# Graph Analysis Toolbox (GAT)

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## GAT Development

GAT was developed by Hadi Hosseini, PhD under supervision of Shelli Kesler, Ph.D. in collaboration with Fumiko Hoeft, MD, PhD in the Department of Psychiatry at Stanford University School of Medicine.

This Matlab based software provides a GUI framework for conducting graph theory analysis with MR data.  It integrates the Brain Connectivity Toolbox by Sporns and Rubinov (https://sites.google.com/a/brain-connectivity-toolbox.net/bct/Home) for quantification of network measures, the REX toolbox for region of interest extraction (http://web.mit.edu/swg/software.htm), the BrainNet Viewer (http://www.nitrc.org/projects/bnv/), with original code for additional analyses and comparing networks between groups.

References regarding GAT are listed below:

Hosseini, S.M.H., Hoeft, F., Kesler, S.R., 2012. GAT: a graph-theoretical analysis toolbox for analyzing between-group differences in large-scale structural and functional brain networks. PLoS ONE 7(7), e40709.  **\*Please cite this paper if you are using GAT**

Hosseini SM, Kesler SR. Comparing connectivity pattern and small-world organization between structural correlation and resting-state networks in healthy adults. NeuroImage 2013;78:402-14.

Hosseini SM, Kesler SR. Influence of choice of null network on small-world parameters of structural correlation networks. PLoS ONE 2013;8:e67354.

We have validated the toolbox in several analyses of both typically developing and neurologically affected children and adults but cannot guarantee that errors will not occur. We do not offer any extensive support for using the toolbox but please report any bugs or suggestions for updates to Hadi Hosseini (hosseiny@stanford.edu) or Shelli Kesler (skesler@stanford.edu).


## Regions of Interest (ROIs)

Before applying network measures, you need to have selected ROIs and created mask images for them or extracted values for them (e.g. functional connectivity z scores).  For example, the WFU Pickatlas software (http://www.fmri.wfubmc.edu/cms/software) allows you to create masks of various regions including those defined by Automated Anatomic Labeling (AAL).  Your masks need to be resliced so they are in the same dimensions as your MR images.  The GAT includes 90 bilateral cortical and subcortical ROIs created using the Automatic Anatomic Labeling (AAL) scheme (Tzourio-Mazoyer N, et al. NeuroImage 2002;15:273-89) in both original dimensions and resliced to SPM image dimensions (121x145x121).

## Installing GAT

1. Download GAT ( http://ncnl.stanford.edu/tools.html ) on your computer and add the directory path with subdirectories to your Matlab path.

2. Ensure you have the SPM8 directory in your Matlab path as well.

3. Start Matlab and type GAT in the command window.

4. Select the type of analysis you wish to run from the GUI window.

   a. Click "Morphometry" if you are analyzing regional brain volume, surface area, or thickness data.

   b. Click "Functional" if you are analyzing functional or resting state MRI data.

   c. Click "Behavioral" if you are analyzing the topology of behavioral data.

   d. Click "Diffusion" if you are analyzing diffusion weighted imaging data.
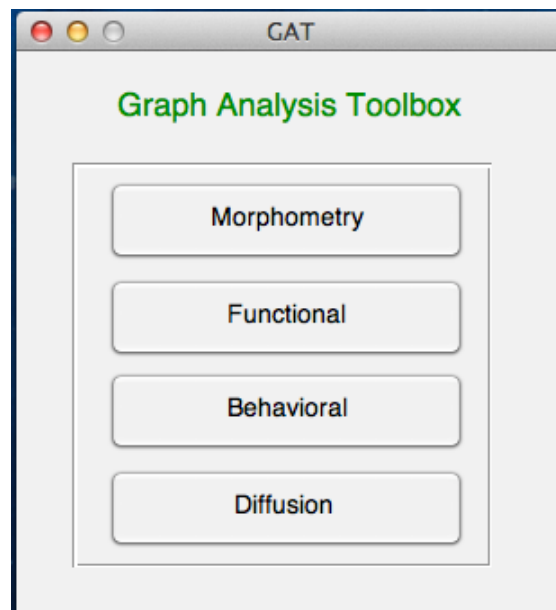


## GAT Morphometry
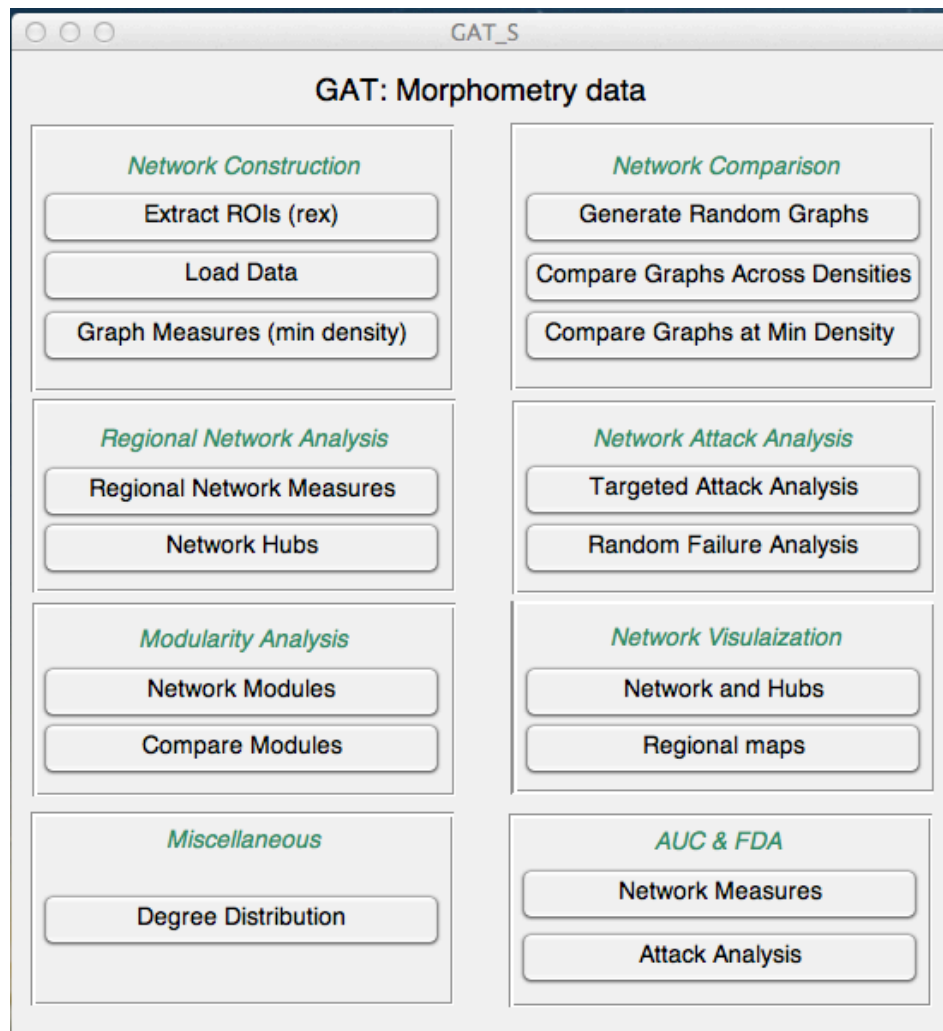
## Network Construction

1. Click on ***Extract ROIs*** and perform the following steps for each group separately:

   a. For sources choose your structural images (e.g. m0wp1*img for gray matter images from VBM8 output).

   b. For ROIs, select all of your mask images.

   c. Choose ROI-level (i.e., extracts one dataset separately for each ROI file)

   d. Choose No conjunction mask.

   e. Choose Extract mean

   f. Choose No scaling

g.  Choose Save Rex Project only.

h.  Click Extract (Caution: the output is written in the current directory so its better to make a new directory for each group's data and change the directory beforehand).

