before, this step will perform segmentation of the structural data in order to define these masks (in this case, after this process is finished come back to *Setup* to inspect the resulting ROIs for possible inconsistencies). Last, this step will also extract the voxel-level and ROI-level time-series for each session (performing PCA on the within-ROI activations when appropriate). All of this information will be then propagated to the *Denoising* tab (see below) which will become active/available allowing you to proceed with the next step in your processing/analysis pipeline (denoising your data).

If, at some later point after having already run this *Setup* step, you need to go back and modify any of the information in the *Setup* tab (with the exception of *Setup.Covariates 2<sup>nd</sup>-level*), after making those changes in the *Setup* tab remember to click on **Done** again in order to propagate those changes to the *Denoising* tab again (you may select the option '*do not overwrite (skip already processed subjects/ROIs)*' if the modifications in the *Setup* tab consisted simply in adding new subjects and/or defining new ROIs, since in those cases there is no need to repeat this step for the already processed subjects/ROIs). Note that the same will need to be done in the *Denoising* tab in order to propagate those changes to the *first-level analysis* tab, etc.

The **Save / Save as** button will save the CONN project setup configurations in a .mat file, which can be loaded later (**Load** button). Note that in addition to your manually saving your project, this .mat file will also be automatically saved each time the *Done* button is pressed on any of the *Setup*, *Denoising*, *First-level analyses*, or *Second-level results* tabs.

## Step two: denoising (define, explore, and remove possible confounds)



Click on the **DENOISING** tab. This step applies linear regression and band-pass filtering in order to remove unwanted motion, physiological, and other artifactual effects from the BOLD signal before computing connectivity measures. 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 and scrubbing parameters); and 3) the *main* condition effects (condition blocks convolved with *hrf*). For each of the selected possible confounds you may change the number of dimensions (specifying how many temporal components are being used), the derivatives order (specifying how many successive orders of temporal derivatives are included in the model), and the order of polynomial expansion (specifying how many powers of each temporal component are to be used). For example, the *realignment* confound (derived from the estimated subject motion parameters) is defined by default by 6 dimensions, and the *derivative order* is set 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 no additional temporal derivative terms).

By default and unless specified otherwise all new projects that use the default CONN preprocessing steps will be automatically set up to use a combination of aCompCor (White and CSF ROIs, 5 components each), scrubbing (as many regressors as identified invalid scans), motion regression (12 regressors: 6 motion parameters + 6 first-order temporal derivatives), and filtering in the Denoising step. In addition for task designs the main effect of task (direct BOLD signal changes associated with the presence/absence of a task) are also regressed out during this step unless specified otherwise. Additional/alternative denoising steps can be specified by adding/substituting effects listed in the ‘confounds’ list. For example GSR is performed when introducing the GrayMatter ROI as an additional regressor; ICA denoising is performed when entering in the confounds list the ROIs associated with all noise components identified during ICA (see ICA analyses below, method (3) of ROI definition).

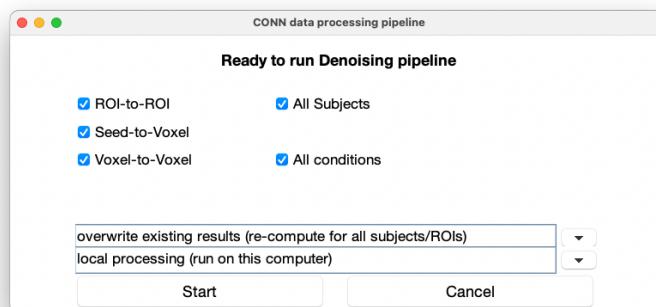
The “**Preview results**” window in the right panel shows the total variance explained (*r*-square) by each of the possible confounding sources (for the selected subject/session data). The histogram plot at the center displays the voxel-to-voxel connectivity (*r* values) before, and after confound removal. 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. Clicking on the “**Quality Control**” button allows the creation of a number of quality control plots

to evaluate the quality of the denoising procedure and easily identify potential outlier subjects. All of the settings in the *Denoising* tab can be modified and the associates changes in the observed histograms and spatial maps are estimated and displayed in real time. Images are displayed in neurological format, hovering over the image will display voxel locations and reference areas<sup>3</sup>.

Enter the **band-pass filter** information in the bottom-left box (two numbers, in Hz, defining the band-pass frequency window of interest), as well as any additional desired denoising options (**detrending** removes linear/quadratic/cubic trends within each functional session, and **despiking** applies a squashing function to reduce the influence of potential outlier scans).

By default CONN uses a *regBP* procedure for denoising (regression followed by band-pass filtering). Choose **simultaneous** if you prefer to use a *simult* procedure (simultaneous regression and band-pass filtering), or select individual elements in the ‘confounds’ list and mark the **Filtered** checkbox to select only a subset of regressors that should be regressed only within the frequency band of interest (using *regBP* and setting all regressors to *filtered* is exactly equivalent to using *simult*)

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 regions of interest, and create condition-specific timeseries (voxel- and ROI- level) by concatenating the samples/scans for each condition across all sessions.

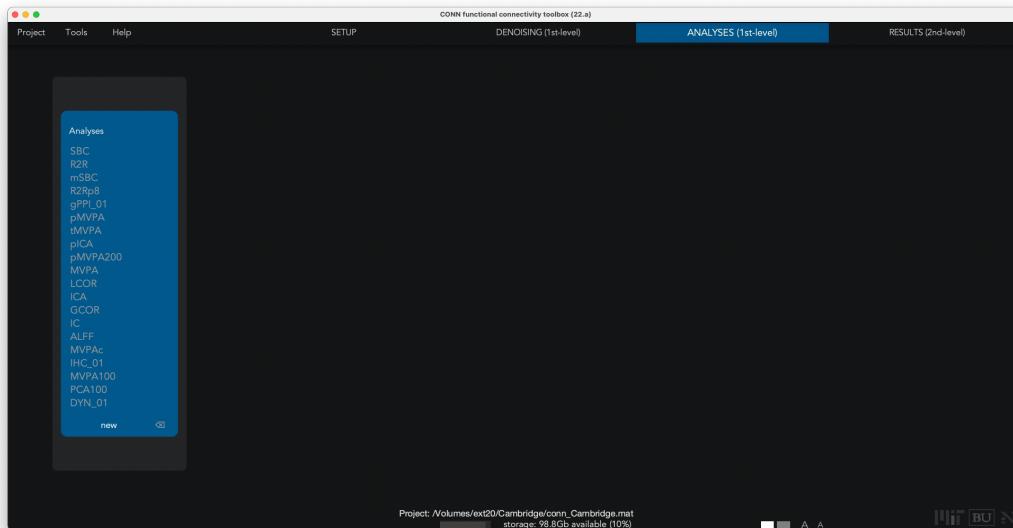


As with other steps, if, at some later point after having already run this *Denoising* step, you need to go back and modify any of the information in the *Setup* or *Denoising* tabs (e.g. adding more subjects, or modifying the denoising settings), remember to click **Done** again in this tab in order to propagate those changes from the *Denoising* to the *First-level analyses* tab again (you may select the option ‘*do not overwrite (skip already processed subjects/ROIs)*’ if the modifications in the *Setup* tab consisted simply in adding new subjects and/or defining new ROIs, since in those cases there is no need to repeat this step for the already processed subjects/ROIs).

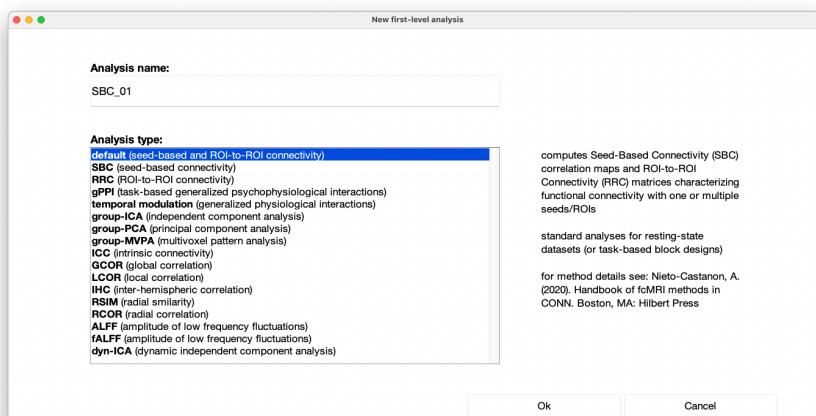
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<sup>3</sup> You may click on *Tools->Gui settings* to change the default atlas used when displaying labels associated with each spatial location (*background reference atlas*), as well as the default background image used in these displays (*background anatomical image*)

## Step three: first-level analyses (define and explore functional connectivity measures for each subject)



On the ‘Analysis’ list select an already-defined first-level analysis (e.g. to view or modify its settings), or click on ‘new’ to add a new first-level analysis to this list. Available analysis types are:



**SBC** (Seed-Based Connectivity): These analyses compute seed-based correlation maps characterizing functional connectivity between one or several seed ROIs and the rest of the brain.

For details about SBC measures in CONN see Nieto-Castanon, A. (2020). Handbook of fcMRI methods in CONN. Boston, MA: Hilbert Press

**RRC** (ROI-to-ROI Connectivity): These analyses compute ROI-to-ROI correlation matrices characterizing functional connectivity between a set of regions of interest.

For details about RRC measures in CONN see Nieto-Castanon, A. (2020). Handbook of fcMRI methods in CONN. Boston, MA: Hilbert Press

**gPPI** (generalized psychophysiological interactions): These analyses compute gPPI maps characterizing

task-related modulation of functional connectivity (e.g. task-related changes in connectivity during event-related designs).

For details about gPPI see McLaren, D. G., Ries, M. L., Xu, G., & Johnson, S. C. (2012). A generalized form of context-dependent psychophysiological interactions (gPPI): a comparison to standard approaches. *Neuroimage*, 61(4), 1277-1286

**temporal modulation** (generalized physiological interactions): These analyses compute gPPI maps characterizing the association between dynamic changes in functional connectivity strength and any user-defined factors/timeseries (e.g. changes in connectivity associated with heart rate).

For details about temporal modulation analyses see Friston, K. J., Buechel, C., Fink, G. R., Morris, J., Rolls, E., & Dolan, R. J. (1997). Psychophysiological and modulatory interactions in neuroimaging. *Neuroimage*, 6(3), 218-229

**group-ICA** (Independent Component Analyses): These analyses compute a series of spatial maps (and associated BOLD timeseries) characterizing the BOLD response across a set of independent spatial components or networks.

For details about group-ICA see Calhoun, V. D., Adali, T., Pearson, G. D., & Pekar, J. J. (2001). A method for making group inferences from functional MRI data using independent component analysis. *Human brain mapping*, 14(3), 140-151

**group-PCA** (Principal Component Analyses): These analyses compute a series of spatial maps (and associated BOLD timeseries) characterizing the BOLD response across a set of orthogonal spatial components or networks.

For details about group-PCA see Andersen, A. H., Gash, D. M., & Avison, M. J. (1999). Principal component analysis of the dynamic response measured by fMRI: a generalized linear systems framework. *Magnetic Resonance Imaging*, 17(6), 795-815

**group-MVPA** (functional connectivity Multi-Variate Pattern Analyses): These analyses compute a series of spatial maps (and associated connectivity patterns) characterizing the connectivity between each voxel and the rest of the brain.

For details about group-MVPA see Nieto-Castanon, A. (2022). Brain-wide connectome inferences using functional connectivity MultiVariate Pattern Analyses (fc-MVPA). *PLOS Computational Biology*

**ICC** (intrinsic connectivity) computes intrinsic connectivity maps characterizing network centrality at each voxel (root mean square of correlation coefficient values between a voxel and the rest of the brain).

For details about ICC see Martuzzi, R., Ramani, R., Qiu, M., Shen, X., Papademetris, X., & Constable, R. T. (2011). A whole-brain voxel based measure of intrinsic connectivity contrast reveals local changes in tissue connectivity with anesthetic without a priori assumptions on thresholds or regions of interest. *Neuroimage*, 58(4), 1044-1050

**GCOR** (global correlation) computes global correlation maps characterizing at each voxel the average of all correlation coefficient values between this voxel and the rest of the brain.

For details about GCOR see Saad, Z. S., Reynolds, R. C., Jo, H. J., Gotts, S. J., Chen, G., Martin, A., & Cox, R. W. (2013). Correcting brain-wide correlation differences in resting-state FMRI. *Brain connectivity*, 3(4), 339-352

**LCOR** (local correlation) computes local correlation maps characterizing local-coherence (average of all short-range connections) at each voxel.

For details about LCOR see Deshpande, G., LaConte, S., Peltier, S., & Hu, X. (2009). Integrated local correlation: a new measure of local coherence in fMRI data. *Human brain mapping*, 30(1), 13-23

**IHC** (inter-hemispheric correlation) computes correlation maps characterizing the connectivity strength between the two hemispheres at each voxel (fisher-transformed correlation between each voxel and the voxel at the same anatomical location in the contralateral hemisphere).

