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# Detecting cluster structure of resting state fMRI brain networks of mice


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# Outline

## Data set

- collected by applying fMRI;
- collected by considering resting-state mice brains.

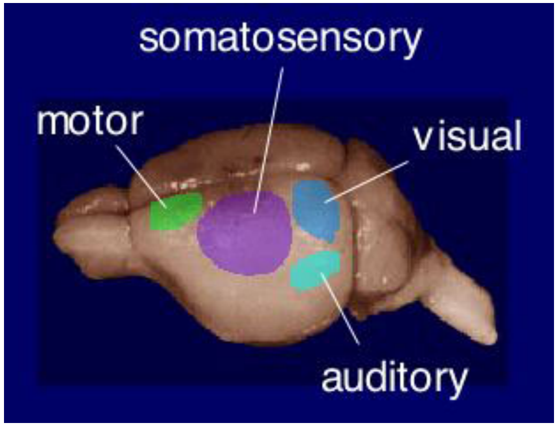
## Our approach (are network theory-based tools useful?)

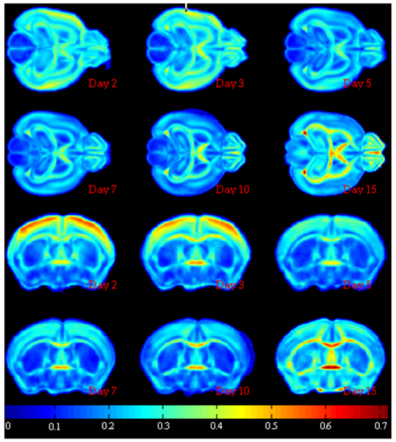
- comparison with null models for. . . ;
- . . . percolation analysis;
- . . . community/modules detection analysis.

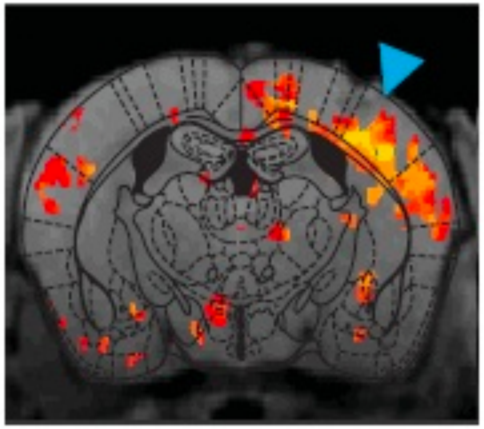
## Results

- modular structure: detectable;
- functional modules: not explained by a null model constraining the correlations distribution;
- better agreement using blockmodels.

# Data set



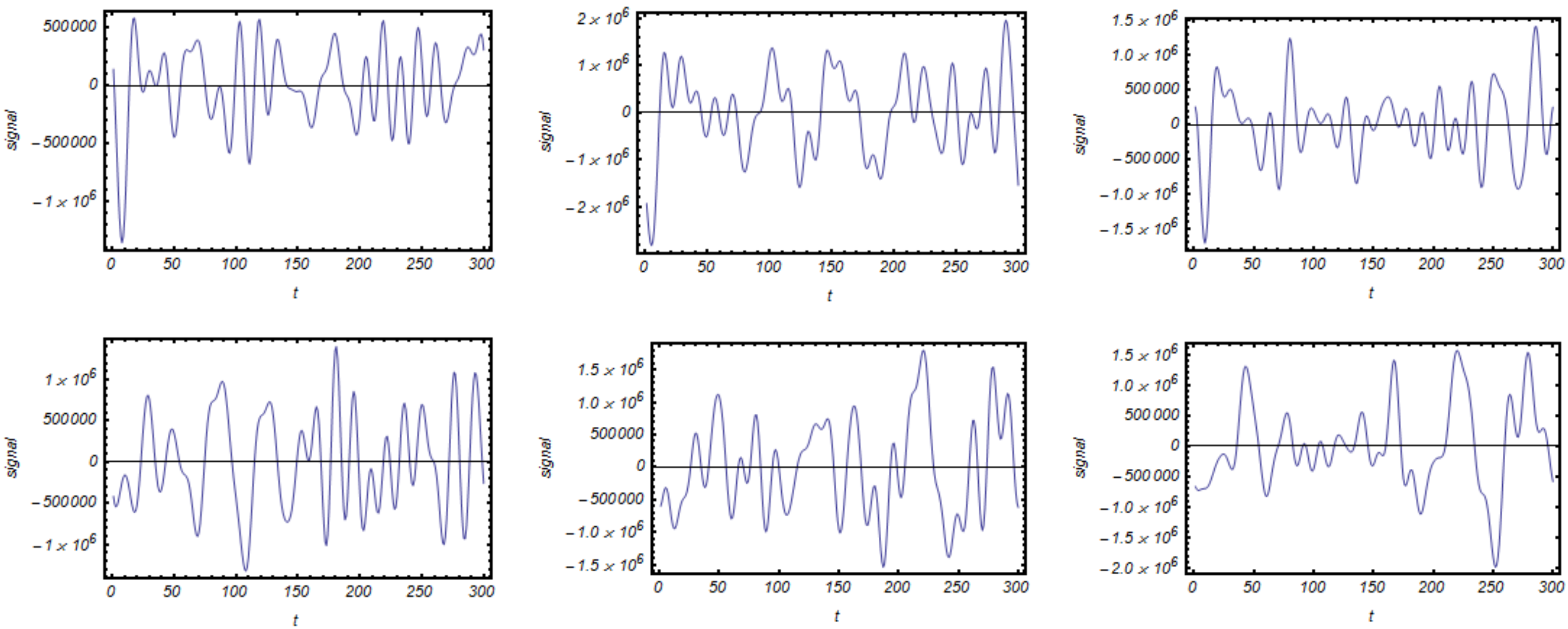


- 41 mice brains [male 20–24 week old C57BL/6J (B6); Charles River, Como, Italy];
- 54 macro-regions (Brodmann's areas) subdivided into left and right part, i.e. 27 regions of interest (ROI) for each hemisphere;
- 1 fMRI time series per region (300 time steps long ~ 300 secs).
- Summing up: 54 time series for each of the 41 mice.