2.  Click on ***Load Data***

a.  Type the name of the first group in single quotation (e.g.  'group1')

b.  Type the name of the second group in single quotation (e.g.  'group2')

c.  Type 1 if you need to correct the data for covariates (e.g. age, total brain volume, etc.),  type 2 if you do not or your data is already corrected.

- If the answer is Yes:

- Type 1 if you want to regress out the interactions between covariates (if you have more than one covariate), type 2 if you do not.

- Then, type 1 if your covariate data is in  .xls format , type 2 if it is in .mat format.

- Select the Excel file (or Mat file) that includes Group1 covariate data. For Excel file input, data should be in the first sheet of the excel file. Names of the covariates come in the first row. The following rows contain covariate values for each subject (caution: the order of the rows should be consistent with the order of subjects' data). For .mat files, just input the covariate values (one column for each covariate, rows represent subjects).

- If the answer is No: jump to the next step.

d.  Click Yes if you want to use REX output (previous step) to extract data. Click No if you already have your data ready (outside of GAT), e.g. you have regional volumes or regional surface-area or regional thickness output from FreeSurfer.

- If the answer is Yes:

- Select Group1 REX.mat file (output from step 3)

- Select Group2 REX.mat file (output from step 3)

- If the answer is No:

> - Select the Excel file that includes Group1 regional data (the data should be in the first sheet of the Excel file. First row should include the names of the regions. The following rows should include subject's regional morphometry data).
>
> - Select the Excel file that includes Group2 regional data.

e. Select the type of null networks you are going to use: Type 2 for "degree distribution preserved null networks", type 3 for "HQS", type 4 for "Correlation matrix randomization". At this moment, there is no gold standard for this selection. We recommend using "degree distribution preserved null networks" (type 2) or HQS (type 3), although they overestimates the clustering coefficient and path length, respectively.

f. Number of null networks: usually a number between 10 and 20 works good. However, with 20, the non-parametric permutation takes longer.

g. Type 1(or 2) for one (or two)-tailed analysis.

h. Input the threshold level (0.05).

i. This outputs mat4GAT.mat file that contains all the above information (each group's data, group names, roi names, covariates (if any), etc.).

3. Click on ***Graph Measures (Minimum Density)***

> *This option allows comparing two graphs at the minimum graph density at which both graphs are fully connected (not fragmented).*

a. Type 1 (or 2) for simple (or partial) correlation analysis. Select partial correlation if your sample size is large (> 50). If you get a lot of NaN, your data are probably over partialed e.g. ROIs include both gray and white matter or structures that remove too much of the variance from others (cancel each other out).

b. Select the output mat4GAT.mat file from "Load Data".

c. This outputs KeepAllNodes_Results.mat file containing:

i. Output1: the thresholded correlation matrix for group 1

ii. Output2: the thresholded correlation matrix for group 2

iii. Output1_Binary: the thresholded binary graph for group 1

iv. Output2_Binary: the thresholded binary graph for group 2

v. Thresh1: the threshold level for group1 graph

vi. Thresh2: the threshold level for group2 graph

vii. Density1: the number of edges of the Output1

viii. Density2: the number of edges of the Output2

ix. NetMes_Bin1: Network measures (degree, clustering coef, etc.) for Output1_Binary

x. NetMes_Bin2: Network measures (degree, clustering coef, etc.) for Output2_Binary

xi. It also reports the minimum density at which both networks are not fragmented. We recommend using this density as the lower bound for FDA and AUC analysis.

*It also plots the final correlation matrices and binary graphs and save it to the current directory.*

## Network Comparison

1. Click on ***Generate Random Graphs***

   *This function makes N random connectivity matrices by randomly assigning each subject's data to a group.  It uses sampling with replacement and the size of the generated samples for each group is the same as the original group size.*

   a. Type the number of random networks to be generated (default: 1000 networks)

   b. Type 1 (or 2) for simple (or partial) correlation analysis. Select the same option that you chose in 13.a.

   c. Select the output mat4GAT.mat file from "Load Data".

    d.   This outputs RandNetworksBootstrap _Results.mat file containing:

         i.   R1: Original unthresholded correlation matrix for group1.

         ii.   R2: Original unthresholded correlation matrix for group2.

         iii.   R_G1: Cell array containing randomly generated unthresholded correlation matrices for group 1

         iv.   R_G2: Cell array containing randomly generated unthresholded correlation matrices for group 2

2.   Click on ***Compare Graphs Across Densities***

      *This function compares the between-group differences in graph measures (e.g. clustering, characteristic path length, small-worldness, global efficiency, etc.) with their difference in randomly-generated graphs and plot the graph measures for each group (as well as between-group differences in graph-measures). It also generates the corresponding 95% confidence interval to see if the observed between-group differences in graph measures are significant or not. In addition, it outputs the p-values for the performed statistics.*

    a.   Select the "Rand Networks Bootstrap_Results.mat " file (output from "Generate Random Graphs").

    b.    Select the output mat4GAT.mat file from "Load Data".

    c.   Input the minimum network density of interest. You can check the KAN_FixedDens_Results.mat (output from Graph Measures (Minimum Density) and use the minimum network density of full connectivity for minimum density of interest here.

    d.   Input the maximum network density of interest. For structural networks, densities above 50% might not have biological meaning.

    e.   Input the density interval (steps): default is 0.02.

    f.   This outputs:

         i.   "NetMes_Bin_group1(2).mat": a .mat file containing NetMes_B1(B2) cell array which includes group1 (group2) network measures across a range of density (default 0.05 to 045).  NetMes_B1(B2) {i} contains group1 (group2) network measures at $i^{th}$ density step.

ii.   "NetMes_Bin_rand_group1(2).mat": a .mat file containing NetMes_B1(B2)_rand cell array which includes network measures for randomly generated graphs associated with group1 (group2) across a range of density (default 0.05 to 045). NetMes_B1(B2)_rand_mat{i}{j} contains graph measures at $i^{th}$ density step for $j^{th}$ random network.

iii.  "NetMesPerDens_group1(2)_mat.mat": a mat file containing network measures for randomly generated graphs (group1(2)) across a range of densities in separate .mat files.

iv.   "NetMesPerDens_OrigNet_group1(2).mat": a mat file containing network measures for group 1(group2) graphs across a range of densities in separate .mat files.

v.    A set of figures showing the difference in network measures (as we1l as network measures themselves) for group1, group2 and randomly-generated graphs. They also include the 95% confidence intervals to check if the observed between-group differences in graph measures are statistically significant or not.

vi.   The p-values for each network measure across a range of densities.

vii.  A set of .mat files containing the thresholded graphs (original and randomly generated ones) named "ThreshDens_group1(2)" and "ThreshDens_rand_group1(2)".

