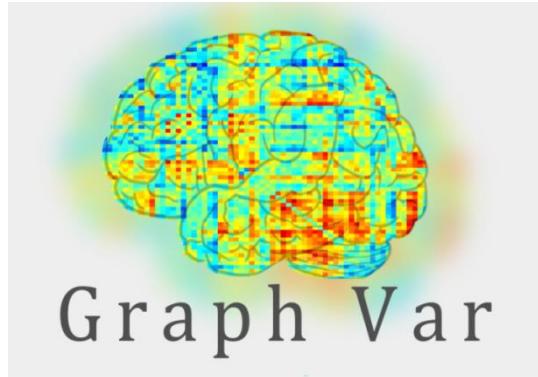
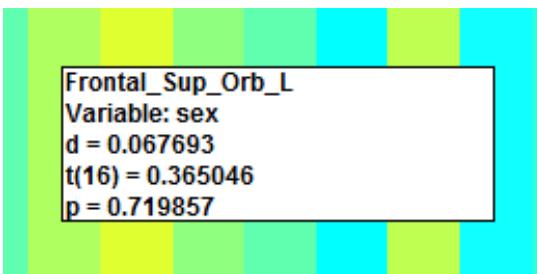


GraphVar: a brief tutorial for getting started

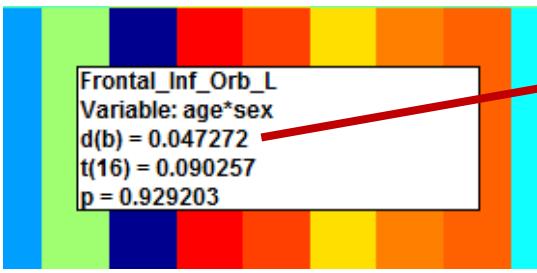


Outline:

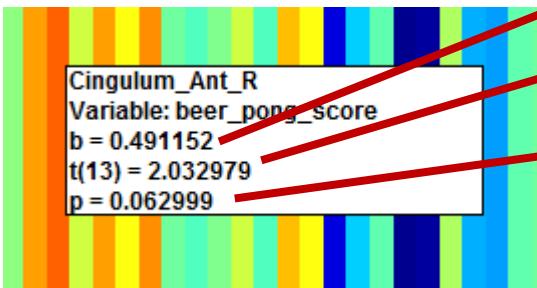
1. ***Example investigation***: „chilli contest“ (global and local efficiency)
2. ***Group comparison*** (global efficiency)
3. ***Raw connectivity matrix*** and ***Network Based Statistics***
4. ***Within design I*** (change of efficiency T1 T2 between sex)
5. ***Within design II*** (change of efficiency and behavior T1 T2)



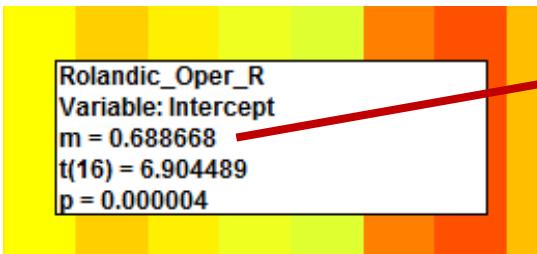
d: Difference between Group Means
F(df1,df2): F-value



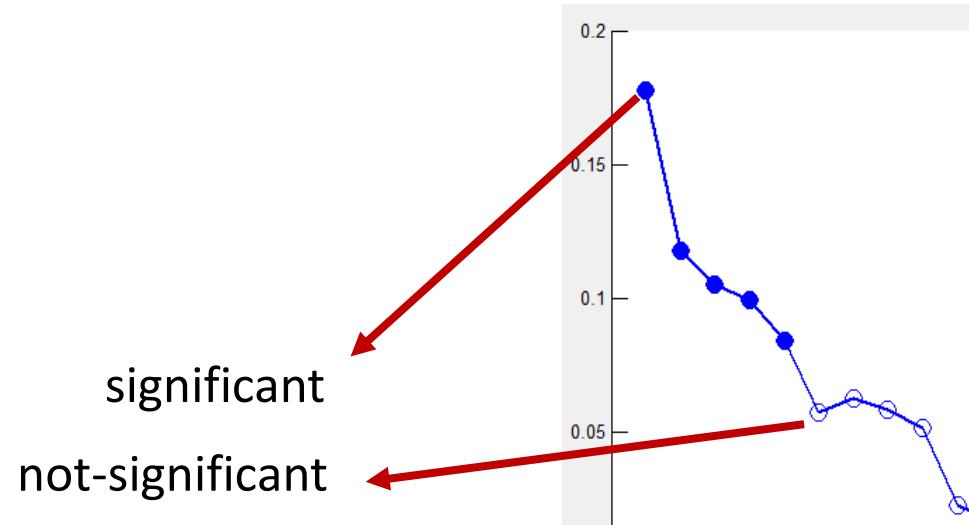
d(b): Difference between Standardized Regression Weights



b: Standardized Regression Weight
t(df): t-Value
p: p-Value



m: Mean



Interpreting GraphVar output

1. Example investigation: „chilli contest“ (global and local efficiency)



Hypothesis:

1. Chilli eating champs probably have more efficient brains... otherwise they could not deal with all the pain!
2. Probably orbito frontal gyrus and supplementary motor area contribute here... something like value representations and motor inhibition (... „don't spit out these delicious chillies“)



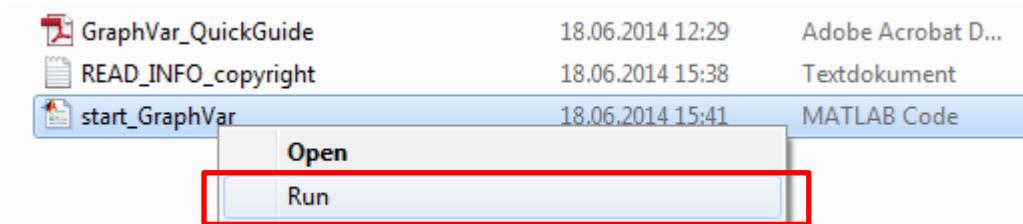


Hypothesis:

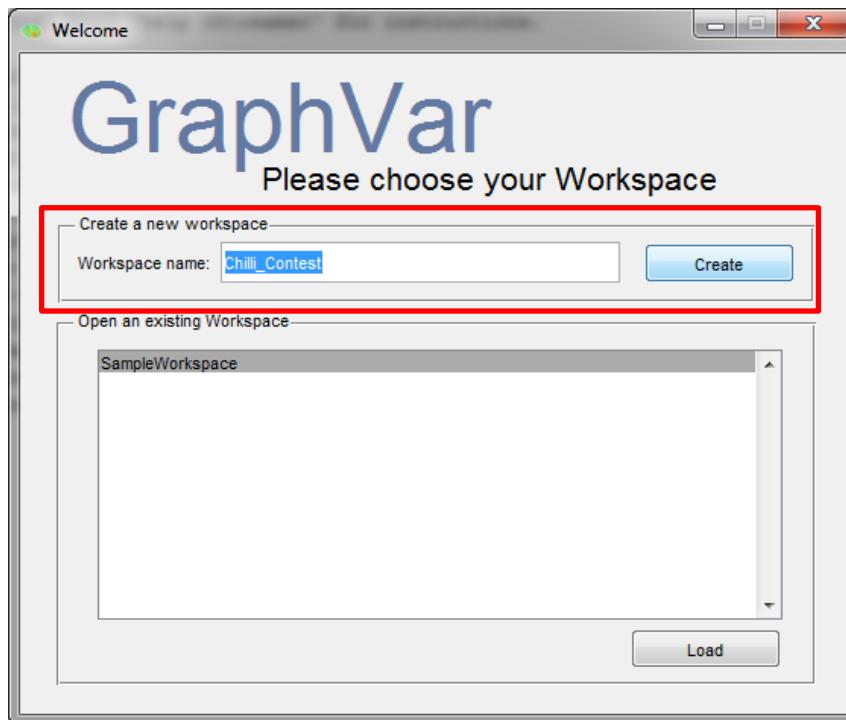
... a potential confound could be how much beer somebody had to drink before (i.e., cooling effect on the brain)

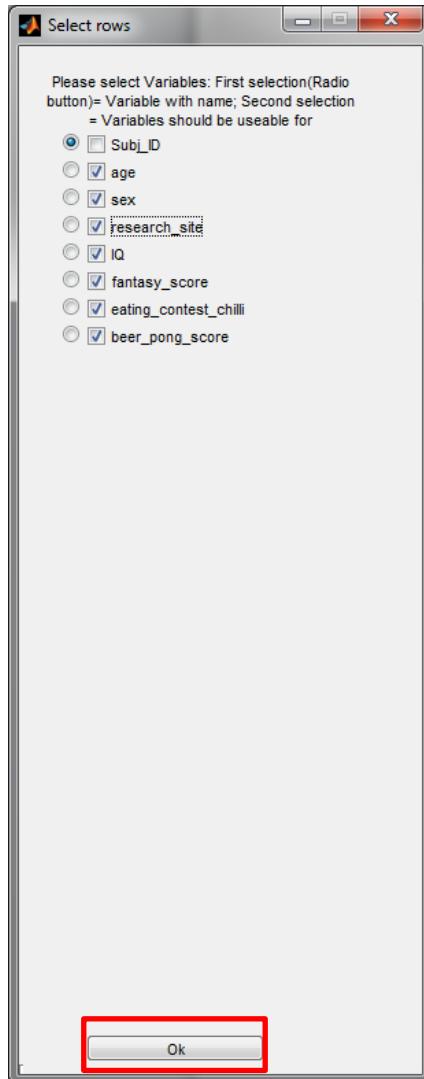


1. Use the right mouse to start GraphVar by clicking RUN on the „start_GraphVar“ script in the main folder

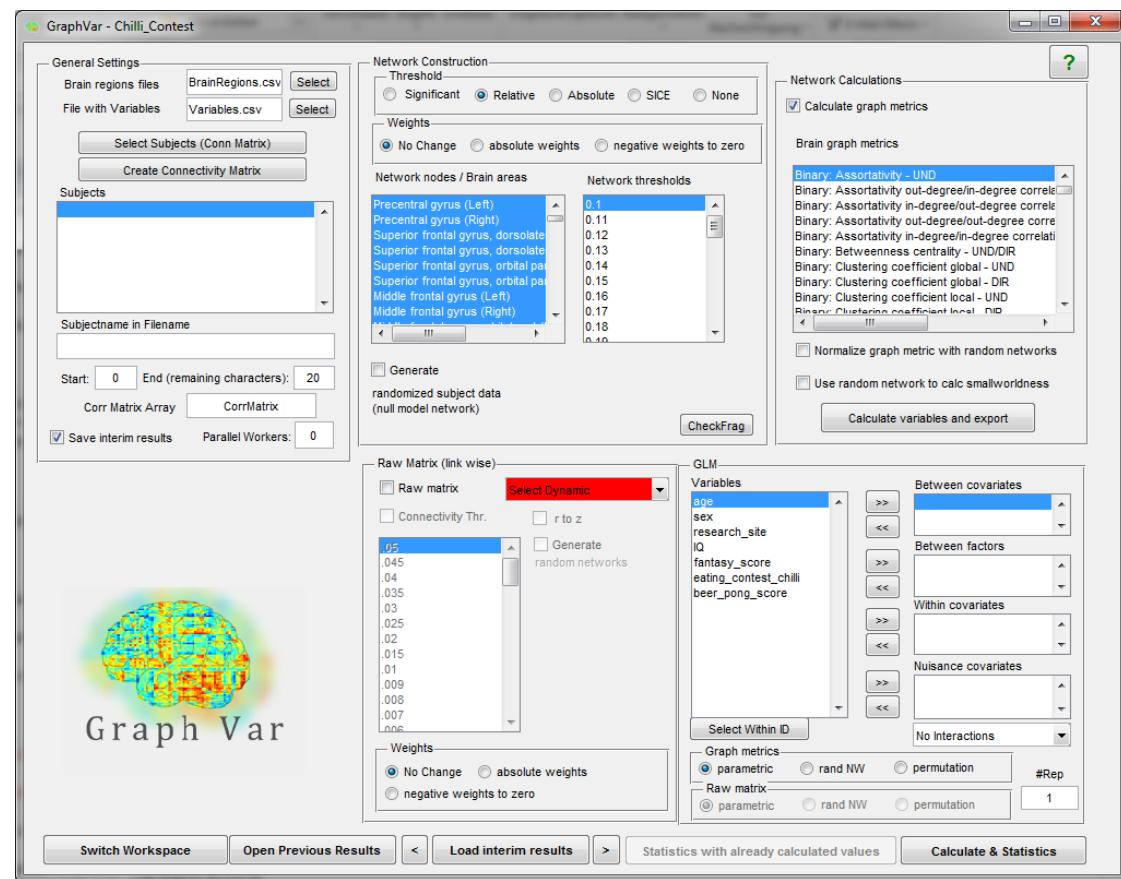


2. Create a new Workspace „Chilli_Contest“

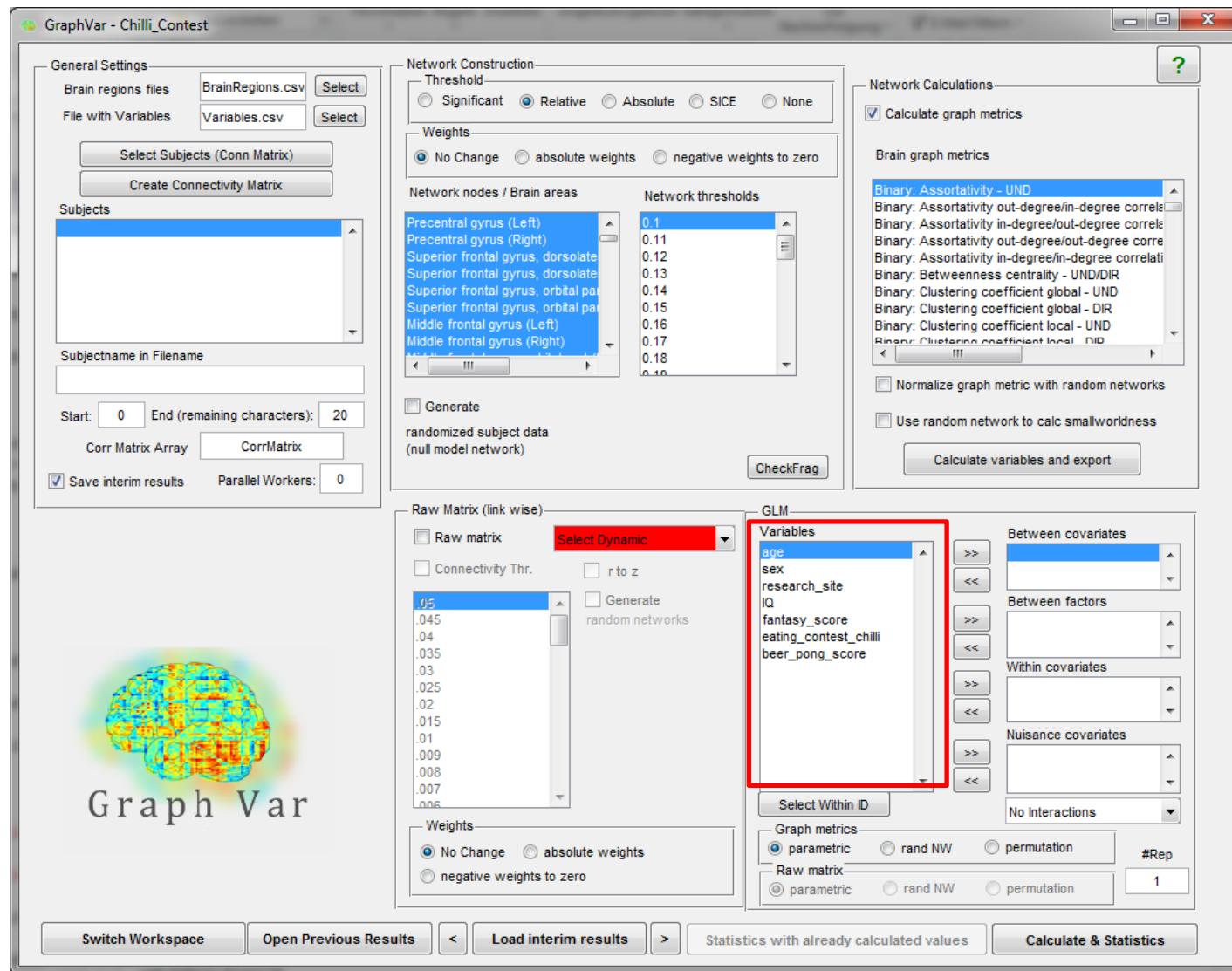




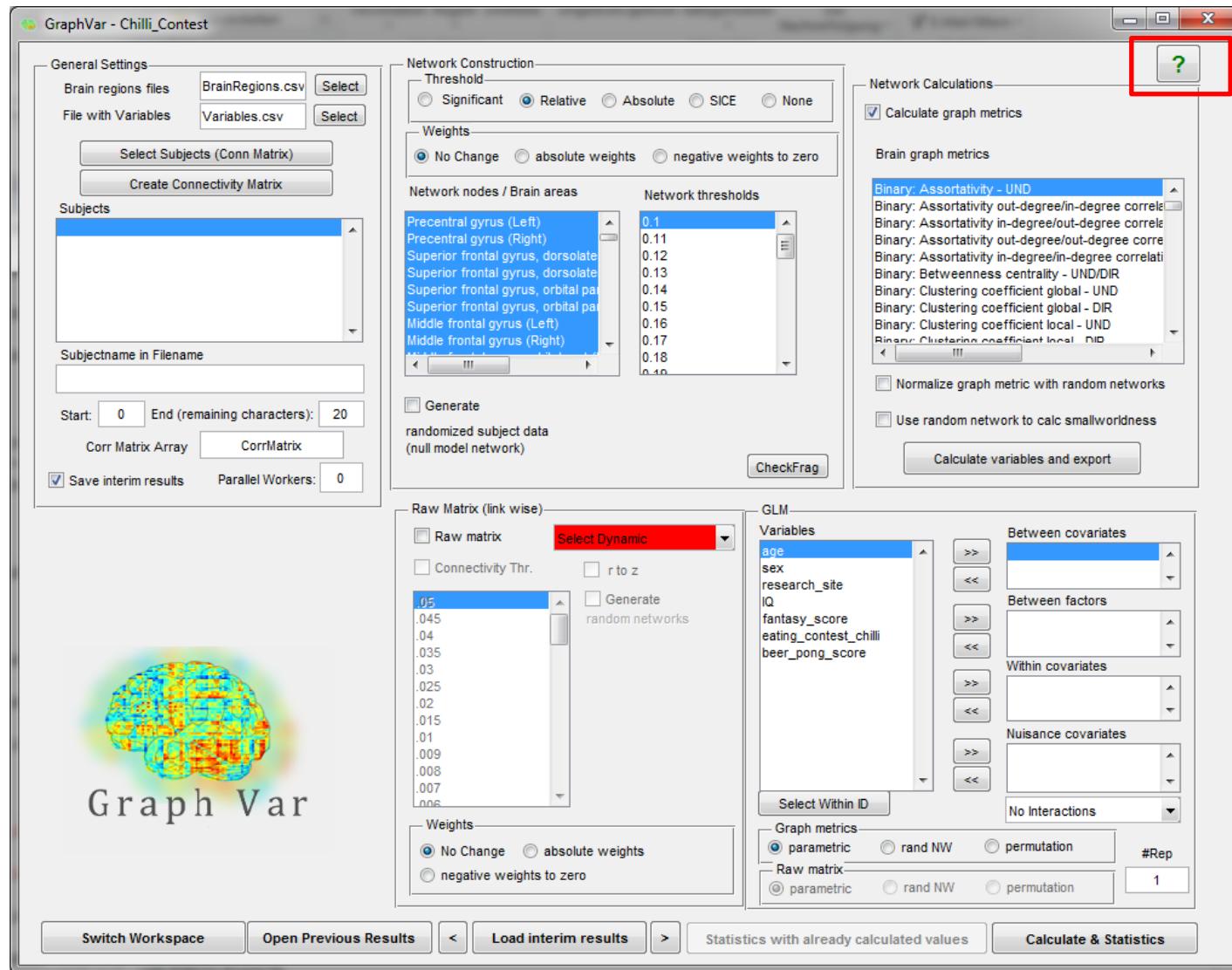
- The demo (default) data selection window appears (refer to the manual for how to change this)
- Research_site and sex are initially not selected as these variables are encoded as strings in the variable spread sheet ... you may select them if you want
- Hit the okay button (or simply close the window)



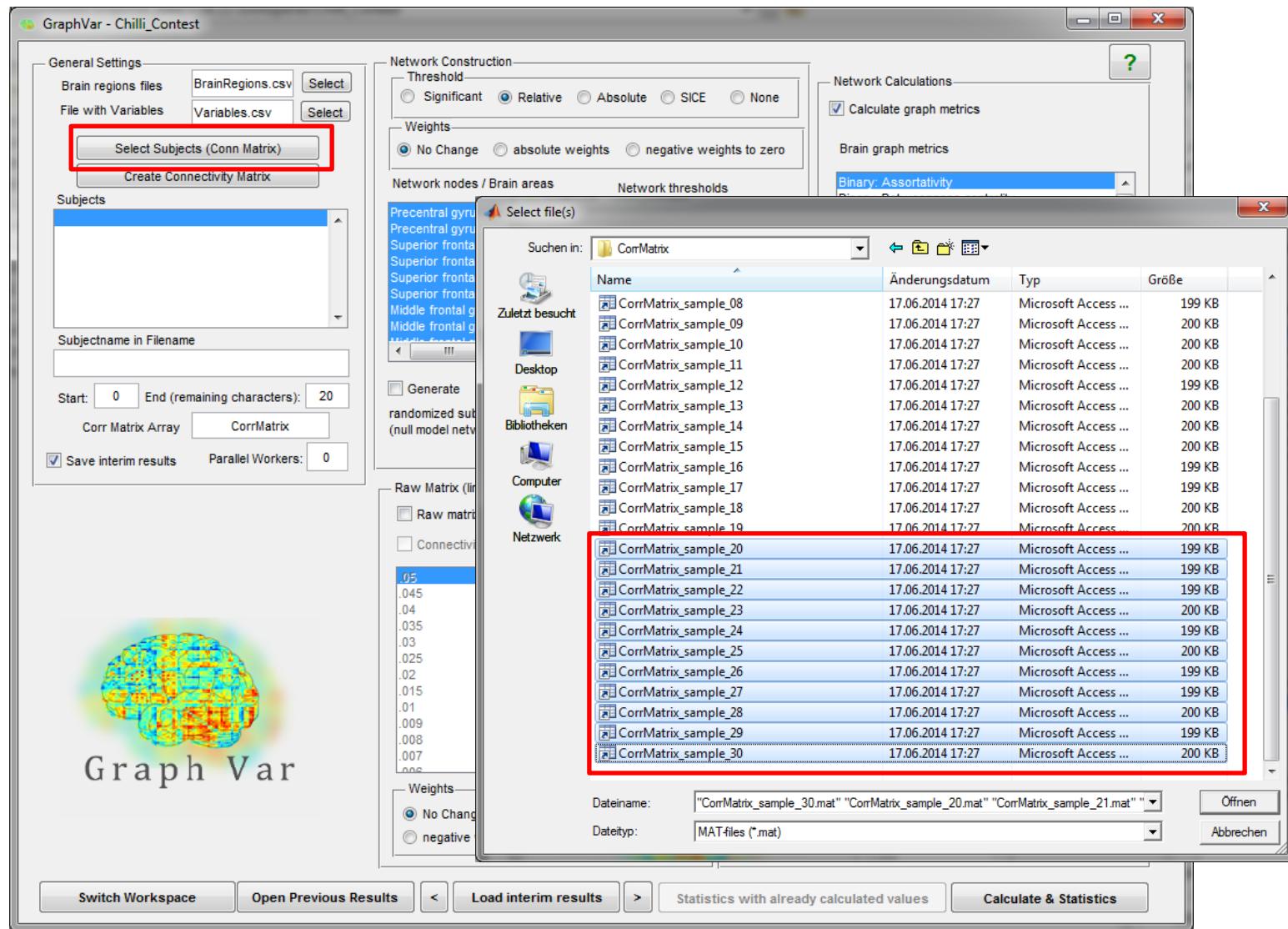
- Selected variables will be loaded in the „statistics window“



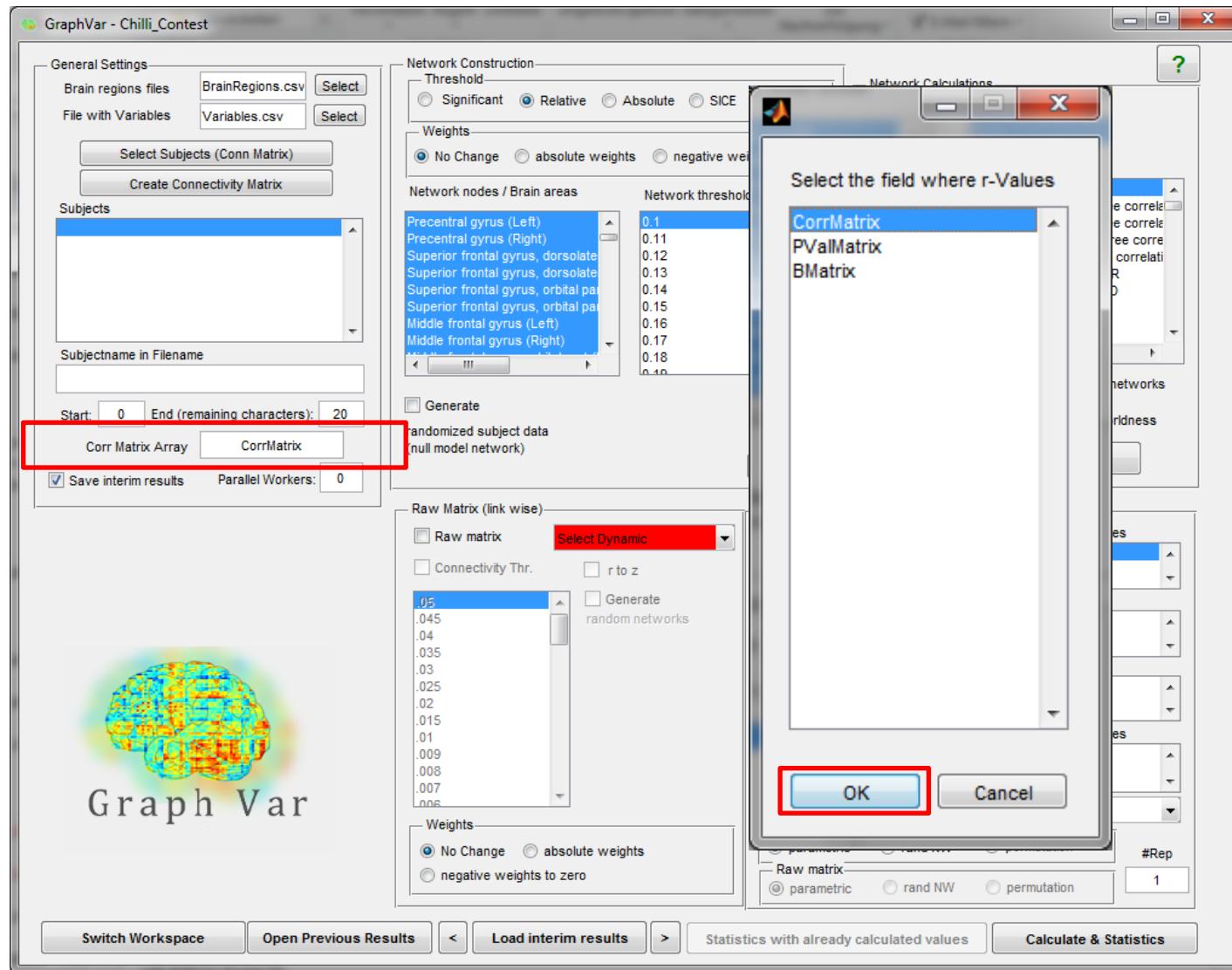
- FYI: there is also a help button in the top right!
- When help is enabled, you will have a mouse over info most functions of the GUI