- Mice were anaesthetized with isoflurane (5% induction), intubated and artificially ventilated under 2% isoflurane maintenance anesthesia. All experiments were performed with a 7.0 T MRI scanner (Bruker Biospin, Milan) using an echo planar imaging (EPI) sequence with the following parameters: TR/TE 1200/15 ms, ␣ip angle 30°, matrix 100 × 100, field of view 2 × 2 cm2, 24 coronal slices, slice thickness 0.50 mm, 300 volumes and a total rsfMRI acquisition time of 6 min.

# Data: fMRI time-series at resting condition

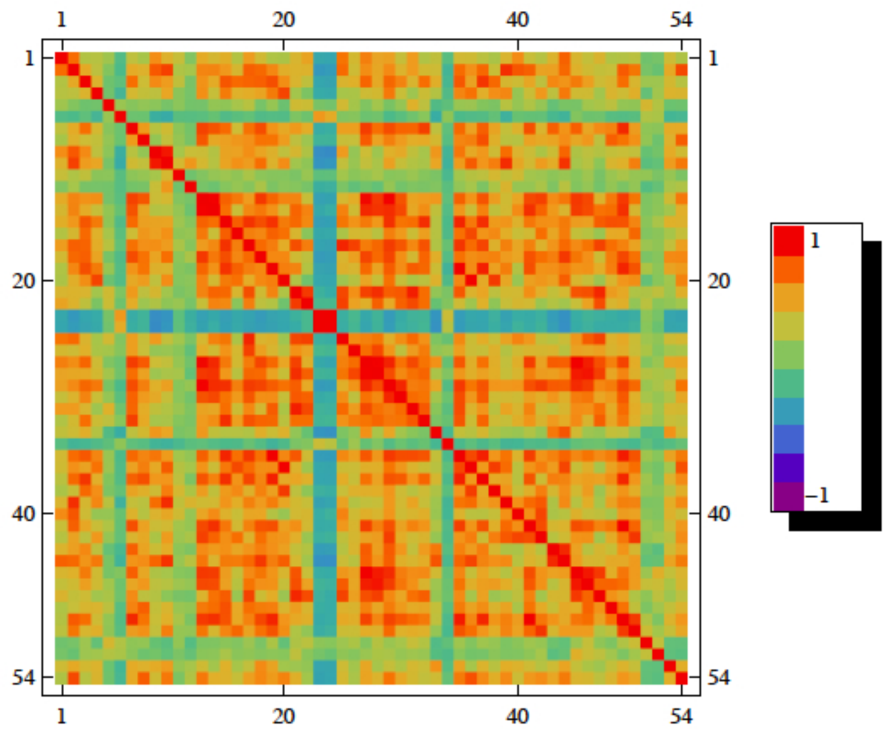
For each region of each mouse a 300 time-steps long BOLD fMRI signal $X^i(t)$ is measured




- accumbens nucleus;
- anterio-dorsal hippocampus;
- amygdala;
- . . .

# Region-region correlation matrix construction (single mouse)

$$\textbf{Pearson coefficient: } C_{ij} = \frac{\mathrm{Cov}[X^i, X^j]}{\sqrt{\mathrm{Var}[X^i] \cdot \mathrm{Var}[X^j]}}$$



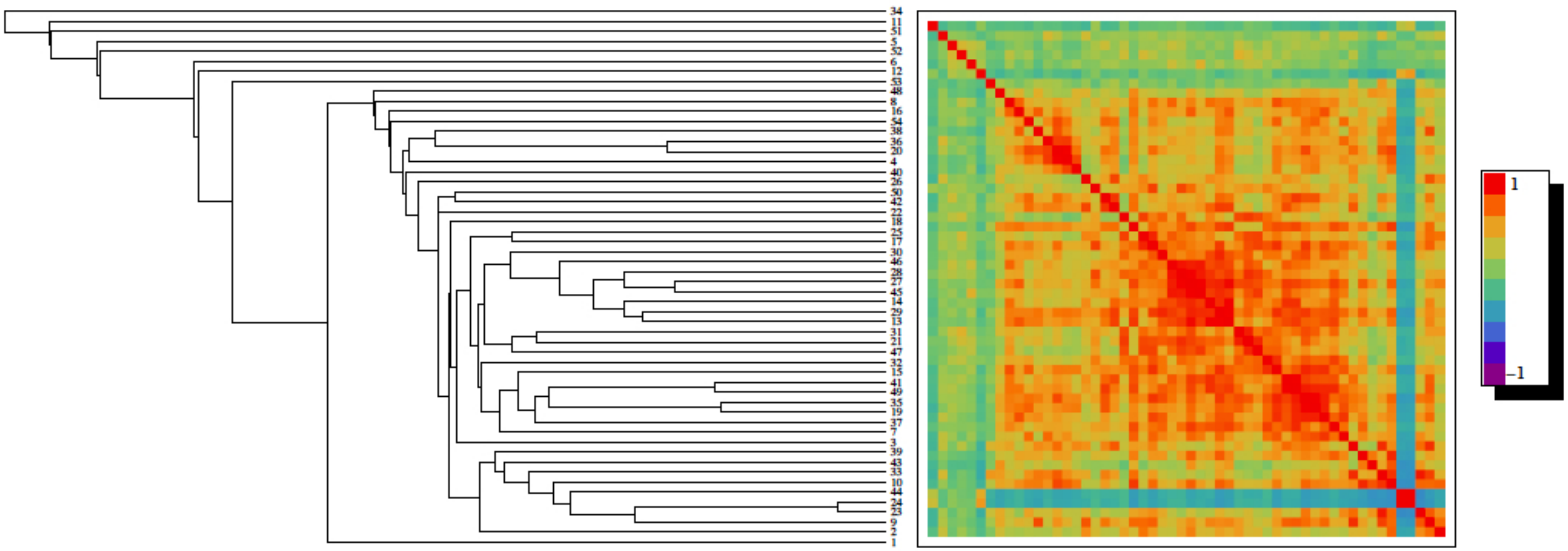

Positive correlations are more numerous than negative correlations and the former are characterized by higher (absolute) values than the latter.