3.  Click on ***Compare Graphs at Minimum Density***

*This option allows computing the p-values of the comparison of network measures at minimum density.*

a.  Select the mat4GAT.mat file from "Load Data".

b.  Select the KAN_FixedDens_Results.mat file (output from "Graph Measures at Minimum Density").

c.  Select the "Rand Networks Bootstrap_Results.mat "file containing original and randomly generated graphs  (output from "Generate Random Graphs")

d.  Select the "ThreshDens_rand*.mat" file for group 1 (output from compare graphs at arranges of densities).

e.  Select the "ThreshDens_rand*.mat" file for group 2 (output from compare graphs at arranges of densities).

f.  This outputs:

   i.  AtMinDens_ NetworkMeasure_pval: The estimated p-values that shows the significance of between group differences in corresponding network measure. Same information is written in a text file named "PvalsAtMinDens.dat".

   ii.  RandNets_ThreshAtMin.mat: the thresholded random networks

## Regional Network Measures

1.  Click on ***Regional Network Analysis***

   *This function gets the outputs from the previous step and calculates the between-group differences in normalized regional network (thresholded at minimum density) measures for each ROI. It also generates the 95% confidence interval for each measure to see if the observed between-group differences in regional measures are statistically significant or not.*

   a.  Select the output mat4GAT.mat file from "Load Data".

   b.  Select the "NetMes_Bin_group1.mat" file (output from Compare Graphs Across Densities)

   c.  Select the "NetMes_Bin_group2.mat" file (output from Compare Graphs Across Densities)

   d.  Select the "NetMes_Bin_rand_group1.mat" file (output from Compare Graphs Across Densities)

   e.  Select the "NetMes_Bin_rand_group2.mat" file (output from Compare Graphs Across Densities)

   f.  Select the KAN_FixedDens_Results.mat  file (output from "Graph Measures at Minimum Density").

   g.  This outputs:

   i.  NetMesReg_group1(2): a .mat containing regional network measures (clustering, degree, and node-betweenness centrality) for group1 (group2) network thresholded at minimum density

ii.  NetMesReg_rand_group1(2): a .mat containing regional network measures (clustering, degree, and node-betweenness centrality) for randomly generated networks associated with group1 (group2) each thresholded at minimum density.

iii.  It also generates a set of figures showing the between-group differences in regional network measures for group1 vs. group2 as well as for randomly-generated graphs. They also include the 95% confidence intervals to check if the observed between-group differences in regional network measures are statistically significant or not.

iv.  In addition, for each regional network measure, four mat files that include the (uncorrected and FDR-corrected) p-values for the test of significance of the difference in (original and normalized) network measures are written in the current directory (e.g. RegDeg_pval.mat, RegDegNorm_pval.mat, fdr_corrected_pval_RegDeg.mat, and fdr_corrected_pval_RegDegNorm.mat are generated for Regional Degree analysis).

2.  Click on ***Network Hubs***

*This function generates the network hubs (based on "degree" and "node-beweeness". Hubs are considered as nodes that their degrees (node-betweenness) are 2\*SD greater than average network degree.*

a.  Select the output mat4GAT.mat file from "Load Data".

b.  Select the KAN_FixedDens_Results.mat file (output from "Graph Measures at Minimum Density").

c.  Type the criterion for calculating hubs: type "*n*" if you want to consider hubs as those nodes that their degree (betweenness) is n\*SD greater than average network degree (betweenness). (2 would be a good candidate).

d.  It outputs:

i.  Net_Hubs.mat that contains the cell array "HubNames" that stores the name of the hubs for the selected network.

# Network Attack Analysis

1. Click on ***Targeted Attack Analysis***

   *This function performs targeted attack analysis on the input graphs (between two groups). It repetitively removes the network nodes in the order of their importance (betweenness centrality, degree, etc.) and calculates the size of the remaining largest component of the network (or diameter, efficiency, etc, of the remaining network). It does the same procedure on the randomly generated networks to see if the observed between-group difference in robustness to attack for the two original networks is significant.*

   a. Select the output mat4GAT.mat file from "Load Data".

   b. Select the "DensityInterval.mat" file.

   c. Select the "CorrMat_group1/2.mat" (output from Compare Graphs Across Densities)

   d. Select the "CorrMat_group1/2_rand.mat" (output from Compare Graphs Across Densities)

   e. Type the measure based on which the nodes are removed:

      - "betw": betweenness cenrality

      - "deg": degree

      - "dist": distance

      - "clust": clustering

      - "leff"; local efficiency

   f. Type the measure based on which the influence of attack is analyzed:

      - "comp": size of the largest component

      - "dist": diameter

      - "deg": mean degree

      - "assort": assortativity

      - "dens": density

- "clust": clustering

- "trans": transitivity

- "geff": global efficiency

- "leff": mean local efficiency

- "mod" (or "modl"): mean modularity

- "charpath": characteristic path length

- "node_betw": node betweenness

- "lambda": normalized path length

- "gamma": normalized clustering

- "sigma": small world index

g. Type the network density of interest (e.g. min density from step 7)

h. It outputs:

   i. "MeanSizeDiff_TargAtt_group1group2.fig" that shows the behavior of group1 and group2 networks in response to targeted attack. The stars show in which points the difference is significant.

   ii. "PvalTargAttack_group2vsgroup1" that contains the p-values of the between-group differences in network response to targeted attack.

   iii. It also writes the attacked network sizes in: MSizeTargAttack_group1/2; MSizeTargAttack_Rand_group1/2

2. Click on ***Random Failure Analysis***

   *This function performs random failure analysis on the input graphs (between two groups). It repetitively removes the network nodes in random order and calculates the size of the remaining largest component of the network. It does the same procedure on the randomly generated networks to see if the observed between-group difference in robustness to random failure for the two original networks is significant.*

a. Select the output mat4GAT.mat file from "Load Data".

b. Select the "DensityInterval.mat" file.

c. Select the "CorrMat_group1/2.mat" (output from Compare Graphs Across Densities)

d. Select the "CorrMat_group1/2_rand.mat" (output from Compare Graphs Across Densities)

e. Type the number of simulations (n). Each step of random node removal procedure will be repeated n times and the average of the remained largest components are calculated.

f. Type the network density of interest (e.g. min density from step 7)

g. It outputs:

   i. "MeanSizeDiff_RandAtt_group1group2.fig" that shows the behavior of group1 and group2 networks in response to random attack. The stars show in which points the difference is significant.

   ii. "PvalRandAttack_group2vsgroup1" that contains the p-values of the between-group differences in network response to random attack.

   iii. It also writes the attacked network sizes in: MSizeRandAttack_group1/2; MSizeRandAttack_Rand_group1/2

## Modularity Analysis

1. Click on ***Network Modules***

*This function performs the modularity analysis on the input graphs (for each group separately). It breaks the network into non-overlapping modules of nodes in a way that maximizes the number of within-module edges, and minimizes the number of between-module edges (i.e. it finds the optimized solution). Note that since this is an optimization problem, you need to repeat it n times.*

a. Select the output mat4GAT.mat file from "Load Data".

b. Select "KAN_FixedDens_Results.mat " file (output from " Graph Measures at Minimum Density).

c. Type number of iterations n (e.g. 100).

     d.   It outputs "ModularCommunity_Group1(2).mat" that contains two .mat files (Com_g1(2) and Coml_g1(2)) for each group networks.  Each of these .mat is a cell array of size (1 * nModule) that contains the names of the nodes (brain regions) in corresponding module.  Each .mat file contains the results for Modularity (ComL_g1/2) and Modularity_Louvian (ComL_g1/2), separately.