For details about IHC see Jin, X., Liang, X., & Gong, G. (2020). Functional integration between the two

brain hemispheres: evidence from the homotopic functional connectivity under resting state. *Frontiers in Neuroscience*, 14

**RSIM** (radial similarity) computes three maps characterizing the spatial gradients (along x/y/z directions) of the seed-based connectivity patterns at each voxel.

For details about RSIM see Whitfield-Gabrieli, S., & Nieto-Castanon, A. (2012). Conn: A functional connectivity toolbox for correlated and anticorrelated brain networks. *Brain connectivity*, 2(3), 125-141

**RCOR** (radial correlation) computes three maps characterizing the spatial gradients (along x/y/z directions) of the short-range connections at each voxel.

For details about RCOR see Goelman, G. 2004. Radial correlation contrast: a functional connectivity MRI contrast to map changes in local neuronal communication. *Neuroimage*, 23(4), 1432-1439

**ALFF** (amplitude of low frequency fluctuations) computes a spatial map characterizing the low-frequency BOLD signal variability at each voxel.

For details about ALFF see Yang, H., Long, X. Y., Yang, Y., Yan, H., Zhu, C. Z., Zhou, X. P., ... & Gong, Q. Y. (2007). Amplitude of low frequency fluctuation within visual areas revealed by resting-state functional MRI. *Neuroimage*, 36(1), 144-152

**fALFF** (fractional amplitude of low frequency fluctuations) computes a spatial map characterizing the ratio between low-frequency and total BOLD signal variability at each voxel.

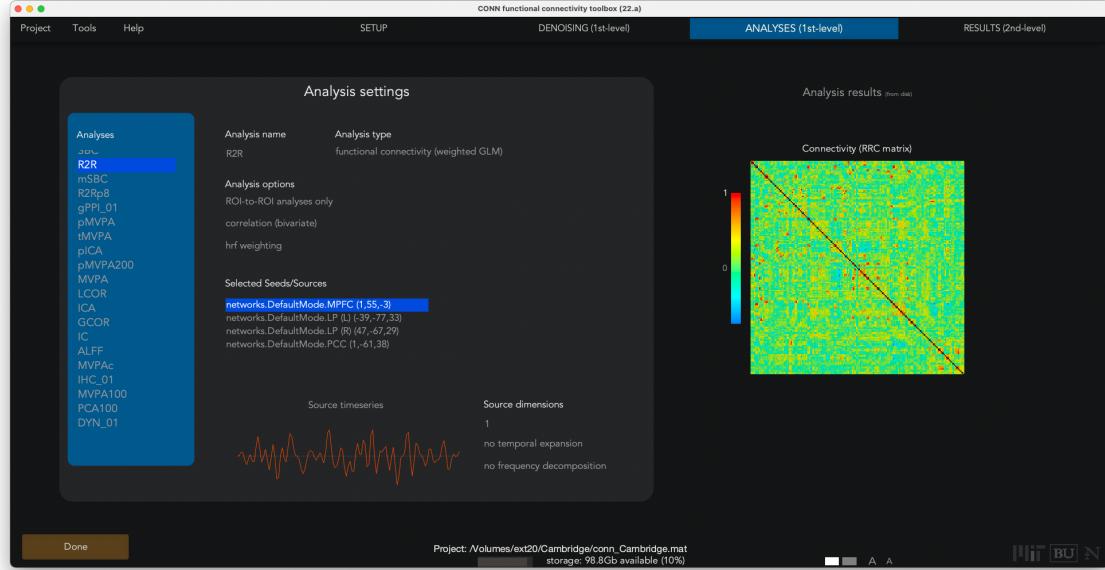
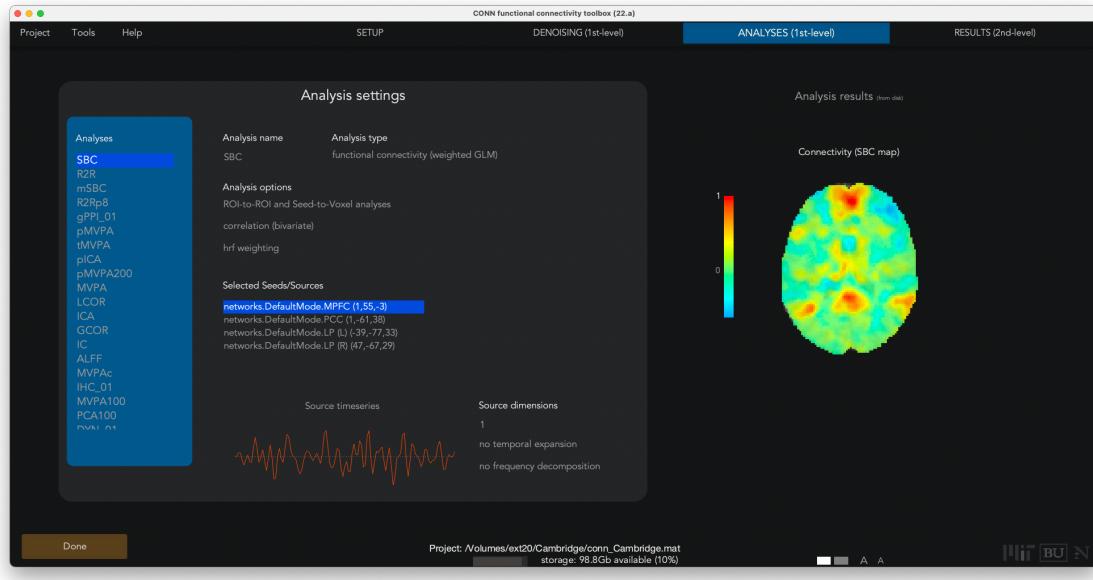
For details about fALFF see Zou, Q. H., Zhu, C. Z., Yang, Y., Zuo, X. N., Long, X. Y., Cao, Q. J., ... & Zang, Y. F. (2008). An improved approach to detection of amplitude of low-frequency fluctuation (ALFF) for resting-state fMRI: fractional ALFF. *Journal of neuroscience methods*, 172(1), 137-141

**dyn-ICA** (dynamic independent component analysis) computes ROI-to-ROI connectivity maps and associated temporal connectivity changes characterizing the dynamic changes in functional connectivity within independent subsets of functional connections.

For details about dyn-ICA see Nieto-Castanon, A. (2020). *Handbook of fcMRI methods in CONN*. Boston, MA: Hilbert Press

See [conn-toolbox.org documentation](http://conn-toolbox.org/documentation) for additional information about these measures/analyses and their implementation in CONN.

## SBC, RRC, gPPI, and temporal modulation analyses



When defining a new **SBC** analysis select from the ‘*all ROIs*’ list the **sources** (seeds) of interest and click the arrow to move these to the ‘*selected seeds/sources*’ list. Similarly, in **RRC** analyses enter in the ‘*selected seeds/sources*’ list all of the ROIs that you would like to be included in the ROI-to-ROI connectivity matrix.

For each seed/source you may optionally also indicate the number of *dimensions* to be analyzed (e.g. when extracting multiple components from a ROI), whether you want to include *derivative* terms, and the number of *frequency bands* if you wish to obtain for this particular ROI/seed a simple decomposition of the connectivity estimates power spectrum.

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 0.25 are shown/colored). For volume-based analyses images are displayed in neurological format (click on the image to see the voxel locations and Brodmann areas). For surface-based analyses images are displayed on the fsaverage brain. After the analyses have been run you may also click on the *Plot subjects* button to access additional display of the computed connectivity measures for each individual subject.

In the context of multiple of a task- design or multiple conditions the default behavior is to use a weighted General Linear Model (**weighted GLM**) for weighted regression/correlation measures of the condition-specific association between the seed/source BOLD timeseries and each voxel or target ROI BOLD timeseries. This model is appropriate both for resting state as well as task-related designs. When using weighted GLM click on **Weights** to change the relative weighting of the scans within each condition (by default condition-specific weights are defined from the hrf-convolved blocks/events for each condition). In addition to the standard bivariate correlation measure for functional connectivity analyses, you may also select regression measures, as well as define whether you want to compute bivariate measures –analyzing individual seed/sources separately-, or semipartial/multivariate –where all of the sources/ROIs are entered jointly into the general linear model to estimate their unique contributions-.

An alternative approach for event-related designs is to select **gPPI** analyses instead. PPI analyses compute the interaction between the seed/source BOLD timeseries and a chosen condition-specific interaction factor when predicting each voxel or target ROI BOLD timeseries. In addition, generalized PPI will include the interaction factors from all conditions simultaneously in the estimation model in order to better account for between-condition overlaps. Click on *gPPI analysis of selected task factors* to define which conditions to be included simultaneously in the gPPI model. You may choose all **task** conditions, for example, to perform standard gPPI analyses, or you may select no conditions (or click Cancel) to perform standard PPI (single-condition) analyses. Click on *source timeseries* and select *first-level design matrix* instead if you want to view the design matrix associated with this gPPI analysis. The term highlighted in red in this display is the one that characterizes the interaction term for the selected subject and condition.

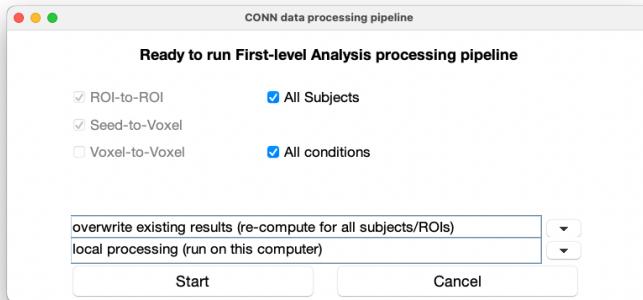
In the context of **temporal modulation** analyses click on *temporal modulation analysis of selected task factors* and select when prompted the desired factor (e.g. select an ROI to perform standard physiophysiological interaction analyses, or select any user-defined first-level covariate to estimate the association between changes in this covariate and functional connectivity strength)<sup>4</sup>. If you enter a first-level covariate that contains multiple timeseries, the model will include equally the main and interaction terms for all of the timeseries, but the last timeseries will be considered the effect-of-interest (the estimated interaction with this term will be passed to the second-level results tab) and the others will be considered control covariates (their interaction are still included in the model and estimated, but their magnitude is not stored/saved). Click on *source timeseries* and select *first-level design matrix* instead if you want to view the design matrix associated with this temporal modulation analysis. The term highlighted in red is the one that characterizes the interaction term for the selected subject and condition.

For all of these analyses you may use the **Analysis options** menu to change the type of connectivity measure to be computed (ROI-to-ROI, seed-to-voxel, or both).

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(s), and/or 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 nifti volumes (one per Subject/Condition/Source combination) in the *results/firstlevel* folder.

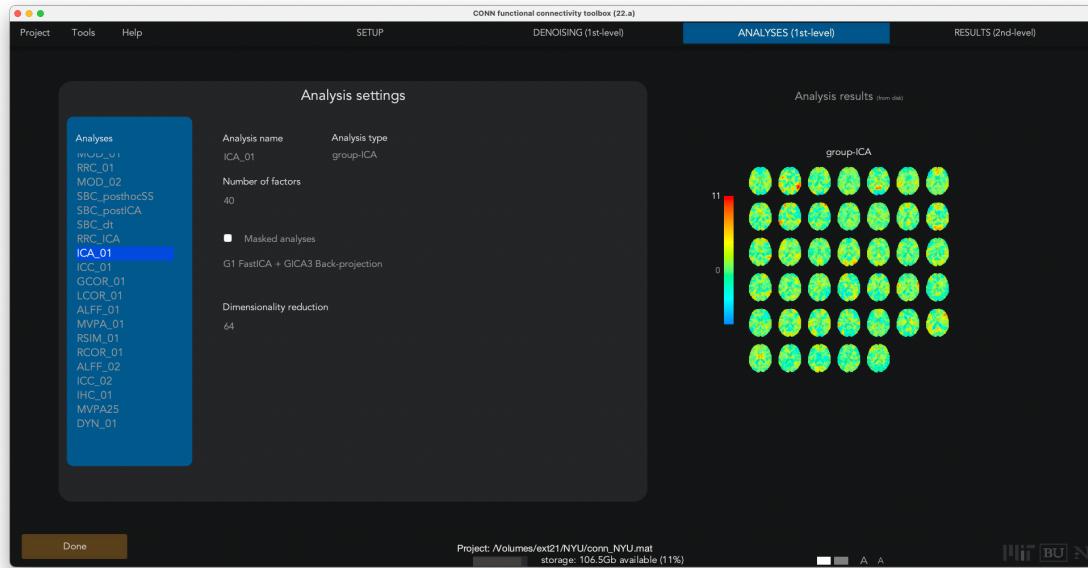
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<sup>4</sup> Note that the PPI/gPPI models used by CONN follow the implementation in FSL, where the interaction factor is convolved with the hemodynamic response function and the linear interactions are modeled on the resulting BOLD-level signal; c.f. SPM-style implementation which deconvolves the BOLD timeseries instead and the interactions are modeled on the resulting "neural"-level signal. Also note that PPI effects (interaction terms in PPI model) are always relative to the baseline state (the baseline state is defined by the zero values of the interaction term), so they provide a *relative* measure of connectivity characterizing differential task-specific effects, rather than an absolute measure of connectivity such as that estimated using standard functional connectivity analyses (e.g. weighted correlation measures).