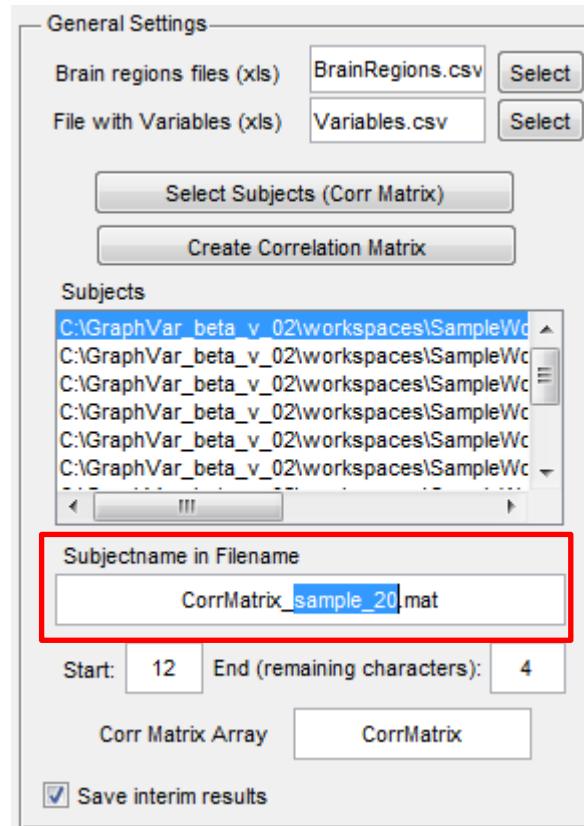
- Now, select the subjects (in general settings)
- Navigate to the „Sample Workspace“ and select subject 20-30
- Path: ...GraphVar/workspaces/SampleWorkspace/data/CorrMatrix



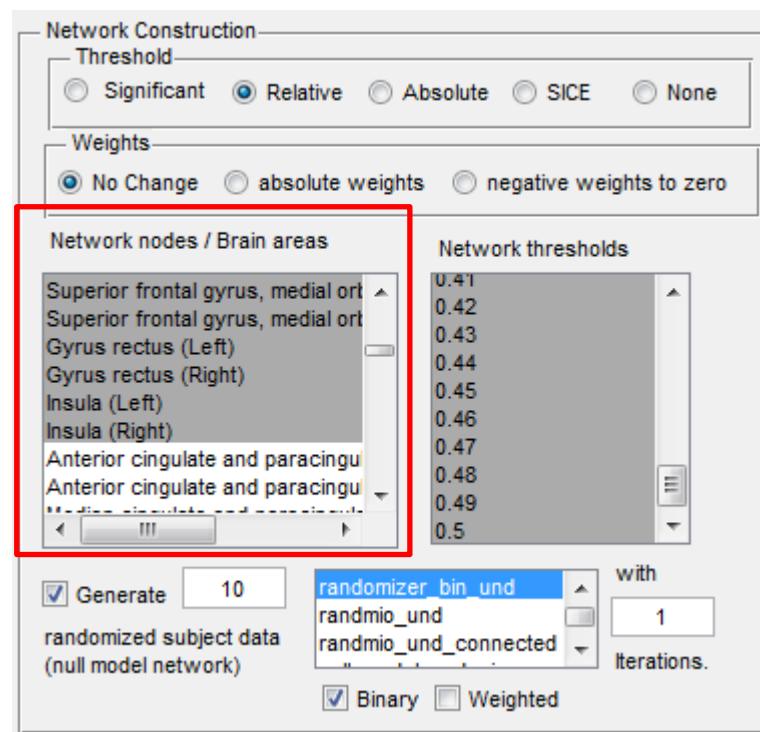
- A selection windows appears asking for the array in the CorrMatrix .mat file in which the correlations are saved (here this is CorrMatrix)
- The name will subsequently appear in the Corr Matrix Array box



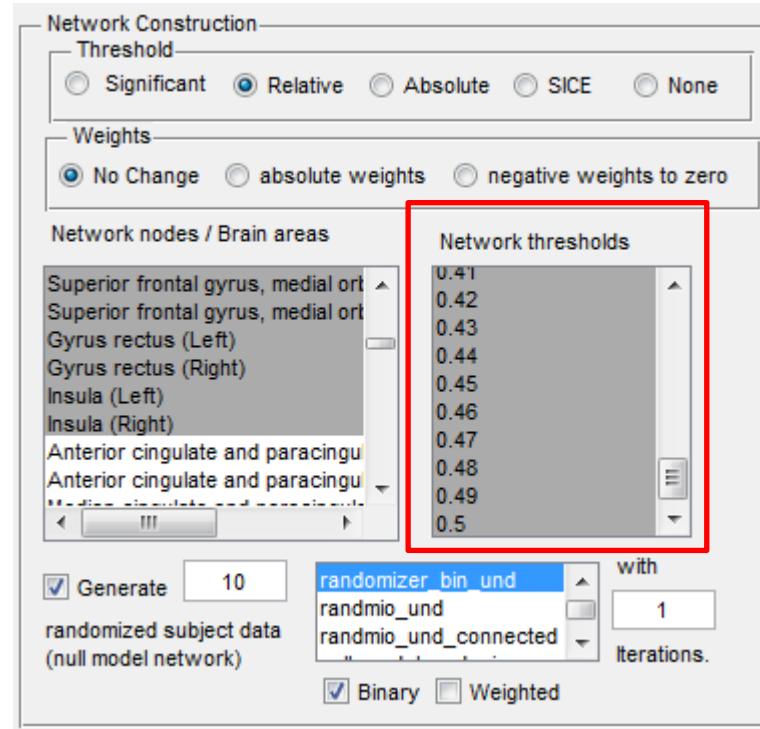
- Highlight the subject ID with the mouse to provide the reference between the CorrMatrices and the subject data in the variable spreadsheet (these should be identical)
- If you don't want to do statistics (only calculation of graph metrics and export) no spreadsheet is required



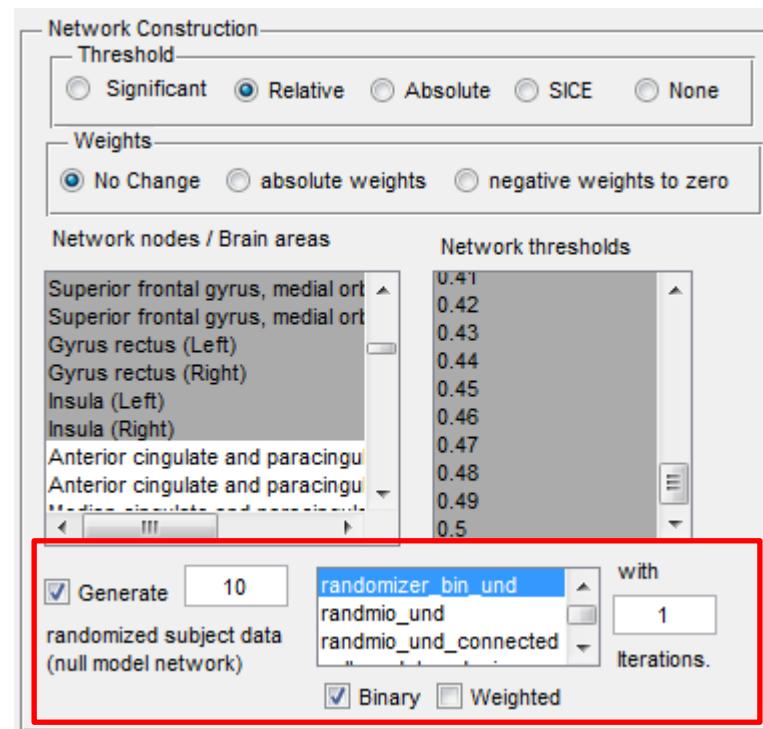
- Now, you'll have to specify a network in the network construction panel (by default AAL labels are loaded)
-> the network nodes/brain areas refer to the „brain regions file“ (see manual)
 - For this tutorial we specify the „**chilly-responsive-network**“:
starting from **Precentral gyrus (left)** until **Insula (right)**
- > select the 30 consecutive nodes with your mouse or keyboard



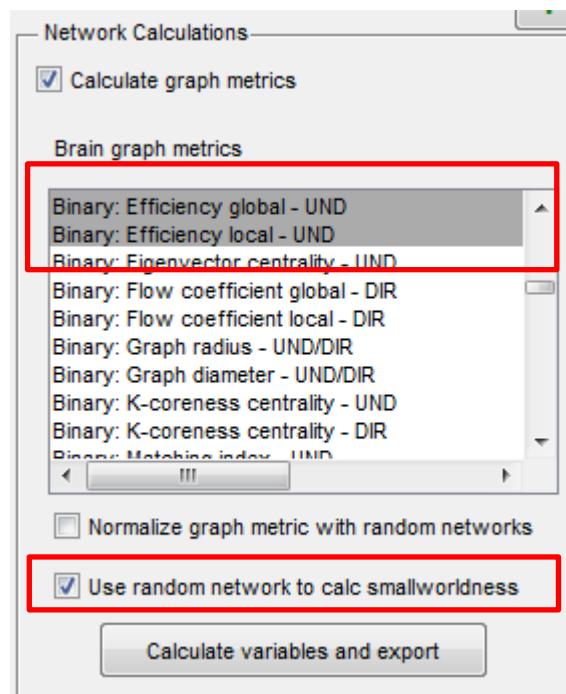
- Here, we want to construct different networks using relative thresholding (i.e., densities)
- Simply select all the thresholds in the box with „ctrl+A“ (see manual for how to add more thresholds)



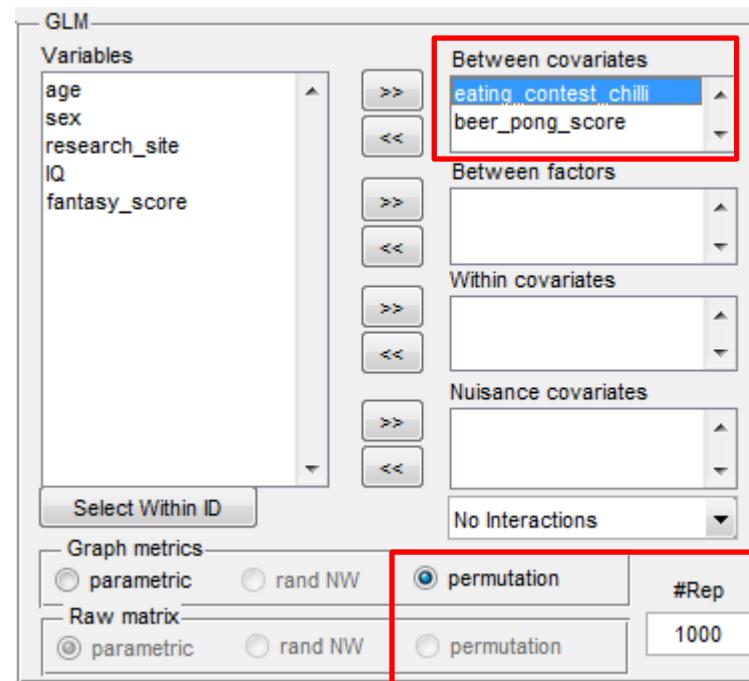
- For this investigation let's construct subject specific null-model networks to calculate „small-worldness“
- Here, we ONLY generate **10 binary random networks** per subject per threshold using the „randomizer_bin_und“ BCT function
 (for small-worldness, normalization purposes, or non-parametric testing you would normally use 100-1000 or even more ...
 but this will take a lot of time)



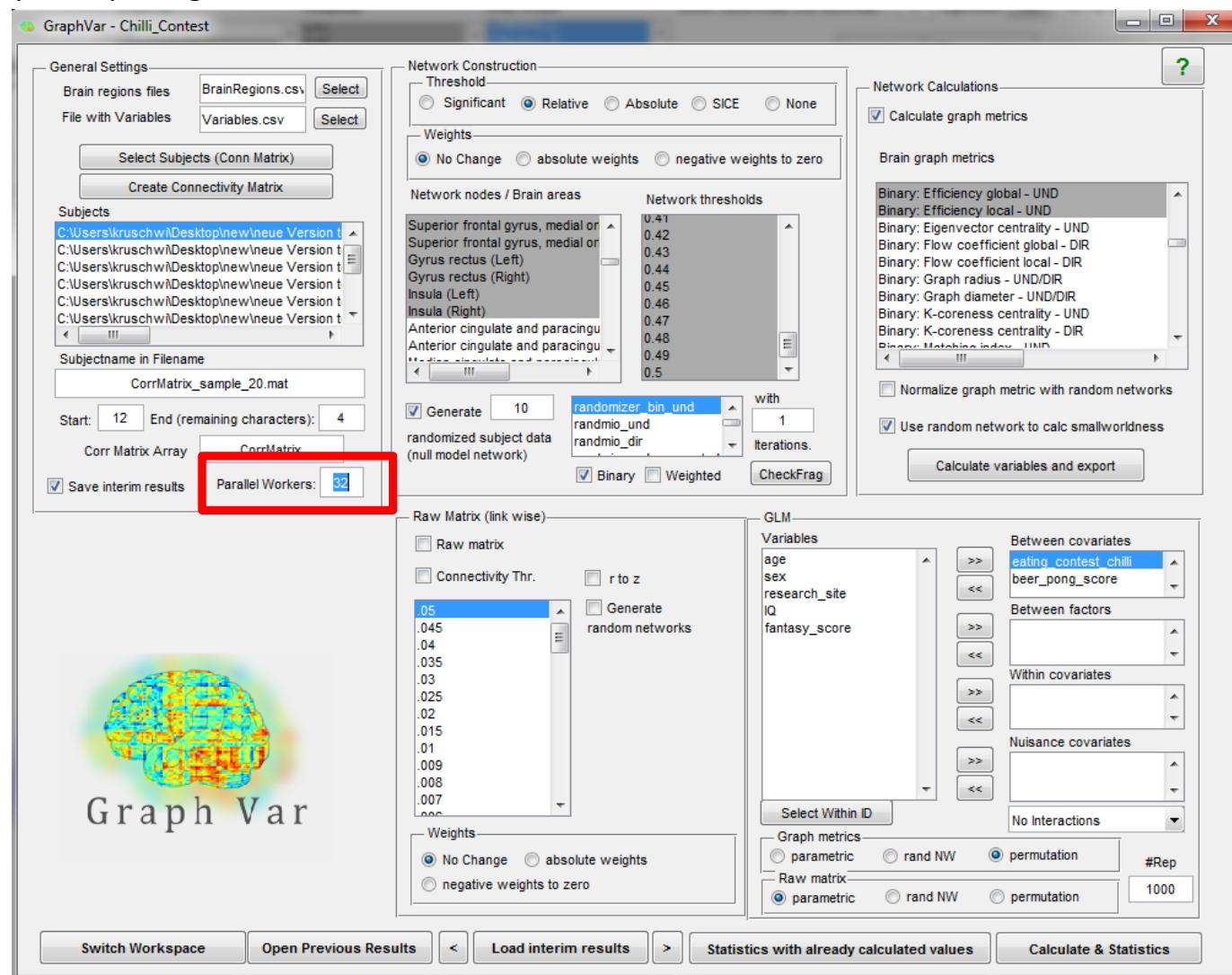
- As we have the hypothesis, that chilli eating champs probably have more efficient brains and that probably insula and orbito frontal cortex may contribute here, we select:
 - Binary: Efficiency global
 - Binary: Efficiency local
- Select also “Use random network to calc smallworldness”
- FYI: you can also add custom functions (see appendix in the manual); also note that for some of the functions it would not make sense to do statistics on (e.g., modularity affiliation vector; get components)



- In the GLM panel add „eating_contest_chilli“ and „beer_pong_score“ as predictors in the between covariates field
- Also select the option to perform permuation testing with 1000 permutations per threshold

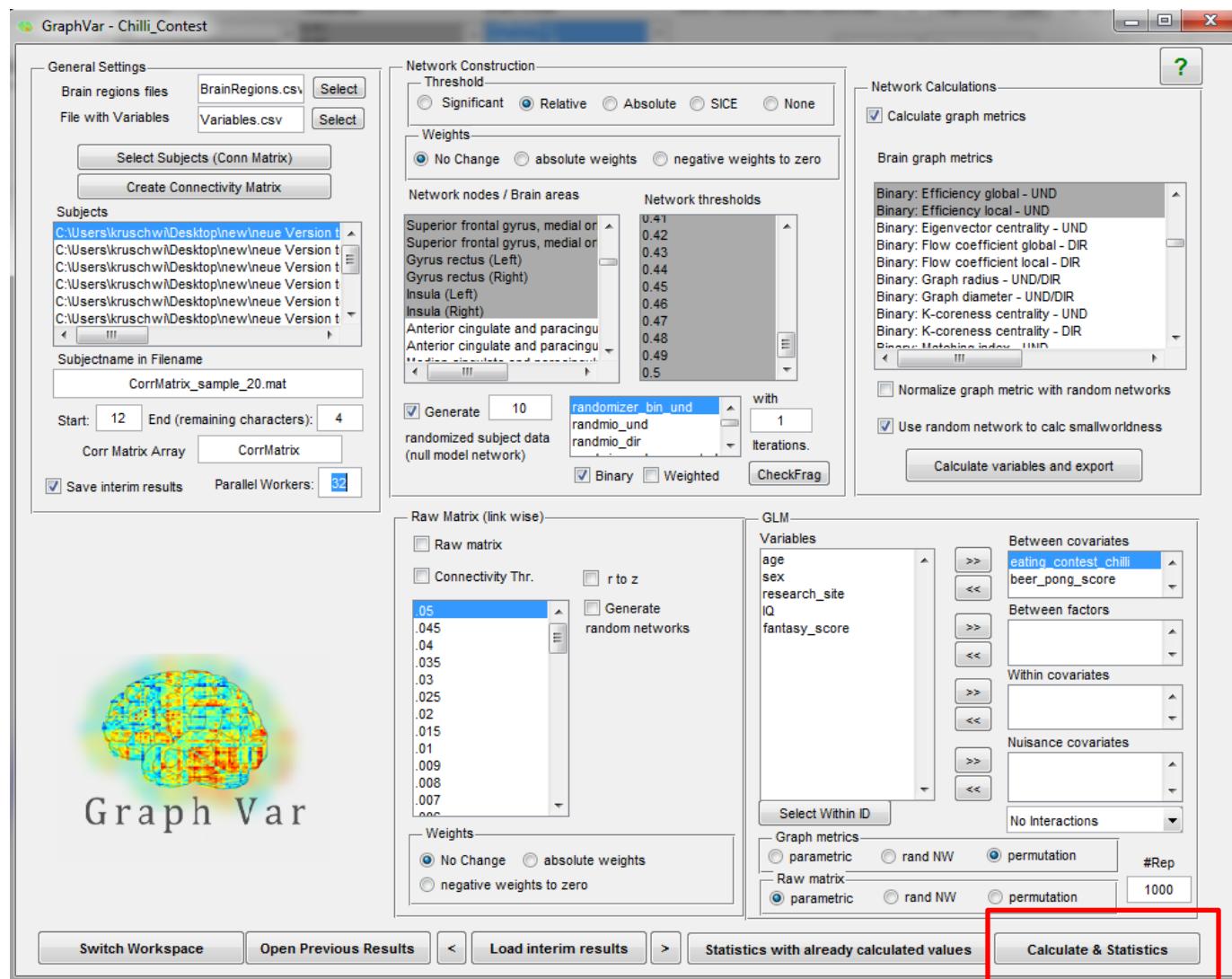


- If you have the parallel computing toolbox installed, you may want to use more workers (cores) to speed up the generation of null-model networks!

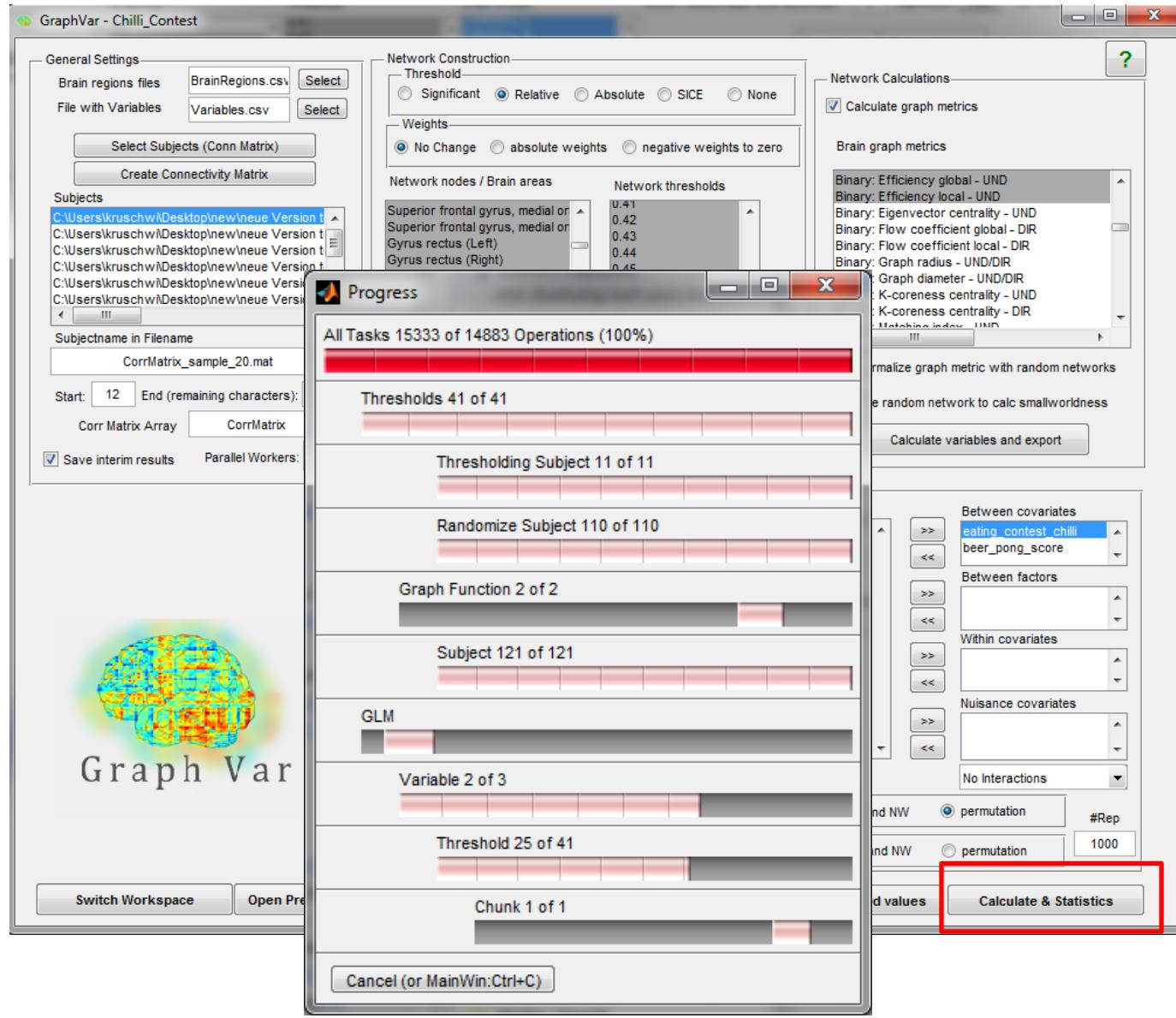


Parallel Computing (with toolbox)

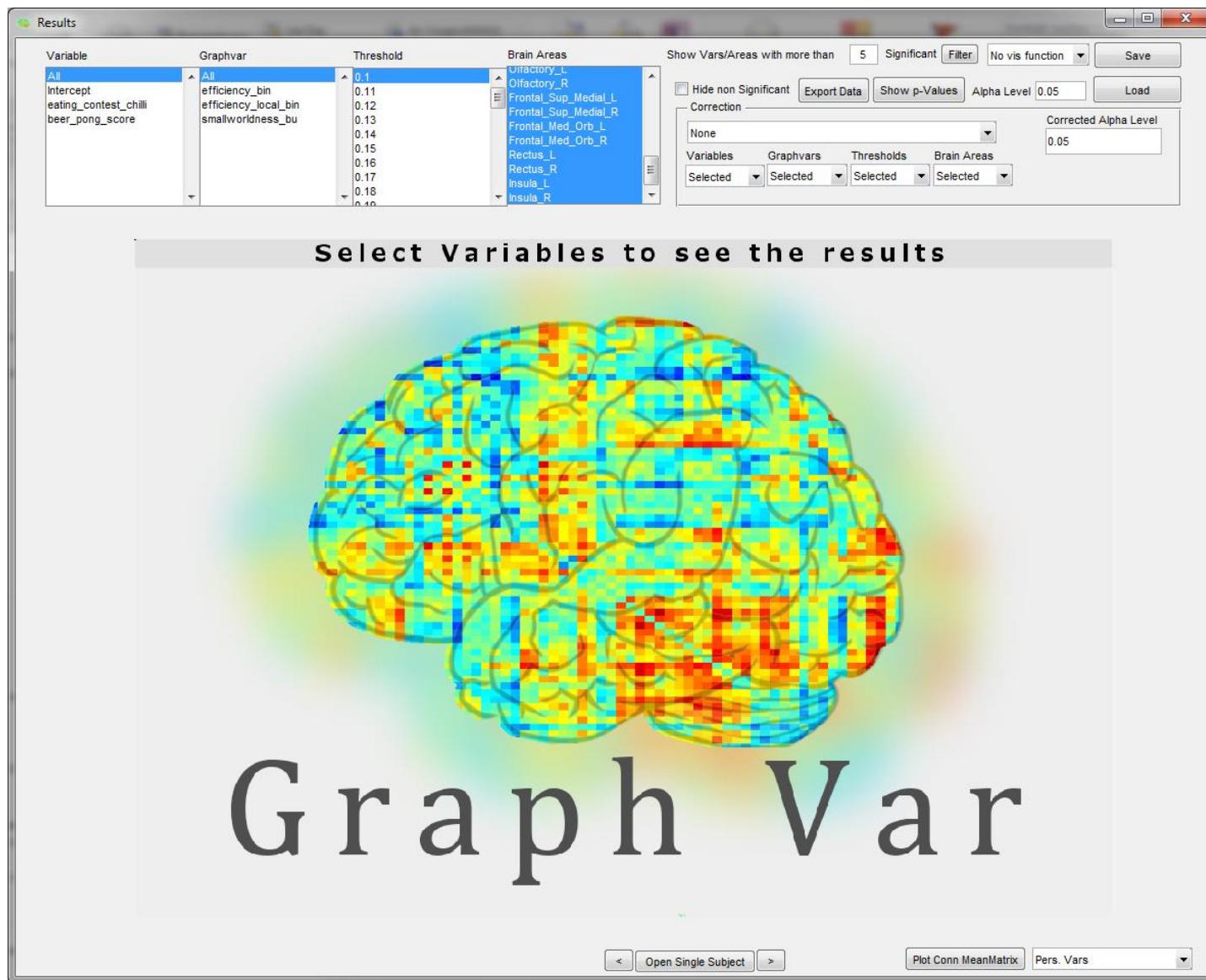
- You are ready to go and thus to test the hypothesis!
- Click on „Calculate & Statistics“



- This computation will take about 2-3 minutes ...