2.   Click on ***Compare Modules*** (not implemented yet!)

## Network Visualization

1.   Click on ***Network & Hubs***

*This function generates the input files required for plotting the network on ICBM152 template using BrainNet Viewer. Note that for visualization, the order of the ROIs (AAL-LR, AAL, and Freesurfer) that you input to GAT should match the order that comes at the end of this manual.*

     a.   Type the ROI scheme used for graph analysis: type 1 for AAL-LR, 2 for AAL original, 3 for FreeSurfer, 4 for manual dimension input.

     b.   Here you indicate that size of each node represents nodal betweenness (type 1) or degree (2) or clustering (3). If you wich o see the nodes all in the same size, type 4.

     c.   Select the output mat4GAT.mat file from "Load Data".

     d.   Select "KAN_FixedDens_Results.mat " file (output from " Graph Measures at Minimum Density).

     e.   If the ROIs that you used are a subset of the ROIs implemented here, you may choose those by clicking on their names.

     f.   The code also asks if you want to display the modular structure in the network. If you want, the code will generate a .node file that comprises the modular structure.

     g.   It outputs two files for each group network: Nodes_Group1(2).nodes and Edge_Group1(2).edge. These files will be used as the input to BrainNet Viewer for visualization.

2.  Click on ***Regional Maps***

*This function generates the regional maps (Betw, Deg, CLust) for overlaying them on ICBM152 template in BrainNet Viewer.*

a.  Indicate the regional map that you want to generate: 1 for Betweenness, 2 for Degree, 3 for Clustering.

b.  Select the output mat4GAT.mat file from "Load Data".

c.  Select the normalized "RegNode* _Pval.mat " file (output from " Regional Network Measures).

d.  The program outputs the list of ROIs that showed significant between-group differences in network measure of interest (Betw/Deg/Clust). You need to select the images corresponding to these ROIs in the pop up dialogue (SPM imcalc) and press Done.

e.  It outputs an image that maps the between-group differences in regional network measure of interest. These files will be used as the input to BrainNet Viewer for visualization.

## AUC and FDA Analyses

1.  Click on ***Network Measures***

*This function performs an area under a curve (AUC) and functional data analyses (FDA) for network measures. It simply compares the between-group differences in area under different network measure curves (e.g. Sigma, Gamma, Lambda, etc.) or summation of curves across a range of densities. These analyses depends on the range of densities. It is suggested to select a density range in which the network is fully connected. The minimum density of full connectivity is one of the outputs of "Graph Measures (min Density)" step that can be used for the lower limit of density for AUC and FDA analysis. The maximum would be a density preferably lower than 0.5.*

a.  Type 1 for AUC or 2 for FDA analysis.

b.  Type 1 for global measures, 2 for regional, and 3 for hubs.

i. For **AUC/FDA + Global**:

- Select the output mat4GAT.mat file from "Load Data".

- Select the "NetMesPerDens_OrigNet_Group1.mat" (output from "Compare Graphs Across Densities").

- Select the "NetMesPerDens_OrigNet_Group2.mat" (output from "Compare Graphs Across Densities").

- Select the "NetMesPerDens_Group1.mat" (output from "Compare Graphs Across Densities").

- Select the "NetMesPerDens _Group2.mat" (output from "Compare Graphs Across Densities").

- Select the "DensityInterval.mat" (output from "Compare Graphs Across Densities").

- Type the minimum density of interest (greater than or equal to the minimum density value that you picked when doing "Compare Graphs Across Densities".

- Type the maximum density of interest (smaller than or equal to the maximum density value that you picked when doing "Compare Graphs Across Densities".

- Input the density interval (steps).

- It will output a p-value for each of the network measures AUC/FDA that shows the significance of the between group differences in AUC/FDA for that measure. The p-values are also saved in mat files named "AUC/fda_[Net Measure]_pval.mat".

ii. For **AUC /FDA + Regional**:

- Type the minimum density of interest (greater than or equal to the minimum density value that you picked when doing "Compare Graphs Across Densities".

- Type the maximum density of interest (smaller than or equal to the maximum density value that you picked when doing "Compare Graphs Across Densities".

- Type the density interval (step) of interest (greater than or equal to the density step that you picked when doing "Compare Graphs Across Densities".

- Select the output mat4GAT.mat file from "Load Data".

- Select the "NetMes_Bin_group1.mat" file (output from Compare Graphs Across Densities)

- Select the "NetMes_Bin_group2.mat" file (output from Compare Graphs Across Densities)

- Select the "NetMes_Bin_rand_group1.mat" file (output from Compare Graphs Across Densities)

- Select the "NetMes_Bin_rand_group2.mat" file (output from Compare Graphs Across Densities)

- Select the DensityInterval.mat file.

- It will output a set of p-values for each of the regional network measure's AUC/FDA. The p-value shows the significance of the between group differences in AUC/FDA for that measure. The p-values are also saved in mat files named "AUC/fda_[NetMesReg]_pval.mat". The code also generates figures showing the differences in AUC of regional network measures between groups.

iii.  For **AUC/FDA + Hubs**:

- Type the criterion for calculating hubs: type "*n*" if you want to consider hubs as those nodes that their degree (betweenness) is n*SD greater than average network degree (betweenness). (2 would be a good candidate).

- Select the output mat4GAT.mat file from "Load Data".

- Select the "AUC/FDA_NetMesReg_NormGroup1.mat" file (output from "AUC/FDA Regional"

- It will output "AUC/fda_Net_Hubs_Betw/Deg_Group1/2.mat" that contains the cell array "HubNames" that stores the name of the hubs for the selected network.

2. Click on ***Attack Analysis***

*This function performs AUC and/or FDA analysis for the curves resulted from random failure and targeted attack analysis, separately.*

    a. Type 1 for AUC or 2 for FDA analysis.

    b. Type 1 for "Targeted attack", 2 for "Random failure", 3 for both.

    c. Select the output mat4GAT.mat file from "Load Data".

    d. Select the "MSizeTarg(orRand)Attack_Group1.mat" (output from "Targeted/Random Attack Analysis").

    e. Select the "MSizeTarg(orRand)Attack_Group2.mat" (output from "Targeted/Random Attack Analysis").

    f. Select the "MSizeTarg(orRand)Attack_Rand_Group1.mat" (output from "Targeted/Random Attack Analysis").

    g. Select the "MSizeTarg(orRand)Attack_Rand_Group2.mat" (output from "Targeted/Random Attack Analysis").

    h. It outputs the p-value of between-group differences in targeted/random attack AUC/FDA ("AUC/FDA_Target/RandAttack_pval.mat").

## Degree Distribution

1. Click on ***Degree Distribution***

*This function generates the degree distribution for each group network and finds an exponential truncated power law function that fits the network degree distribution.*

    a. Type 1 for the input type.

    b. Select the output mat4GAT.mat file from "Load Data".

    c. Type 1 to explore degree distribution at Dmin.

    d. Select "KAN_FixedDens_Results.mat " file (output from " Graph Measures at Minimum Density).

e.  It will generate three plots and displays the parameters for the fitted function for the first group network in Matlab command window. Check the "Log Plot of Cumulative Degree Distribution" figure and if the fitted line is satisfactory, type 1 to continue. Otherwise, you can try to play with "a" and "b" manually to see how the fit works.

f.  Follow the above procedure for group2 network.

g.  It outputs degree distribution histogram, and normal (and log) plot of cumulative degree distribution for each network.