As with other steps, if, at some later point after having already run this first-level analysis, you need to go back and modify any of the information in the *Setup*, *Denoising*, or this analysis' options in the *first-level analysis* tab, remember to select this first-level analysis and click **Done** again in this tab in order to propagate those changes from the *First-level analyses* tab to the *Second-level results* tab again (you may select the option '*do not overwrite (skip already processed subjects/ROIs)*' if the modifications in the *Setup* tab consisted simply in adding new subjects and/or defining new ROIs, since in those cases there is no need to repeat this step for the already processed subjects/ROIs).

## Group-ICA, group-PCA, group-MVPA, and other network analyses



Unlike most connectivity measures (such as SBC, RRC, IC, LCOR, GCOR, etc.), where connectivity measures can be computed separately and independently for each subject, group-ICA/PCA/MVPA analyses use information from the entire sample to then derive meaningful subject-specific measures.

**Group-ICA** (Independent Component Analyses) identifies a number of networks of highly functionally-connected areas. CONN's implementation uses Calhoun's group-level ICA approach (Calhoun et al. 2001), with variance normalization pre-conditioning, optional subject-level dimensionality reduction, subject/condition concatenation of BOLD signal data along temporal dimension, group-level dimensionality reduction (to the target number of dimensions/components), fastICA for estimation of independent spatial components, and GICA3 backprojection for individual subject-level spatial map estimation.

When defining a new **group-ICA** analysis you may edit the *number of factors* field to modify the number of independent components estimated. Optionally you may also edit the 1st-level *dimensionality reduction* field to specify the degree of subject-level dimensionality reduction desired (number of subject-specific SVD components retained when characterizing the voxel-to-voxel connectivity matrix for each subject/condition; set to *inf* for no dimensionality reduction). Optionally check the *masked analysis* checkbox and select a mask image file for masked-ICA analyses (in masked ICA the data is masked to consider only the connectivity between voxels within the mask and voxels across the entire brain). You may also edit the choice of non-linear contrast function in fastICA (G1 for tanh, G2 for gauss, and G3 for pow3) and the choice of back-projection algorithm (GICA1 or GICA3).

**Group-PCA** (Principal Component Analyses) analyses are identical to group-ICA above, but skipping the fastICA reorientation of group-level components and using instead the maximal-variance spatial components determined by a group-level dimensionality reduction step. All other steps, including back-projection, are performed identically to group-ICA.

**Group-MPVA** (functional connectivity MultiVariate Pattern Analyses) create a set of fc-MVPA eigenpattern score maps characterizing the connectivity patterns between each individual voxel and the rest of the brain for each individual subject and condition (this representation is defined by performing separately for each voxel a Singular Value Decomposition of the variability in connectivity patterns between this voxel and the rest of the brain across all subjects and conditions; see Nieto-Castanon 2022 for details). The resulting low-dimensional representation optimally characterizes arbitrary connectivity patterns, and it allows you to investigate connectivity differences across subjects simply running second-level multivariate analyses on the resulting eigenpattern maps.

When defining a new **fc-MVPA** analysis edit the *number of factors* field to modify the number of eigenpattern

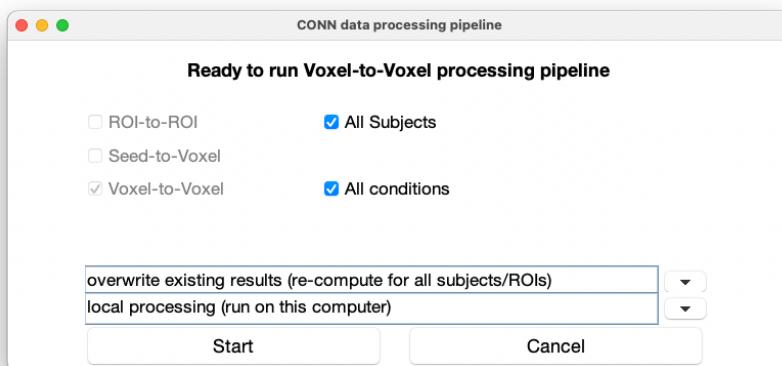
scores computed. Keep in mind that later (during second-level analyses) you may select all or a subset of the estimated eigenpatterns to be included in your second-level analyses. Optionally edit the 1st-level *dimensionality reduction* field to modify the degree of dimensionality reduction used to characterize the voxel-to-voxel connectivity matrix for each subject (number of subject-specific SVD components retained when characterizing this matrix; set to *inf* for no dimensionality reduction). Optionally check the *masked analysis* checkbox and select a mask image file for masked-MVPA analyses (in masked MVPA the data is masked to consider only, at each voxel across the entire brain, the connectivity between this voxel and voxels within the mask).

For all other connectivity measures derived from the voxel-to-voxel correlation matrix (**IC**, **GCOR**, **LCOR**, **RCOR**, **RSIM**) you may optionally edit the 1st-level *dimensionality reduction* field to specify the degree of subject-level dimensionality reduction desired (number of subject-specific SVD components retained when characterizing the voxel-to-voxel connectivity matrix separately for each subject/condition; set to *inf* for no dimensionality reduction).

For all connectivity measures (except group-ICA/group-PCA/group-MVPA) you may also optionally select the *normalization* option to convert the distribution of computed measures across all voxels to a N(0,1) Gaussian distribution separately for each subject and condition (this is useful, for example, when computing centrality measures and wishing to control for potential global network-cost differences between subjects or conditions, but keep in mind that this control will remove global differences in these measures across subjects or conditions so it may be detrimental if such global differences are meaningful).

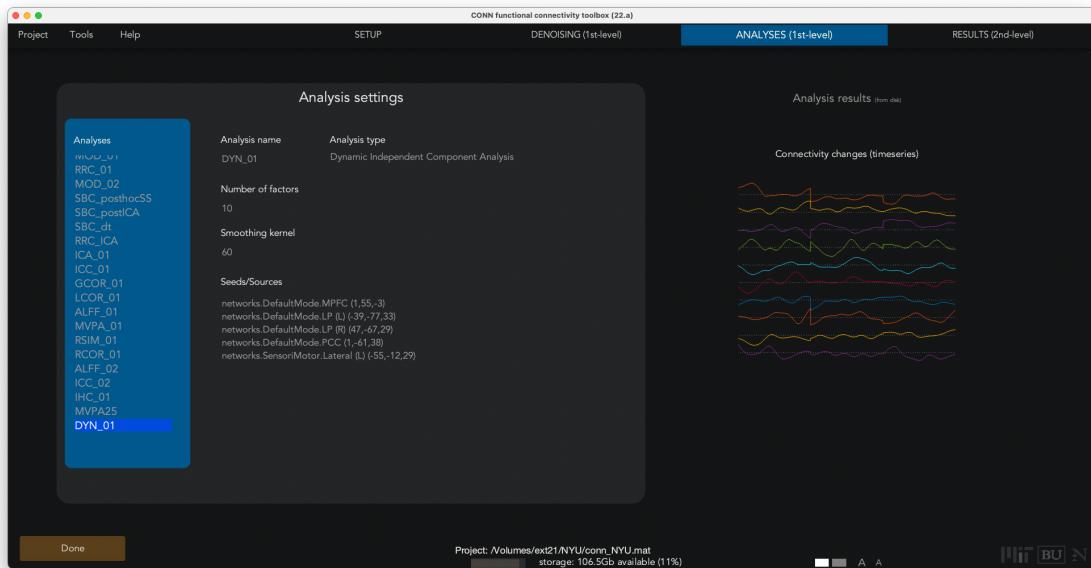
In addition, for **LCOR** analyses you may specify the level of locality by editing the *kernel size (mm)* field. In these analyses each voxel neighbourhood is defined as a probabilistic region (an isotropic Gaussian kernel with user-defined width), and you may define in the *kernel size* field the size of the local averaging window (Gaussian FWHM in mm).

When finished defining/exploring the connectivity analyses press **Done**. This will perform the defined analyses for all subjects, estimating the resulting maps for each subject and for each condition. First-level results (beta maps) are also exported as NIFTI volumes (one per Subject/Condition/Measure combination) in the *results/firstlevel* folder.



As with other steps, if, at some later point after having already run this first-level analysis, you need to go back and modify any of the settings or previous information (in Setup/Denoising tabs), after propagating those changes to this level click **Done** again in this tab in order to propagate those changes from this *First-level analyses* tab to the *Second-level results* tab again.

## Dynamic ICA analyses

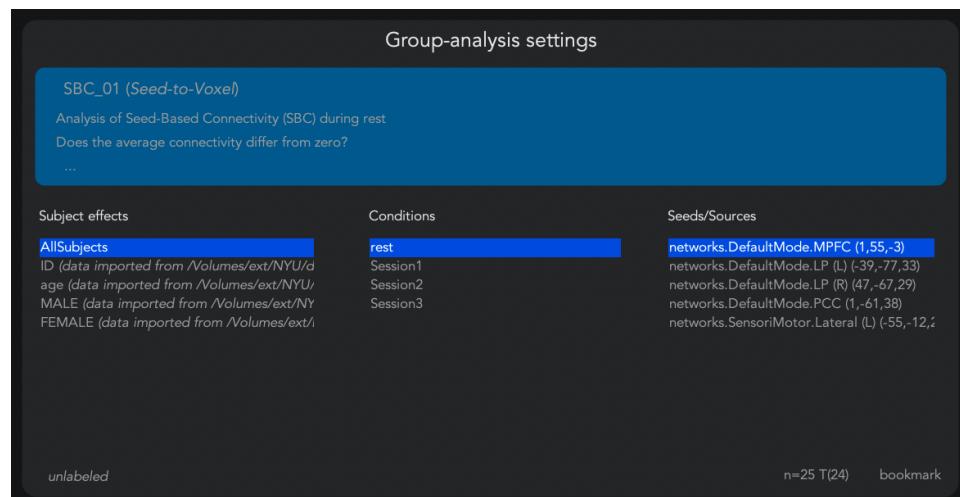


Dynamic connectivity analyses (Nieto-Castanon, 2016) explore dynamic properties (temporal modulation) of the ROI-to-ROI connectivity matrix identifying a number of circuits of connections that are similarly-modulated across time. Dyn-ICA performs an Independent Component Analysis of the connectivity timeseries (strength of connectivity between each pair of ROIs at any given timepoint), returning a number of independent components/circuits, and associated connectivity-changes timeseries, best characterizing the observed functional connectivity modulation across time.

When defining a new **dyn-ICA** analysis, select the ROIs to be included in this analyses in the allROIs list, and click on ‘<’ to enter these into the **Sources** list. Define the number of desired factors in *Number of factors*. Enter an optional low-pass filter threshold value (in seconds) in *smoothing kernel* in order to focus the estimated temporal modulation factors on the desired low-frequency range (similar to window-length in sliding-window approaches; set to *0s* for no filtering).

When finished defining the dynamic analyses press **Done**. This will estimate the dynamic-ICA components and propagate the resulting measures to the second-level analysis tab.

## Step four: second-level results (define and explore contrasts of interest and group-level analyses)



Second-level analysis can be defined in the **RESULTS (2<sup>nd</sup>-level)** tab. CONN's second-level analyses use a multivariate General Linear Model (GLM) for modeling your data across multiple subjects, and a likelihood ratio test (LRT) for testing specific hypothesis about the data. For a mathematical description of these analyses see Nieto-Castanon, A. (2020). General Linear Model. In Handbook of functional connectivity Magnetic Resonance Imaging methods in CONN (pp. 63–82). Hilbert Press.

The options below apply to *all* second-level analyses in CONN, irrespective of the type of first-level connectivity measure used (e.g. for the analyses of SBC connectivity maps, RRC connectivity matrices, etc.)

A second-level model can be defined in CONN by selecting the corresponding descriptive question in the blue area. For example:

- '***does the average connectivity differ from zero?***' will perform a one-sample t-test across all subjects
- '***does the average connectivity within MALE subjects differ from zero?***' will perform a one-sample t-test across all male subjects (this question will be available only when you have defined in the *Setup.Covariates (2<sup>nd</sup>-level)* tab one group covariate -with 0/1 values only -, in this example a MALE group covariates)
- '***does the average connectivity differ between MALE and FEMALE subjects?***' will perform a two-sample t-test (this question will be available only when you have defined in the *Setup.Covariates (2<sup>nd</sup>-level)* tab two non-overlapping group covariates, in this example MALE and FEMALE group covariates)
- '***does the correlation between connectivity and AGE differ from zero?***' will perform a bivariate regression analysis (this question will be available only when you have defined in the *Setup.Covariates (2<sup>nd</sup>-level)* tab a continuous covariate, in this example AGE)
- '***does the connectivity differences between MALE and FEMALE subjects depend on AGE?***' will perform a test of sex-by-age interactions (this question will be available only when the three variables above have been defined in *Setup.Covariates (2<sup>nd</sup>-level)* as well as their interactions terms -e.g. by selecting those three covariates and then click on 'covariate tools. Create interaction(s) of selected covariates' in the same *Setup.Covariates (2<sup>nd</sup>-level)* tab)

In addition, you may also select additional control variables for your analyses by clicking on the ‘...’ menu within the blue area and selecting the associated 2<sup>nd</sup>-level covariates (defined in *Setup.Covariates (2<sup>nd</sup>-level)*, and characterizing any continuous or group variable).