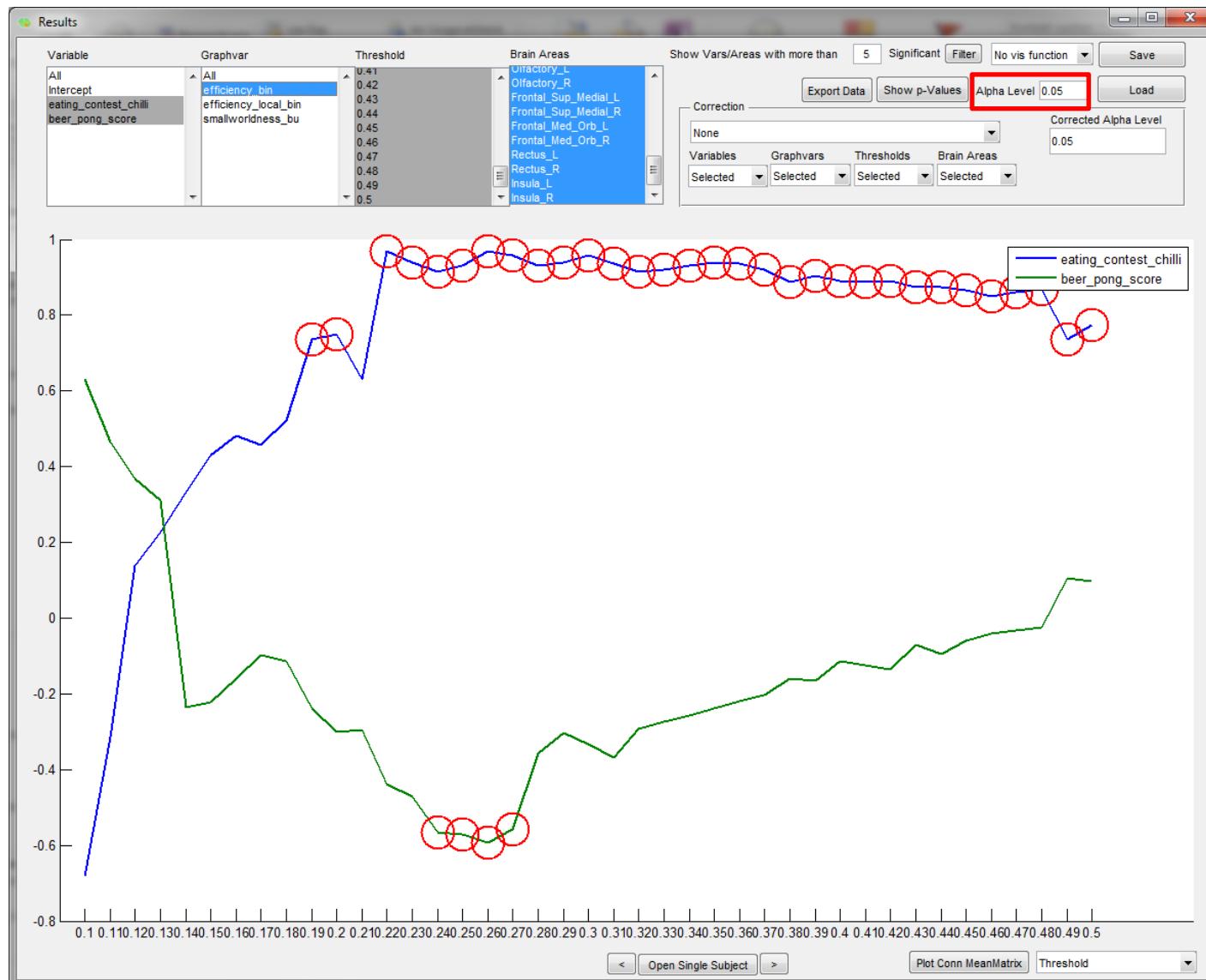
- This is the results viewer



- To see the results for global efficiency across thresholds, select the „eating_contest_chilli“, „beer_pong_score“, „efficiency_bin“, and all thresholds (ctrl+A)
- Selection of brain areas does not have an effect on global variables
- FYI: the „Intercept“ is the constant in the GLM (i.e., expected mean value of Y when all X=0)

Variable	Graphvar	Threshold	Brain Areas
All	All	0.41	Olfactory_L
Intercept	efficiency_bin	0.42	Olfactory_R
eating_contest_chilli	efficiency_local_bin	0.43	Frontal_Sup_Medial_L
beer_pong_score	smallworldness_bu	0.44	Frontal_Sup_Medial_R
		0.45	Frontal_Med_Orb_L
		0.46	Frontal_Med_Orb_R
		0.47	Rectus_L
		0.48	Rectus_R
		0.49	Insula_L
		0.5	Insula_R

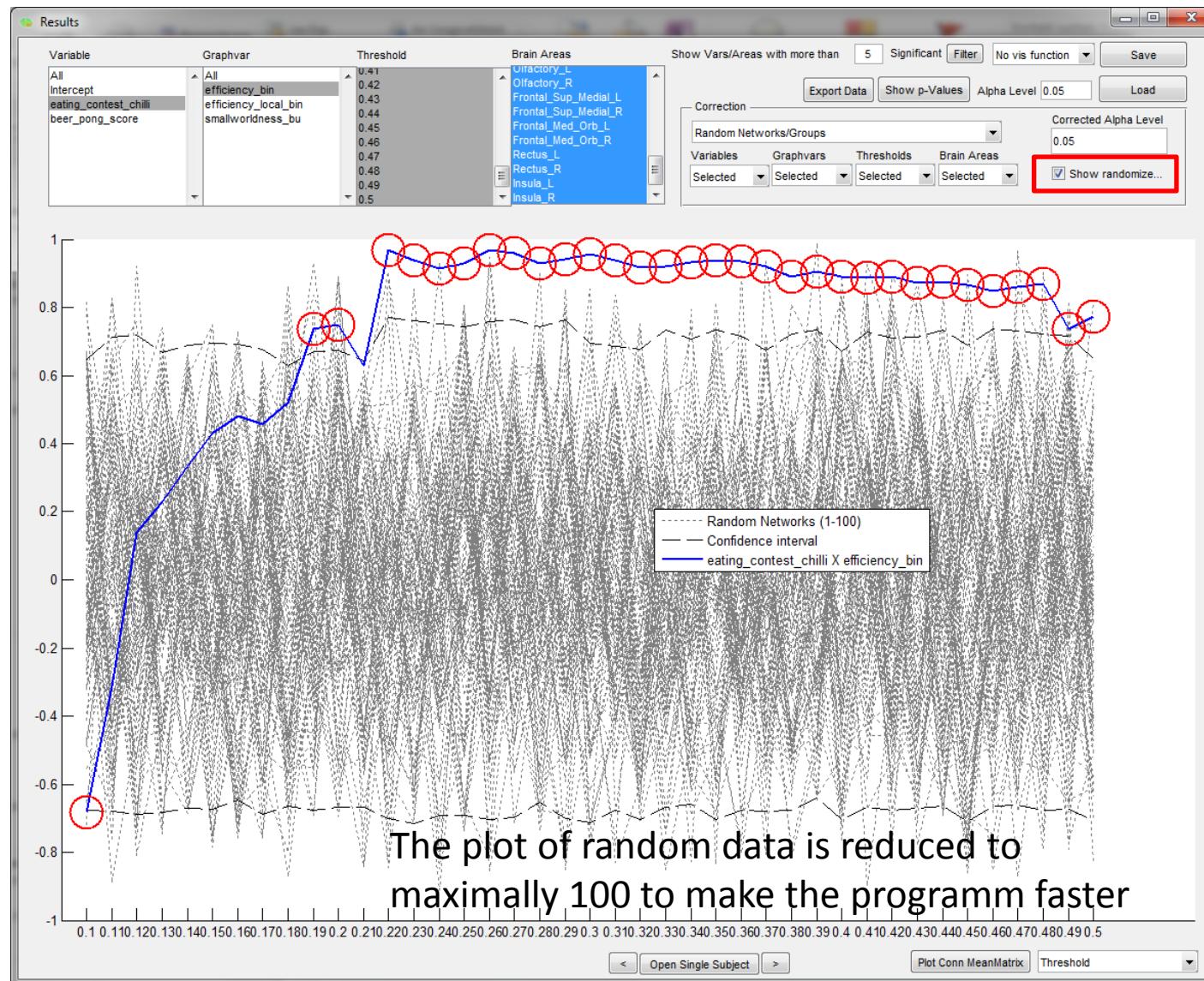
- Now, you should see the association of chilli eating contest scores, beer pong scores to global efficiency
- The red dots indicate where the correlation is significant according to the desired alpha level (which you can change here)



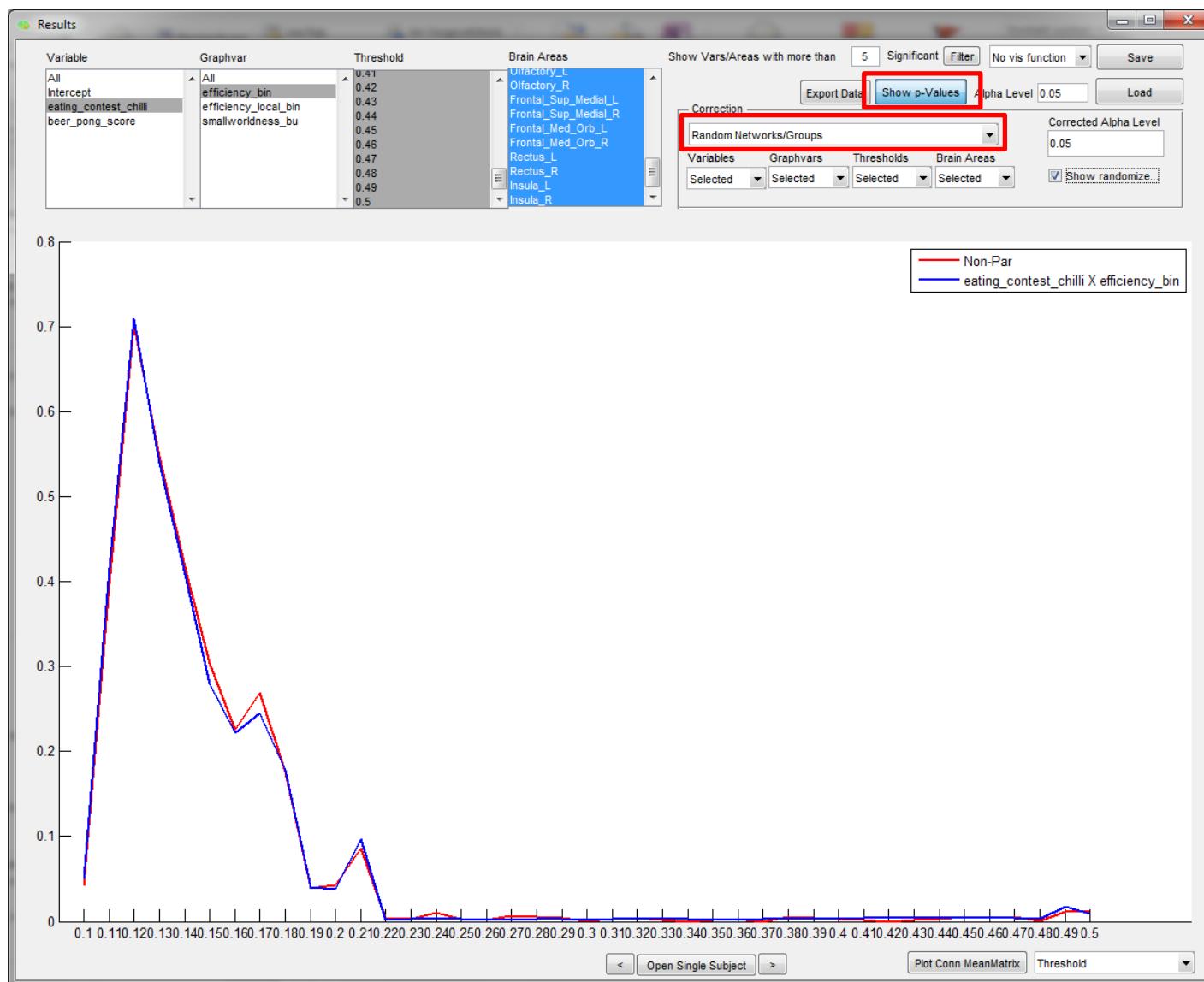
- To explore the p-values across thresholds select the „show p-Values“ button
- By hitting the button again the correlation appears again



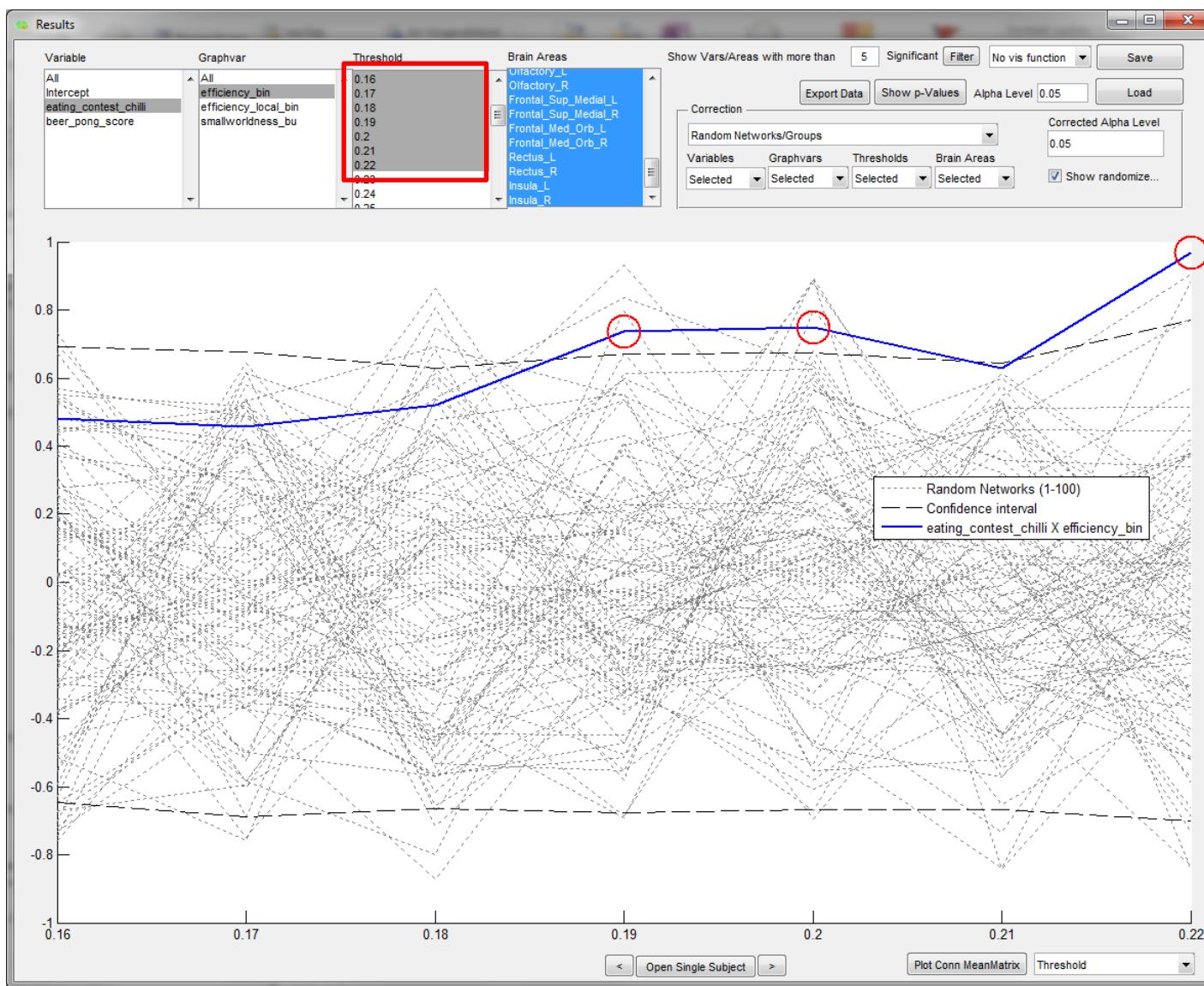
- To plot the non-parametric prediction of chilli scores and global efficiency derived from the random data (null-model distribution with 1000 permutations per threshold), select only „eating_contest_chilli“, select the correction method „Random Networks/Groups“ and click in „show randomize...“
- The confidence intervall (according to the selected alpha) and the null-model distribution appears
- You can drag and drop the legend box



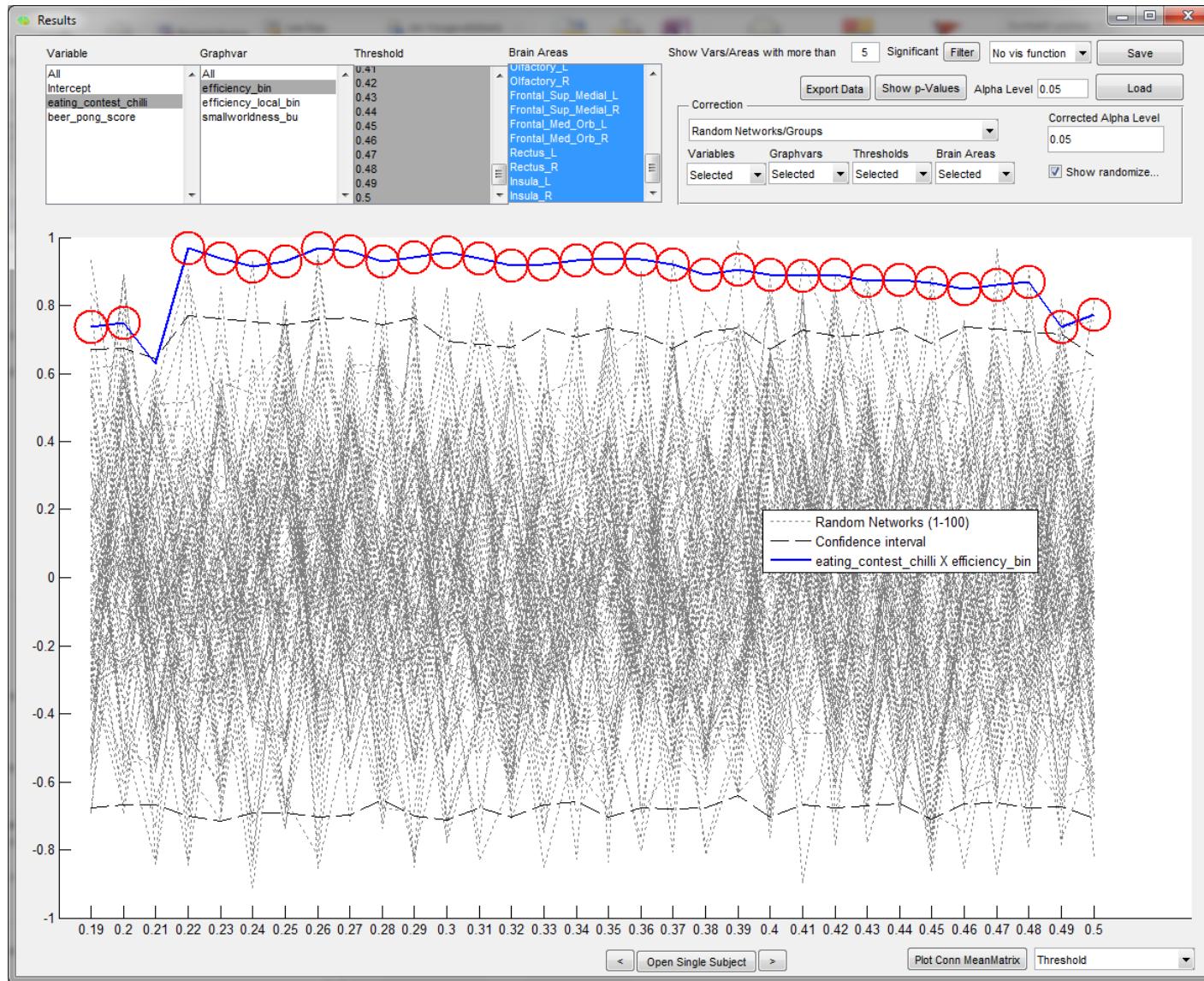
- You can also explore the overlap of the parametric und non-parametric p-values
- Here, non-parametric p-values are nearly identical to the parametric distribution



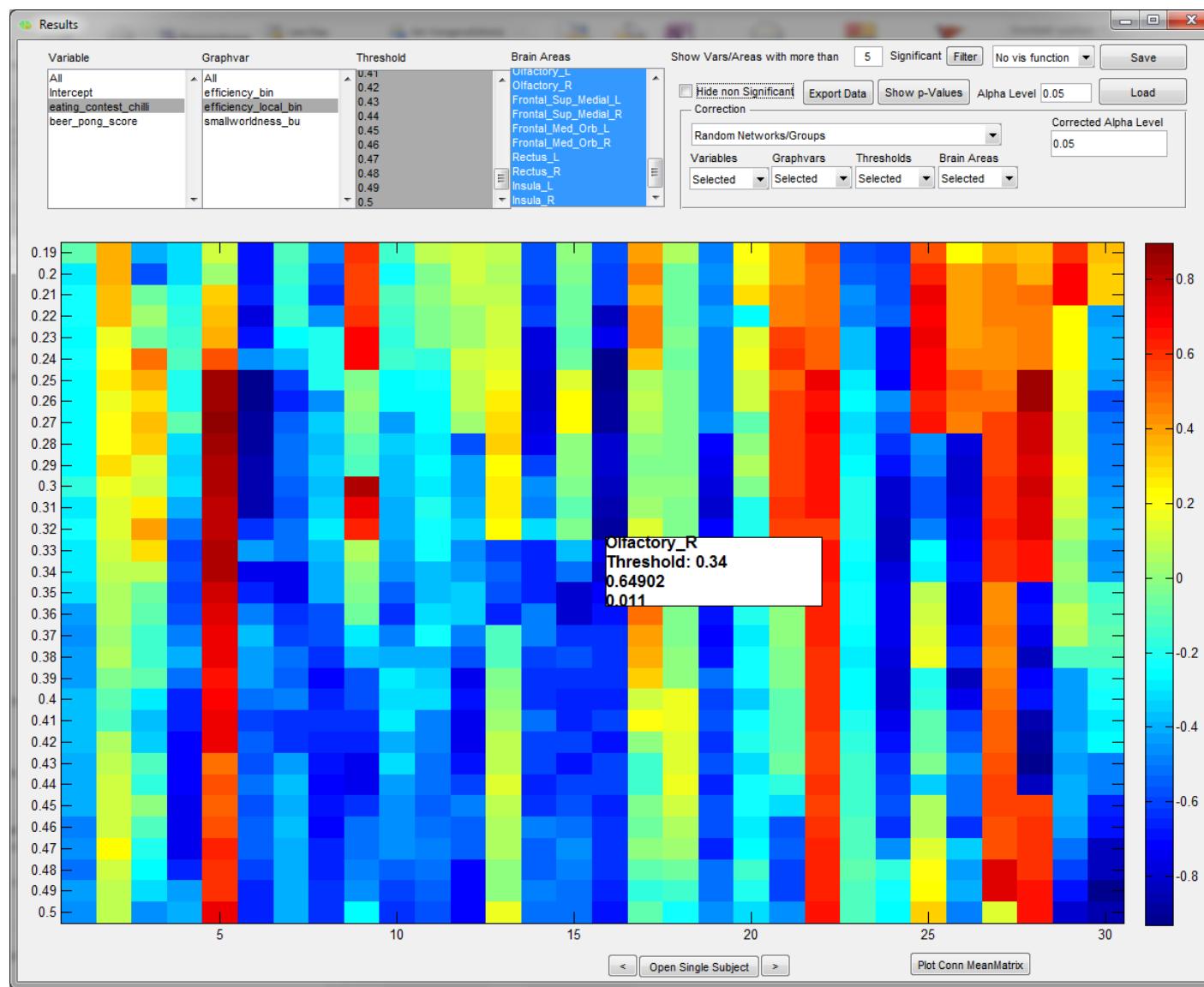
- By scaling down the thresholds, we notice that the association of global efficiency and chilli eating scores starts at a threshold of 0.19