## GAT Functional

The GAT functional works with the output from the CONN toolbox, which conducts functional connectivity analysis (http://www.nitrc.org/projects/conn Whitfield-Gabrieli S, et al. Brain connectivity 2012;2:125-41). You may also do the functional connectivity analysis using other tools and put the connectivity matrices in an array of size N*N*M (N: number of rois, M: number of subjects).
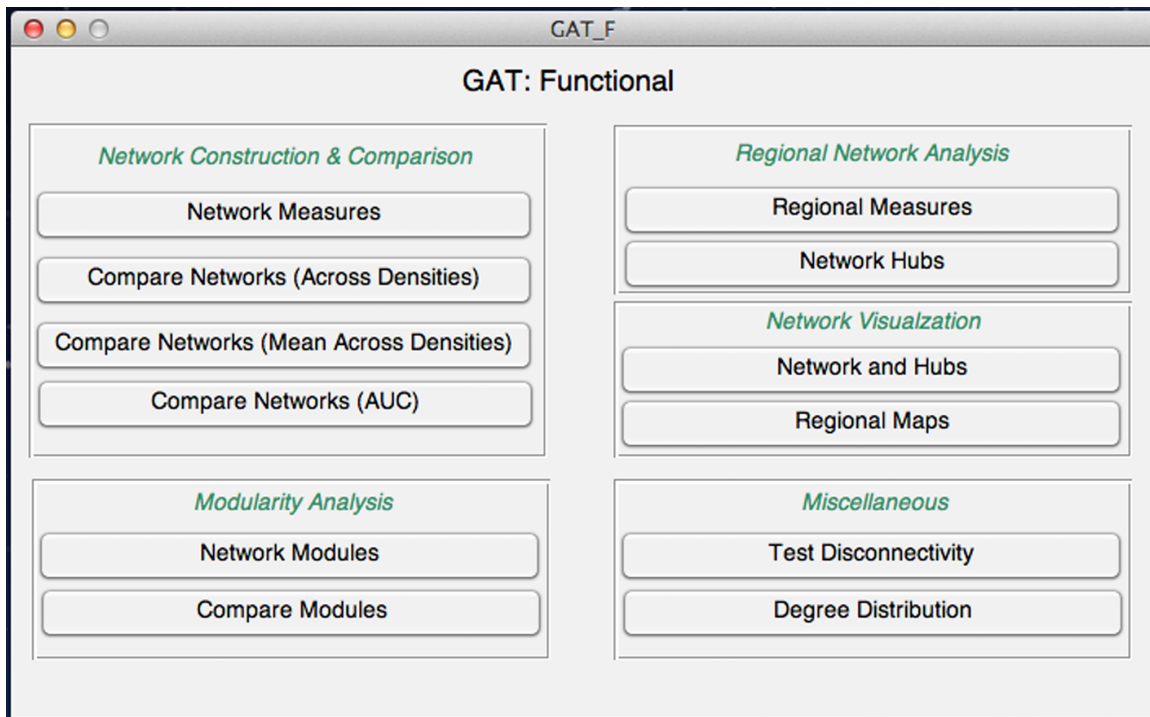


## Network Construction and Comparison

1. Click on ***Network Measures***

    *This function gets the output from CONN toolbox ("resultsROI_Condition*.mat") and calculates individual functional network measures for each group at a range of densities. Note that the output "resultsROI_Condition*.mat" file should have the correlation maps for both groups. (e.g. the CONN output mat file would be a 90*90*48 where 90 is the number of ROIs and 48 is the total number of subjects in both groups:  group1 (1:24) and group2 (25:48)).*

    a. Type name of the dataset in single quotation (e.g. 'Group1&Group2_data').

b.  Type the lowest density of interest (e.g. 0.1).

c.  Type the highest density of interest (e.g. 0.6).

d.  Type the density intervals (steps) of interest (e.g. 0.02).

e.  Type 1 if you want to regress out the effect of covariates, and 2 if you do not. Note that here you may need to correct for between-group covariates (e.g. age, gender, etc.) and not intra-subject covariates (e.g. movement params). The intra-subject covariates should have already taken into account in CONN.

f.  If you typed 1 (regress out the covariates of no interest) in previous step,

    i.  Type 1 if you want to regress out the interactions between covariates (if you have more than one covariate), type 2 if you do not.

    ii.  Then, type 1 if your covariate data is in  .xls format , type 2 if it is in .mat format.

    iii.  Select the Excel file (or Mat file) that includes covariate data for Group 1. Group1 is the group whose data comes first in the CONN output file. (for Excel inputs, data should be in the first sheet of the excel file. Names of the covariates come in the first row. The following rows contain covariate values for each subject) (caution: the order of the rows should be consistent with the order of subjects' data in CONN output).

    iv.  Select the Excel file (or Mat file) that includes covariate data for Group 2.

g.  Select the "ResultsROI_Condition*.mat" file (output from CONN toolbox)

h.  Here you may select the ROIs that you are interested in. Otherwise, all the ROIs will be included in the analysis.

i.  Number of null networks: usually a number between 10 and 20 works good.

j.  Type 1(or 2) for one (or two)-tailed analysis.

k.  Input the threshold level (0.05).

l.  Next, the code will extract the thresholded correlation matrices for each subject across the specified range of densities. Then it will ask for group specific information:

      i.   Type the name of the first group in single quotation (e.g. 'group1').

      ii.   Type the name of the second group in single quotation (e.g. 'group2')

      iii.   Type number of subjects in group1.

      iv.   Type number of subjects in group2.

m.  It outputs:

      i.   "mat4GATf.mat" file that contains group information (each group's name, roi names, and covariates (if any)).

      ii.   A set of mat files "NetMesBin_f_Group1(2)_FinalThrRange.mat" (or "NetMesBin_f_Adjusted_Group1(2)_FinalThrRange.mat") that includes the individual network measures thresholded across the range of densities of interest.

2.   Click on ***Compare Networks (Across Densities)***

*This function gets the outputs from previous step ("network measures") and compares the network measures between groups. It tests the significance of the between-group differences in network measures using 2-sample t-test as well as using permutation analysis.*

a.  Type the number of random network to be generated (at least 1000).

b.  Select the output "mat4GATf.mat" file from "Network Measures".

c.  Select the "NetMesBinf_(adjusted)_Group1(2)_FinalThrRange.mat" file; output from "Network Measures".

d.  After generating plots of network measures across densities, the program will ask you for new density range values before doing 2-sample t-test. You can check the graphs and select a range in which the networks are small-worlds.

e.  It outputs:

      i.   "NetMesPerDens_f.mat": a mat file containing network measures for randomly generated graphs (group1(2)) and original networks thresholded across a range of densities.

ii. A set of figures showing the differences in network measures (as well as network measures themselves) for group1, group2 and randomly-generated graphs. They also include the 95% confidence intervals to check if the observed between-group differences in graph measures are statistically significant or not. Note that these results are based on permutation analysis.

iii. The p-values for comparing between-group differences in each network measure across a range of densities.

iv. It also makes a folder named "TTest_Results" and saves the p-values for 2-sample t-test results ("pVal_TTest.mat") in there.

3. Click on ***Compare Networks (Mean Across Densities)***

   *This function gets the outputs from previous step ("Compare Networks Across Densities") and compares the average network measures between groups.*

   a. Select the output "mat4GATf.mat" file from "Network Measures".

   b. Select the "NetMesPerDens_f.mat" file; output from previous step.

   c. Type the minimum threshold.

   d. Type the maximum threshold.

   e. It will output:

      i. The p-values for comparing between-group differences in average network measure across a range of densities (permutation testing) (e.g. Sigma_*_pval.mat).

      ii. The p-values for 2-sample t-test results ("pVal_TTest.mat").