# $1^{st}$ level clustering analysis: dendrogram plot

Usually binarization implies the introduction of ad hoc thresholds: we analyze directly $C_{ij}$

Dendrogram built by a correlations-induced absolute distance

**Dissimilarity:** $D_{ij} = 1 - |C_{ij}|$ (Jaccard distance, attractive anticorr.)




A first hint of modular structure appears as a nested structure

The dendrogram tool make evident a coherent and nested cluster structure

## Example

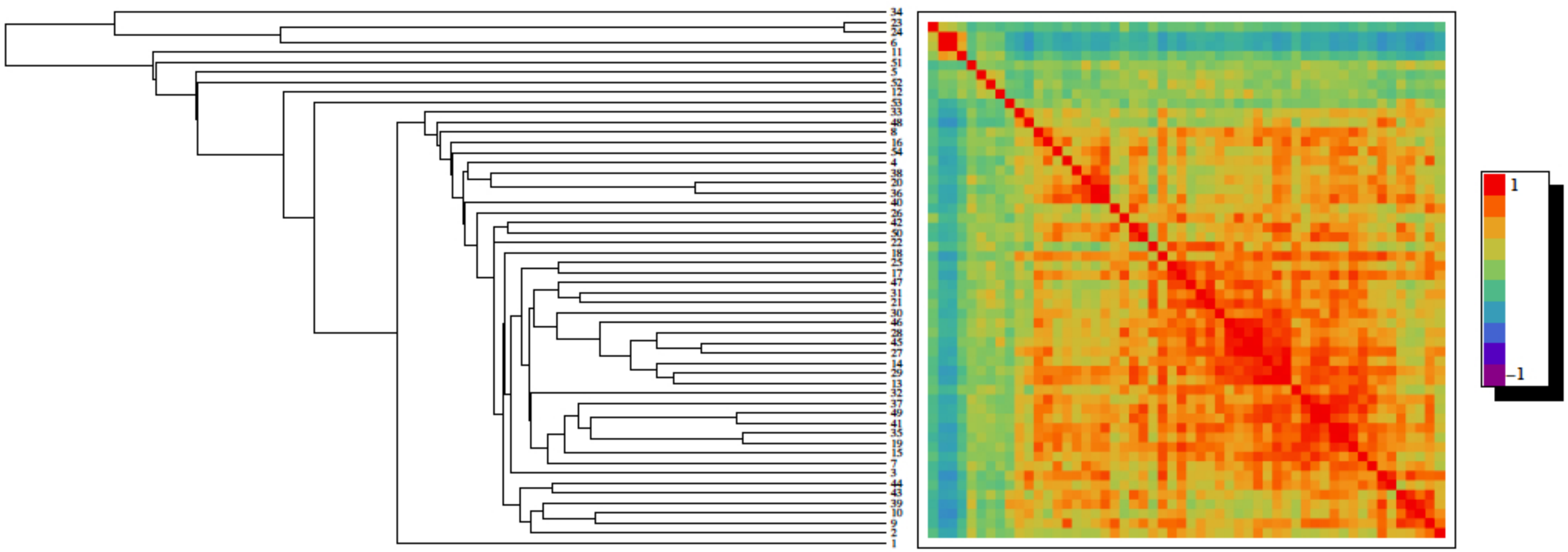
- An example is provided by areas 13, 14, 27, 28, 29, 30, 45 and 46 (i.e. the whole cingulate cortex, the whole motor cortex, the whole medial prefrontal cortex and the whole primary somatosensory cortex, respectively).
- While they give origin to a 8X8 matrix whose average value is approximately 0.85, the two subgroups composed respectively by 13, 14, 27, 29 and 28, 30, 45, 46 constitute two 4X4 sub-matrices whose average value is around 0.95.
- This can be rephrased by saying that, within the same group of areas responding coherently to some stimulus, there exist subgroups responding maximally coherently, thus identifying functionally correlated brain modules.

The modular structure of the brain clearly appears as a nested structure of highly correlated areas, the latter emerging as sub-matrices of smaller size characterized by higher correlations values than the background

We can increase information on hierarchical clustering by introducing a dissimilarity induced by correlation with sign (repulsive anti-correlations)

Dendrogram built by a correlations-induced distance

**Dissimilarity:** $D_{ij} = 1 - C_{ij}$



- Retaining the information on the correlations sign allows one to clearly distinguish positively correlated groups of areas from the negatively correlated ones, thus improving the detection of brain modules.

## Example

- Areas 6, 23 and 24 (i.e. the left part of amygdala and the whole hypothalamus, respectively) are recognized as forming a group of highly positively correlated areas, interacting with the rest of the brain via quite large negative correlations: this suggests that they should be considered as (part of) a separate module.

Non-trivial to observe a hierarchical structure in less evolved animals (e.g. in C. elegans non sign of hierachical organization of modules)

# Modularity approach

[See M. MacMahon, D. Garlaschelli, Phys. Rev. X **5**, 021006 (2015).
M. E. J.Newman, PNAS, **103**, 8577 (2006)]

Newman modularity with corrected null model for correlation networks (Louvain's detection algorithm)

Only three modules detected

1. Sensory macro-region
   - 7-Au: auditory cortex_dx;
   - 8-Au: auditory cortex_sx;
   - 11-BNST: bed nucleus of stria terminals_dx;
   - 13-Cg: cingulate cortex_dx;
   - 14-Cg: cingulate cortex_sx;
   - 27-M: motor cortex_dx;
   - 28-M: motor cortex_sx;
   - 29-mPFC: medial prefrontal cortex_dx;
   - 30-mPFC: medial prefrontal cortex_sx;
   - 33-Parietal_Ass: parietal association cortex_dx;
   - 34-Parietal_Ass: parietal association cortex_sx;
   - 39-Pir: piriform cortex_dx;
   - 41-Rhinal: rhinal cortex_dx;
   - 42-Rhinal: rhinal cortex_sx;
   - 45-S1: primary somatosensory cortex_dx;
   - 46-S1: primary somatosensory cortex_sx;
   - 47-S2: secondary somatosensory cortex_dx;
   - 48-S2: secondary somatosensory cortex_sx;
   - 49-TeA: temporal association cortex_dx;
   - 50-TeA: temporal association cortex_sx;
   - 53-Vctx: visual cortex_dx;
2. Sensory integration macro-region
   - 1-Acb: accumbens Nucleus_dx;
   - 2-Acb: accumbens Nucleus_sx;
   - 10-BF: basal forebrain_sx;
   - 12-BNST: bed nucleus of stria terminals_sx;
   - 17-Cpu: caudate putamen_dx;
   - 18-Cpu: caudate putamen_sx;
   - 21-FrA: frontal association cortex_dx;
   - 22-FrA: frontal association cortex_sx;
   - 23-Hypo: hypothalamus_dx;
   - 24-Hypo: hypothalamus_sx;
   - 26-Ins: insular cortex_sx;
   - 31-OFC: orbitofrontal cortex_dx;
   - 32-OFC: orbitofrontal cortex_sx;
   - 44-RS: retrosplenial cortex_sx;
   - 51-Th: thalamus_dx;
   - 52-Th: thalamus_sx;
   - 54-Vctx: visual cortex_sx;
3. Limbic system
   - 3-AdHC: anterio-dorsal hippocampus_dx;
   - 4-AdHC: anterio-dorsal hippocampus_sx;
   - 5-Amy: amygdala_dx;
   - 6-Amy: amygdala_sx;
   - 9-BF: basal forebrain_dx;
   - 15-Collicoli: collicoli_dx;
   - 16-Collicoli: collicoli_sx;
   - 19-DG: dentate gyrus_dx;
   - 20-DG: dentate gyrus_sx;
   - 25-Ins: insular cortex_dx;
   - 35-pDG: posterior dentate gyrus_dx;
   - 36-pDG: posterior dentate gyrus_sx;
   - 37-pHC: posterio-ventral hippocampus_dx;
   - 38-pHC: posterio-ventral hippocampus_sx;
   - 40-Pir: piriform cortex_sx;
   - 43-RS: retrosplenial cortex_dx.