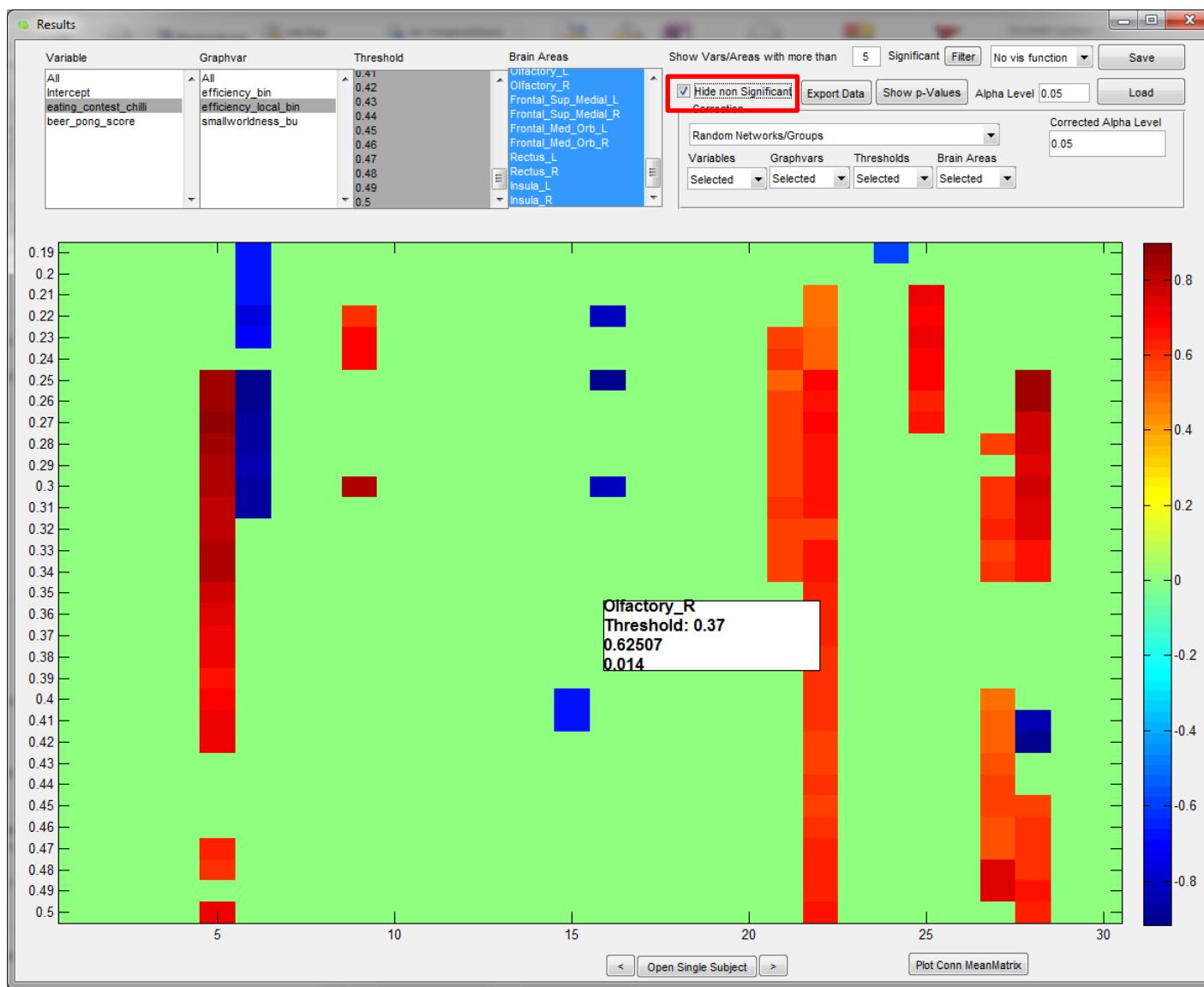
- Driven by this beautiful association of global efficiency and chilli eating scores, we now decide to explore the local efficiencies of all the regions in our chilli-responsive-network across the threshold range 0.19-0.5
- Specify the threshold range and select the Graphvar „efficiency_local_bin“**



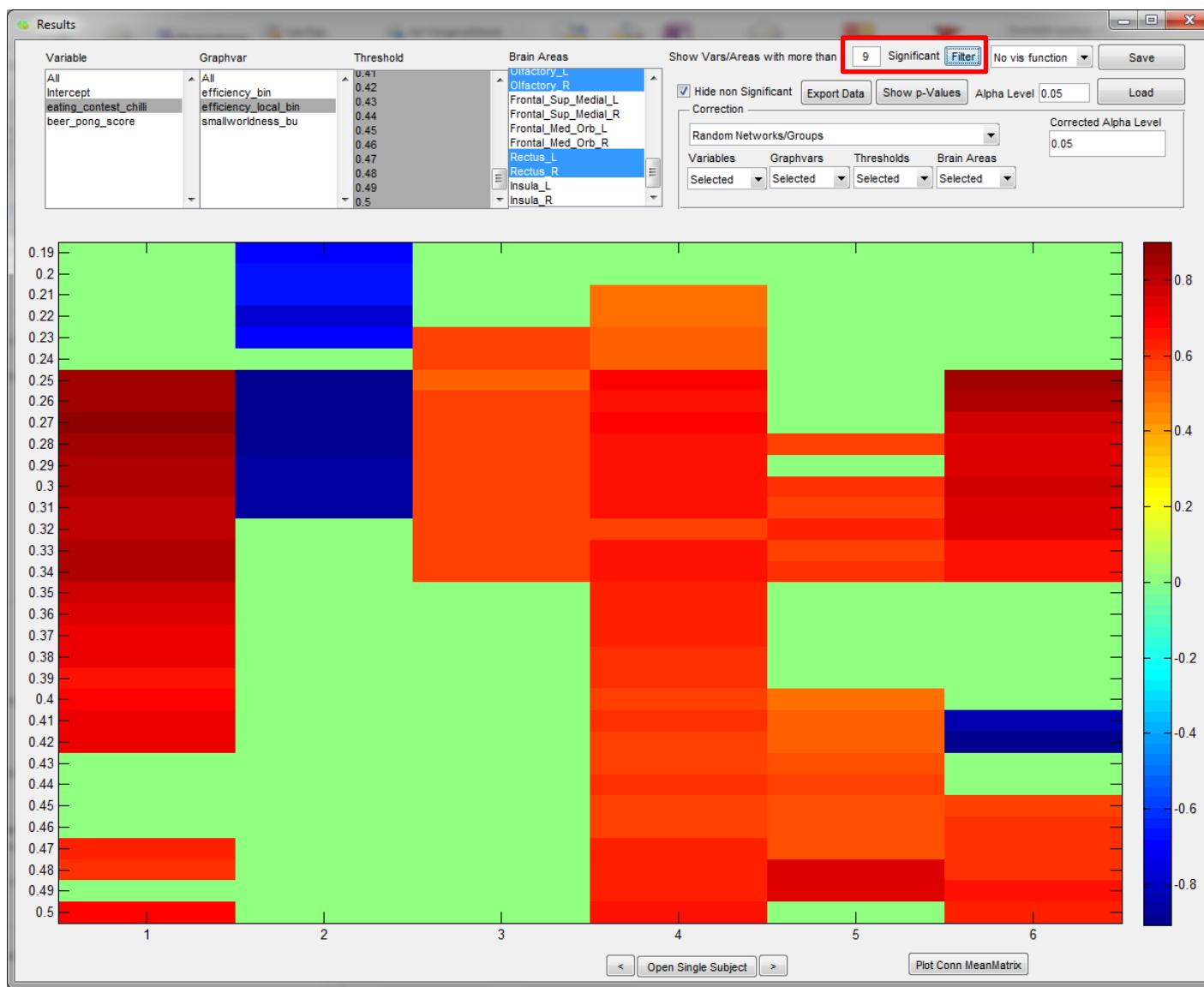
- Here you see the association of local efficiency of each of the 30 regions in the network to the chilli score
- Notice the mouse over box telling you the area, threshold, beta, and p-value



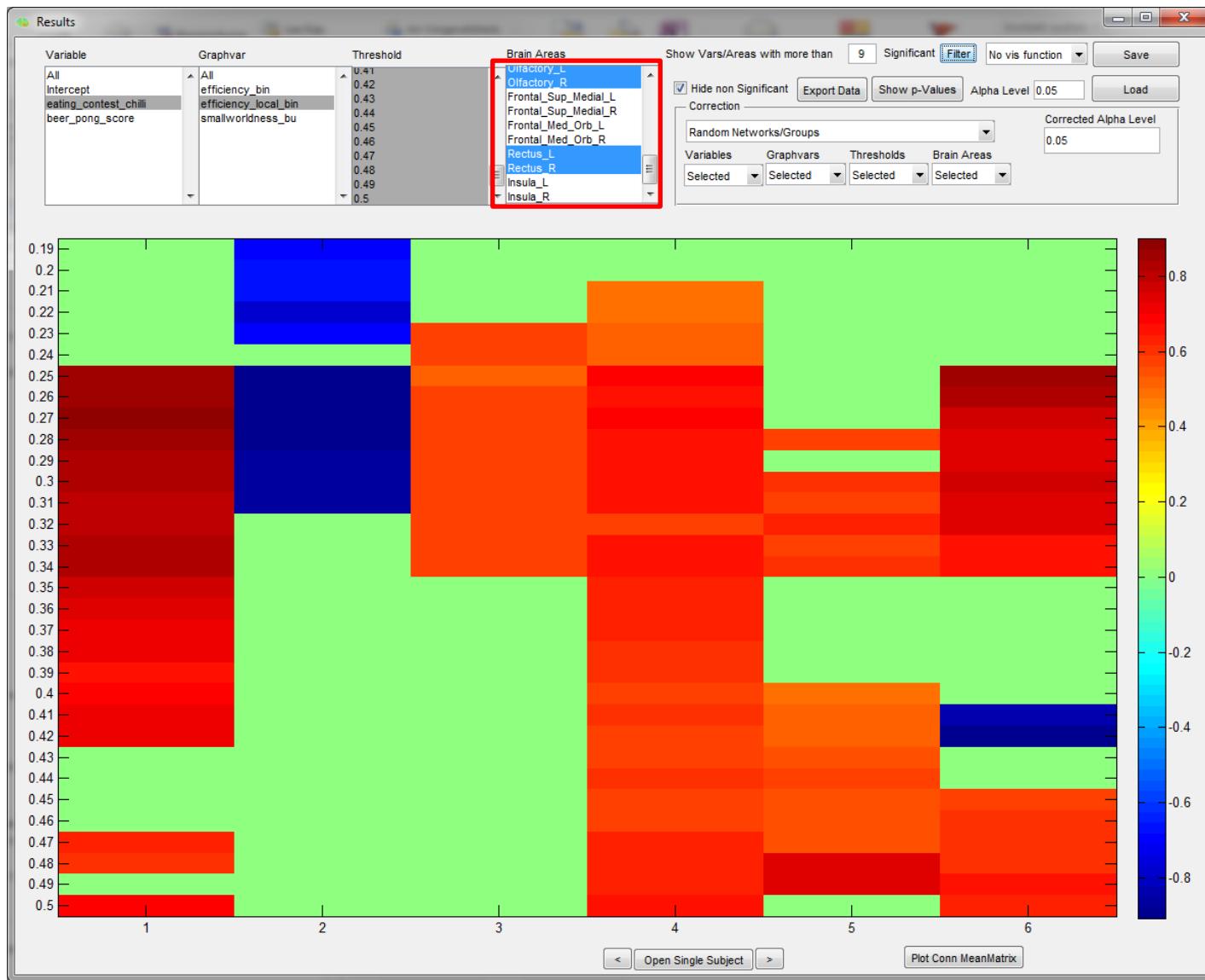
- FYI: it is possible to hide all non-significant associations



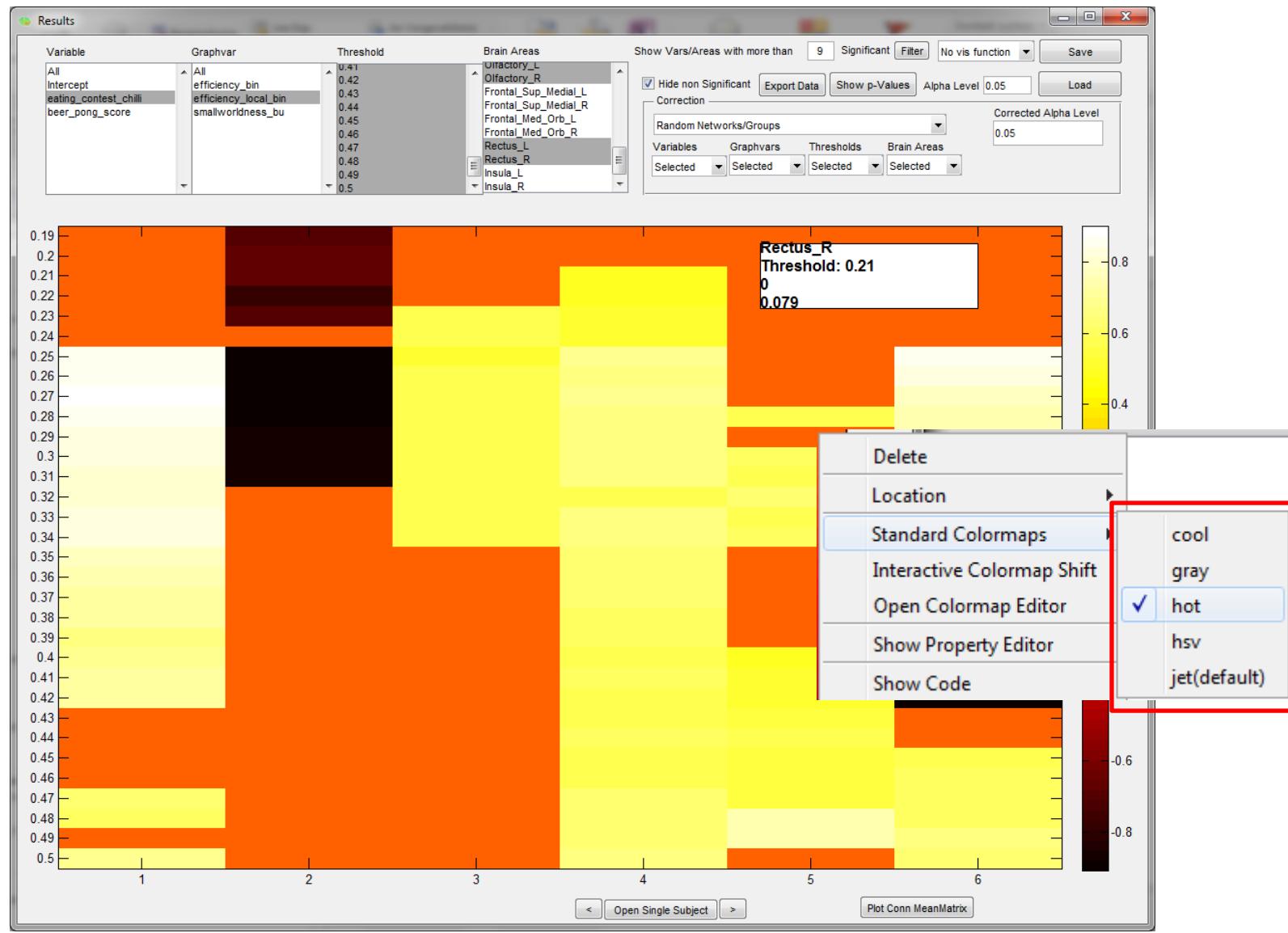
- If we a-priori determined that only significant associations on minimally 10 thresholds would be meaningful, we can use the build in filter function and set the number on 9 (i.e., thr > 9)



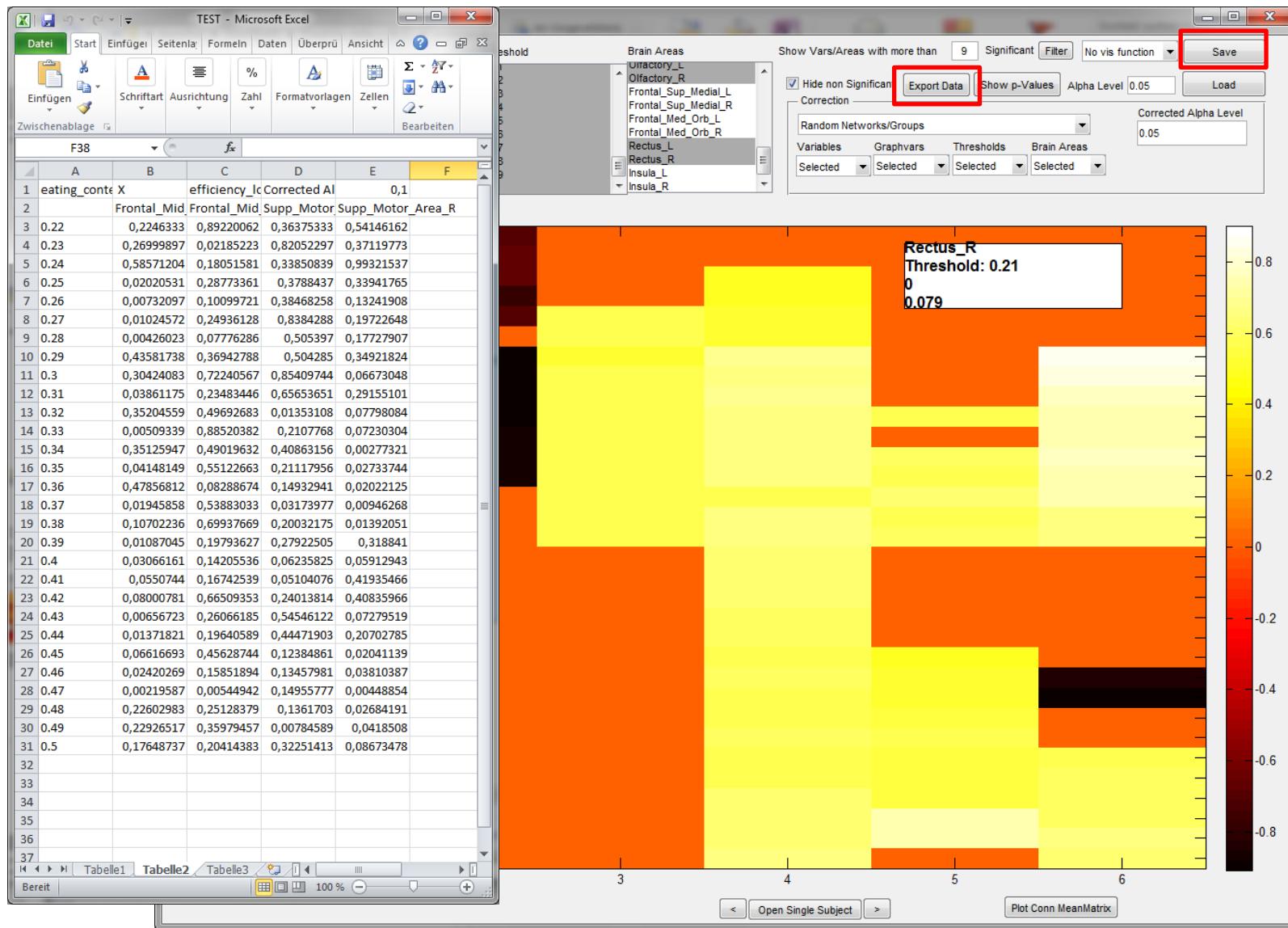
- Subsequently, the viewer will only show the areas with the specified criteria
- Note that these regions are highlighted in the „Brain Areas“ field.
- All subsequent actions will only apply to these regions (e.g. filtering)
- If you want the full network again you will have to select all nodes (ctrl+A)
- If you have an a-priori hypothesis on specific structures you can also simply select those in the „Brain Areas“ field



- You can also change the properties of the colour map (right mouse click on the colour map)
- Also use the colour map editor to set the range of correlations in the colour bar (e.g., -1 to 1)



- We think that these results are meaningful and decide to save and to export these to a csv file (which we open with excel later on)
- Only things that are visible in the results window will be exported
Everything we have computed (global efficiency and local efficiency across thresholds) may be saved

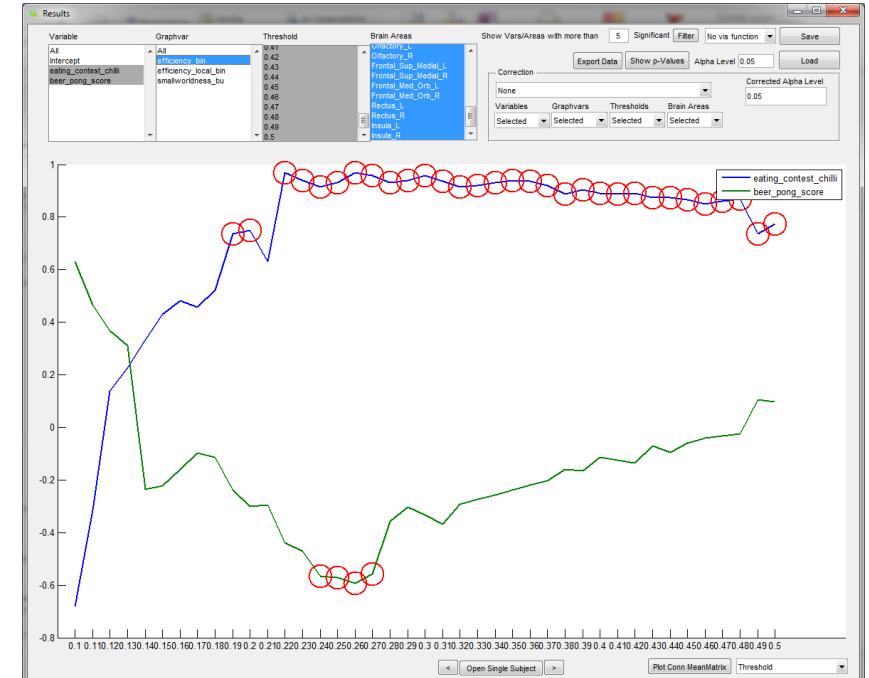
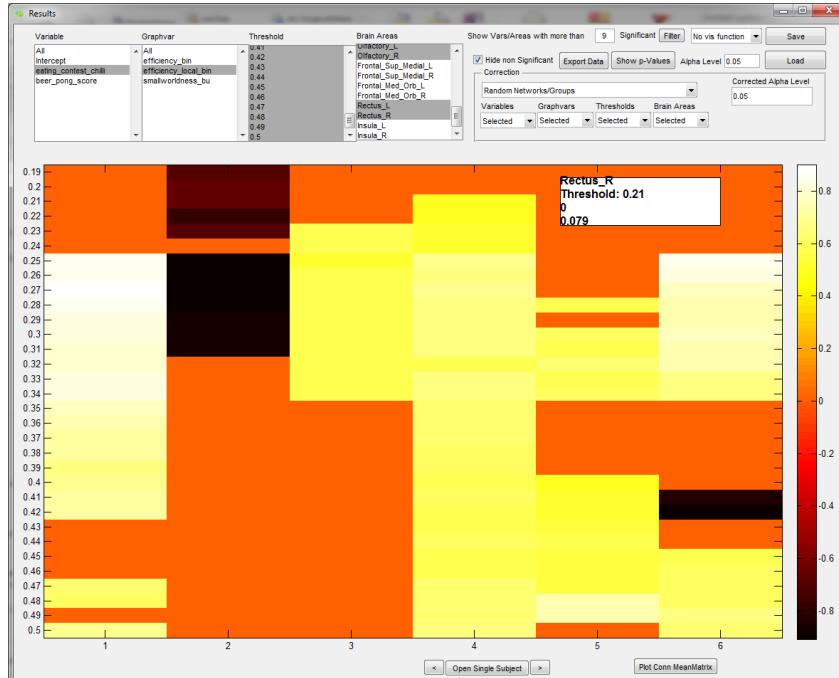


Interpretation



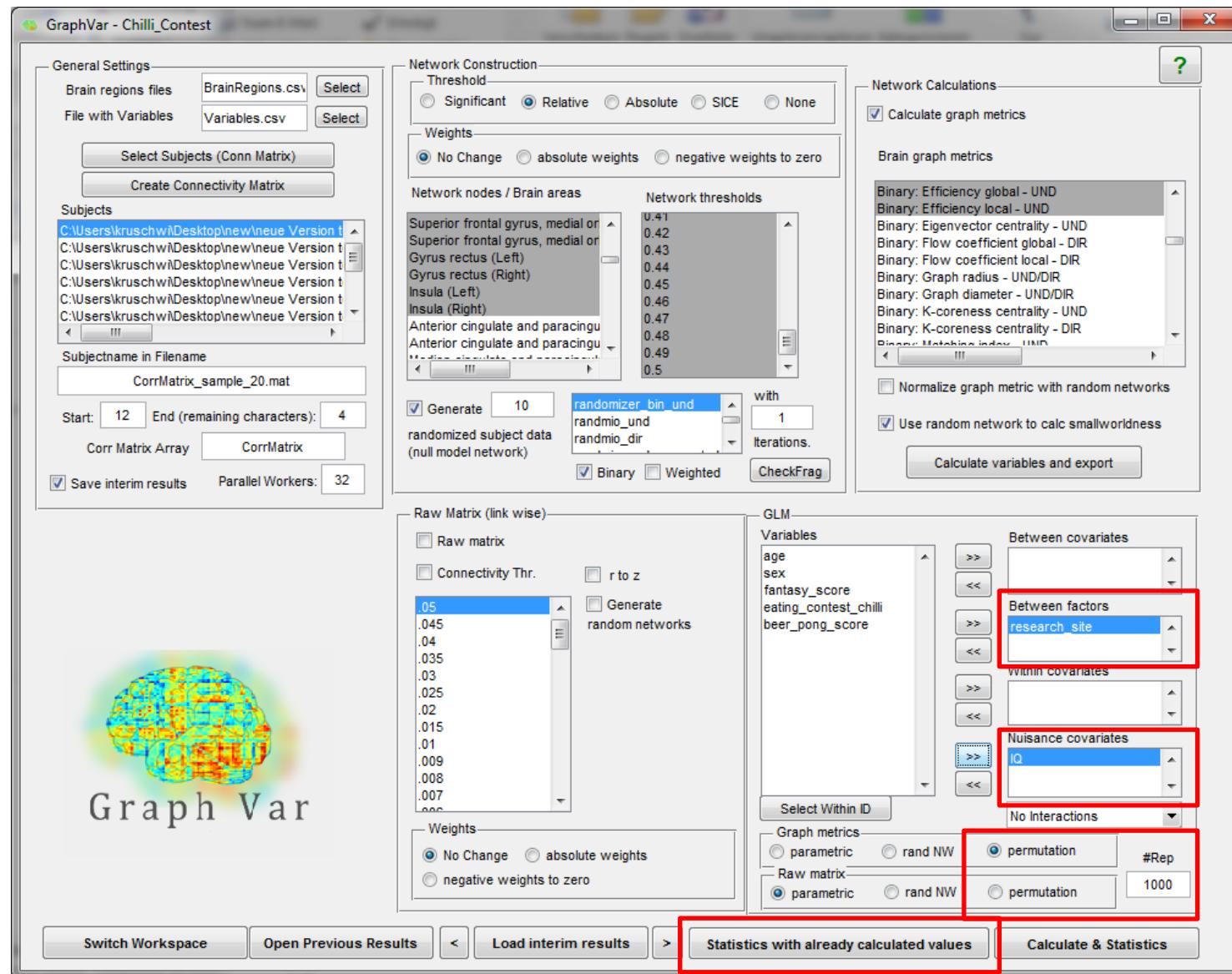
Hypothesis – confirmed!