In addition, if you have multiple conditions, you may also select a description of what aspects of your conditions you would like to test in the second prompt within the blue area. For example:

- ‘*Analysis of seed-based connectivity change between PRE and POST*’ together with ‘*does the average connectivity-change differ from zero?*’ will perform a paired t-test comparing connectivity across the PRE and POST conditions (this question will be available only when your project has defined in *Setup.Conditions* two different conditions, in this example PRE and POST conditions)
- ‘*Analysis of seed-based connectivity change between PRE and POST*’ together with ‘*does the average connectivity-change differ between MALE and FEMALE subjects?*’ will test the time-by-sex interaction in a mixed within/between subjects ANOVA (this question will be available only when your project has defined in *Setup.Conditions* two different conditions, in this example PRE and POST conditions, as well as two non-overlapping groups covariates in *Setup.Covariates (2<sup>nd</sup>-level)*, in this example MALE and FEMALE covariates)

Last, if you have multiple first-level analyses, you may also select the name of your 1<sup>st</sup>-level analysis in the first prompt of the blue area to apply the same test to different connectivity measures.

Alternatively, if you prefer, you may define the same analyses (instead of by selecting the corresponding descriptive question in the blue area) by manually selecting the 2<sup>nd</sup>-level covariates that you would like to include in your model in the ‘*subject effects*’ list below that, and specifying a contrast across these selected covariates in the ‘*between-subjects contrast*’ field (e.g. select PATIENTS and CONTROLS and enter a [-1 1] contrast to compare the two groups). If you have multiple conditions, you may also manually select the conditions that you would like to include in your analysis in the ‘*conditions*’ list and specify the corresponding contrast across the selected conditions in the ‘*between-conditions contrast*’ field (e.g. select conditions TASK and REST and enter a [1 -1] contrast to compare the two conditions). Similarly, in SBC analyses, multiple ROIs/sources can be selected simultaneously in order to analyze connectivity results across several seeds/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, or enter [1,0; 0,1] contrast to investigate regions functionally connected to any of these two seeds).

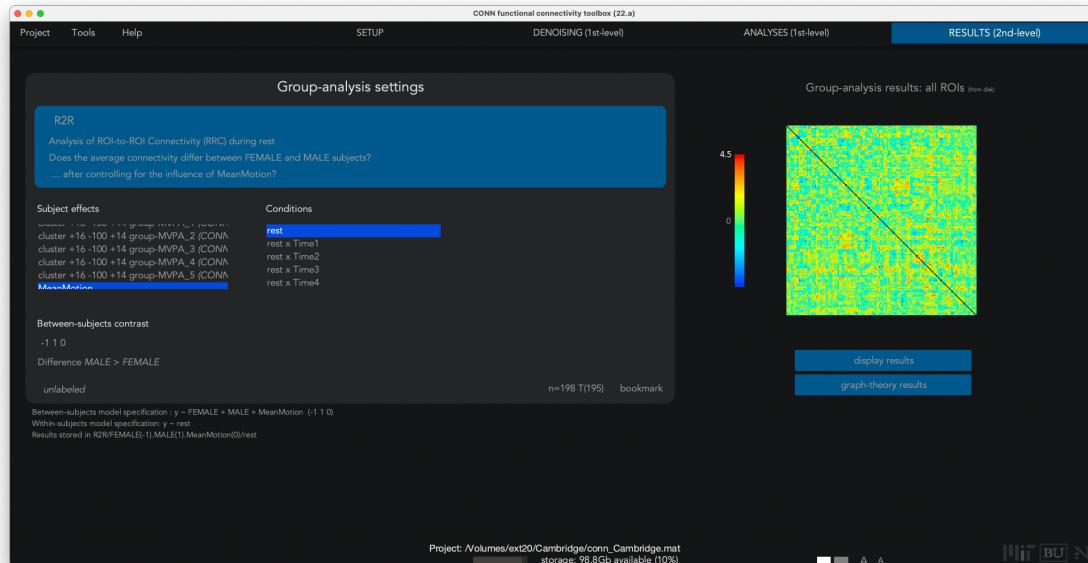
Each of these contrasts (between-subjects, between-conditions, and between-sources) can be defined as a single vector (e.g. [1,-1], for T-statistics) or as matrix (e.g. [1,0; 0,1], for F-statistics, where multiple contrasts are entered separated by ‘;’). In general, contrast matrices containing several rows are equivalent to an OR conjunction test on any of the individual row contrasts.

Both between-condition and between-source contrasts represent within-subject effects and the second-level analyses will correspond to multivariate/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(s) 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). For F-contrasts, voxel-level analyses are implemented as repeated-measures analyses using ReML estimation of covariance components and evaluated through F-statistical parameter maps, and ROI-level analyses are implemented as multivariate analyses and evaluated through F- or Wilks lambda statistics depending on the dimensionality of the within- and between- subjects contrasts. The following table illustrates a few common second-level design examples:

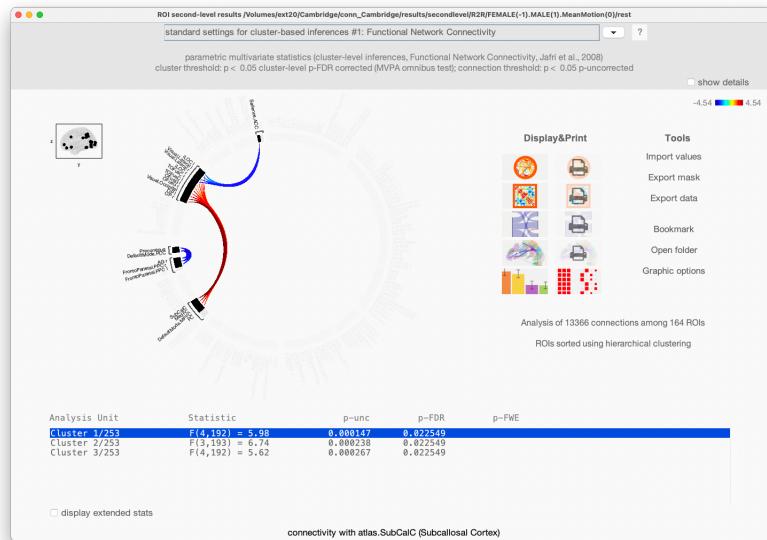
<b>Analysis type</b>	<b>Subject-effects [Between-subjects contrast]</b>	<b>Conditions [Between-conditions contrast]</b>
one-sample t-test	AllSubjects [1]	Rest [1]
two-sample t-test	Patients, Controls [1 -1]	Rest [1]
paired t-test	AllSubjects [1]	Pre, Post [-1 1]
regression	AllSubjects, behavioral [0 1]	Rest [1]
multiple regression (individual/unique effect)	AllSubjects, behav, age [0 1 0]	Rest [1]
multiple regression (joint effect)	AllSubjects, behav1, behav2 [0 1 0; 0 0 1]	Rest [1]
2x2 between-subjects ANOVA interaction	PatientsTreat, PatientsSham, ControlTreat, ControlSham [1 -1 -1 1]	Rest [1]
2x2 within-subjects / repeated measures ANOVA interaction	AllSubjects [1]	PreTask, PreRest, PostTask, PostRest [-1 1 1 -1]
2x2 mixed ANOVA interaction	Patients, Controls [1 -1]	Pre, Post [-1 1]
3x2 mixed ANOVA interaction	PatientsA, PatientsB, PatientsC [1 -1 0; 0 1 -1]	Pre, Post [-1 1]
2x2x2 mixed ANOVA interaction	PatientsTreat, PatientsSham, ControlTreat, ControlSham [1 -1 -1 1]	Pre, Post [-1 1]
one-way ANCOVA covariate control	Patients, Controls, age [1 -1 0]	Rest [1]
one-way ANCOVA covariate interaction (comparing regression between groups)	Patients, Controls, behavPatients, behavControls [0 0 1 -1]	Rest [1]
<i>General Linear Model data fit: <math>\mathbf{Y} = \mathbf{X}^* \mathbf{B}</math> hyp test: <math>\mathbf{C}^* \mathbf{B}^* \mathbf{M}' = 0</math></i>	Columns of X (effects) [C]	Columns of Y (conditions) [M]

Second-level design examples

## ROI-to-ROI analyses (ROI-to-ROI functional connectivity matrices)



When selecting **ROI-to-ROI Connectivity** analyses in the *results (2<sup>nd</sup>-level)* tab (or when selecting any other first-level connectivity measure that also computes ROI-to-ROI maps), the default behavior is to perform analyses encompassing the entire ROI-to-ROI connectivity matrix. After selecting the desired type of analyses (e.g. specifying the subject-effects, between-subject contrasts, etc.) select the option **Group-analysis results (all ROIs)** and click on **compute results** to compute this 2<sup>nd</sup>-level analysis (or **display results** to display the results if these analyses have already been computed). That will launch a *results explorer* window where you can specify your choice of inferential method and error rate threshold used to display the results:



*ROI-to-ROI results explorer window*

By default the results explorer window uses FNC (functional network connectivity) to identify significant effects within clusters of connections, while appropriately correcting for multiple comparisons in the context of the entire set of ROI-to-ROI connections evaluated. Click on ‘standard settings for cluster-based

*inferences #1: functional network connectivity*' if you prefer to select a different approach. Available inferential methods are:

- *standard settings for cluster-based inferences #1: functional network connectivity.* Significance is determined at the level of **clusters/groups of connections**. Clusters of connections are defined using a hierarchical clustering approach to first group ROIs into meaningful networks (groups of ROIs), and then grouping all connections between the same two networks (for each pair of networks, including within- and between- network connectivity). Results are corrected across multiple comparisons using FDR across the entire set of possible pairwise clusters.
- *standard settings for cluster-based inferences #2: spatial pairwise clustering.* Significance is determined at the level of **clusters/groups of connections**. Clusters of connections are defined as connected components in the 2D binary image resulting from thresholding the ROI-to-ROI connectivity matrix at some user-defined connection-level threshold. The ROI-to-ROI matrix is sorted automatically to take into account functional similarity between ROIs and anatomical proximity. Results are corrected across multiple comparisons by performing randomization/permuation analyses.
- *standard settings for cluster-based inferences #3: threshold free cluster enhancement.* Significance is determined at the level of **clusters/groups of connections**. Clusters of connections are defined as connected components in the 2D binary image resulting from thresholding the ROI-to-ROI connectivity matrix at a TFCE threshold (TFCE scores at each connection combine connection and cluster statistics into a single measure). The appropriate TFCE threshold is determined using randomization/permuation analyses, to guarantee the desired maximum familywise error rate.
- *Alternative settings for connection-level inferences: parametric univariate statistics.* Significance is determined at the level of **individual connections**. Results are corrected across multiple comparisons using FDR across the entire set of possible pairwise connections.
- *Alternative settings for ROI-level inferences: parametric multivariate statistics.* Significance is determined at the level of **individual ROIs** (considering the cluster/group of connections between a given ROI and all other ROIs). Results are corrected across multiple comparisons using FDR across the entire set of ROIs.
- *Alternative settings for network-level inferences: network based statistics.* Significance is determined at the level of **individual networks** (considering the cluster/group of connections defined as connected subgraphs within the graph of all suprathreshold ROI-to-ROI connections). Results are corrected across multiple comparisons using randomization/permuation analyses.

For any of the above methods you may click on the 'show details' checkbox to display and modify any of the individual parameters used in these options (e.g. individual connection-level thresholds, cluster-level p-values, etc.). For more mathematical details about any of the above methods, see Nieto-Castanon, A. (2020). Cluster-level inferences. In Handbook of functional connectivity Magnetic Resonance Imaging methods in CONN (pp. 83–104). Hilbert Press. doi:10.56441/hilbertpress.2207.6603

Click on '*analysis of ### connections among ### ROIs*' if you want to restrict the ROI-to-ROI matrix to only consider the connections between a subset of ROIs.

Click on '*ROIs sorted using hierarchical clustering*' for methods #1-#3 above if you prefer to sort (for methods #2 or #3) or cluster (for method #1) the ROIs in your ROI-to-ROI connectivity matrix differently.