# Standard Percolation approach

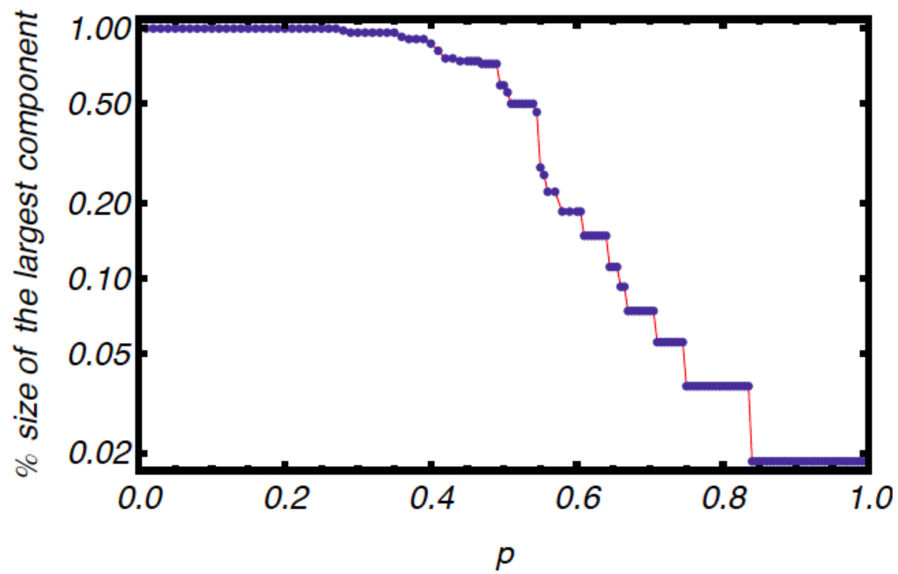
[see Gallos et al., PNAS **109**, 2825 (2012) for “standard” perc. under training conditions]

- The abs values of the measured correlations are listed in increasing order;
- starting from the lowest one, each of them is chosen as a threshold;
- the links corresponding to the correlations below the threshold are removed;
- the size of the giant (largest) component is measured at each step.

In Gallos et al. on human brain at voxel level under strong external audio-visual stimuli a step-wise behavior is observed suggesting a hierarchical multiple transition behavior

Very different from Erdos Renyi networks: STEPS!

- We observe a similar behavior in our case
- The coarse-grained nature of ROI permits multiple transition detection also at resting condition



# Our (variation of standard) percolation analysis

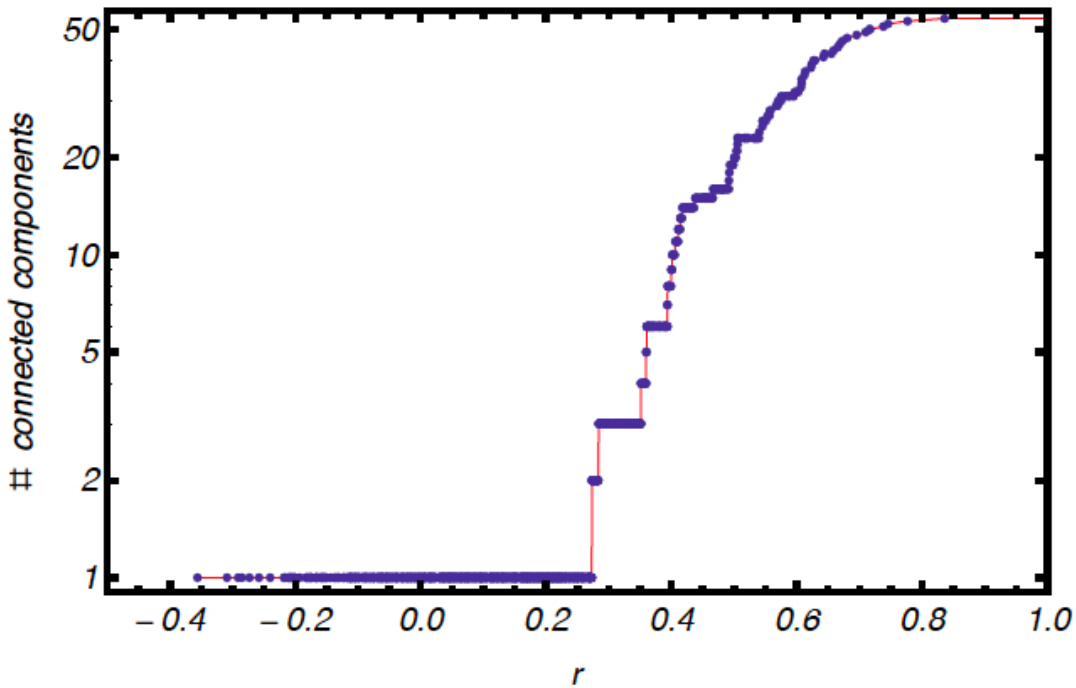
Better to detect the hierarchical organization of clusters/modules
In general better for small networks where giant component can be problematic