1. YES - Chilli eating champs probably have more efficient brains... otherwise they could not deal with all the pain!
2. YES - orbito frontal gyrus and the olfactory gyrus contribute here...with a positive correlation of local efficiency to chilli eating ... much more spicy information transfer here!

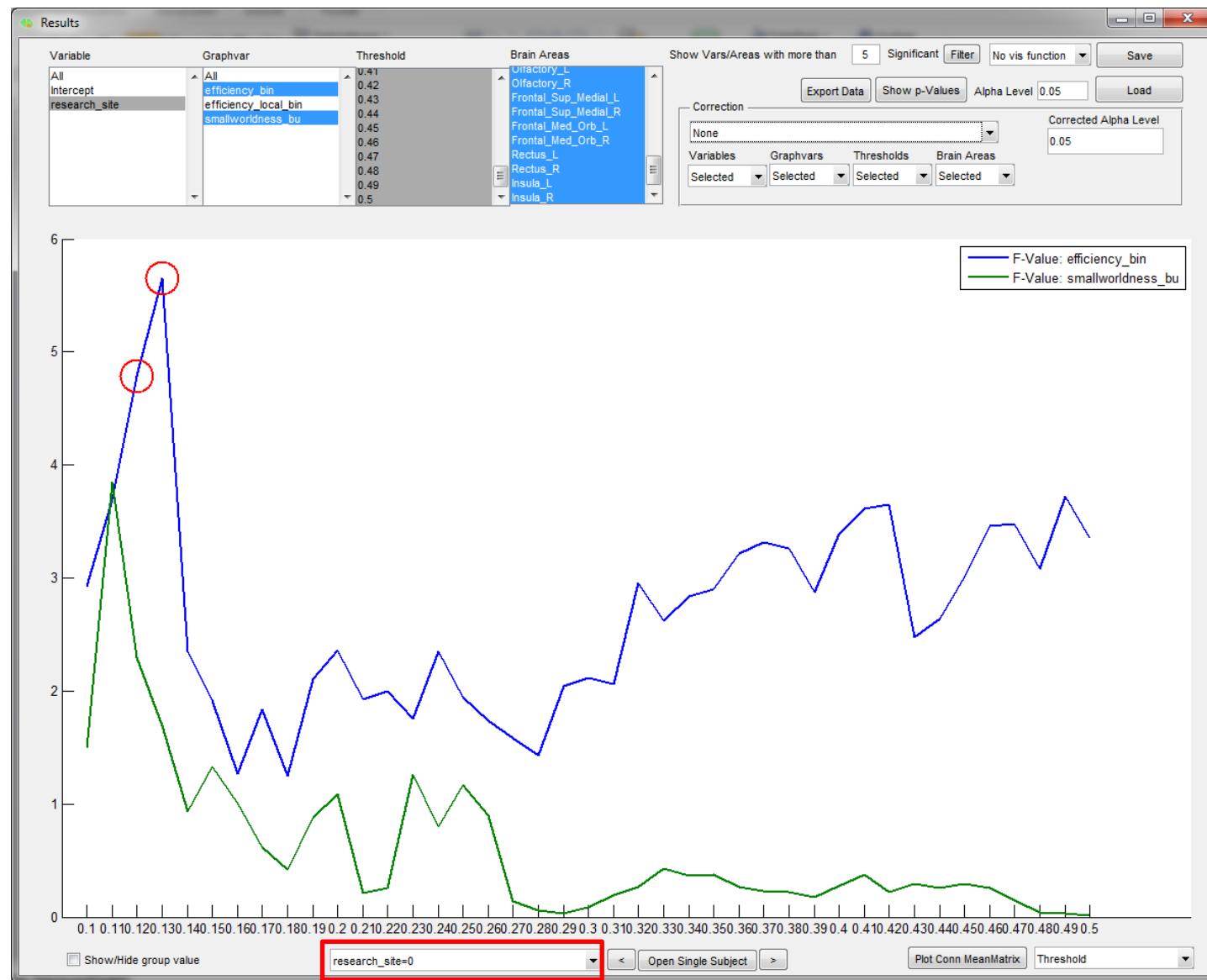


2. Group comparison (global efficiency)

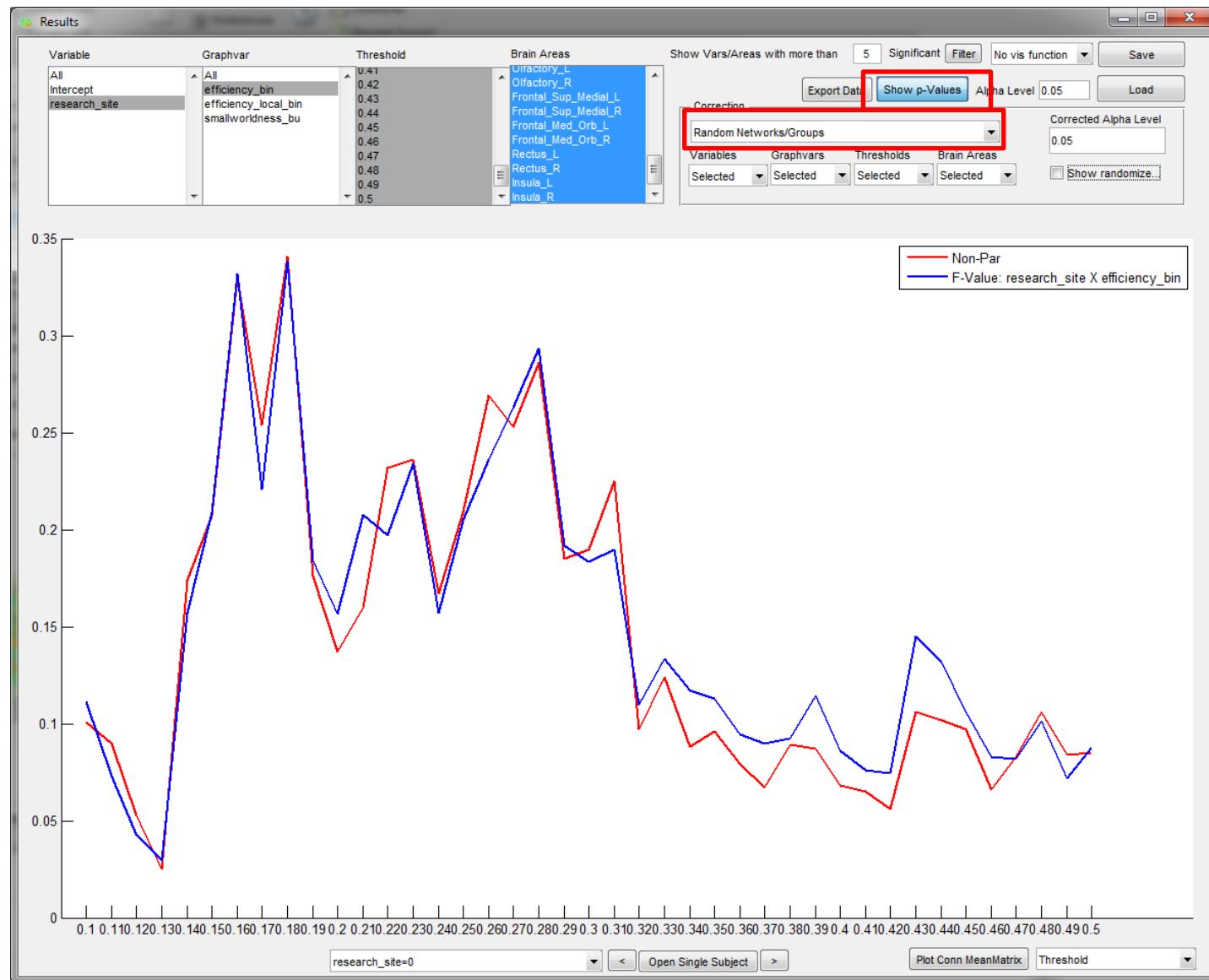
- Now, we can also go back and decide to do a group comparison (ANOVA) on the previously calculated efficiencies, where we regress out the influence of IQ and compute the model on the residuals
- Add „research site“ as a „between factor“, add „IQ“ as „nuisance covariate“ and „test against 1000 perm“
- Finally hit „Statistics with already calculated values“



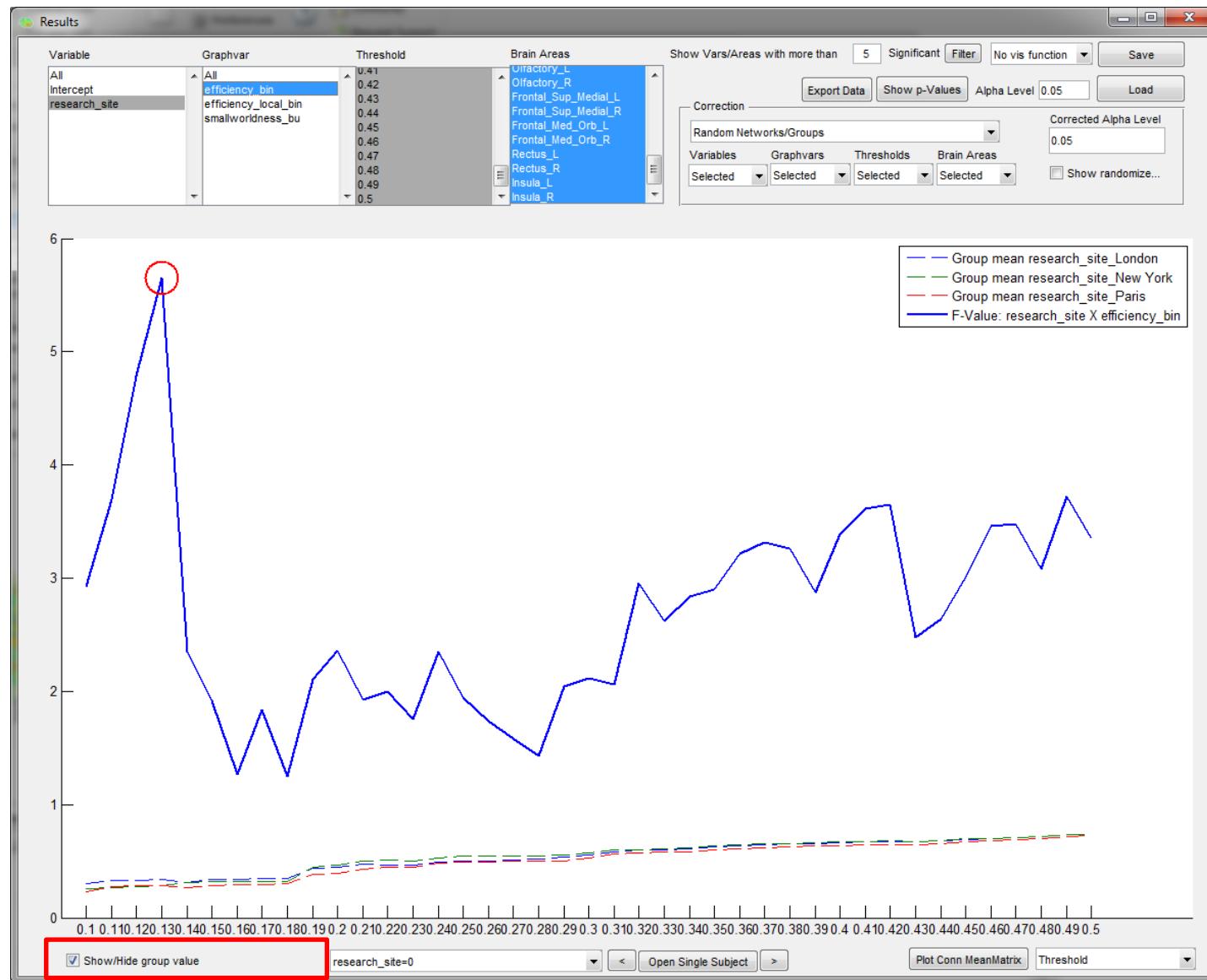
- You see the distribution of F values across thresholds for the group differences (i.e., factor research site) on global efficiency and small-worldness
- Red dots again indicate significance



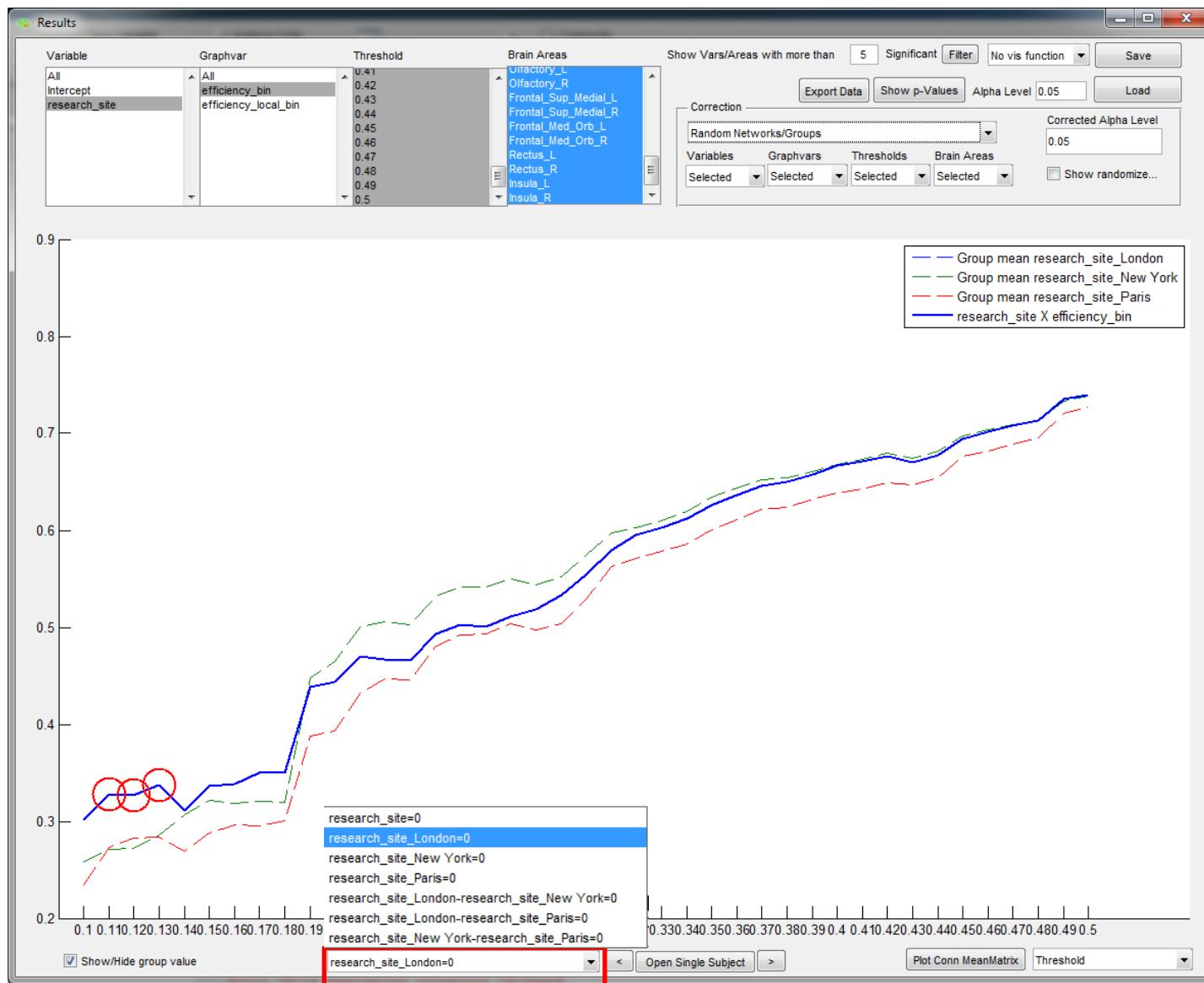
- Plot the parametric and non-parametric p-values ...



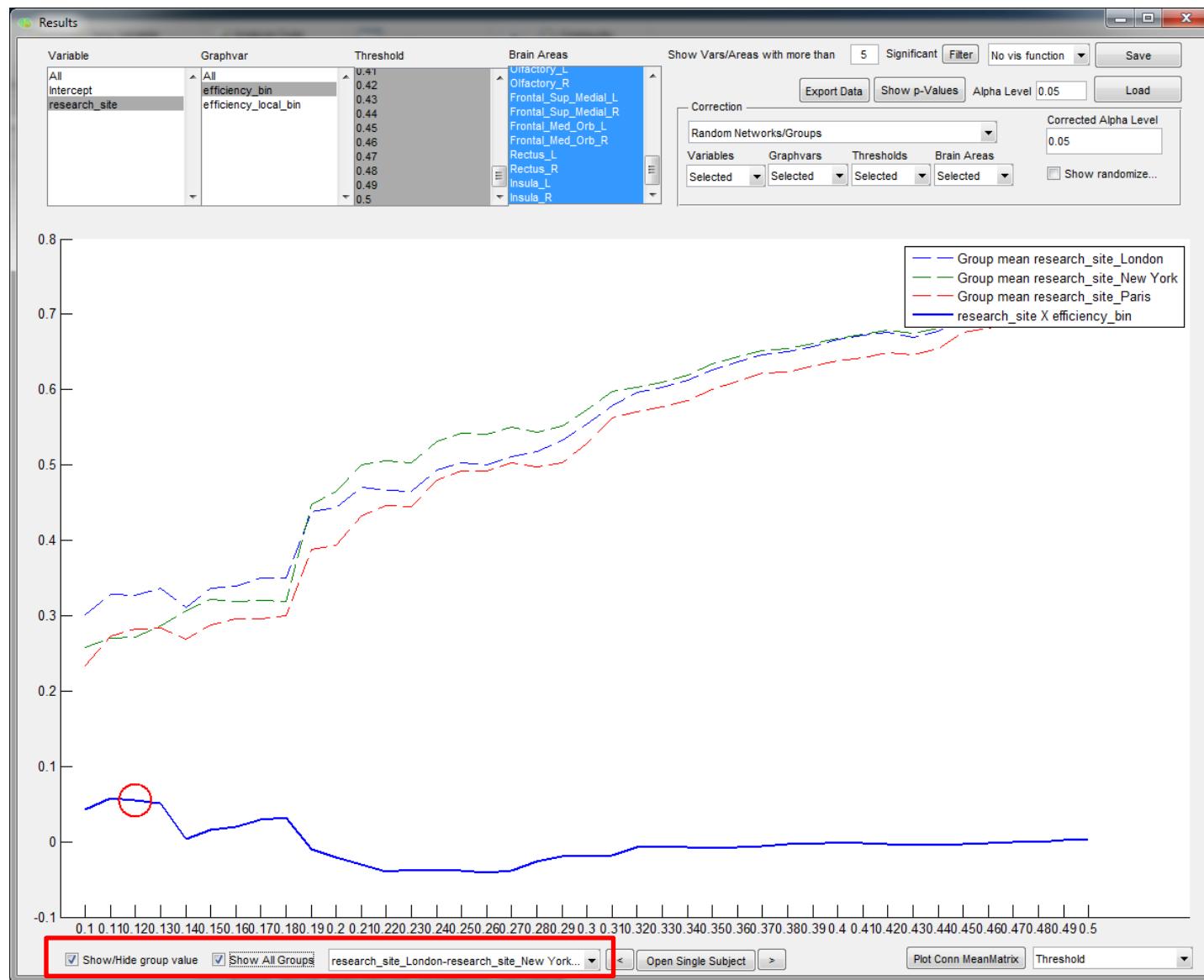
- Select only „efficiency_bin“
- Explore how the groups contribute to this effect by selecting „Show/Hide group value“



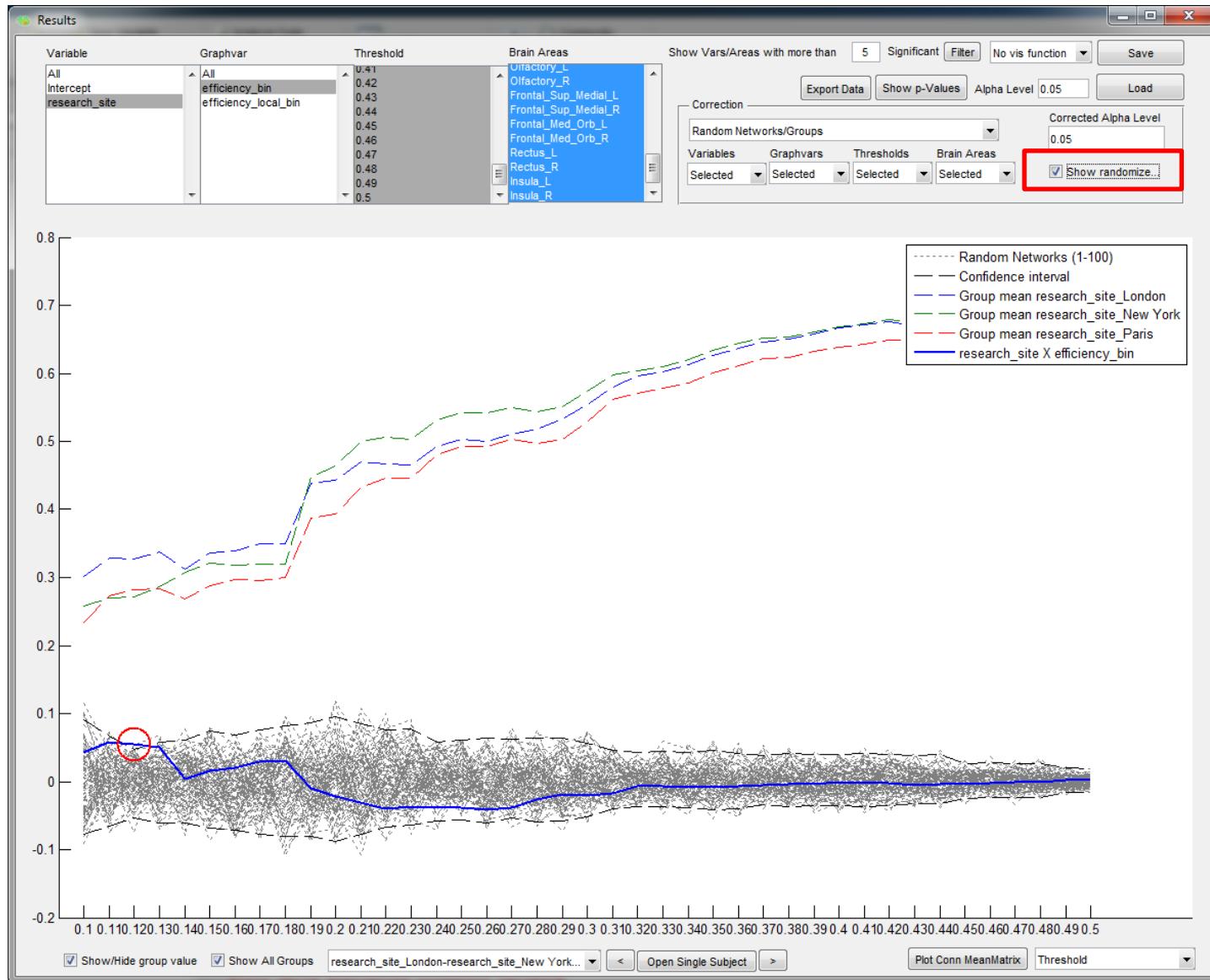
- Examine the mean of „efficiency_bin“ for each single group (i.e., significance test against zero)
- The blue line shows the group mean for the selected group



- Perform pair-wise comparisons by switching through the group contrasts (here: London vs. NewYork)
- The blue line shows the group difference for the specific contrast