Click on individual clusters in the bottom list to select individual clusters of significant connections. The following methods are available for displaying only the selected significant clusters / connections:



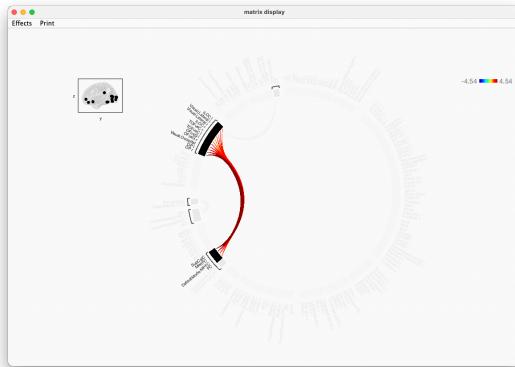
**ring display** shows connections among all ROIs distributed in a ring

**matrix display** shows the ROI-to-ROI connectivity matrix

**connections display** shows a graphical list of all significant connections

**brain display** shows connections as lines between ROIs displayed in the brain

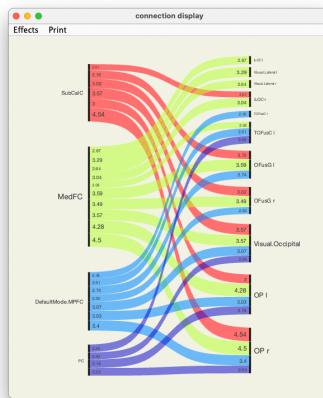
ring display



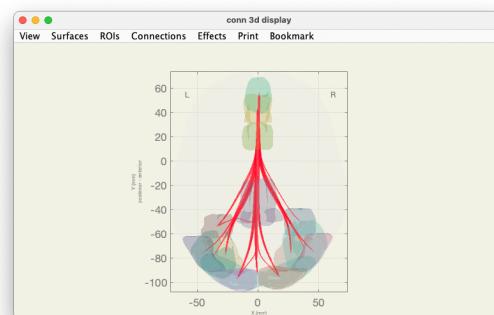
matrix display



connections display



brain display



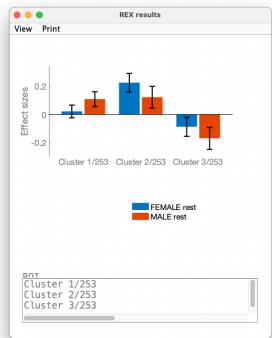
Click on ‘*import values*’ to import individual connectivity values within each significant connection separately for each subject into your CONN project as a set of new second-level covariates.

Click on ‘*export mask*’ to export a matrix NIFTI file containing the mask of suprathreshold connections in these second-level analysis results

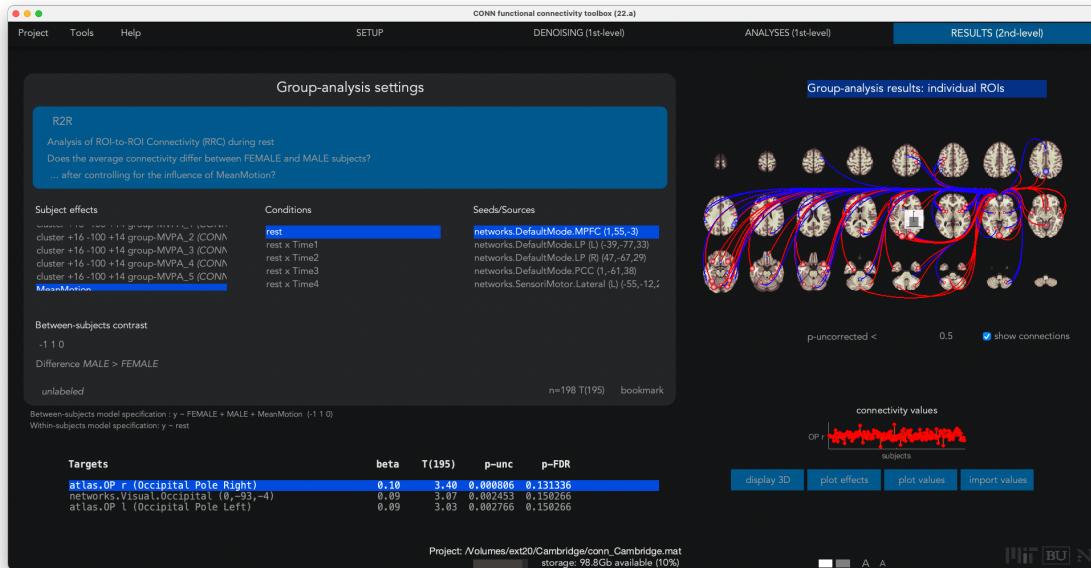
Click on ‘*export data*’ to export this analysis’ data as a 4D matrix NIFTI file the data (ROI-to-ROI connectivity matrices for each subject and condition)



Click on ‘*plot effects*’ to compute effect-sizes (e.g. connectivity values within each group) averaged across all connections within each significant cluster



## ROI-to-ROI analyses (connectivity with individual ROIs)

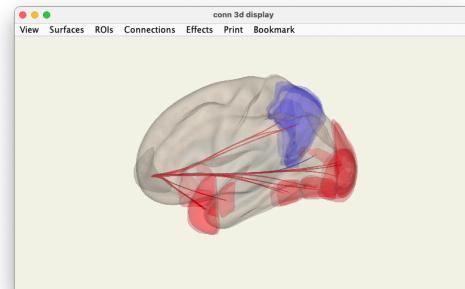


When selecting **ROI-to-ROI** analyses in the *results (2<sup>nd</sup>-level)* tab, it is also possible to perform analysis restricted to the connectivity between an individual ROI and all of the other ROIs. After selecting the desired type of analyses (e.g. specifying the subject-effects, between-subject contrasts, etc.) select the option **Group-analysis results (individual ROIs)** to compute this 2<sup>nd</sup>-level analysis.

In the ‘Seed/Sources’ list select the individual seed that you would like to analyze. The brain display at the right shows an axial view of the ROI-to-ROI second-level analysis results estimated in real time for the selected seed ROI. These results can be thresholded at the desired **p-value** threshold, using uncorrected p-values or FDR-corrected p-values (corrected for multiple comparisons across all of the selected target ROIs; use p-FDR<0.05 for appropriate multiple comparisons correction only for this individual analysis).

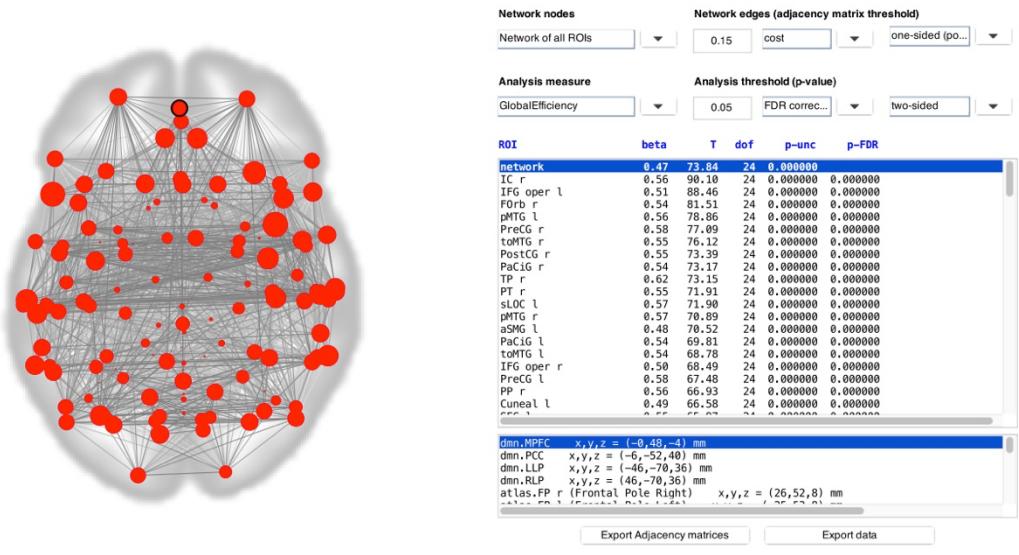
The list at the bottom of this figure shows the statistics associated with the analysis of the connectivity between the chosen seed ROI and each individual target ROI. Right-click on this list if you wish to restrict the list of target ROIs being included in this analysis to a different set of ROIs (this choice affects the FDR-corrected p-values of the results).

Images are displayed in neurological format. Select **Display 3d** to view a 3d-rendered display of the supra-threshold ROI-level results. For each target ROI the list at the bottom of the figure displays the connectivity contrast effect sizes (between the selected source –or linear combination of sources- and each target), as well as T/F/X- values, uncorrected p-values, and FDR-corrected p- values for the specified second-level analysis. Right-clicking on this table allows you to export this table to a .txt, .csv, or .mat file. Select **Display Values** or **Import Values** to display/import the estimated ROI-to-ROI connectivity values for each subject and for each selected condition between the selected seed and target ROIs. Importing these values will create new second-level covariates containing these connectivity values for each subject for further analyses.



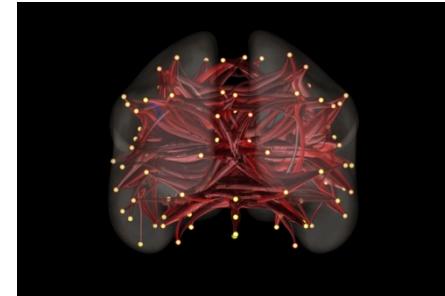
## ROI-to-ROI analyses (graph measures)

When selecting **ROI-to-ROI** analyses in the *results (2<sup>nd</sup>-level)* tab, it is also possible to perform graph-analysis of the resulting ROI-to-ROI connectivity matrices. After selecting the desired type of analyses (e.g. specifying the subject-effects, between-subject contrasts, etc.) click on **Graph theory results** to compute this 2<sup>nd</sup>-level analysis on the available graph measures.



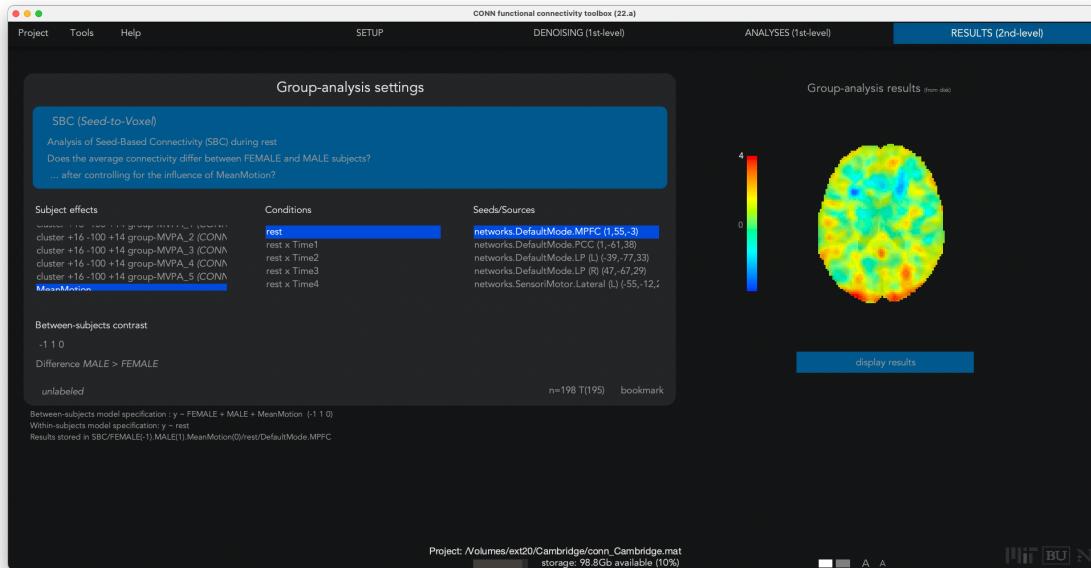
graph theory results explorer window

Last by clicking **graph-theory** a graphical display is shown allowing the users to test (for the selected between-subjects and between-condition contrasts) measures of efficiency, centrality, and cost/degree, associated with an ROI-to-ROI connectivity network. Click on 'Network nodes' to limit the ROI-to-ROI network analyzed to that defined by a subset of ROIs. The 'Network edges' option allows the definition of the connectivity threshold above which two ROIs are considered connected, and it can be defined based on correlation scores, z-scores, or cost values. For each ROI the list at the bottom of the figure displays the corresponding measure effect size (global efficiency, local efficiency, or cost), as well as T- values, uncorrected p-values, and FDR-corrected p- values for the specified second-level analysis. The graphical display and second-level results can be thresholded using either uncorrected or FDR-corrected p- values, and it can be set to display one-sided or two-sided results. Right-clicking on the brain display shows additional display options, including 3d-rendered views of the analyzed network of connectivity.