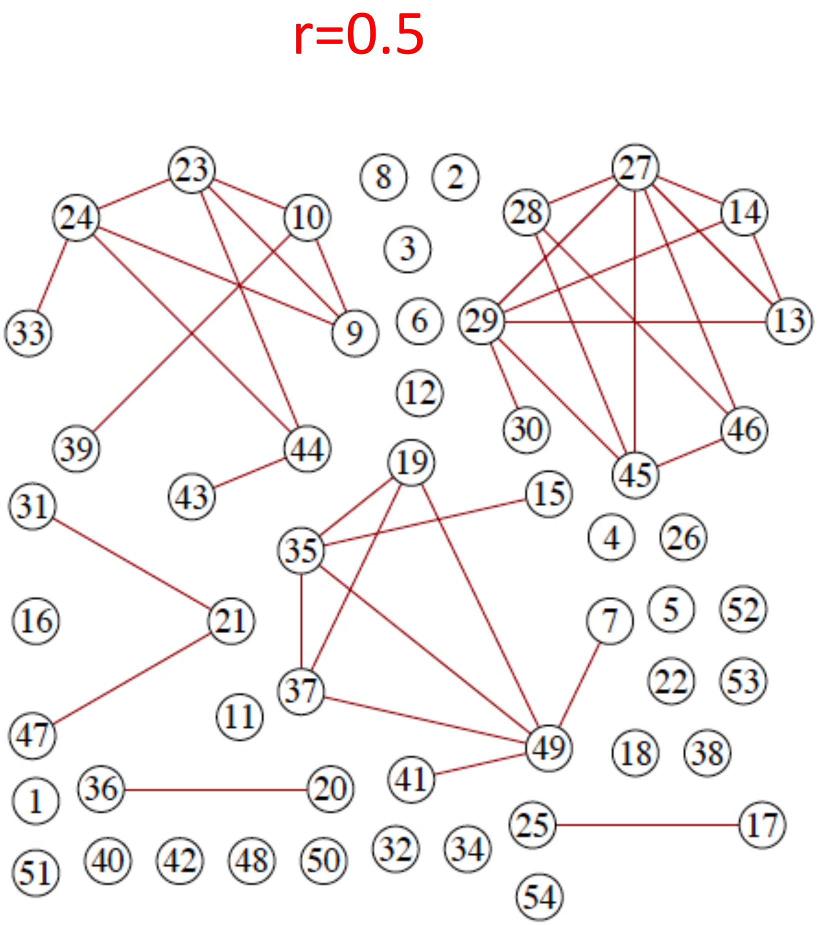
- the measured correlations values *r* are listed in increasing order;
- starting from the lowest one, each of them is chosen as a threshold;
- the links corresponding to the correlations below the threshold are removed;
- the number of connected components is computed.

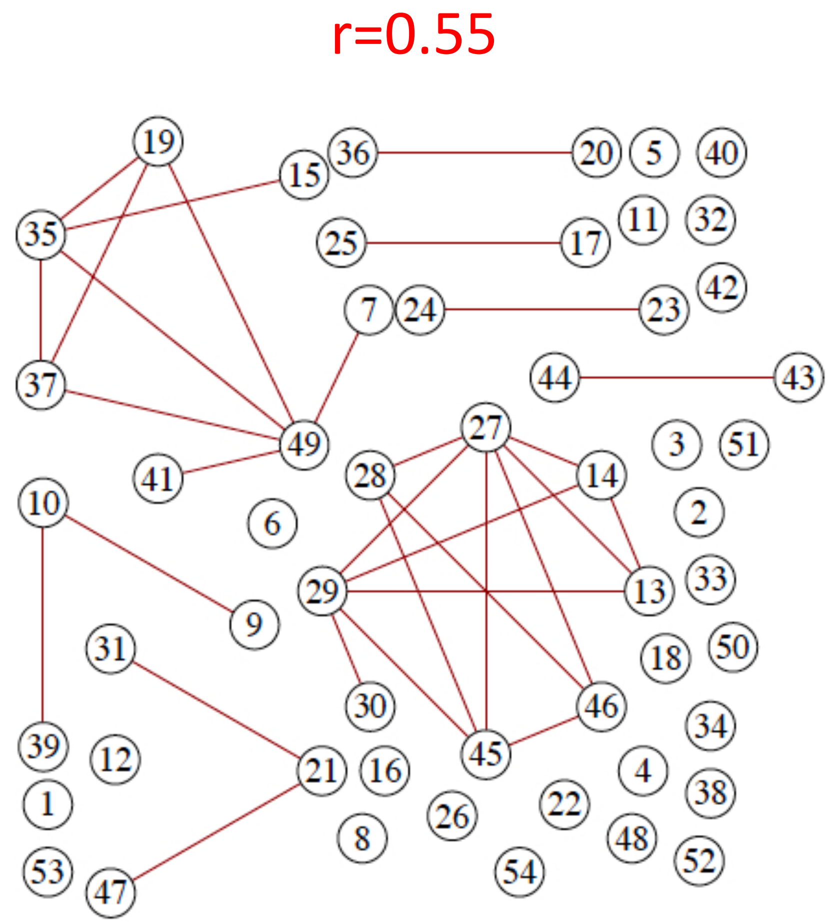
Step-like behavior: at a step the number of connected components do not increase by increasing *r*

→ *The connected components are "robust" and may indicate neurophysiological modules/ clusters (to be validated)*

**Multiple steps feature**
→ *Hierarchical organization of such modules/clusters*






One can check that the use of absolute values of $C_{ij}$ leads to spurious clusters