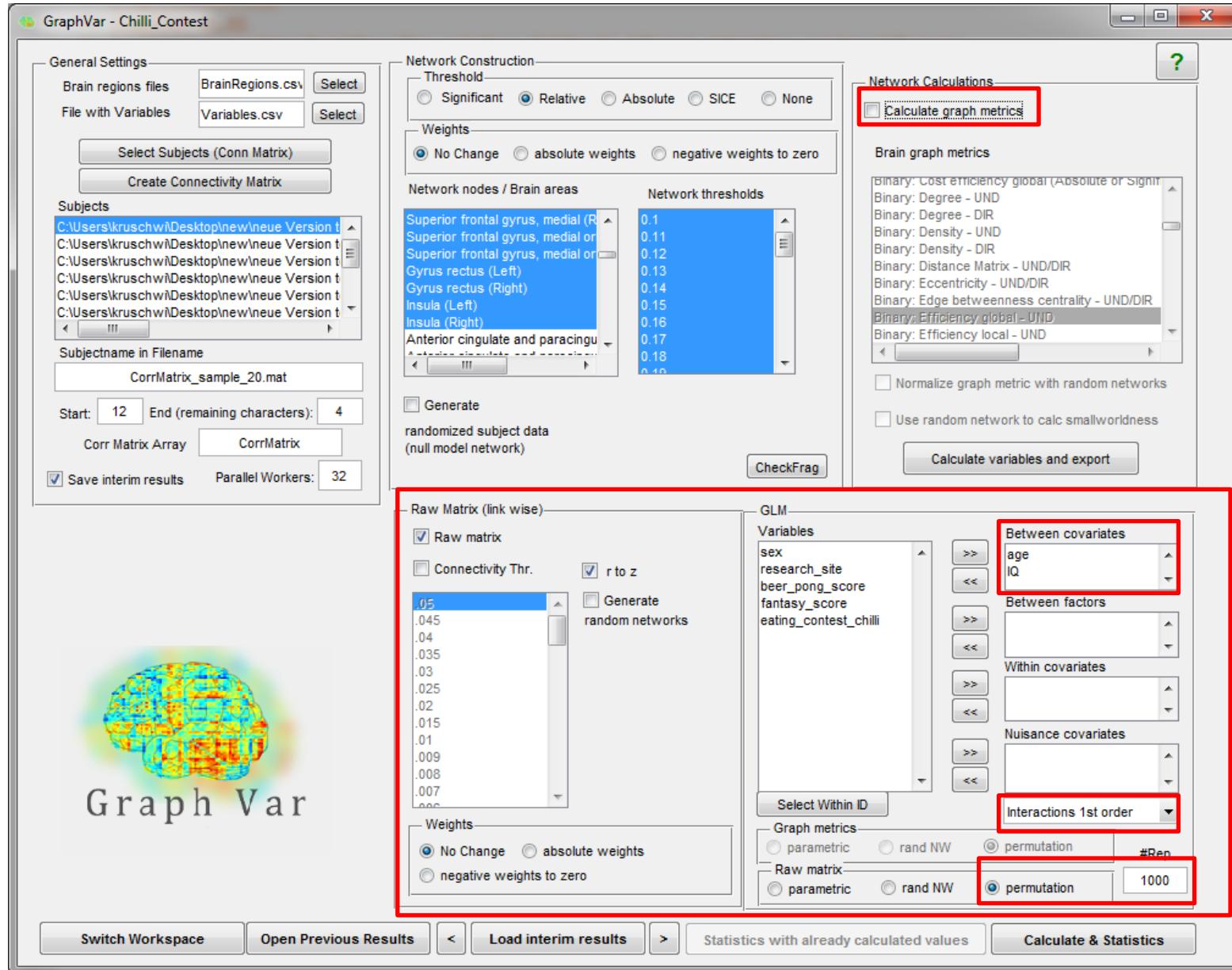


- Examine the permutation testing derived null-model distribution for the selected contrast
- You can see how the confidence interval will change with decreasing alpha values (e.g. 0.001)

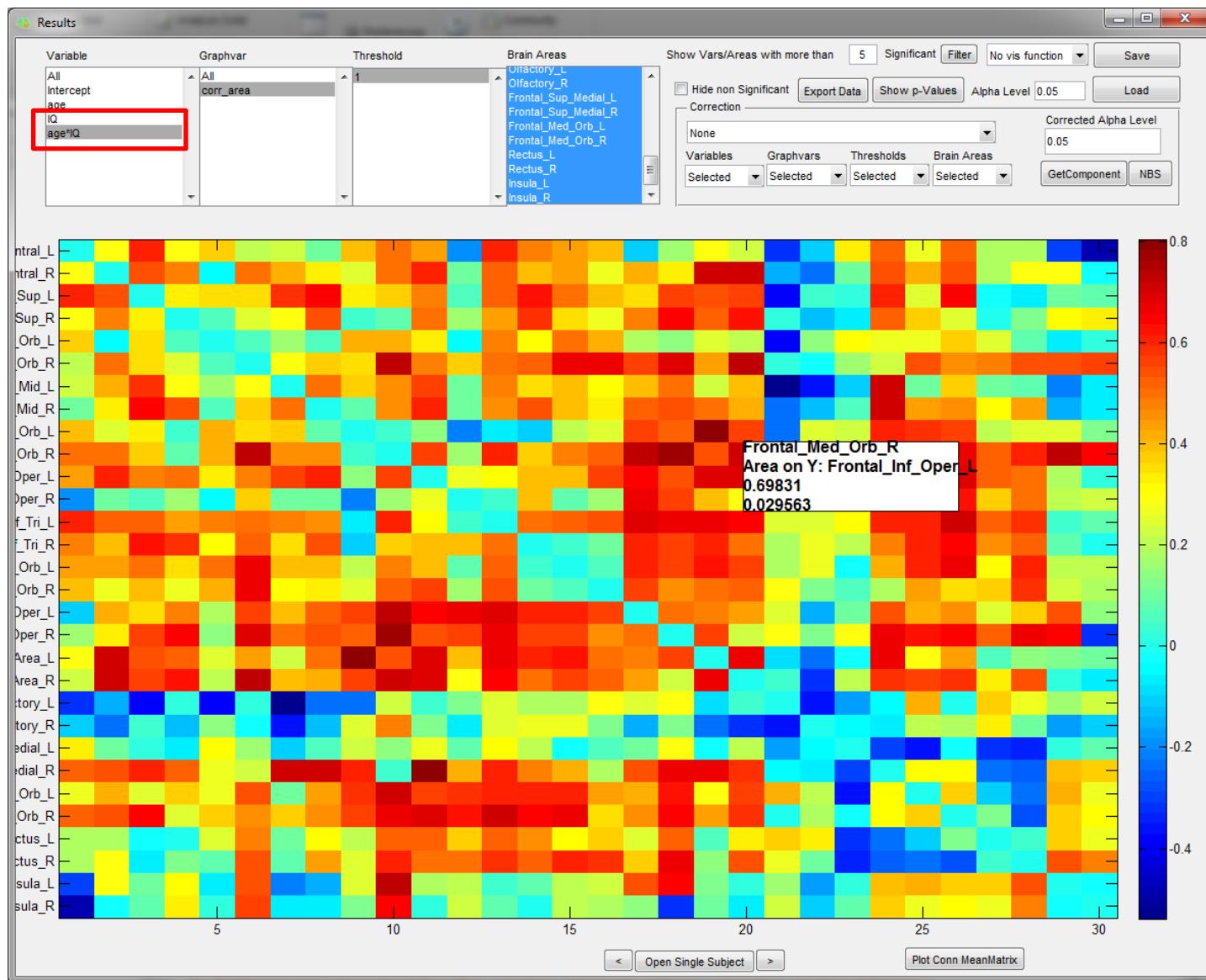


3. Raw Conn Matrix and Network Based Statistics

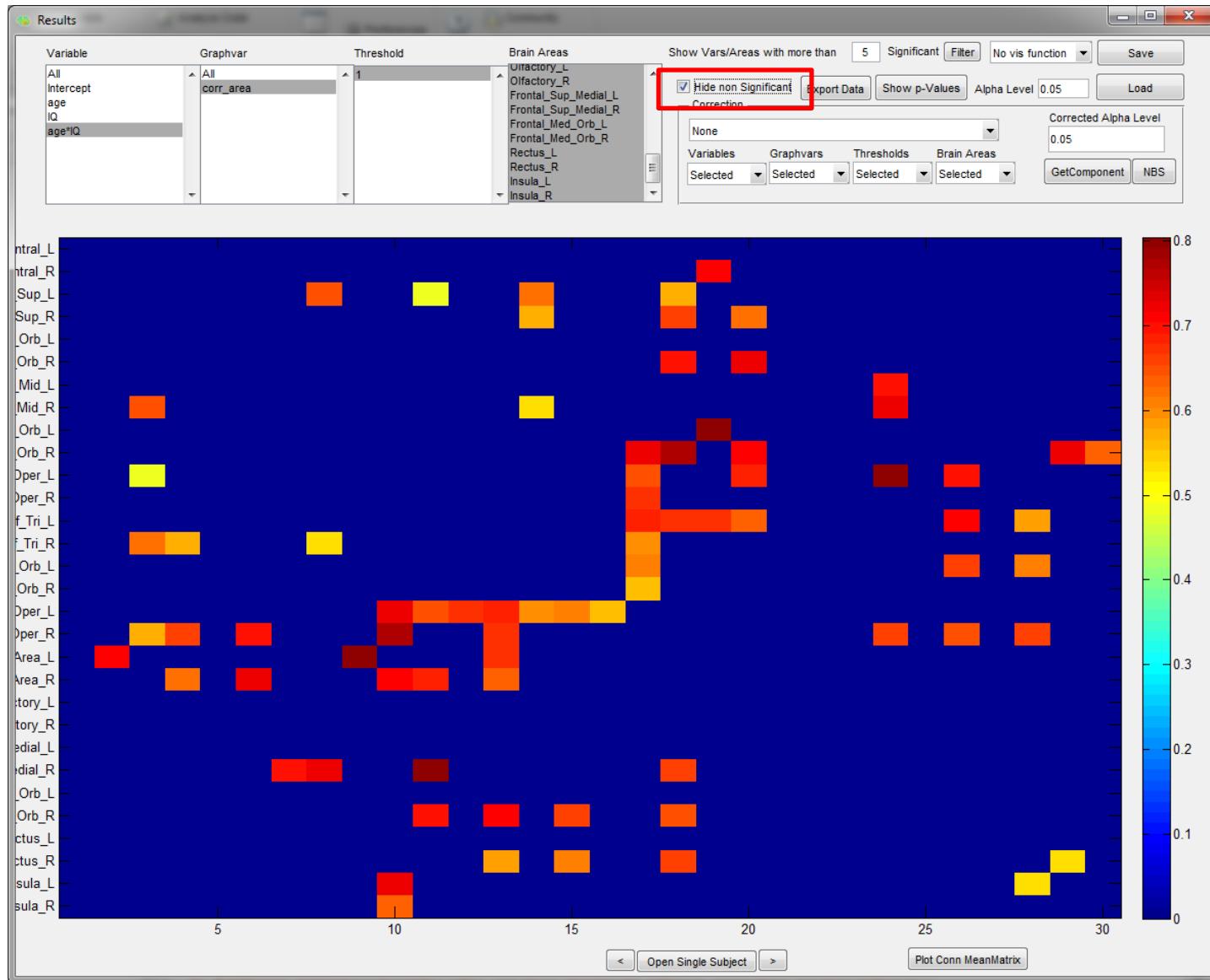
- Let's try some computations on the raw correlation matrices!
- Deselect „Calculate graph metrics“ and only use the following setting in:



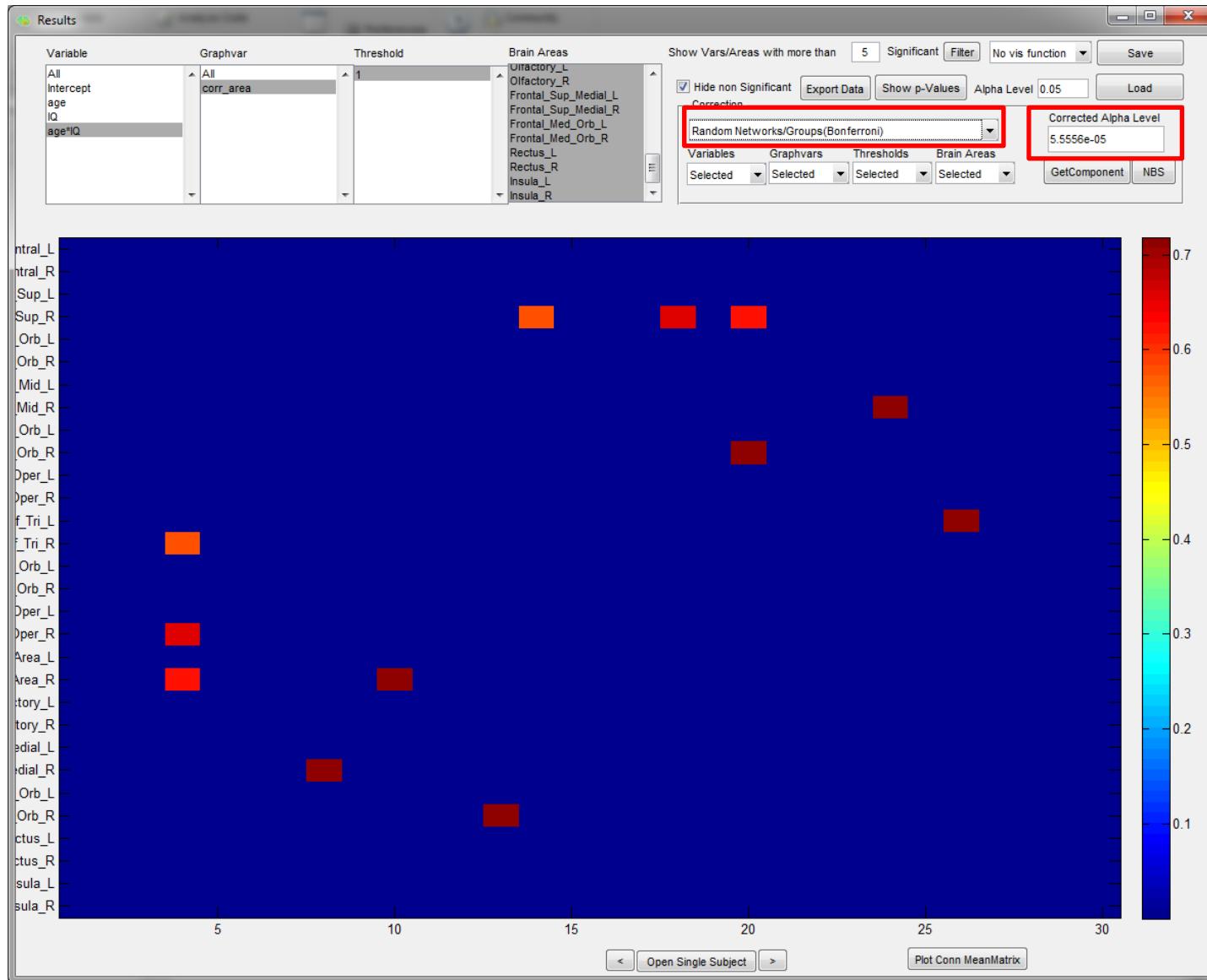
- This is the interaction of age and IQ and the raw connectivities between the nodes in the 30x30 matrix



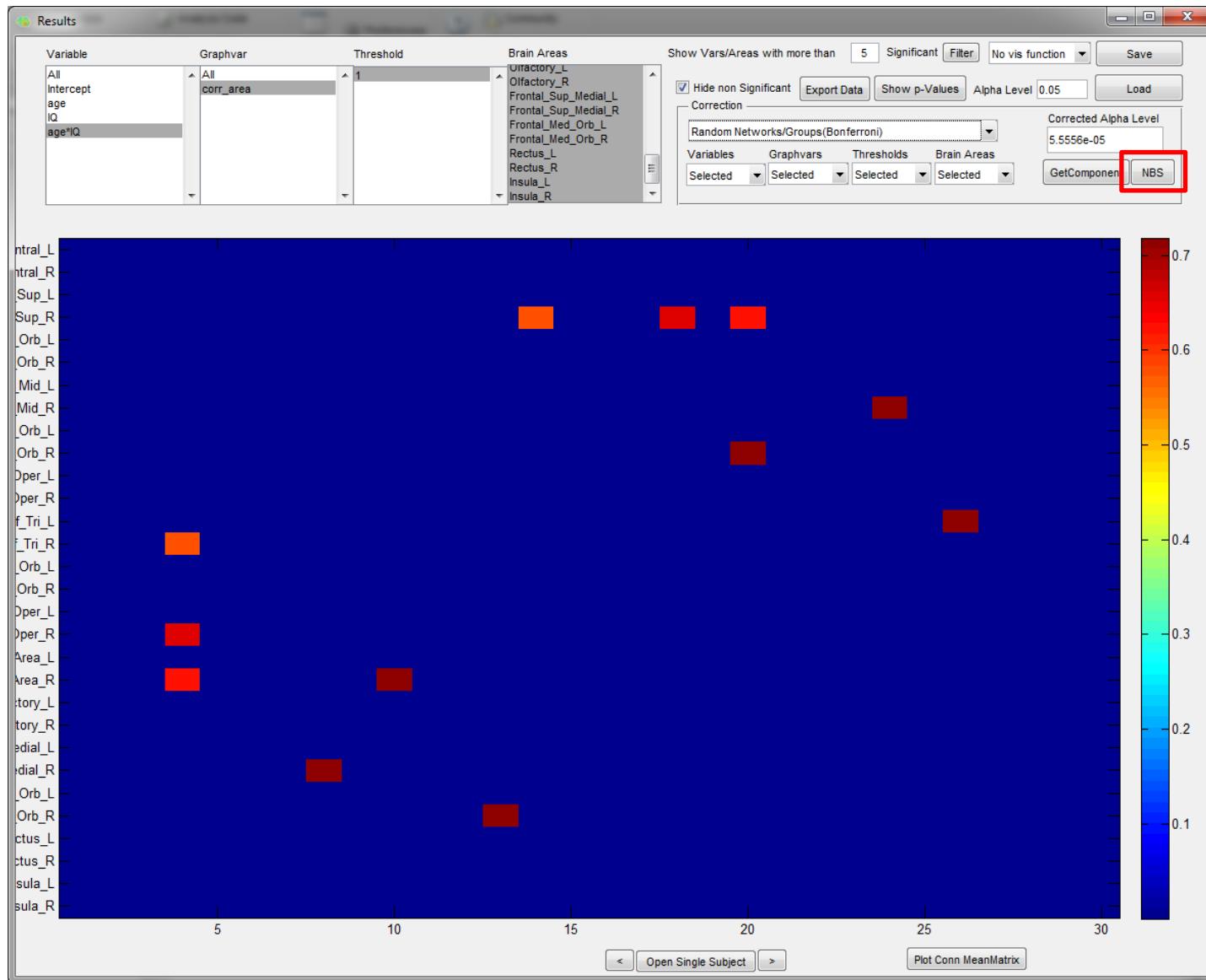
- Show only the significant brain areas for the selected interaction by selecting the „hide non significant“ button
- FYI: in the „brain areas field“ you can also select only some areas to be plotted



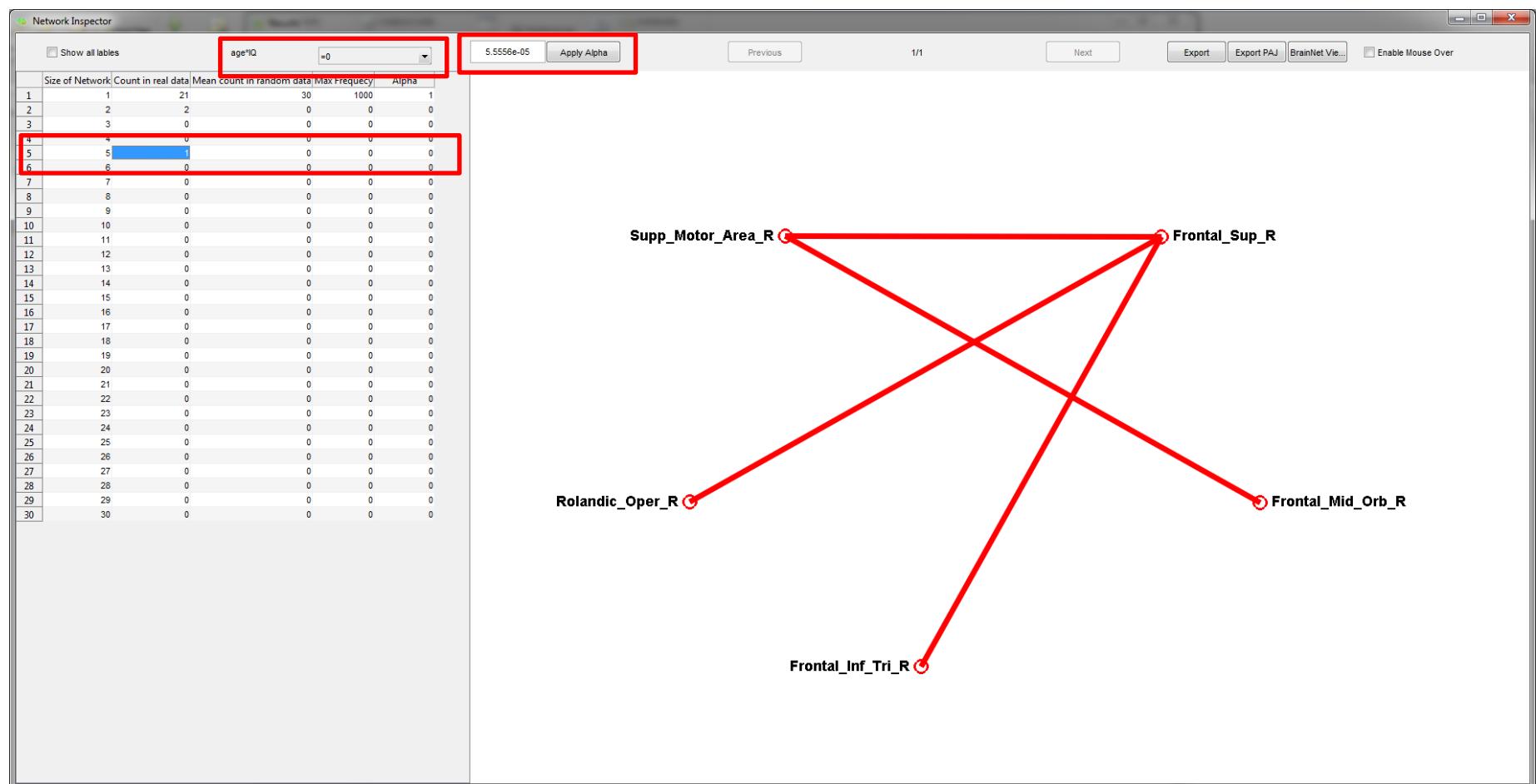
- Select the non-parametric results and apply the Bonferroni correction method: observe also the corresponding corrected alpha level (you may also try FDR correction)



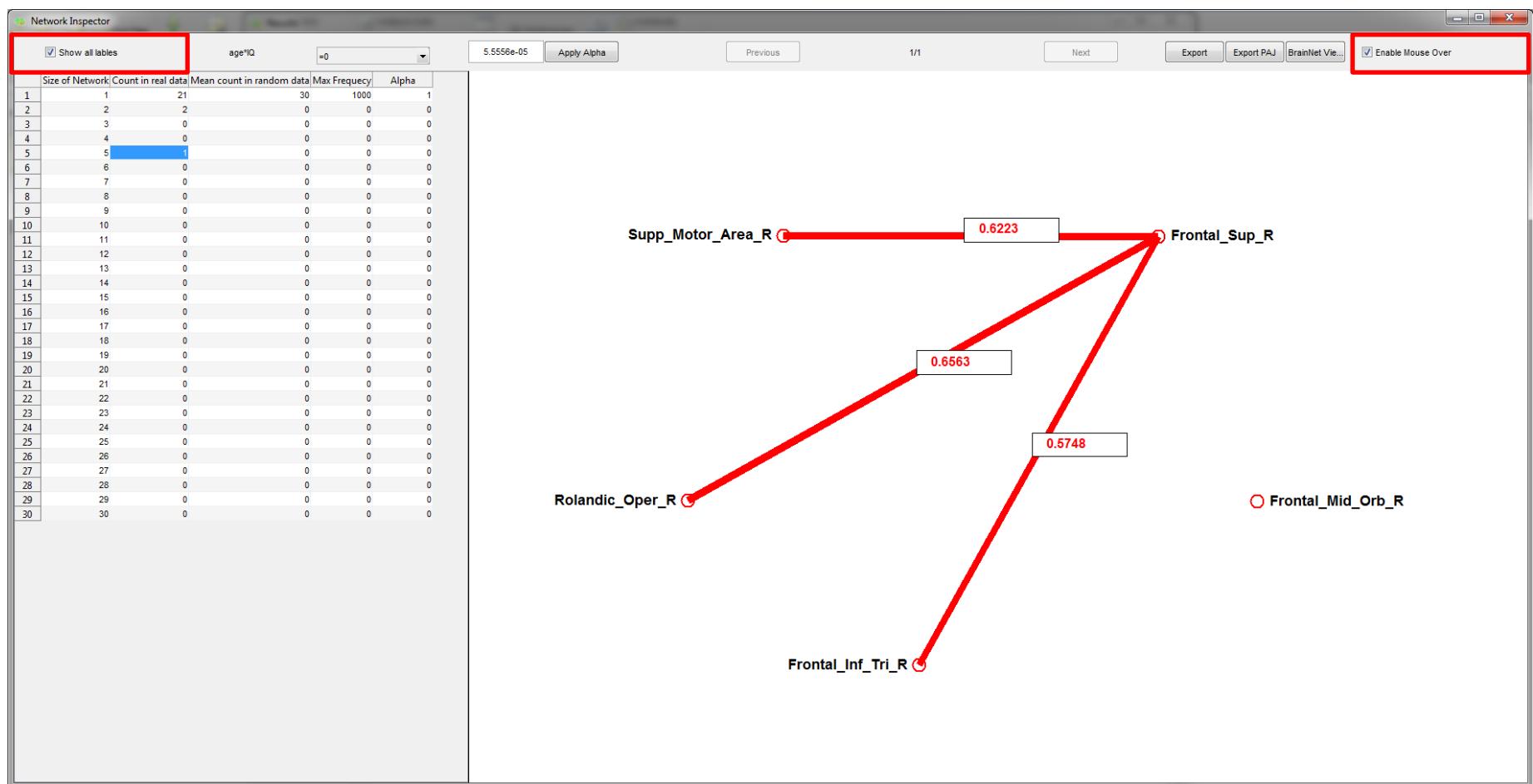
- You can now perform NBS! Note that for NBS the selected correction method will provide the p-values for the „initial-link-thresholding“ (here we take the non-parametric Bonferroni corrected p-values)
- Hit the „NBS“ button (for the „get component button please refer to the PDF „new features beta 0.62“)