4. Click on ***Compare Networks (AUC & FDA)***

   *This function gets the outputs from previous step ("Compare Networks Across Densities") and compares the average network measures between groups.*

   a. Type 1 for AUC or 2 for FDA analysis.

b.  Select the output "mat4GATf.mat" file from "Network Measures".

c.  Select the "NetMesPerDens_f.mat" file; output from step 2.

d.  Type the minimum threshold.

e.  Type the maximum threshold.

    i.  It will make a folder named AUC/FDA_Results and output the p-values for comparing between-group differences in AUC/FDA of network measures (permutation testing) (e.g. AUC(FDA)_Sigma_*_pval.mat).

## Regional Network Analysis

1.  Click on ***Regional Measures (AUC & FDA)***

*This function gets the outputs from previous steps and compares the regional network measures between groups (AUC and FDA analyses).*

a.  Type the number of random network to be generated (at least 1000).

b.  Select the output "mat4GATf.mat" file from "Network Measures".

c.  Select the "NetMesBinf_(adjusted)_Group1(2)_FinalThrRange.mat" file; output from "Network Measures".

d.  Type the minimum threshold.

e.  Type the maximum threshold.

f.  It outputs:

    i.  A set of p-values showing the significance of the difference in AUC/FDA of regional network measures (e.g. AUC_RegDegNorm*_pval.mat, fda_RegDegNorm_*_pval.mat).

    ii.  A set of figures showing between-group differences in regional network measures.

2.   Click on ***Network Hubs (AUC & FDA)***

*This function gets the outputs from previous steps and quantifies hubs based on AUC/FDA.*

h.   Type the criterion for calculating hubs: type "*n*" if you want to consider hubs as those nodes that their degree (betweenness) is n*SD greater than average network degree (betweenness). (2 would be a good candidate).

i.   Select the output "mat4GATf.mat" file from "Network Measures".

j.   Select the "AUC/FDA_NetMesReg_Group1.mat" file (output from "Regional Measures AUC/FDA ".

k.   Select the "AUC/FDA_NetMesReg_Group2.mat" file (output from "Regional Measures AUC/FDA ".

l.   It will output "Net_Hubs_Betw/Deg_Group1/2.mat" that contains the cell array "HubNames" that stores the name of the hubs (based on AUC/FDA) for the selected networks.

## Network Visualization

1.   Click on ***Network & Hubs***

*This function generates the input files required for plotting the networks on ICBM152 template using BrainNet Viewer.*

a.   Type the ROI scheme used for graph analysis: type 1 for AAL-LR, 2 for AAL original, 3 for FreeSurfer, 4 for manual dimension input.

b.   Next you indicate that size of each node represents nodal betweenness (type 1) or degree (2) or clustering (3). If you want to display the nodes all in the same size, type 4.

c.   Select the output "mat4GATf.mat" file from "Network Measures".

d.   Select the "NetMesBinf_(unadjusted)_Group1(2)*.mat" file; output from "Network Measures".

e.   Type the minimum threshold.

f.   Type the maximum threshold.

g.   If the ROIs that you used are a subset of the ROIs implemented here, you may choose those by clicking on their names.

h.   The code also asks if you want to display the modular structure in the network. If you want, the code will generate a .node file that comprises the modular structure.

i.   It outputs two files for each group network: Nodes_Group1(2).nodes and Edge_Group1(2).edge. These files will be used as the input to BrainNet Viewer for visualization.

2.   Click on ***Regional Maps*** *(not implemented yet!)*


## Modularity Analysis

1.   Click on ***Network Modules***

*This function performs the modularity analysis on the input functional graphs. It breaks the network into non-overlapping modules of nodes in a way that maximizes the number of within-module edges, and minimizes the number of between-module edges. Note that since this is an optimization problem, you need to repeat it* <u>*n*</u> *times.*

a.   Select the output "mat4GATf.mat" file from "Network Measures".

b.   Select the unadjusted  "NetMesBinf_Group1(2)_FinalThrRange.mat" file; output from "Network Measures".

c.   Type number of iterations <u>n</u> (e.g. 100).

d.   Type the minimum threshold.

e.   Type the maximum threshold.

f.   It outputs "ModularCommunity_Group1(2).mat" that contains two .mat files (Com_g1(2) and Coml_g1(2)) for each group networks.  Each of these .mat is a cell array of size (1 * nModule) that contains the names of the nodes (brain regions) in corresponding module.  Each .mat file contains the results for Modularity (Com_g1/2) and Modularity_Louvian (ComL_g1/2), separately.

2. Click on ***Compare Modules*** (not implemented yet!)

## Test Disconnection

1. Click on ***Test Disconnection***

*This function finds the density at which the networks get fragmented. It is always good to compare the functional networks between groups in a density range at which the networks are not fragmented. You need to test the disconnections for each group network separately.*

    a. Select the "NetMesBinf_Group1(2)_*.mat" file that you want to test (output from "Network Measures").

    b. It outputs an array (DiscResults.mat) showing the disconnection density associated with individual's network. It also outputs the minimum network density at which no individual's networks is fragmented.

2. Click on ***Degree Distribution***

*This function generates the degree distribution for each group network (by averaging individual network degree) and finds an exponential truncated power law function that fits the network degree distribution.*

    a. Select the output "mat4GATf.mat" file from "Network Measures".

    b. Select the unadjusted  "NetMesBinf_Group1(2)_FinalThrRange.mat" file; output from "Network Measures".

    c. Type number of iterations $\underline{n}$ (e.g. 100).

    d. Type the minimum threshold.

    e. Type the maximum threshold.

    f. It will generate three plots and displays the parameters for the fitted function for the first group network in Matlab command window. Check the

"Log Plot of Cumulative Degree Distribution" figure and if the fitted line is satisfactory, type 1 to continue. Otherwise, you can try to play with "a" and "b" manually to see how the fit works.

g.  Follow the above procedure for group2 network.

h.  It outputs degree distribution histogram, and normal (and log) plot of cumulative degree distribution for each network.