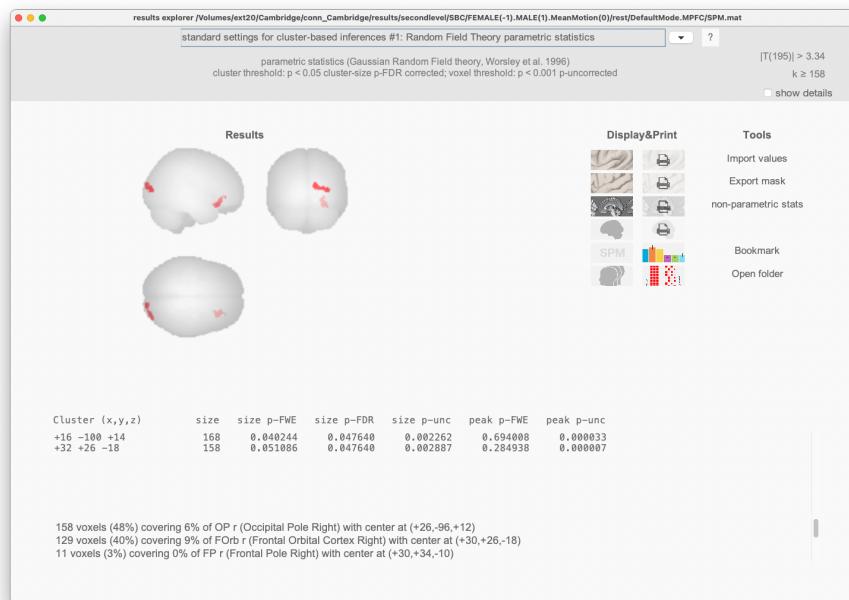


graph theory 3d-view figure

## Seed-Based Connectivity analyses (functional connectivity maps)



When selecting **Seed-Based Connectivity** analyses in the *results (2<sup>nd</sup>-level)* tab (or when selecting any other first-level connectivity measure that also computes voxel-level maps), after selecting the desired type of analyses (e.g. specifying the subject-effects, between-subject contrasts, etc.) click on **compute results** to compute this 2<sup>nd</sup>-level analysis (or **display results** to display the results if these analyses have already been computed). That will launch a *results explorer* window where you can specify your choice of inferential method and error rate threshold used to display the results:



Voxel-level results explorer window

By default the results explorer window uses RFT (random field theory) parametric statistics to identify significant effects within clusters of voxels, while appropriately correcting for multiple comparisons across

the entire brain. Click on ‘*standard settings for cluster-based inferences #1: random field theory parametric statistics*’ if you prefer to select a different approach. Available inferential methods are:

- ***standard settings for cluster-based inferences #1: random field theory parametric statistics.*** Significance is determined at the level of clusters/groups of voxels. Clusters are defined as connected components in the 3D binary image resulting from thresholding the voxel-level statistics at some user-defined voxel-level threshold. Results are corrected across multiple comparisons using FWE or FDR across the entire set of voxels within the analysis mask (whole brain).
- ***standard settings for cluster-based inferences #2: permutation/randomization analyses.*** Same options as above, but cluster-level statistics and multiple comparison corrections are obtained using randomization/permuation analyses.
- ***standard settings for cluster-based inferences #3: threshold free cluster enhancement.*** Same options as above, but voxels are thresholded using a TFCE threshold (TFCE scores combine voxel and cluster statistics into a single measure). The appropriate TFCE threshold is determined using randomization/permuation analyses, to guarantee the desired maximum familywise error rate.

For any of the above methods you may click on the ‘show details’ checkbox to display and modify any of the individual parameters used in these options (e.g. individual voxel-level thresholds, cluster-level p- values, etc.). For more mathematical details about any of the above methods, see Nieto-Castanon, A. (2020). Cluster-level inferences. In Handbook of functional connectivity Magnetic Resonance Imaging methods in CONN (pp. 83–104). Hilbert Press. doi:10.56441/hilbertpress.2207.6603

Click on individual clusters in the bottom list to select significant clusters. The following methods are available for displaying only the selected significant clusters / areas:



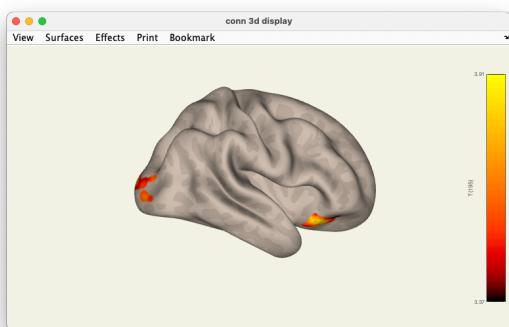
***surface display*** shows areas projected on the cortical surface of an ICBM template brain

***volume display*** shows areas as 3D ‘blobs’

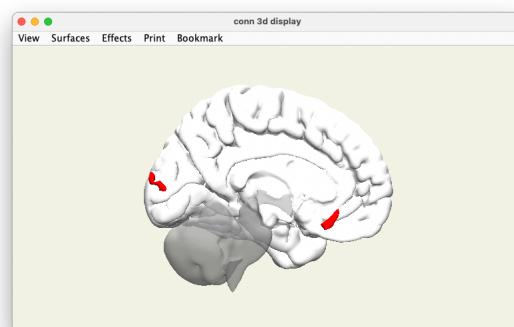
***slice display*** shows areas across individual slices of an ICBM template T1w structural image

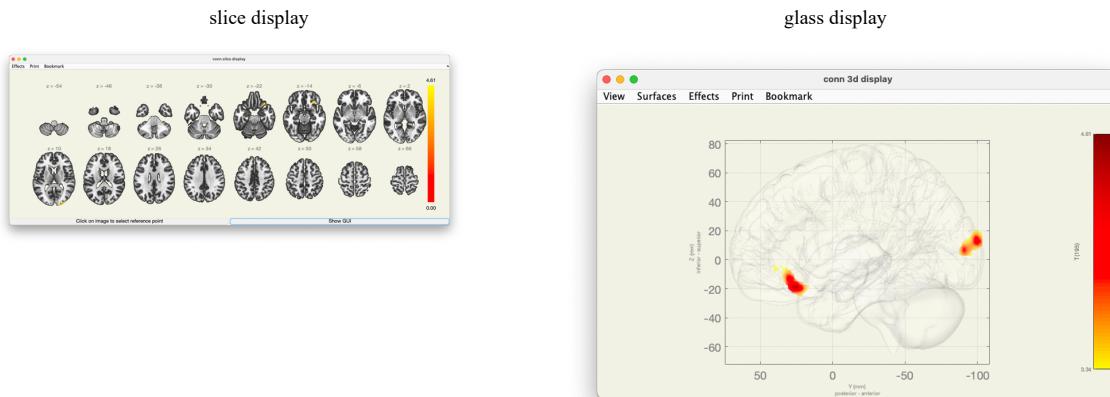
***glass display*** shows areas in MIP glass-brain display

surface display



volume display

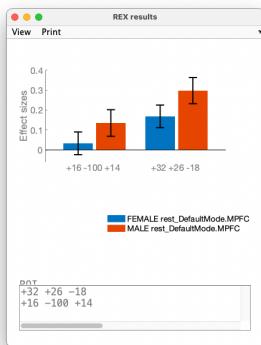




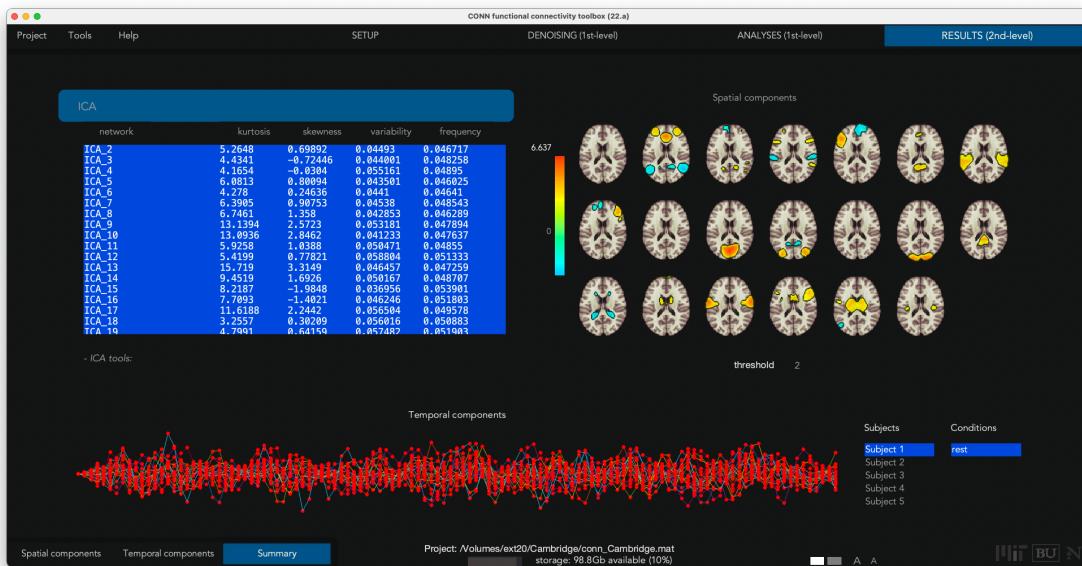
Click on ‘*import values*’ to import individual connectivity values within each significant cluster separately for each subject into your CONN project as a set of new second-level covariates.

Click on ‘*export mask*’ to export a volume NIFTI file containing the mask of suprathreshold clusters in these second-level analysis results

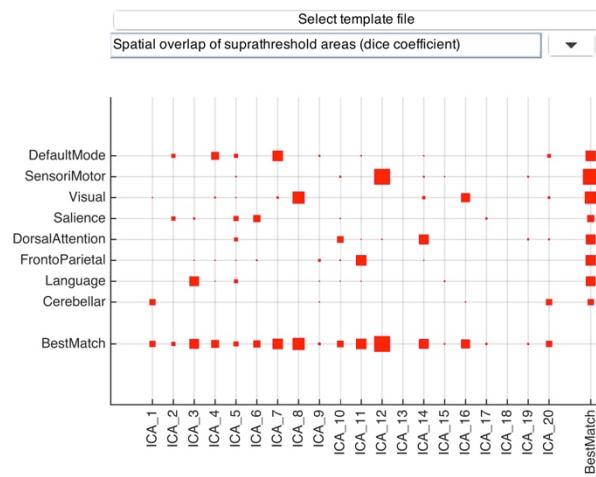
Click on ‘*plot effects*’ to compute effect-sizes (e.g. connectivity values within each group) averaged across all voxels within each significant cluster



## Group-ICA analyses



Select **ICA.Summary** to explore the estimated ICA networks/components. The top-left table lists several properties of each network (spatial variability/kurtosis/skewness, and temporal variability/frequency). The top-right display shows the group-level maps (*ICA spatial components*) for the selected networks/components. These maps may be thresholded based on a z-score threshold for display purposes (factor loadings are centered and normalized across all voxels to compute z-scores). The bottom display shows the BOLD signal timeseries associated with each network (*ICA temporal components*) for the selected subject/condition. By default ICA components are sorted by their relative overlap with gray matter areas (last networks/components may be loading more heavily on white or CSF areas, which are often identified as noise components)



networks match-to-template summary display

In addition selecting **ICA tools. Spatial match to template** computes the correlation between each group-level spatial map and a CONN's default networks file or a user-defined mask file, which can be used to identify networks of interest. In addition, hovering over the top-right plot displays the factor loadings at each

voxel, as well as the networks/components with the highest loadings at each voxel, which is another common way to identify networks of interest. The top-right display can also be selected to show the associated *ICA parcellation* maps. Parcellations are computed by finding, among the selected networks/components, the one that shows the highest factor loading at each voxel, and thresholding the resulting maps using the chosen z-score threshold. Parcellations may be computed across all networks/components or only across a subset of the estimated networks simply by selecting the desired subset of networks in the top-left table. The resulting parcellations can also be exported to a file (e.g. to be used as ROIs) by selecting the ***ICA tools. Create ICA parcellation ROI file*** option.