# Minimal Spanning Forest

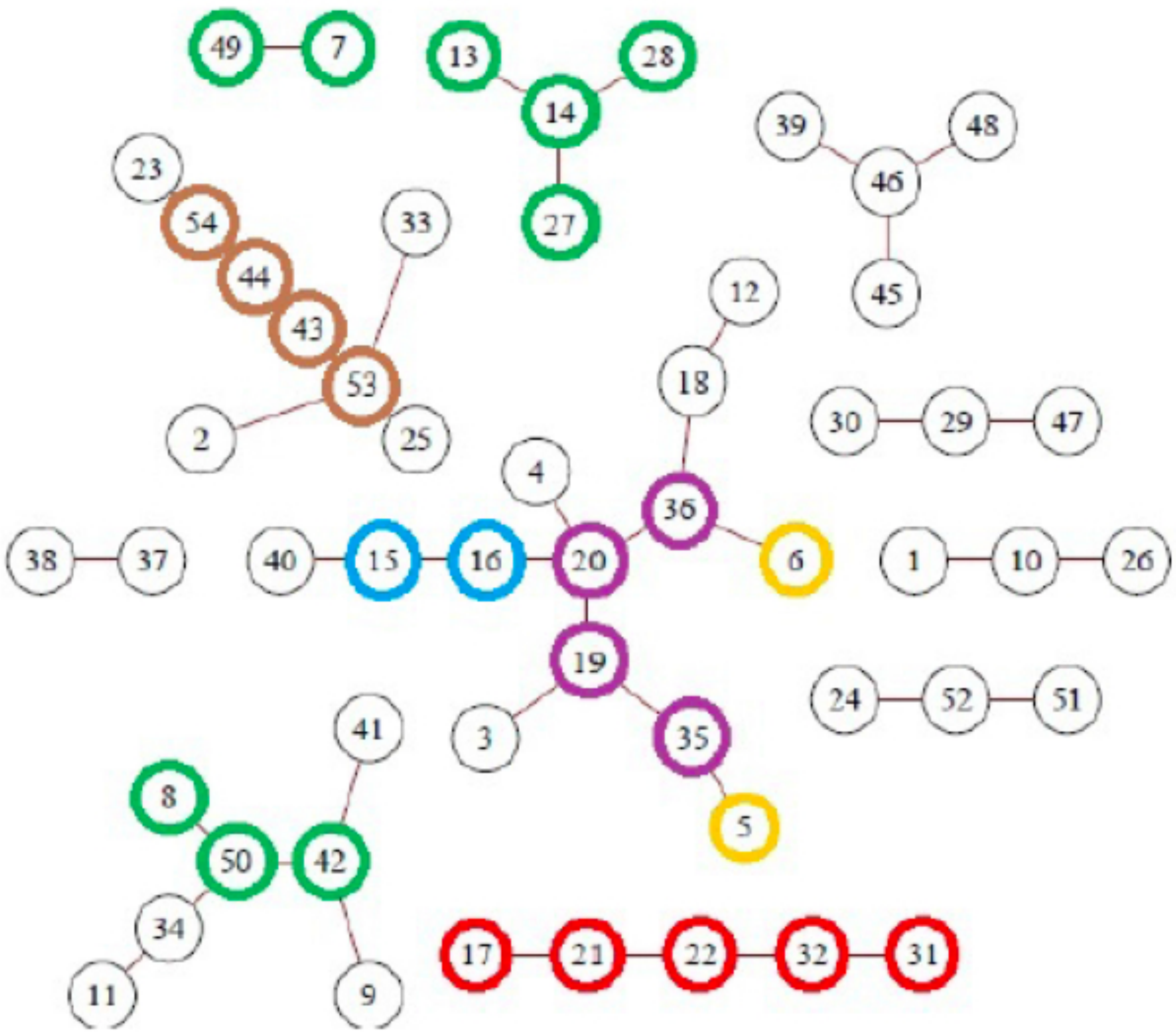
First step of Kruskal's algorithm for the Minimal Spanning Tree

- Region pair *(i,j)* are ordered in decreasing $C_{ij}$
- One starts by drawing a graph from the pair with the highest $C_{ij}$
- One add consecutively other pairs following the order of decreasing $C_{ij}$
- A pair is not added to the graph is the two nodes already appear in the graph

In this way a set of disconnected trees maximally correlated is obtained

It correspond to the Minimal Spanning Tree by removing the "weak" links connecing clusters more strongly connected

# Typical MSF partition in a single mouse



# Which areas are the most important?

- the *right caudate putamen* (i.e. **17**), the *frontal association cortex* (i.e. **21**, **22**), the *orbitofrontal cortex* (i.e. **31**, **32**) frequently recovered for the mice in our sample;
- the *auditory cortex* (i.e. **7**, **8**), the *left rhinal cortex* (i.e. **42**) and the *temporal association cortex* (i.e. **49**, **50**), often found to be linked via the pair **7-49** and the triple **8-42-50**;
- the *cingulate cortex*, the *motor cortex*, the *medial prefrontal cortex*, the *parietal association cortex* (i.e. **13**, **14**, **27**, **28**, **29**, **30**, **33**, **34**): believed to form the *default mode network*;
- the *gyrus area* (dentate and posterior - i.e. **19**, **20**, **35**, **36**) often found to be linked via the pairs **19-35** and **20-36**;
- the *retrosplenial cortex* (i.e. **43**, **44**) and the *visual cortex* (i.e. **53**, **54**) often found to be linked together;
- the *collicoli areas* (i.e. **15**, **16**) often found to be linked together;
- the *amygdala areas* (i.e. **5**, **6**) never found to be linked together.

# Comparison with the Modularity analysis

**Sensory macro-region**

- *Auditory cortex*;
- *motor cortex*;
- *rhinal cortex*;

...

**Sensory integration macro-region**

- *Frontal association cortex*;
- *orbitofrontal cortex*;

...