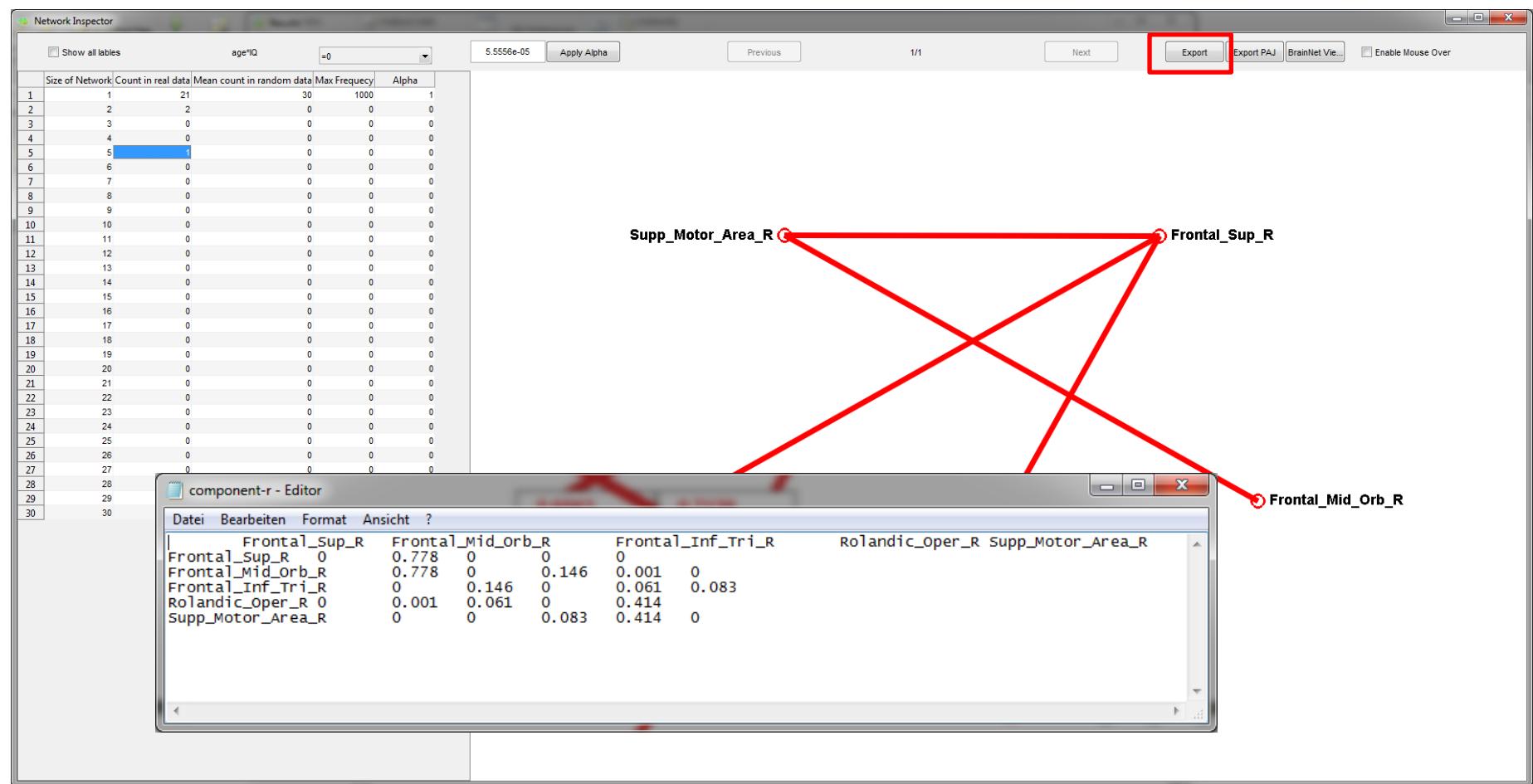
- The initial-link threshold is carried over from the results viewer (here: non-parametric)
- And observe a significant graph component comprised of 12 nodes for the interaction
- The selection box allows you to plot positive and/or negative associations (here we only have positive)
- Please refer to the Manual for how to interpret and to use the Network Inspector



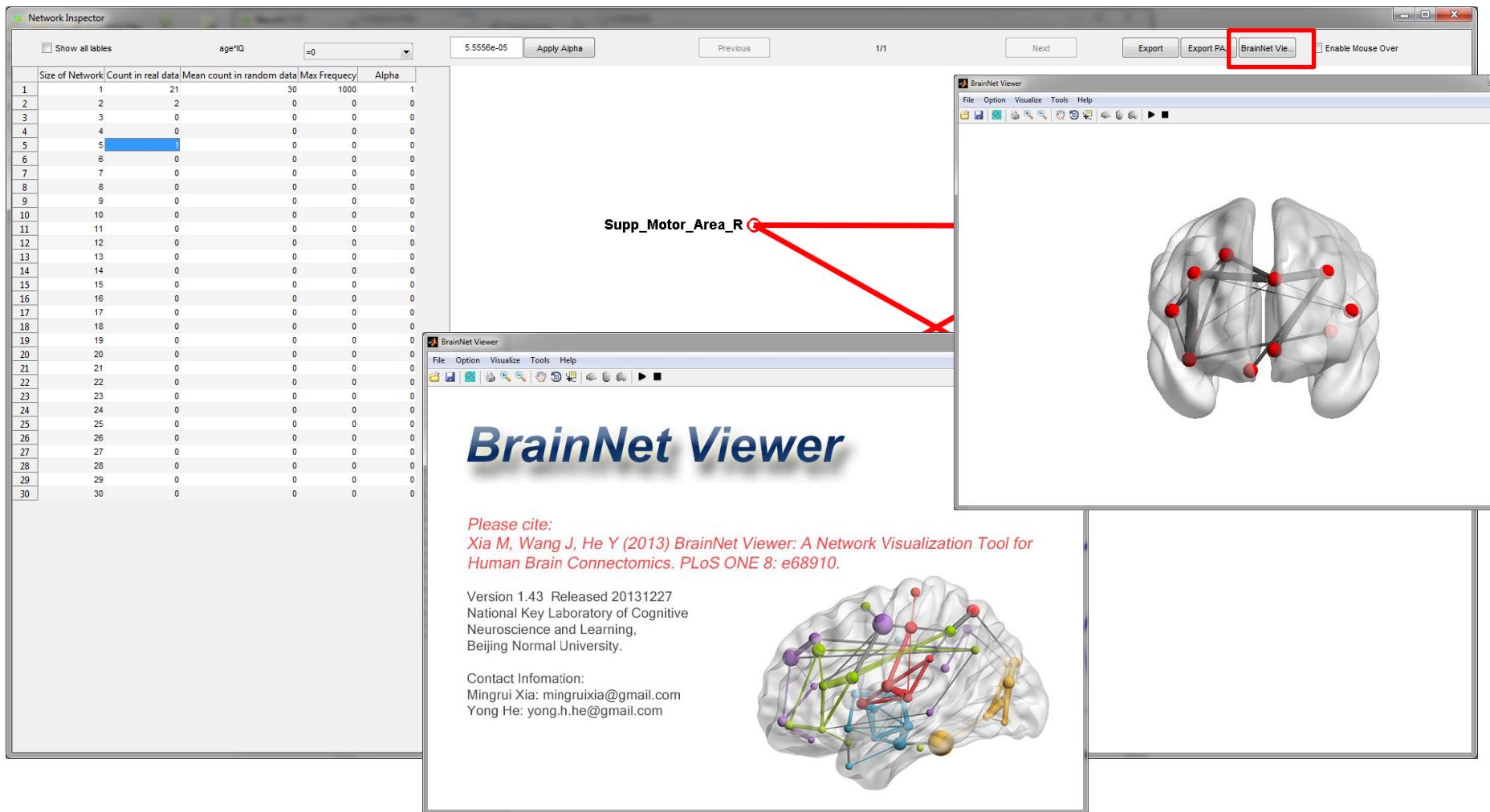
- Also use the „show all labels“ and „Enable Mouse Over“ to explore this component



- You can export the graph component in matrix format (.csv) but also in Pajek (PAJ) format for other visualization purposes



- You can also directly open the graph component in BrainNetViewer (Xia et al.) if this nice viewer is installed
- INFO: BrainNetViewer must be added to the MATLAB path with subfolders



4. Within design I (change of efficiency T1 T2)

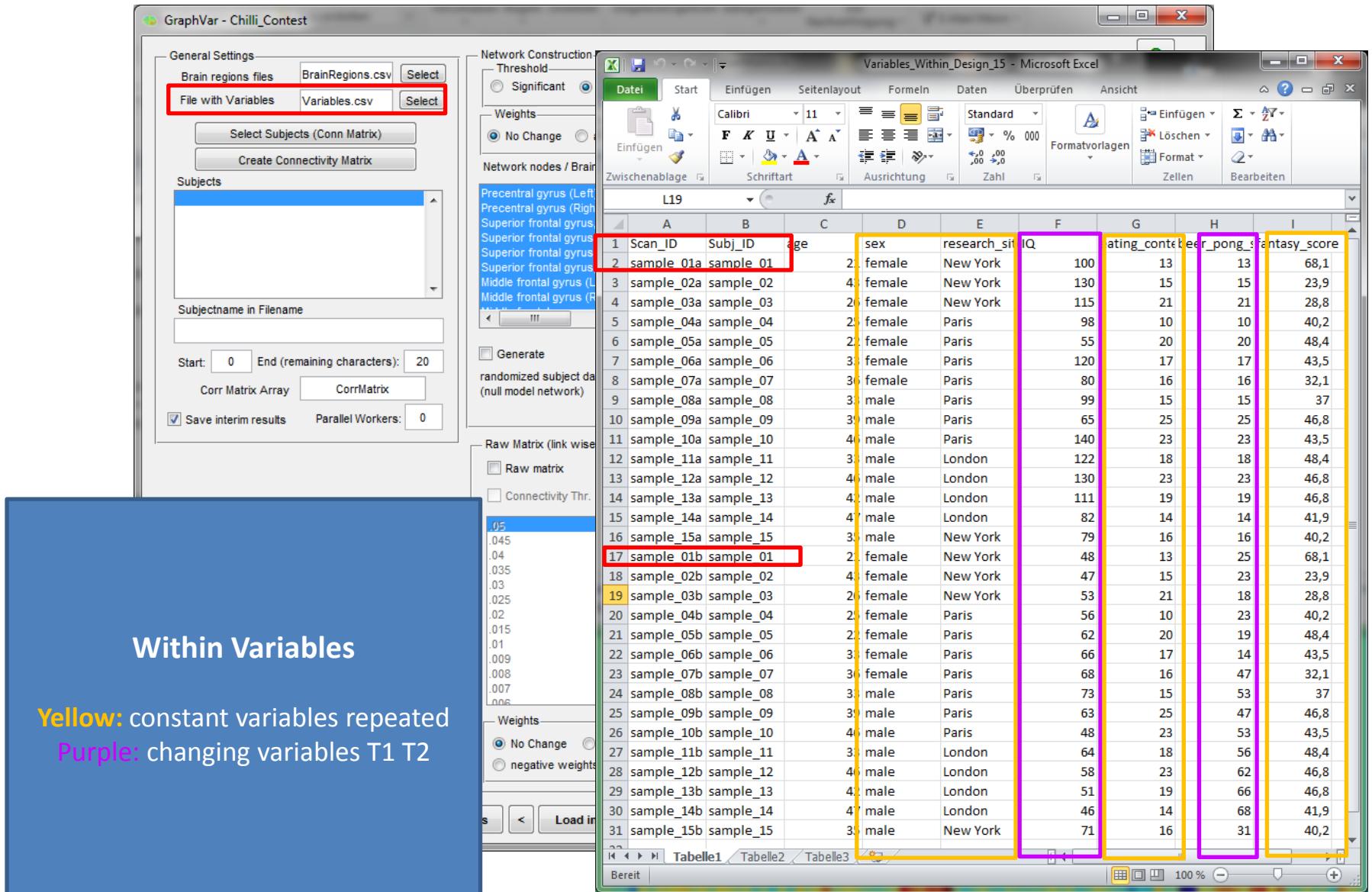
Research option 1 - example:

investigate the association between a constant independent variable (e.g. sex or genotype) and a changing dependent network variable (e.g. efficiency in T1 and T2)

- Select the „Variables_Within_Design“ sheet under .../workspaces/SampleWorkspace
- Notice: ***Scan_ID*** (one subject = T1: *sample_01a*, T2: *sample_01b*) and ***Subj_ID*** (*sample 1*)

Within Variables

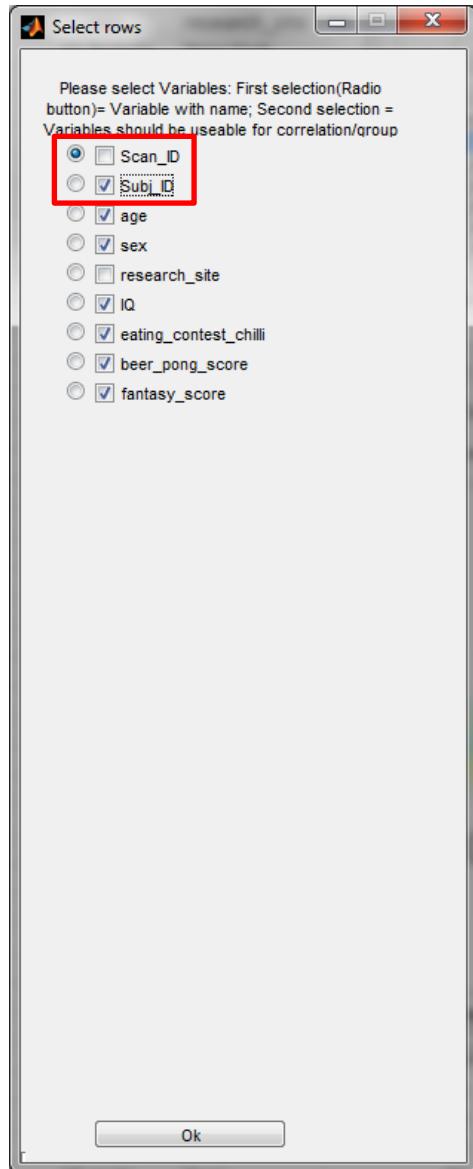
Yellow: constant variables repeated
 Purple: changing variables T1 T2



The GraphVar software interface shows settings for "Brain regions files" (BrainRegions.csv) and "File with Variables" (Variables.csv). The Excel spreadsheet "Variables_Within_Design_15" displays data across multiple sheets (Tabelle1, Tabelle2, Tabelle3). The columns represent variables: Scan_ID, Subj_ID, age, sex, research_site, IQ, rating_contest, beer_pong_score, fantasy_score. The data rows show 31 entries, each corresponding to a different subject (sample_01a to sample_15b). The "sex" column is highlighted in yellow, indicating constant variables. The "rating_contest" and "beer_pong_score" columns are highlighted in purple, indicating changing variables. The "fantasy_score" column is highlighted in green.

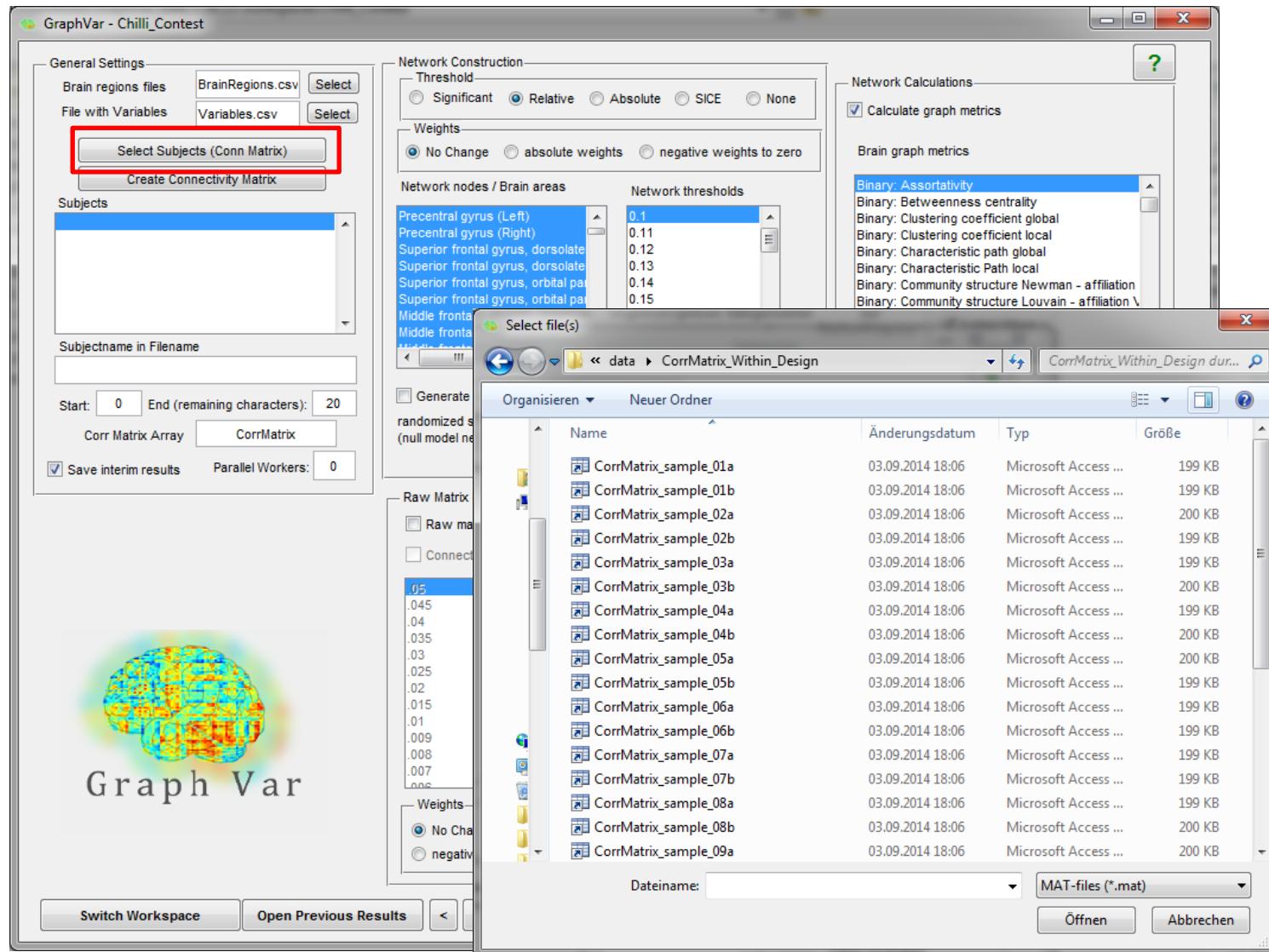
	A	B	C	D	E	F	G	H	I
1	Scan_ID	Subj_ID	age	sex	research_site	IQ	rating_contest	beer_pong_score	fantasy_score
2	sample_01a	sample_01	21	female	New York	100	13	13	68,1
3	sample_02a	sample_02	41	female	New York	130	15	15	23,9
4	sample_03a	sample_03	20	female	New York	115	21	21	28,8
5	sample_04a	sample_04	21	female	Paris	98	10	10	40,2
6	sample_05a	sample_05	21	female	Paris	55	20	20	48,4
7	sample_06a	sample_06	31	female	Paris	120	17	17	43,5
8	sample_07a	sample_07	30	female	Paris	80	16	16	32,1
9	sample_08a	sample_08	31	male	Paris	99	15	15	37
10	sample_09a	sample_09	31	male	Paris	65	25	25	46,8
11	sample_10a	sample_10	40	male	Paris	140	23	23	43,5
12	sample_11a	sample_11	31	male	London	122	18	18	48,4
13	sample_12a	sample_12	40	male	London	130	23	23	46,8
14	sample_13a	sample_13	41	male	London	111	19	19	46,8
15	sample_14a	sample_14	41	male	London	82	14	14	41,9
16	sample_15a	sample_15	31	male	New York	79	16	16	40,2
17	sample_01b	sample_01	21	female	New York	48	13	25	68,1
18	sample_02b	sample_02	41	female	New York	47	15	23	23,9
19	sample_03b	sample_03	20	female	New York	53	21	18	28,8
20	sample_04b	sample_04	21	female	Paris	56	10	23	40,2
21	sample_05b	sample_05	21	female	Paris	62	20	19	48,4
22	sample_06b	sample_06	31	female	Paris	66	17	14	43,5
23	sample_07b	sample_07	31	female	Paris	68	16	47	32,1
24	sample_08b	sample_08	31	male	Paris	73	15	53	37
25	sample_09b	sample_09	31	male	Paris	63	25	47	46,8
26	sample_10b	sample_10	40	male	Paris	48	23	53	43,5
27	sample_11b	sample_11	31	male	London	64	18	56	48,4
28	sample_12b	sample_12	40	male	London	58	23	62	46,8
29	sample_13b	sample_13	41	male	London	51	19	66	46,8
30	sample_14b	sample_14	41	male	London	46	14	68	41,9
31	sample_15b	sample_15	31	male	New York	71	16	31	40,2

- Select the „Variables_Within_Design“ sheet under .../workspaces/SampleWorkspace
- Notice: ***Scan_ID*** (one subject = T1: *sample_01a*, T2: *sample_01b*) and ***Subj_ID*** (*sample 1*)

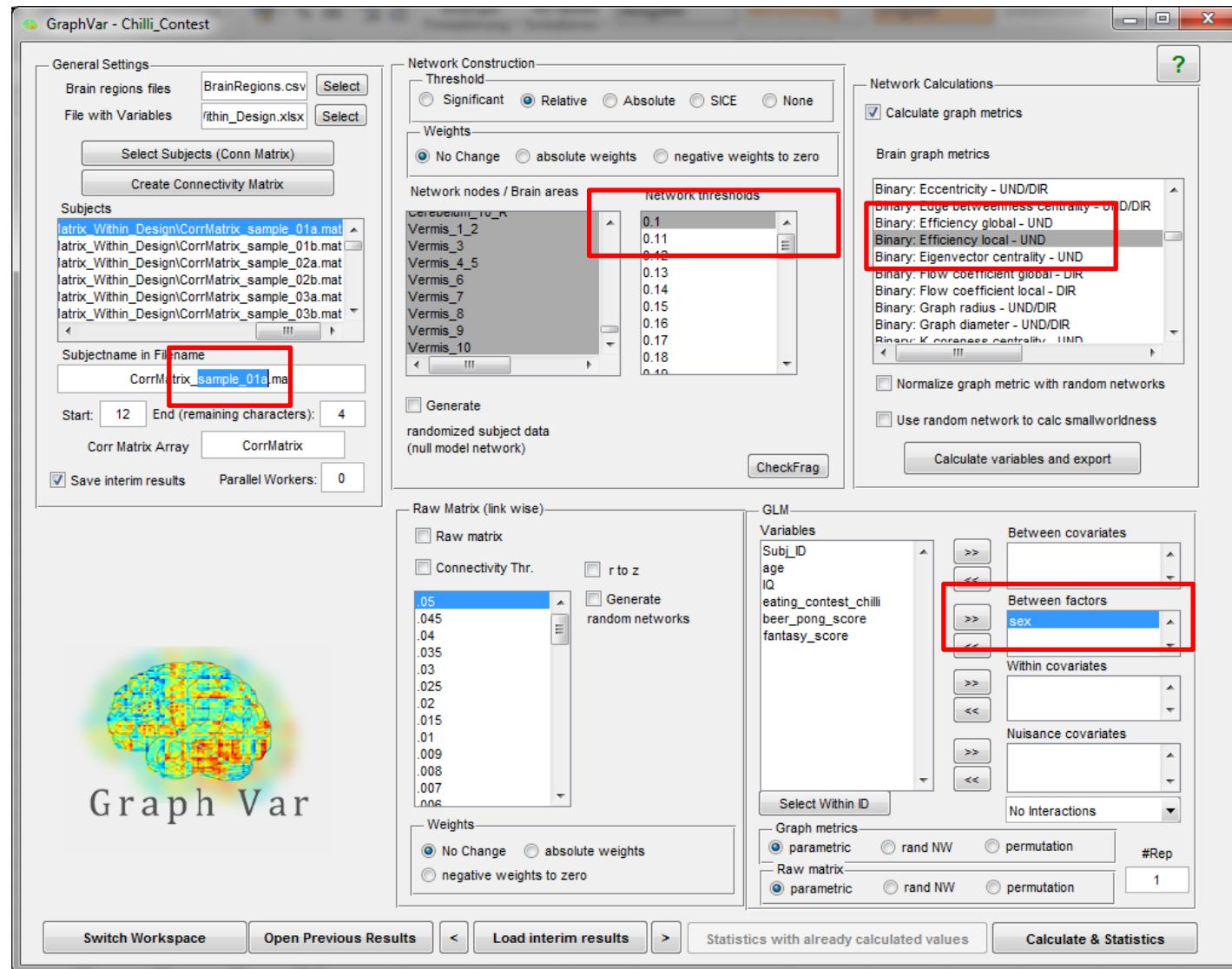


- Select the „Scan_ID“ as file identifier
- Select also „Subj_ID“ as subject ID
- Select: age, sex, and IQ