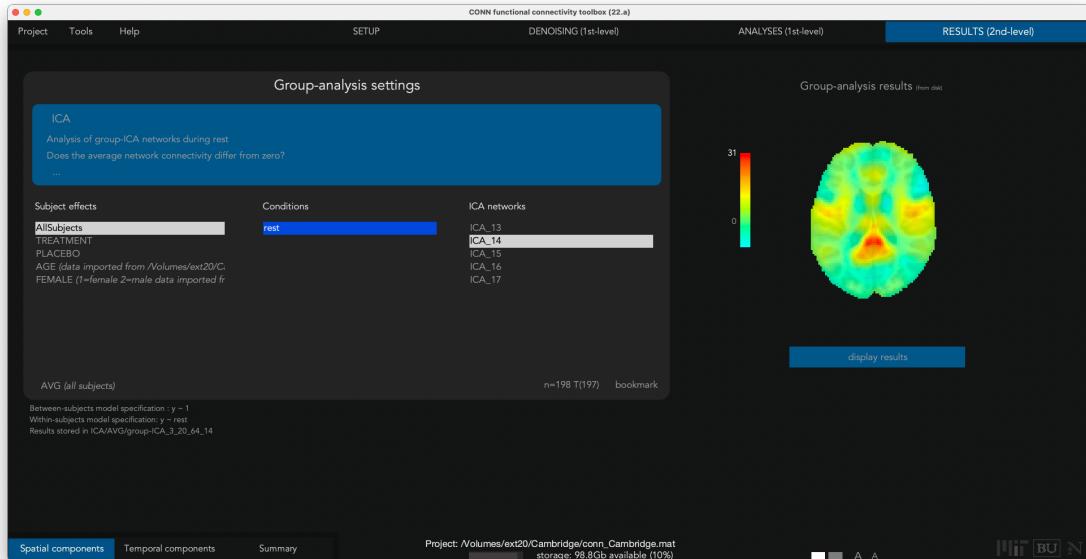
**Tip:** some times it is desirable to define ROIs from some or all of the ICA networks for additional analyses (e.g. within- and between- network connectivity analyses, ICA denoising, etc.) There are three ways in CONN to define meaningful ROIs from ICA results (and all of these methods are commonly used in the literature):

1) thresholded second-level analyses: from *ICA.SpatialComponent* analyses (see section below), in the *results explorer* window define the desired thresholds (e.g. in terms of T- statistics from a one-sample t-test of the individual subject-level maps) and use ‘*export mask*’ to create a mask of suprathreshold areas, then in *Setup.ROIs* import the resulting mask.ROI.nii file as a new ROI atlas file

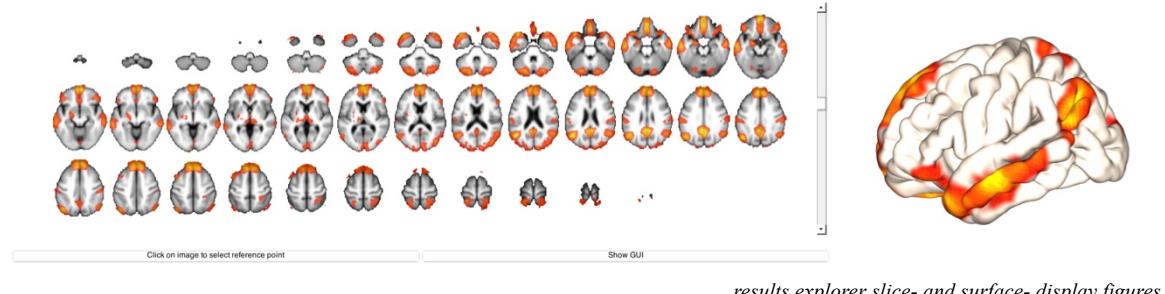
2) thresholded group-level maps: from the *ICA.Summary* tab, select one or several networks, define the desired z-score threshold, and use ‘*ICA tools. Create ICA parcellation file*’ to create a new ROI file, then again import the resulting file in *Setup.ROIs* as a new ROI atlas file

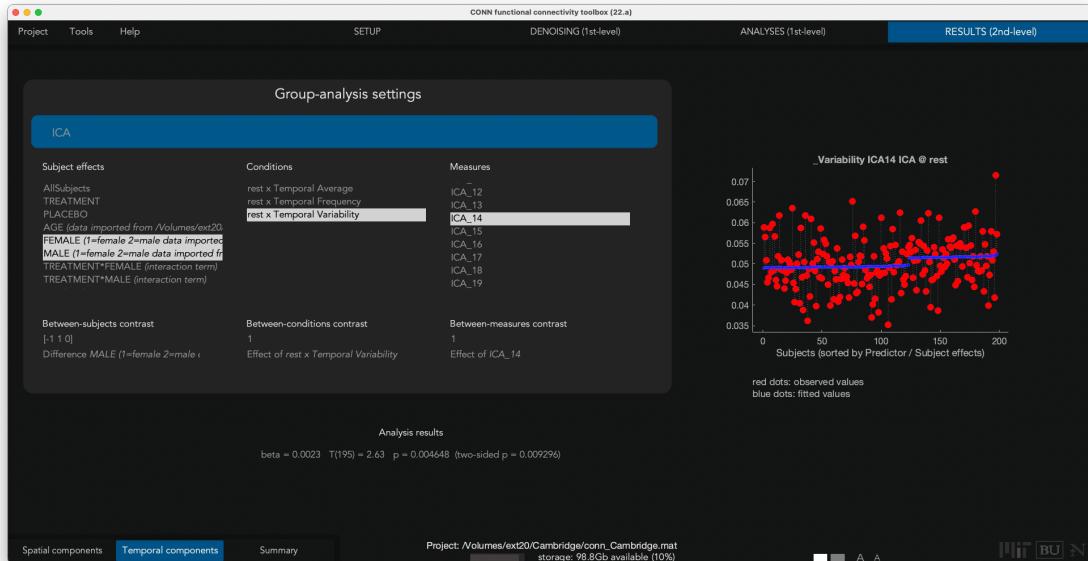
and 3) weighted/unthresholded group-level maps: in *Setup.ROIs* select ‘*ROI tools. Add ICA-network ROIs*’ to define a new set of ROIs directly form the group-level spatial masks, using these maps as weighted/probabilistic-ROIs (one ROI per network)

Generally the advantage of method (3) is that the resulting ROI timeseries and connectivity maps more closely represent the properties of the networks identified in the ICA procedure (when using this method, the extracted ROI timeseries for each network will be exactly the same as the ICA temporal component timeseries, and if using these ROIs as seeds in multivariate analyses the resulting seed-to-voxel maps will be exactly the same as the individual subject-level spatial maps estimated in the ICA procedure for each network), while the advantage of methods (1) and (2) is that they allow breaking individual networks into potentially multiple separate clusters/areas (e.g. for additional within-network or between-network connectivity analyses).



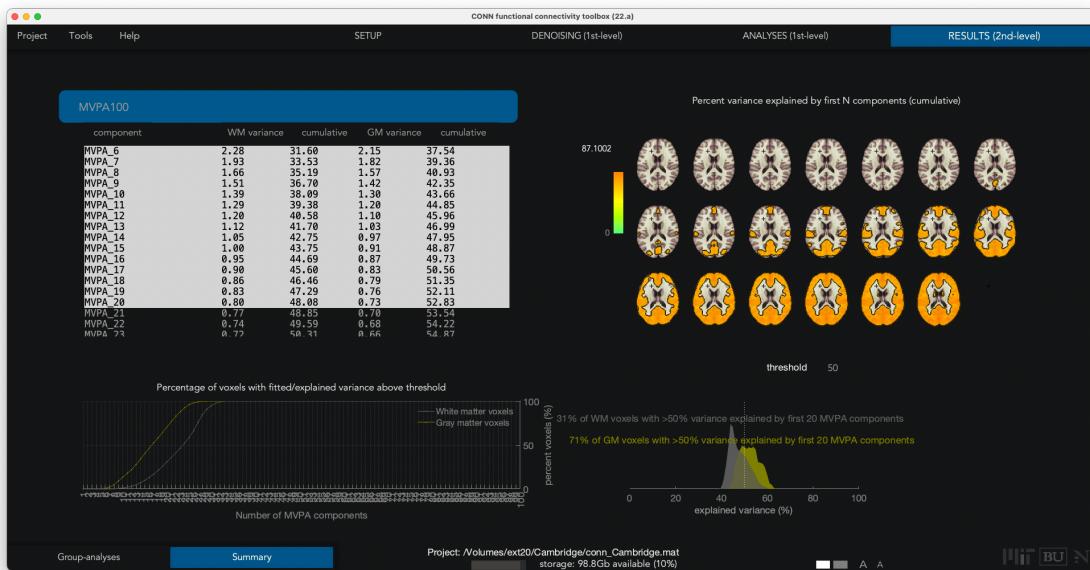
Select **ICA.SpatialComponents** to analyze the subject-level maps for each network/component. Second-level analyses can be defined in the same way as for any other voxel-level measure (e.g. seed-to-voxel connectivity maps). These analyses enter the estimated subject-level spatial maps for each subject/condition/network into standard second-level analyses in order, for example, to display the average map across all subjects for a given network, or compare these networks across subjects and/or conditions. Select **compute results** to perform the desired second-level analyses, threshold the resulting statistical maps using a combination of height- and extent- thresholds, perform non-parametric analyses, or additional display and analysis options.



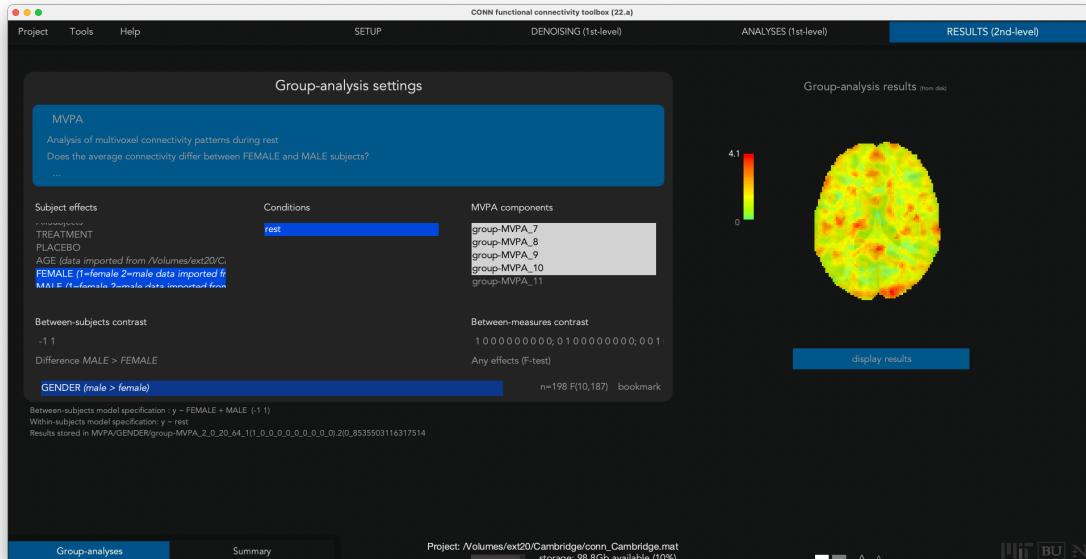


Select **ICA.TemporalComponents** to analyze properties of the BOLD signal timeseries associated with each network/component. For each network/component, the temporal variability as well as the frequency (center of mass in spectral power) of these BOLD signal timeseries are computed for each subject and for each condition. Second-level analyses can be defined in the same way as for any other individual measure, for example, to compute the average of these measures across all subjects or to compare these measures across subjects or conditions.

## fc-MVPA analyses

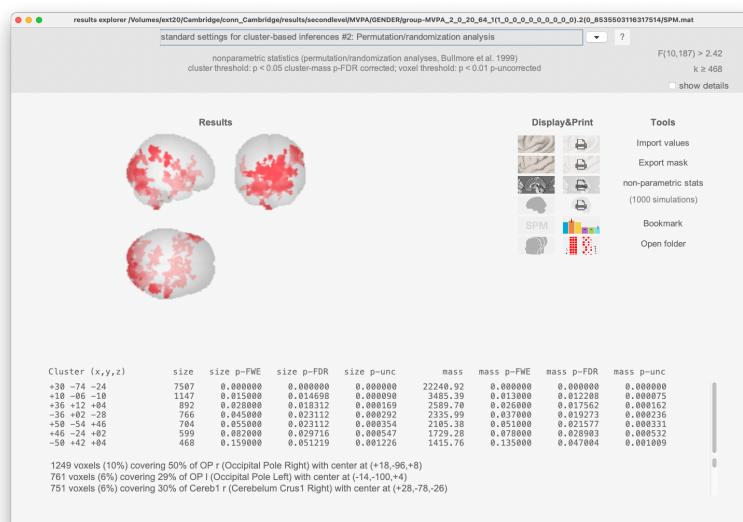


Select **MVPA.Summary** to explore the estimated MVPA eigenpatterns/components. The top-left table lists several properties of each eigenpattern (percentage  $\xi_i(x)$  of intersubject covariance in connectivity patterns associated with each eigenpattern, averaged within grey and white matter voxels separately). The top-right display shows the cumulative percentages  $\sum_{i \leq k} \xi_i(x)$  for each voxel over the first  $k$  eigenpatterns. These maps may be thresholded based on a user-defined threshold (e.g. 50%) for display purposes. The bottom displays show the distribution of the cumulative percentages  $\sum_{i \leq k} \xi_i(x)$  across voxels within grey and white matter areas separately, for varying number of eigenpatterns  $k$ .

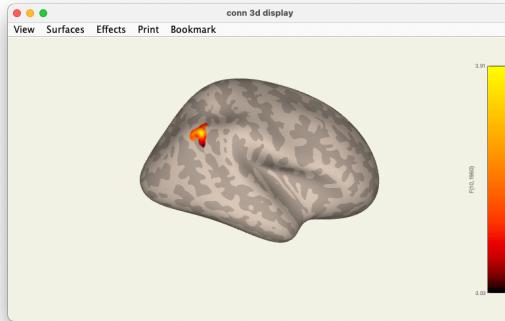


Select **MVPA.Group-analyses** to perform brain-wide connectome inferences using the estimated fc-MVPA eigenpattern scores. Second-level analyses can be defined in the same way as for any other voxel-level measure (e.g. seed-to-voxel connectivity maps). These analyses enter the estimated subject-level eigenpattern score maps for each subject/condition/network into standard second-level analyses in order analyze the connectivity patterns at each voxel across subjects and/or conditions.