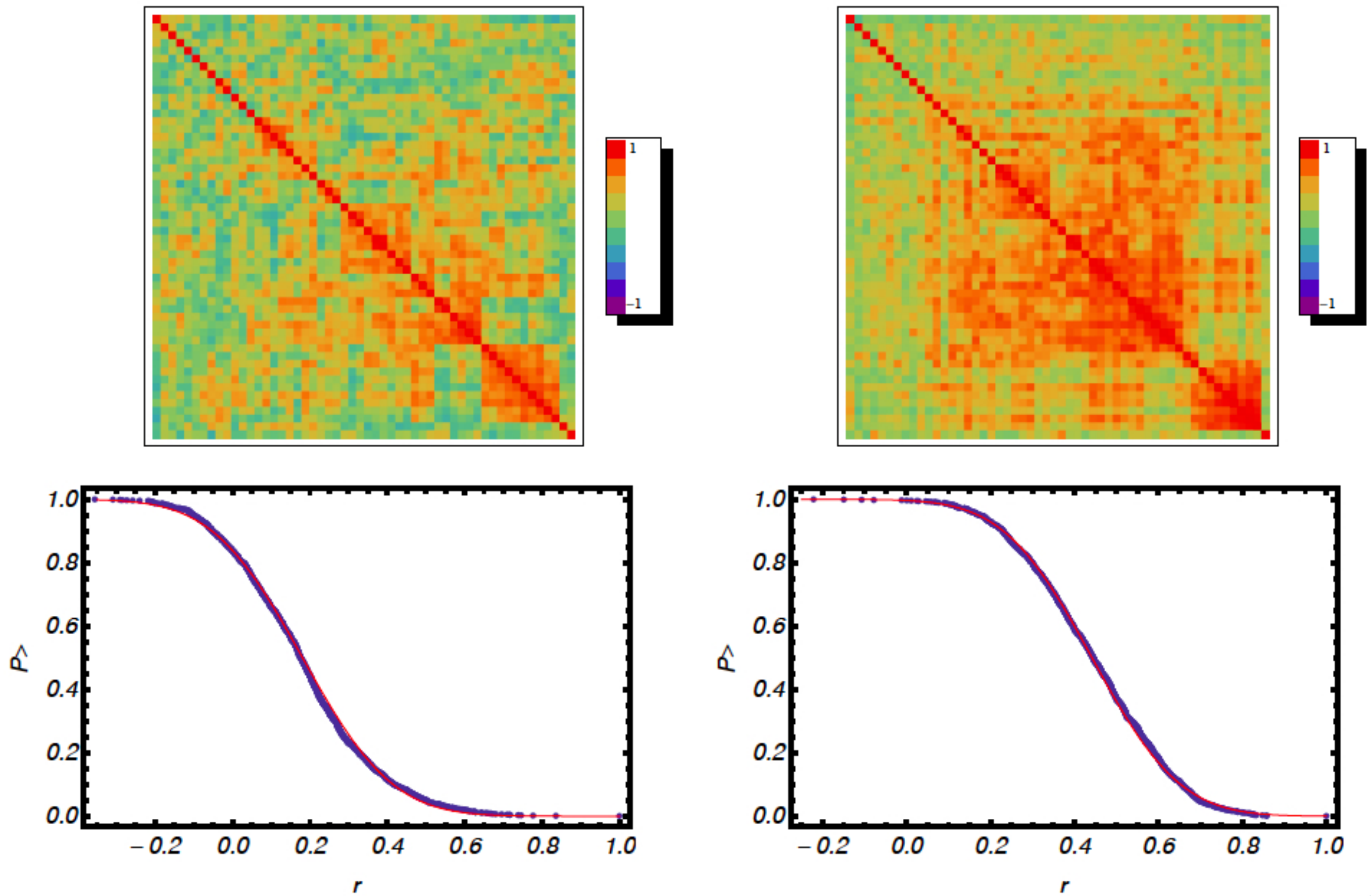
**Limbic system**

- *Amygdala*;
- *dentate gyrus* and *posterior dentate gyrus*;
- *collicoli*;

...

# Validation of results vs "randomized" samples

Our real correlation matrices present a quasi-Gaussian distribution of entries



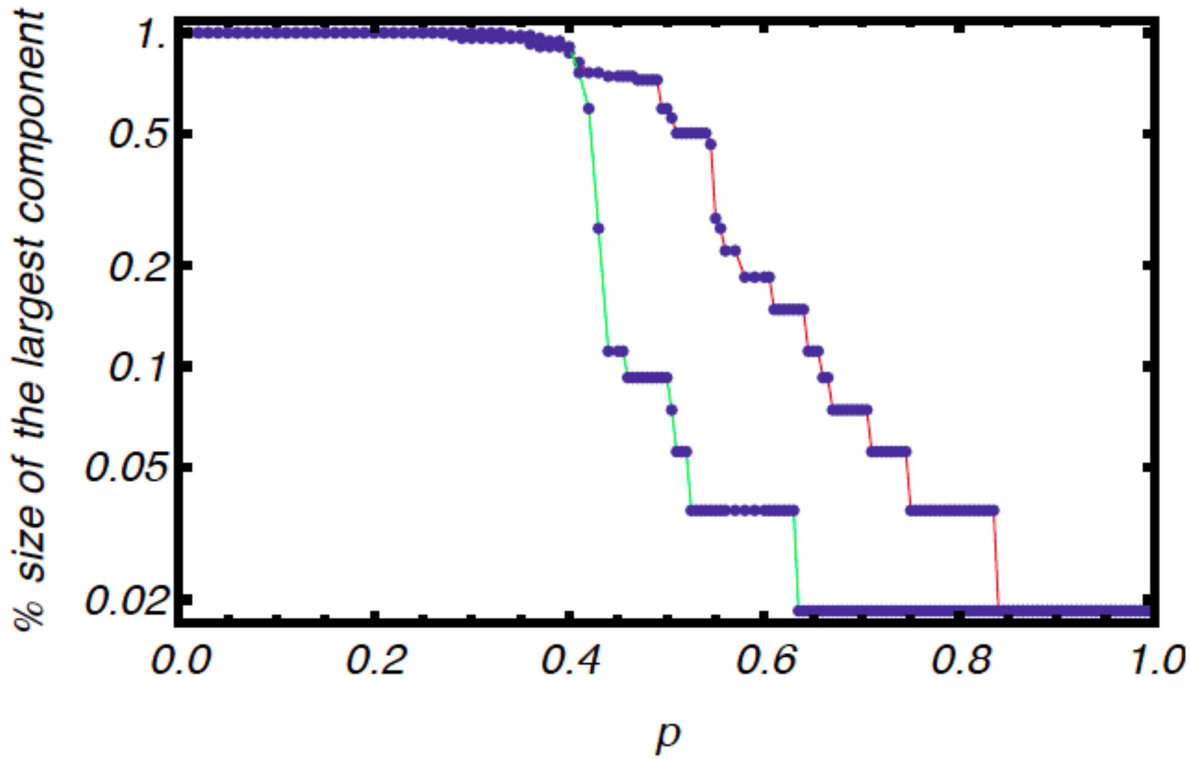
# Generating a "synthetic" randomized brain

- draw the pairwise correlations from the chosen gaussian distribution. . .
- . . . in such a way to create a **symmetric** matrix. . .
- . . . with 54 "**one**" on the diagonal;
- process this matrix according to the paper:

  N. J. Higham, *Computing the nearest correlation matrix - |a problem from finance*, IMA Journal of Numerical Analysis **22**, 329-343 (2002);
- repeat the percolation analysis.