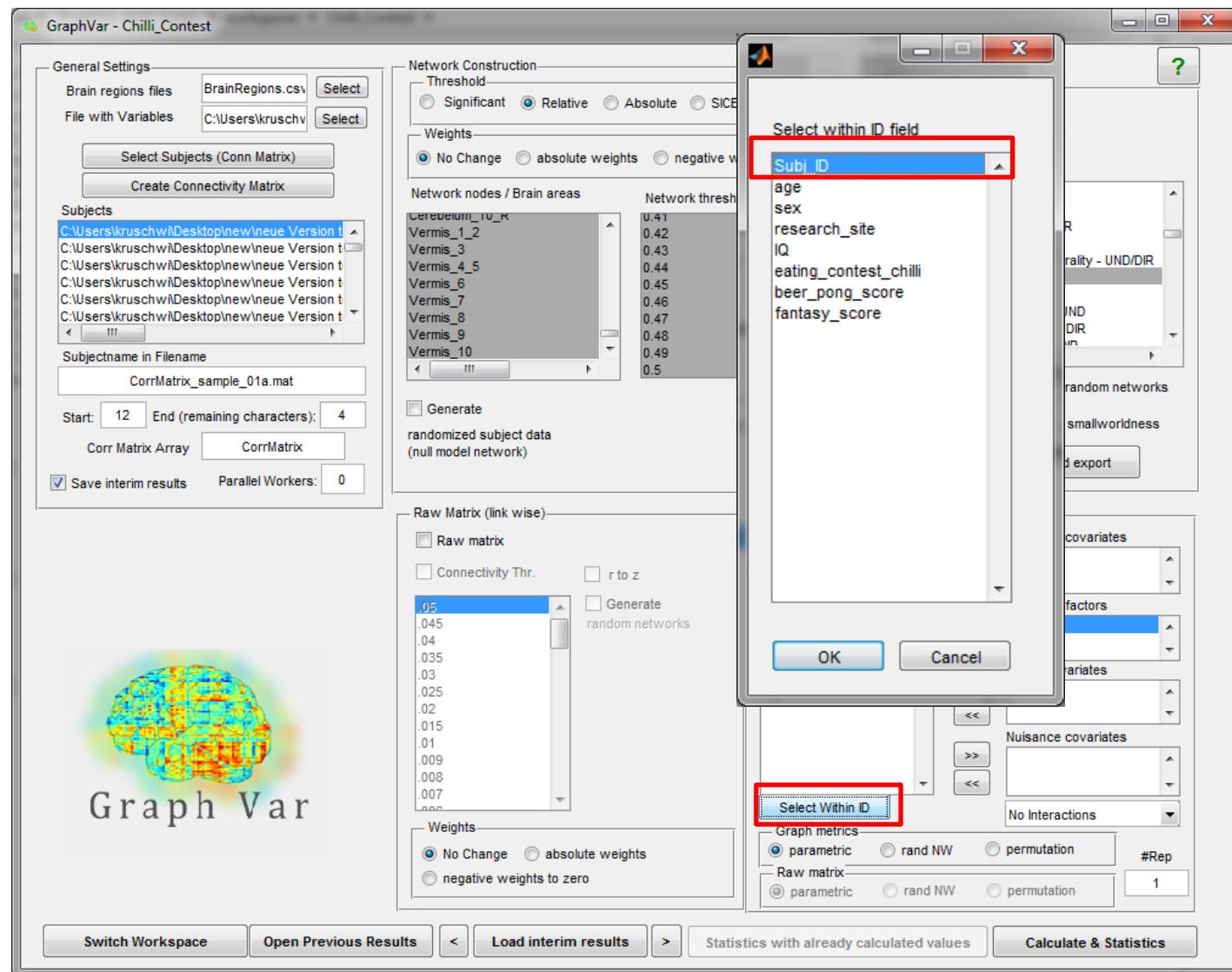
- Navigate to GraphVar/workspaces/SampleWorkspace/data/CorrMatrix_Within_Design
- Select all 15 subjects (i.e., 30 files as 1 subject = T1: sample_01a, T2: sample_01b)



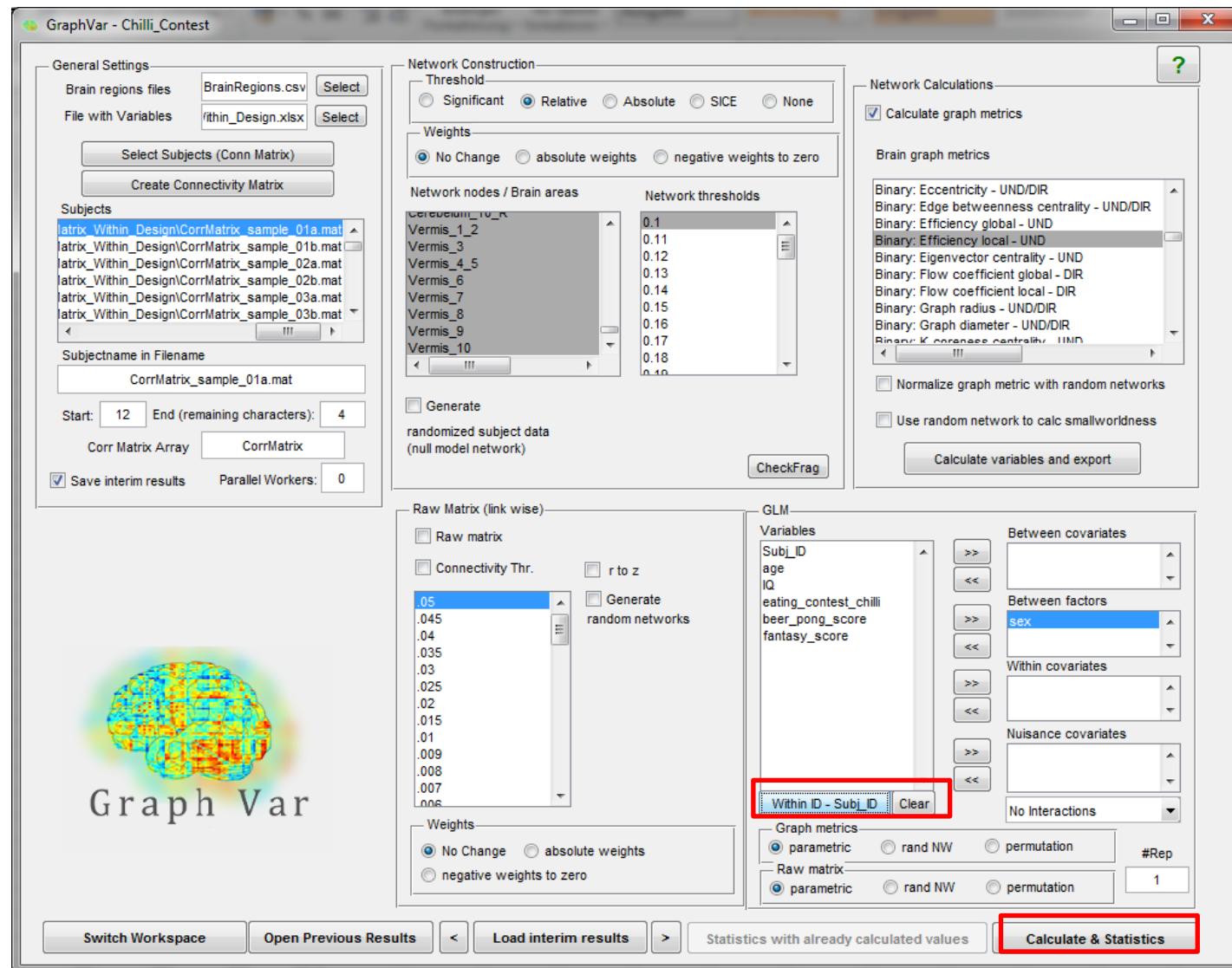
- Highlight the Scan_ID
- Select all nodes, threshold 0.1, and local efficiency
- GLM: sex as between factor



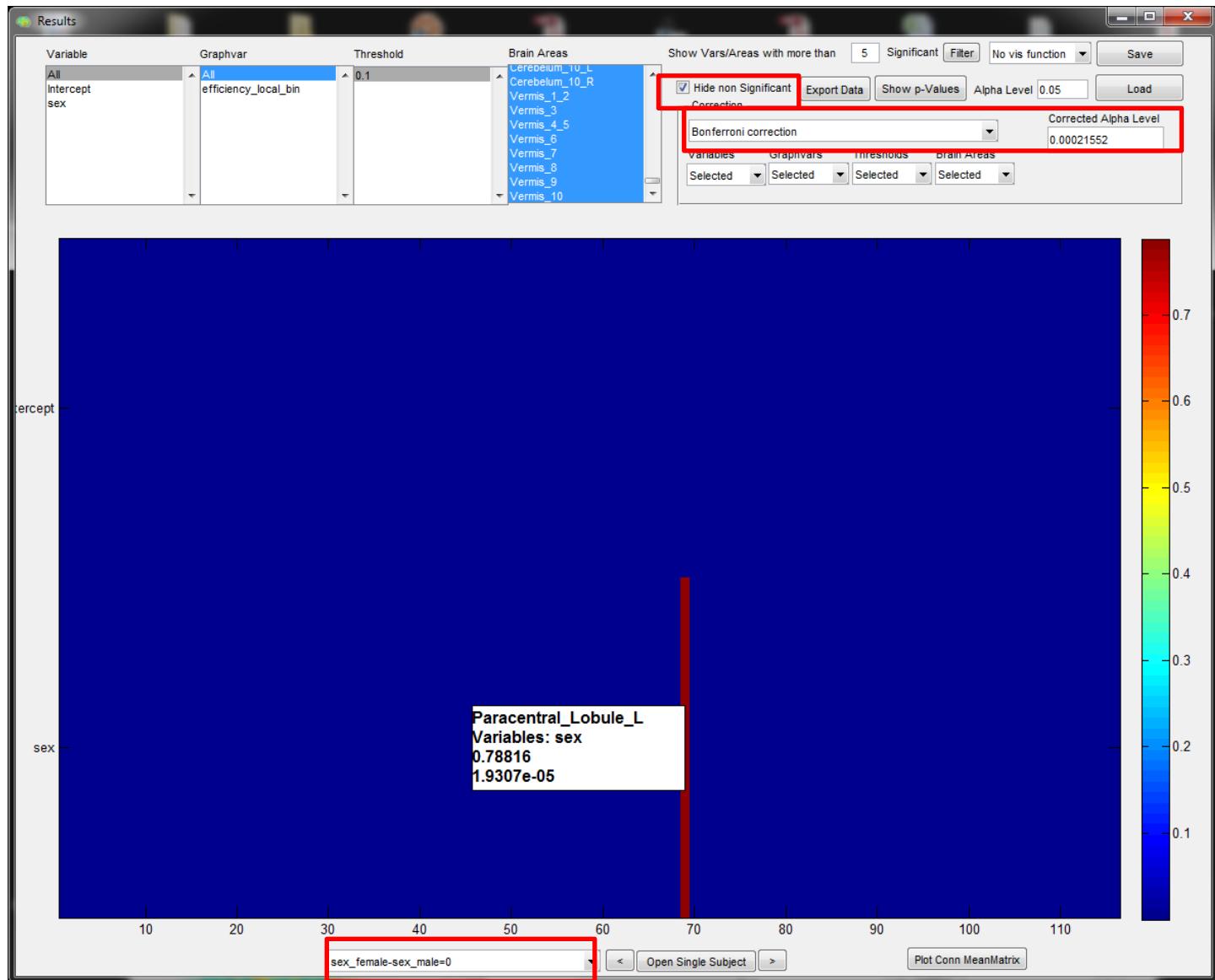
- Click on „Select Within ID“
- And highlight the „Subj_ID“



- The Within deviding field will appear as such on the button
- „Calculate & Statistics“



- After Bonferroni correction we can observe a significant difference in change of local efficiency between T1 and T2 as a function of sex (i.e., females as compared to males have a stronger change in local efficiency between T1 and T2)

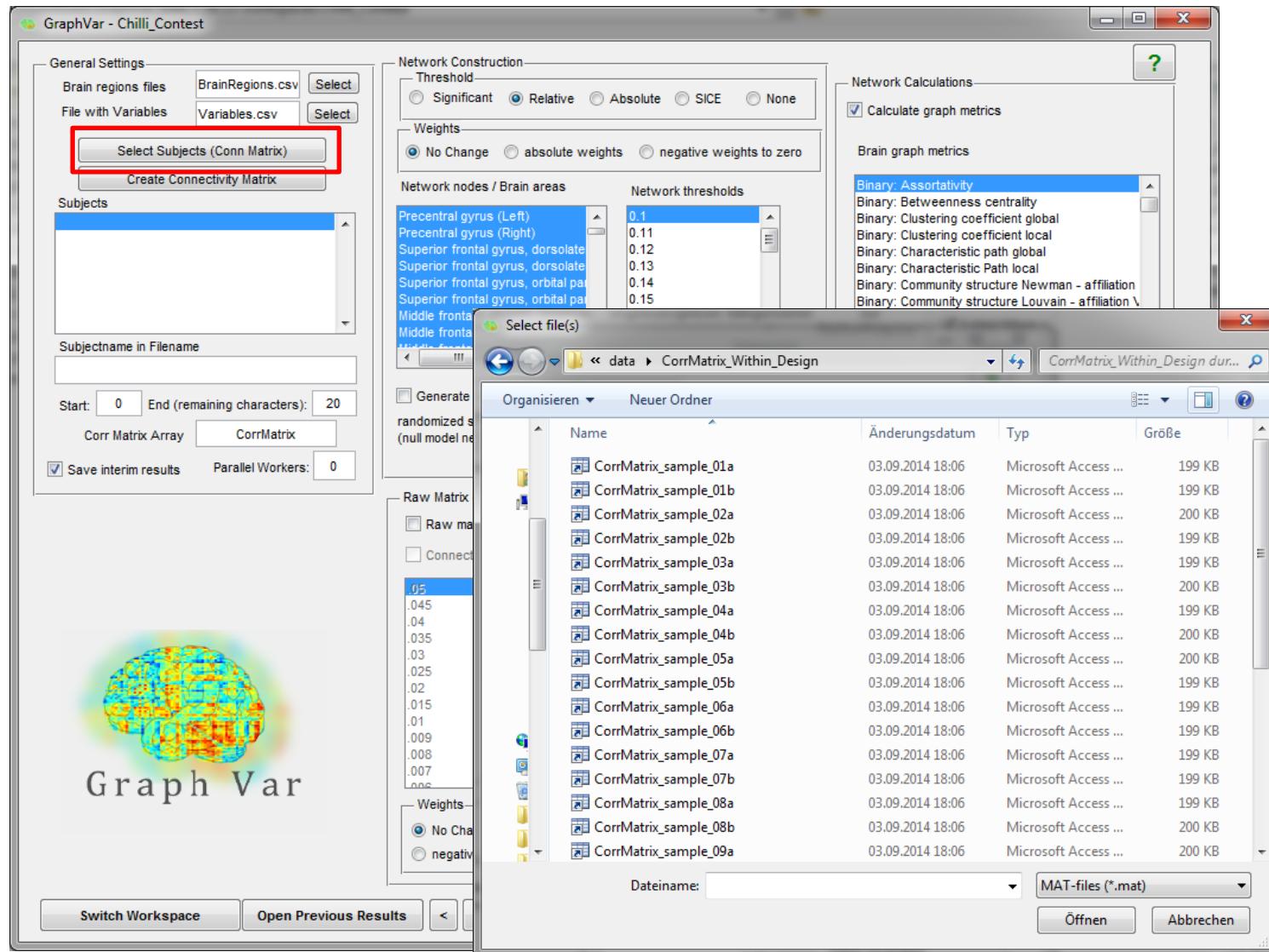


4. Within design II (change of efficiency and behavior T1 T2)

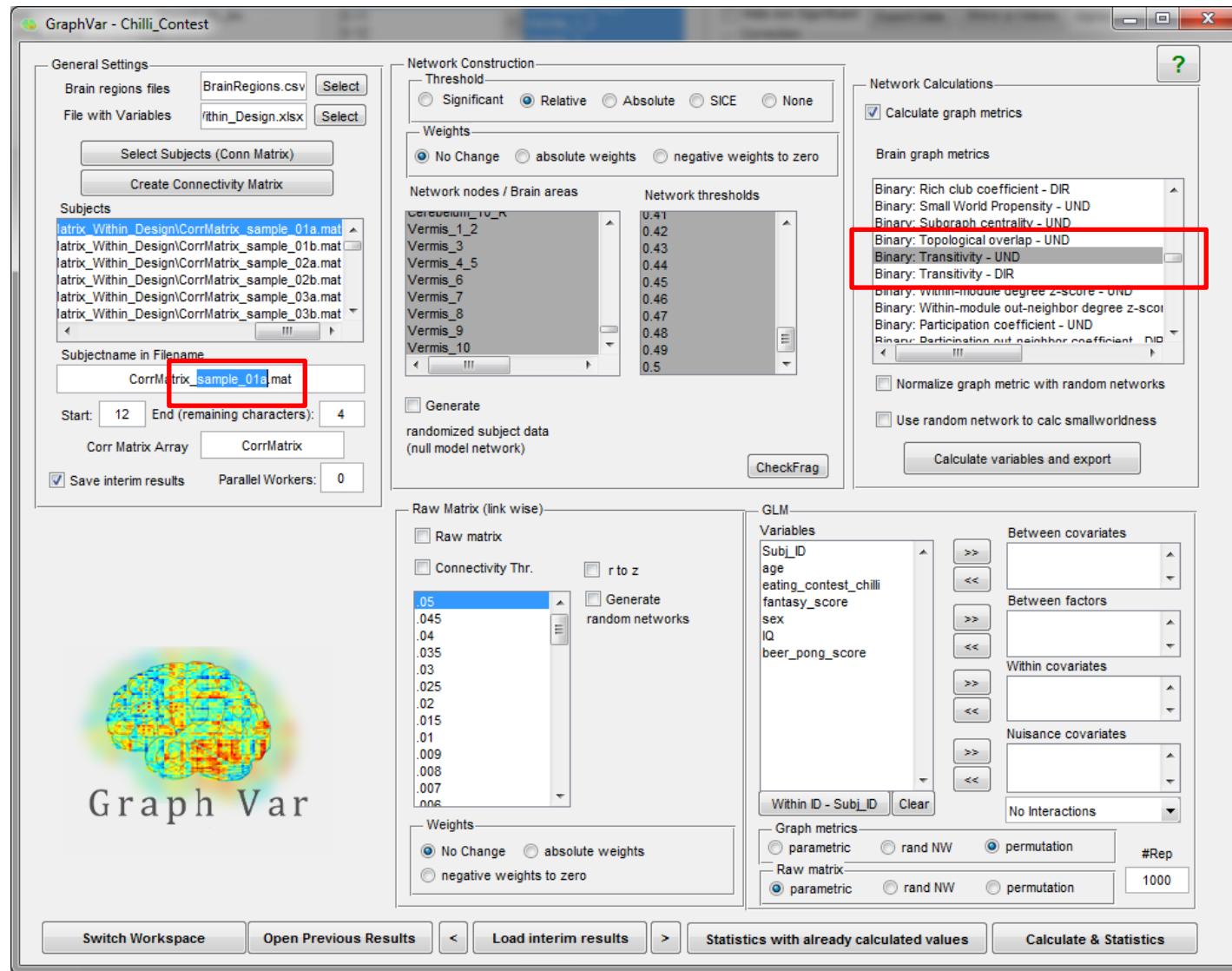
Research option 2 - example:

investigate the association between a changing independent variable (e.g. cognitive function in T1 and T2) and the change of a dependent network variable (e.g. network efficiency in T1 T2)

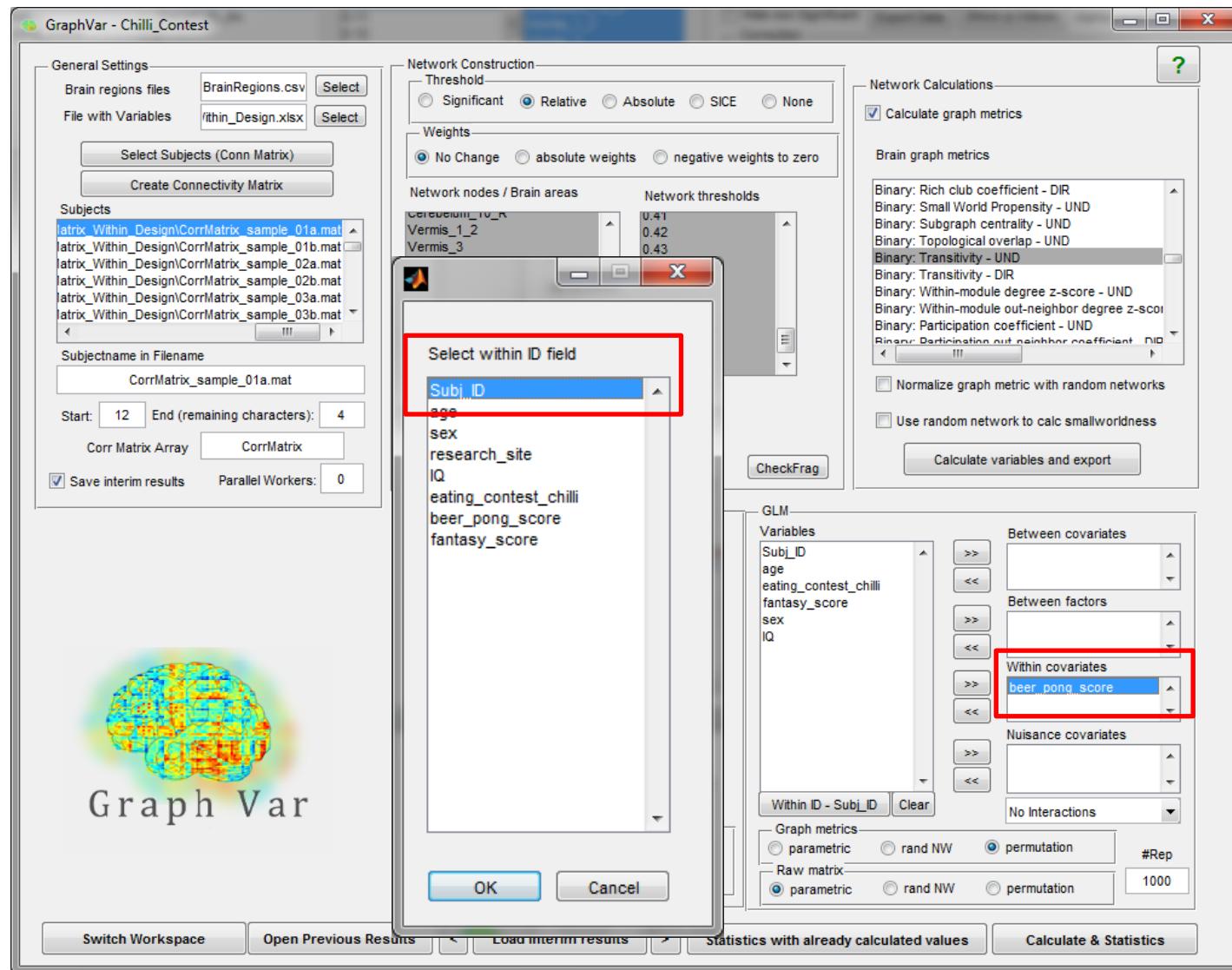
- Navigate to GraphVar/workspaces/SampleWorkspace/data/CorrMatrix_Within_Design
- Select all 15 subjects (i.e., 30 files as 1 subject = T1: sample_01a, T2: sample_01b)



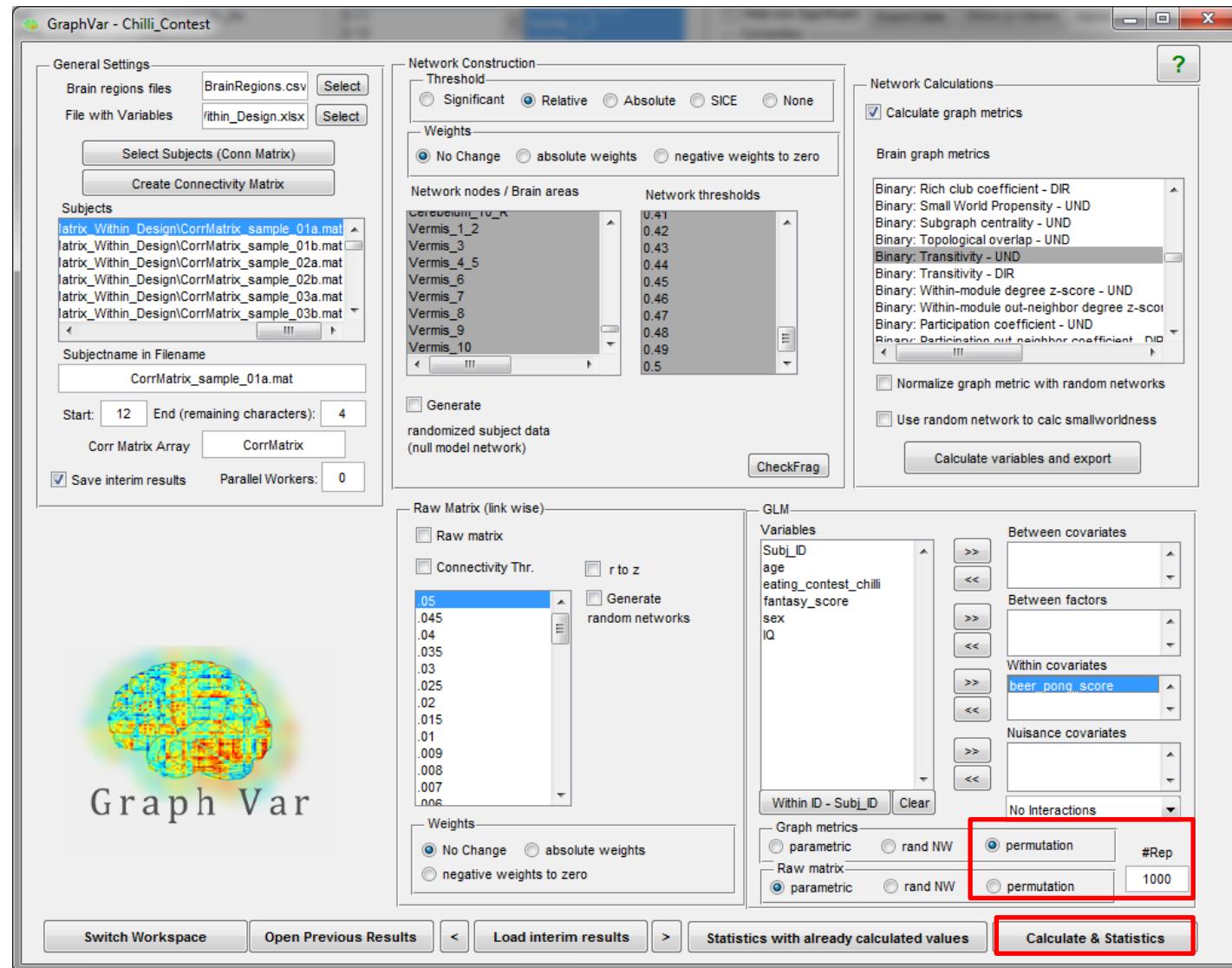
- Highlight the Scan_ID
- Select all nodes, all thresholds, binary „Transitivity“



- 1. GLM: select beer_pong_score as within covariate
- 2. Select „Subj_ID“ as within dividing field in the automatic pop-up window



- Run 1000 permutations
- Calculate & Statistics



- You will observe a significant positive association between change in behavior and a change in transitivity in the low threshold range