Select the desired **number of eigenpatterns** in the *MVPA components* list (e.g. selecting the component 10 will enter the first 10 components in your fc-MVPA second-level analysis). After selecting the desired type of analyses (e.g. specifying the subject-effects, between-subject contrasts, etc.) click on **compute results** to compute this 2<sup>nd</sup>-level analysis (or **display results** to display the results if these analyses have already been computed). That will launch a *results explorer* window where you can specify your choice of inferential method and error rate threshold used to display the fc-MVPA results (the recommended setting for fc-MVPA inferences are the non-parametric options '*standard settings for cluster-based inferences #2: permutation/randomization analyses*' or '*standard settings for cluster-based inferences #3: threshold free cluster enhancement*' )

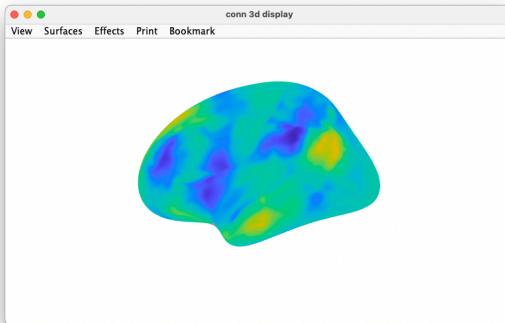


Click on individual clusters in the bottom list to select significant clusters, and use any of the surface/volume/slice/glass display options to display those clusters.

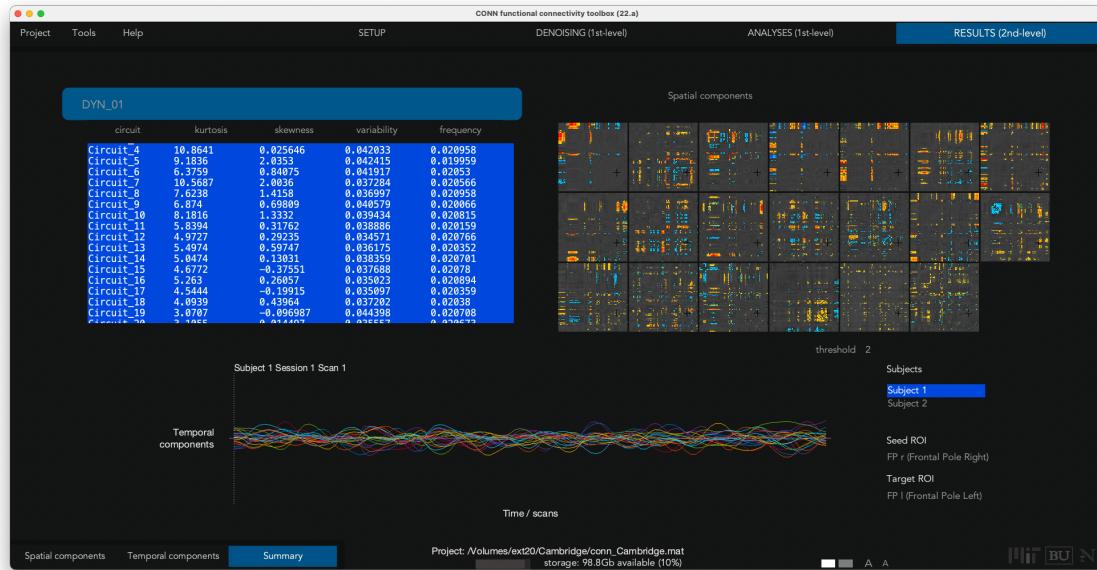


To display effect-sizes associated with each individual cluster you may click on ‘*plot effects*’(for effect-sizes across each individual eigenpattern).

Alternatively, to compute effect-sizes expanded across all voxels you may click on ‘*export mask*’ to export these clusters, and then import them back in *Setup ROIs* as a new set of ROIs, then define a new SBC 1<sup>st</sup>-level analysis (e.g. named SBC\_posthocMVPA) using these clusters as seeds, and last compute the same 2<sup>nd</sup>-level analysis (same model and contrasts as the original fc-MVPA analysis) now on this SBC\_posthocMVPA measures separately for each seed.

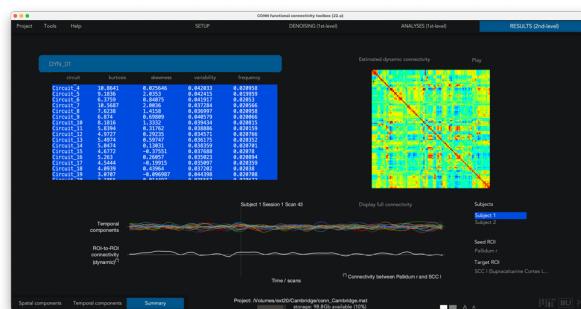


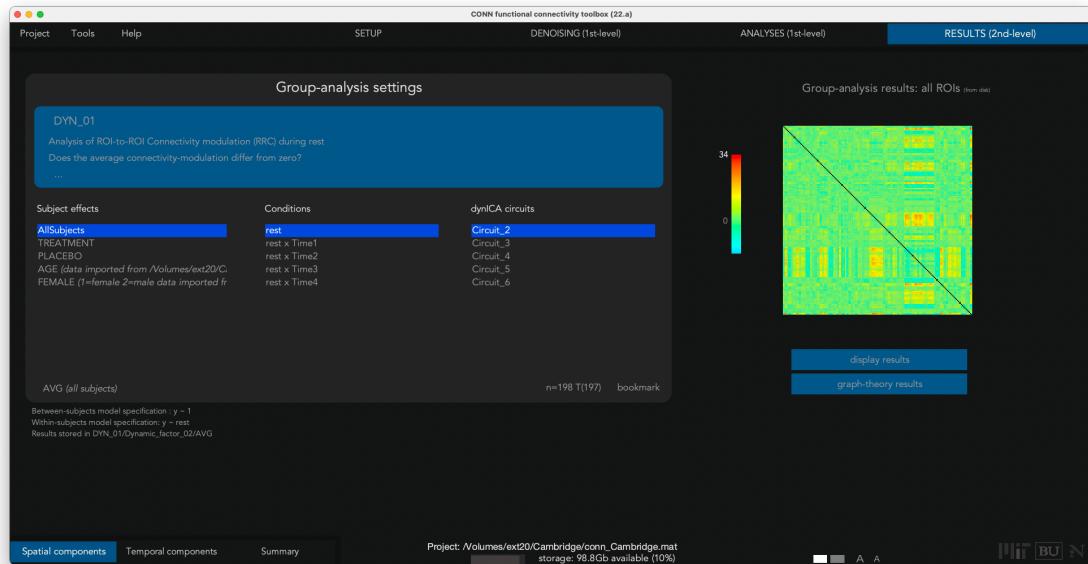
## Dynamic ICA analyses



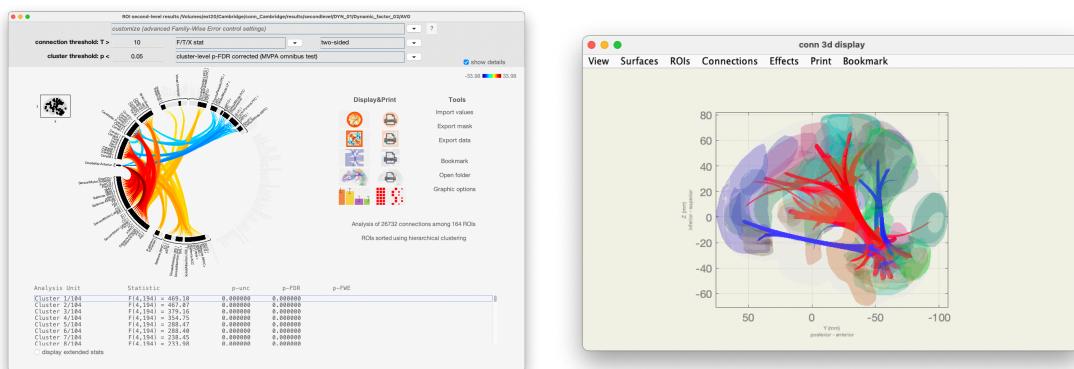
Select **dyn-ICA.Summary** to explore the estimated dynamic circuits/components. The top-left table lists several properties of each circuit (spatial variability/kurtosis/skewness, and temporal variability/frequency). The top-right display shows the group-level maps (*ICA spatial components*) for the selected circuits/components. These maps represent an ROI-to-ROI connectivity matrix with the factor loadings associated with each individual connection for each circuit color coded. These maps may be thresholded based on a z-score threshold for display purposes (factor loadings are centered and normalized across all connections to compute z-scores). The bottom display shows the connectivity modulation timeseries associated with each circuit (*ICA temporal components*) for the selected subject/condition. Hovering over the top-right plot displays the factor loadings at each connection, as well as the circuits/components with the highest loadings for each connection, which can be used to identify circuits of interest.

The top-right display can also be selected to show the associated *estimated dynamic connectivity*. From the estimated ICA decomposition the connectivity timeseries can be reconstructed showing the ROI-to-ROI connectivity matrix at each timepoint during each subject scan. The top-right display shows in this case the estimated ROI-to-ROI connectivity matrix modulation over time, and the bottom plot shows the ROI-to-ROI connectivity values over time for any selected connection (ROI pair). Select in the ‘Subjects’ list the desired subject to display. You may switch between displaying the full connectivity at each timepoint or only the contribution from one or a subset of circuits/components (by selecting the ‘display the selected circuit(s) contribution only’ option). Click on ‘Play’ to browse through the selected subject scans displaying the observed dynamic connectivity matrix at each timepoint.

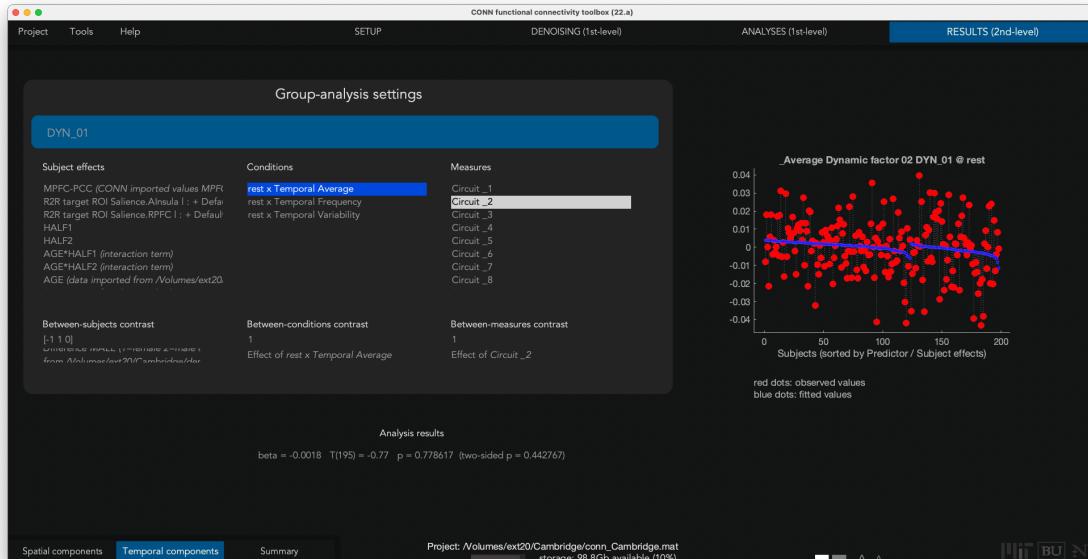




Select **dyn-JCA.SpatialComponents** to analyze the subject-level connectivity matrices for each circuit/component. These connectivity matrices define circuits / groups of connections, that share similar changes in functional connectivity across time. Second-level analyses can be defined in the same way as for any other ROI-to-ROI measure. These analyses enter the estimated subject-level spatial maps (representing ROI-to-ROI connectivity modulation matrices) for each subject/condition/circuit into standard second-level analyses in order, for example, to display the average map across all subjects for a given circuit, or compare these circuits across subjects and/or conditions. Select **compute results** to perform the desired second-level analyses across all ROIs, threshold the resulting statistical maps using a combination of connection- and seed- or network- level thresholds, perform non-parametric analyses, or additional display and analysis options. You may also select **Graph Theory** to perform additional analyses of network-theoretical measures of the resulting circuits across subjects and/or conditions.



ROI-to-ROI results explorer 3d-view figure and connectome display



Select **dyn-ICA.TemporalComponents** to analyze properties of the connectivity-modulation timeseries associated with each circuit/component. For each circuit/component, the temporal average, temporal variability, as well as the frequency (center of mass in spectral power) of these connectivity-modulation timeseries are computed for each subject and for each condition. Second-level analyses can be defined in the same way as for any other individual measure to, for example, compute the average of these measures across all subjects or compare these measures across subjects or conditions.

For more information about the CONN toolbox visit:

NITRC conn site: <http://www.nitrc.org/projects/conn>

Help forum: [http://www.nitrc.org/forum/forum.php?forum\\_id=1144](http://www.nitrc.org/forum/forum.php?forum_id=1144)

FAQ: <http://www.alfnie.com/software/conn>