# GAT Diffusion
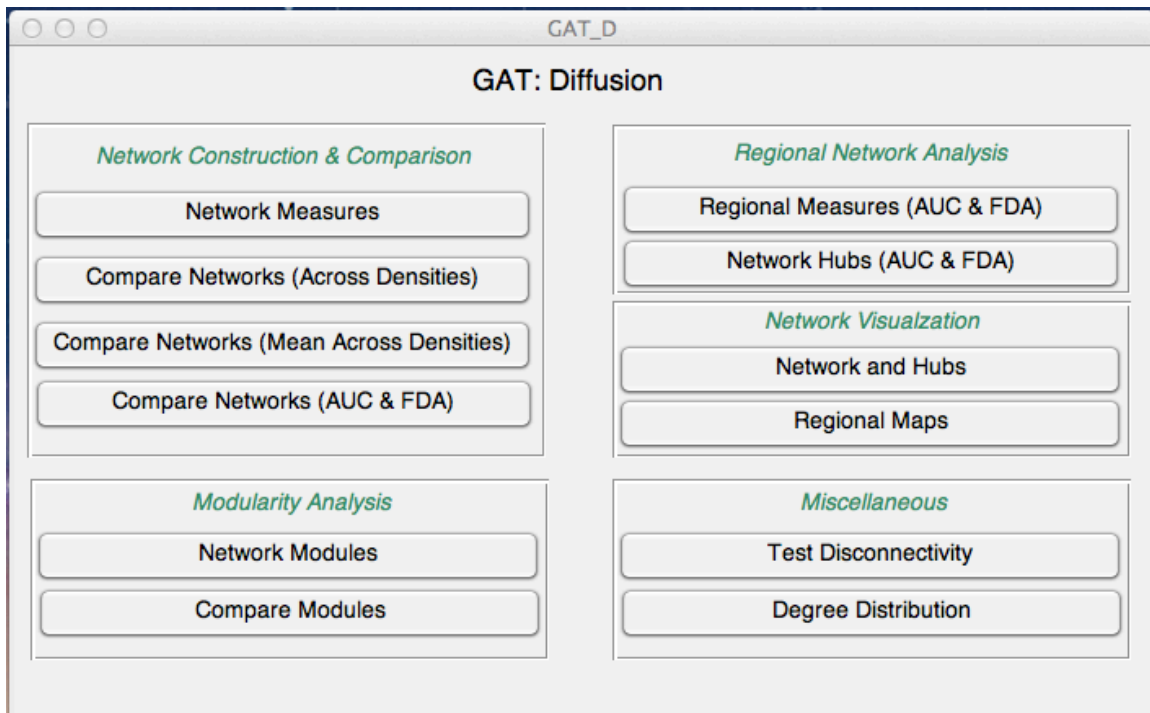


The procedure for performing graph analysis on diffusion networks is similar to that of functional networks. You need to have a network of size Nroi*Nroi for each individual. The only difference is that you need to make the input in the following format. A Matlab array "Z" of size Nroi*Nroi*Nsbj containing individual networks and a cell array "names" of size 1*Nroi that includes the names of ROIs. These two variables need be saved in a .mat file named "results_diff*.mat". The GUI asks you to choose the "results_diff*.mat" file to kick off the analysis. The Z array should include both subject's data (ordered by groups).

# GAT Behavioral

The procedure for performing graph analysis on behavioral data is similar to that of morphometric networks.
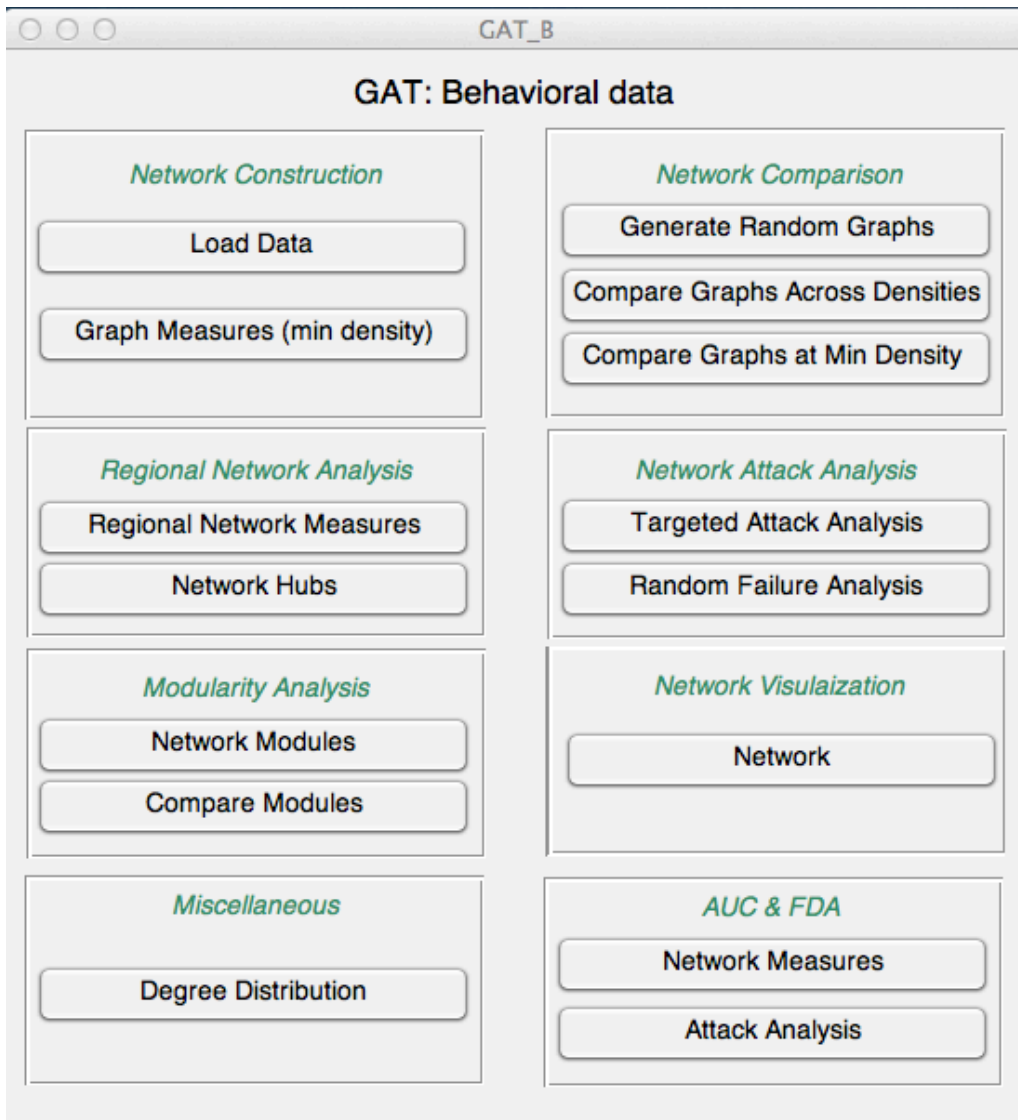
# Notes

lambda = normalized path length

gamma = normalized clustering coefficient

sigma = small worldness

- The p-value for each network measure is a vector corresponding to the number of density thresholds (e.g. for 0.05:0.01:0.45, p(1) is the p-value for comparing networks at density=0.05).

- A positive p value means group2 network measure is significantly (p<0.05) greater than that of group 1.  A negative p value means group1 network measure is significantly (p<0.05) greater than that of group 2.

# ROI Schemes

| AAL-LR ROIs | AAL ROIs | Free Surfer ROIs |
|---|---|---|
| 'L.Amygdala' | 'L.Amygdala' | 'L.Cerebellum' |
| 'R.Amygdala' | 'R.Amygdala' | 'R.Cerebellum' |
| 'L.Angular' | 'L.Angular' | 'L.Caudate' |
| 'R.Angular' | 'R.Angular' | 'L.Putamen' |
| 'L.Calcarine' | 'L.Calcarine' | 'L.Pallidum' |
| 'R.Calcarine' | 'R.Calcarine' | 'L.Thalamus' |
| 'L.Caudate' | 'L.Caudate' | 'L.Hippocampus' |
| 'R.Caudate' | 'R.Caudate' | 'L.Amygdala' |
| 'L.Cingulum_Ant' | 'L.Cingulum_Ant' | 'L.Accumbens' |
| 'R.Cingulum_Ant' | 'R.Cingulum_Ant' | 'L.VentralDC' |

| | | |
|---|---|---|
| 'L.Cingulum_Mid' | 'L.Cingulum_Mid' | 'R.Thalamus' |
| 'R.Cingulum_Mid' | 'R.Cingulum_Mid' | 'R.Caudate' |
| 'L.Cingulum_Post' | 'L.Cingulum_Post' | 'R.Putamen' |
| 'R.Cingulum_Post' | 'R.Cingulum_Post' | 'R.Pallidum' |
| 'L.Cuneus' | 'L.Cuneus' | 'R.Hippocampus' |
| 'R.Cuneus' | 'R.Cuneus' | 'R.Amygdala' |
| 'L.Frontal_Inf_Oper' | 'L.Frontal_Inf_Oper' | 'R.Accumbens' |
| 'R.Frontal_Inf_Oper' | 'R.Frontal_Inf_Oper' | 'R.VentralDC' |
| 'L.Frontal_Inf_Orb' | 'L.Frontal_Inf_Orb' | 'L.bankssts' |
| 'R.Frontal_Inf_Orb_R' | 'R.Frontal_Inf_Orb_R' | 'L.caudalanteriorcingulate' |
| 'L.Frontal_Inf_Tri' | 'L.Frontal_Inf_Tri' | 'L.caudalmiddlefrontal' |
| 'R.Frontal_Inf_Tri' | 'R.Frontal_Inf_Tri' | 'L.cuneus' |
| 'L.Frontal_Med_Orb' | 'L.Frontal_Med_Orb' | 'L.entorhinal' |
| 'R.Frontal_Med_Orb' | 'R.Frontal_Med_Orb' | 'L.fusiform' |
| 'L.Frontal_Mid' | 'L.Frontal_Mid' | 'L.inferiorparietal' |
| 'R.Frontal_Mid' | 'L.Frontal_Mid_Orb' | 'L.inferiortemporal' |
| 'L.Frontal_Mid_Orb' | 'R.Frontal_Mid_Orb' | 'L.isthmuscingulate' |
| 'R.Frontal_Mid_Orb' | 'R.Frontal_Mid' | 'L.lateraloccipital' |
| 'L.Frontal_Sup' | 'L.Frontal_Sup' | 'L.lateralorbitofrontal' |
| 'R.Frontal_Sup' | 'L.Frontal_Sup_Medial' | 'L.lingual' |
| 'L.Frontal_Sup_Medial' | 'R.Frontal_Sup_Medial' | 'L.medialorbitofrontal' |
| 'R.Frontal_Sup_Medial' | 'L.Frontal_Sup_Orb' | 'L.middletemporal' |
| 'L.Frontal_Sup_Orb' | 'R.Frontal_Sup_Orb' | 'L.parahippocampal' |
| 'R.Frontal_Sup_Orb' | 'R.Frontal_Sup' | 'L.paracentral' |
| 'L.Fusiform' | 'L.Fusiform' | 'L.parsopercularis' |

| | | |
|---|---|---|
| 'R.Fusiform' | 'R.Fusiform' | 'L.parsorbitalis' |
| 'L.Heschel' | 'L.Heschel' | 'L.parstriangularis' |
| 'R.Heschl' | 'R.Heschl' | 'L.pericalcarine' |
| 'L.Hippocampus' | 'L.Hippocampus' | 'L.postcentral' |
| 'R.Hippocampus' | 'R.Hippocampus' | 'L.posteriorcingulate' |
| 'L.Insula' | 'L.Insula' | 'L.precentral' |
| 'R.Insula' | 'R.Insula' | 'L.precuneus' |
| 'L.Lingual' | 'L.Lingual' | 'L.rostralanteriorcingulate' |
| 'R.Lingual' | 'R.Lingual' | 'L.rostralmiddlefrontal' |
| 'L.Occipital_Inf' | 'L.Occipital_Inf' | 'L.superiorfrontal' |
| 'R.Occipital_Inf' | 'R.Occipital_Inf' | 'L.superiorparietal' |
| 'L.Occipital_Mid' | 'L.Occipital_Mid' | 'L.superiortemporal' |
| 'R.Occipital_Mid' | 'R.Occipital_Mid' | 'L.supramarginal' |
| 'L.Occipital_Sup' | 'L.Occipital_Sup' | 'L.frontalpole' |
| 'R.Occipital_Sup' | 'R.Occipital_Sup' | 'L.temporalpole' |
| 'L.Olfactory' | 'L.Olfactory' | 'L.transversetemporal' |
| 'R.Olfactory' | 'R.Olfactory' | 'L.insula' |
| 'L.Pallidum' | 'L.Pallidum' | 'R.bankssts' |
| 'R.Pallidum' | 'R.Pallidum' | 'R.caudalanteriorcingulate' |
| 'L.Paracentral_Lobule' | 'L.Paracentral_Lobule' | 'R.caudalmiddlefrontal' |
| 'R.Paracentral_Lobule' | 'R.Paracentral_Lobule' | 'R.cuneus' |
| 'L.Parahippocampal' | 'L.Parahippocampal' | 'R.entorhinal' |
| 'R.Parahippocampal' | 'R.Parahippocampal' | 'R.fusiform' |
| 'L.Parietal_Inf' | 'L.Parietal_Inf' | 'R.inferiorparietal' |
| 'R.Parietal_Inf' | 'R.Parietal_Inf' | 'R.inferiortemporal' |

| | | |
|---|---|---|
| 'L.Parietal_Sup' | 'L.Parietal_Sup' | 'R.isthmuscingulate' |
| 'R.Parietal_Sup' | 'R.Parietal_Sup' | 'R.lateraloccipital' |
| 'L.Postcentral' | 'L.Postcentral' | 'R.lateralorbitofrontal' |
| 'R.Postcentral' | 'R.Postcentral' | 'R.lingual' |
| 'L.Precentral' | 'L.Precentral' | 'R.medialorbitofrontal' |
| 'R.Precentral' | 'R.Precentral' | 'R.middletemporal' |
| 'L.Precuneus' | 'L.Precuneus' | 'R.parahippocampal' |
| 'R.Precuneus' | 'R.Precuneus' | 'R.paracentral' |
| 'L.Putamen' | 'L.Putamen' | 'R.parsopercularis' |
| 'R.Putamen' | 'R.Putamen' | 'R.parsorbitalis' |
| 'L.Rectus' | 'L.Rectus' | 'R.parstriangularis' |
| 'R.Rectus' | 'R.Rectus' | 'R.pericalcarine' |
| 'L.Rolandic_Oper' | 'L.Rolandic_Oper' | 'R.postcentral' |
| 'R.Rolandic_Oper' | 'R.Rolandic_Oper' | 'R.posteriorcingulate' |
| 'L.Supp_Motor_Area' | 'L.Supp_Motor_Area' | 'R.precentral' |
| 'R.Supp_Motor_Area' | 'R.Supp_Motor_Area' | 'R.precuneus' |
| 'L.SupraMarginal' | 'L.SupraMarginal' | 'R.rostralanteriorcingulate' |
| 'R.SupraMarginal' | 'R.SupraMarginal' | 'R.rostralmiddlefrontal' |
| 'L.Temporal_Inf' | 'L.Temporal_Inf' | 'R.superiorfrontal' |
| 'R.Temporal_Inf' | 'R.Temporal_Inf' | 'R.superiorparietal' |
| 'L.Temporal_Mid' | 'L.Temporal_Mid' | 'R.superiortemporal' |
| 'R.Temporal_Mid' | 'R.Temporal_Mid' | 'R.supramarginal' |
| 'L.Temporal_Pole_Mid' | 'L.Temporal_Pole_Mid' | 'R.frontalpole' |
| 'R.Temporal_Pole_Mid' | 'R.Temporal_Pole_Mid' | 'R.temporalpole' |
| 'L.Temporal_Pole_Sup' | 'L.Temporal_Pole_Sup' | 'R.transversetemporal' |

| | | |
|---|---|---|
| 'R.Temporal_Pole_Sup' | 'R.Temporal_Pole_Sup' | 'R.insula' |
| 'L.Temporal_Sup' | 'L.Temporal_Sup' | |
| 'R.Temporal_Sup' | 'R.Temporal_Sup' | |
| 'L.Thalamus' | 'L.Thalamus' | |
| 'R.Thalamus | 'R.Thalamus' | |