# "Usual" percolation keeps the step-wise feature

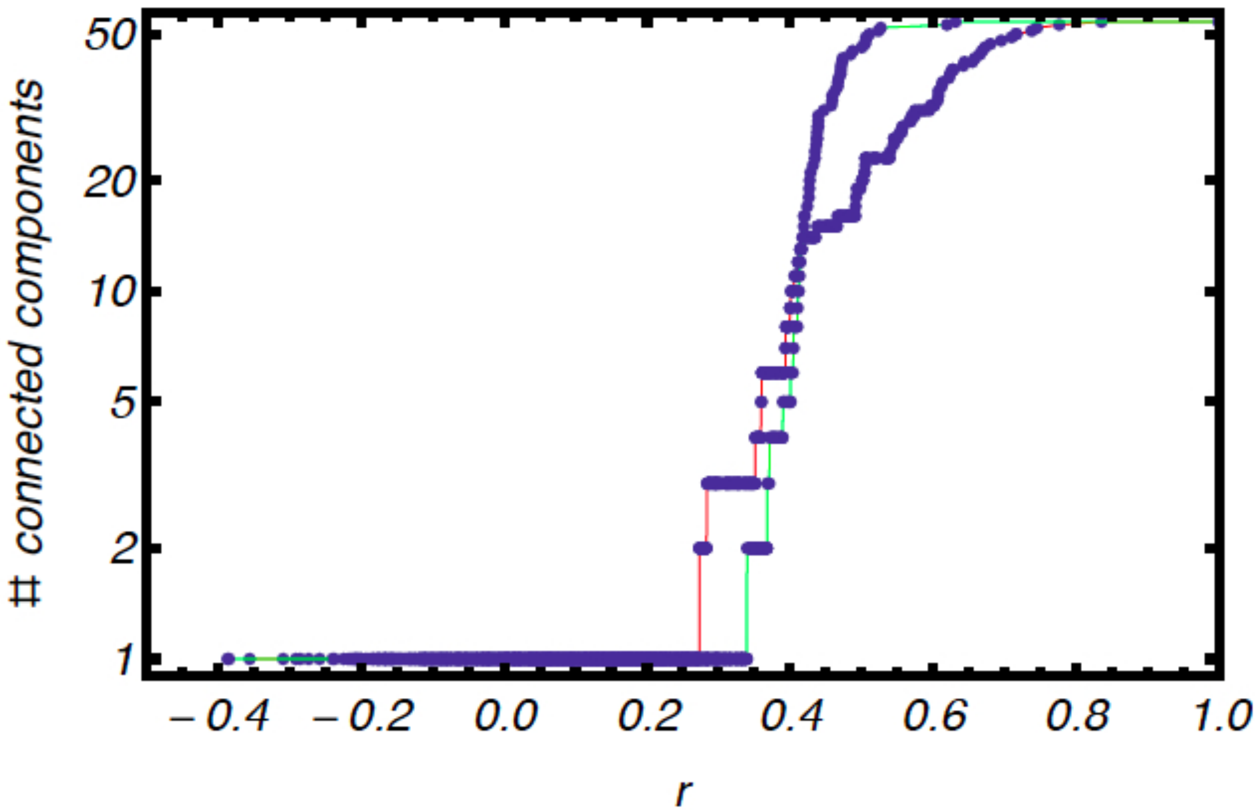
Our null model does not reproduce the percolation trend.




<u>But it is still step-wise!</u>

# Validation of percolation results vs null model

Our null model does not reproduce the percolation trend.



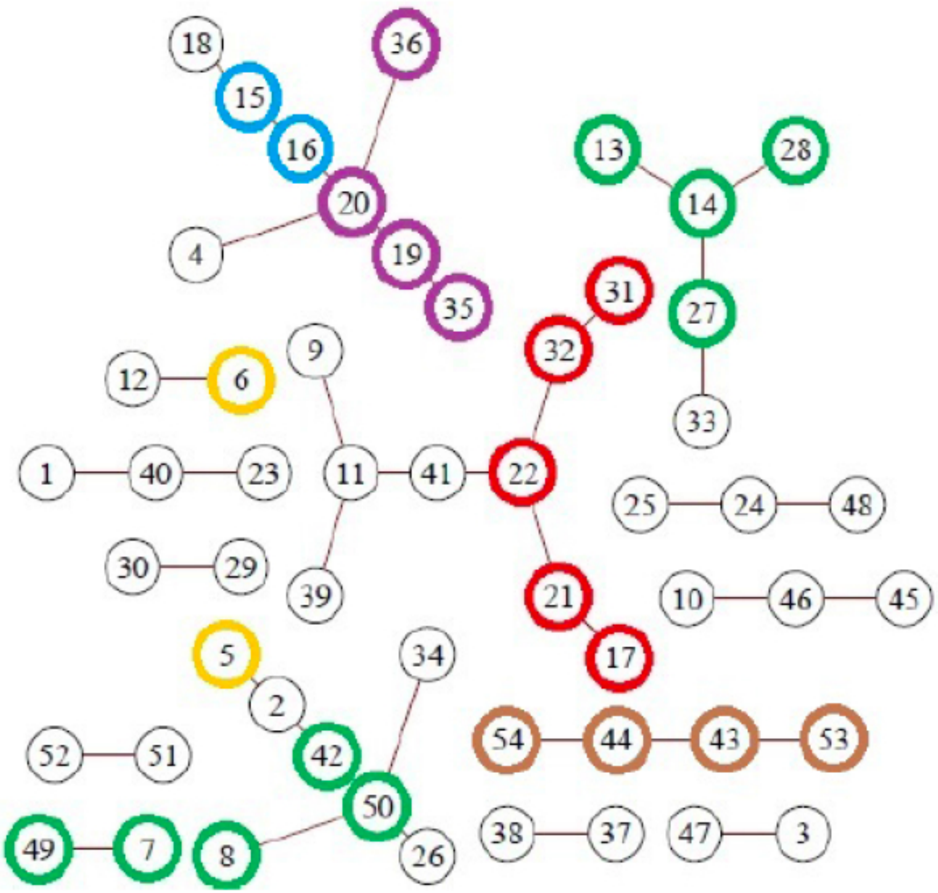

Stepwise trend: **genuine sign of self-organization!**

# Statistical validation of the MSF

- draw the pairwise correlations from the chosen gaussian distribution;
- generate 1000 synthetic brains;
- compute the ensemble distribution of each pair of areas;
- perform a pair-specific one-tailed test;
- compute the **statistically validated** MSF.

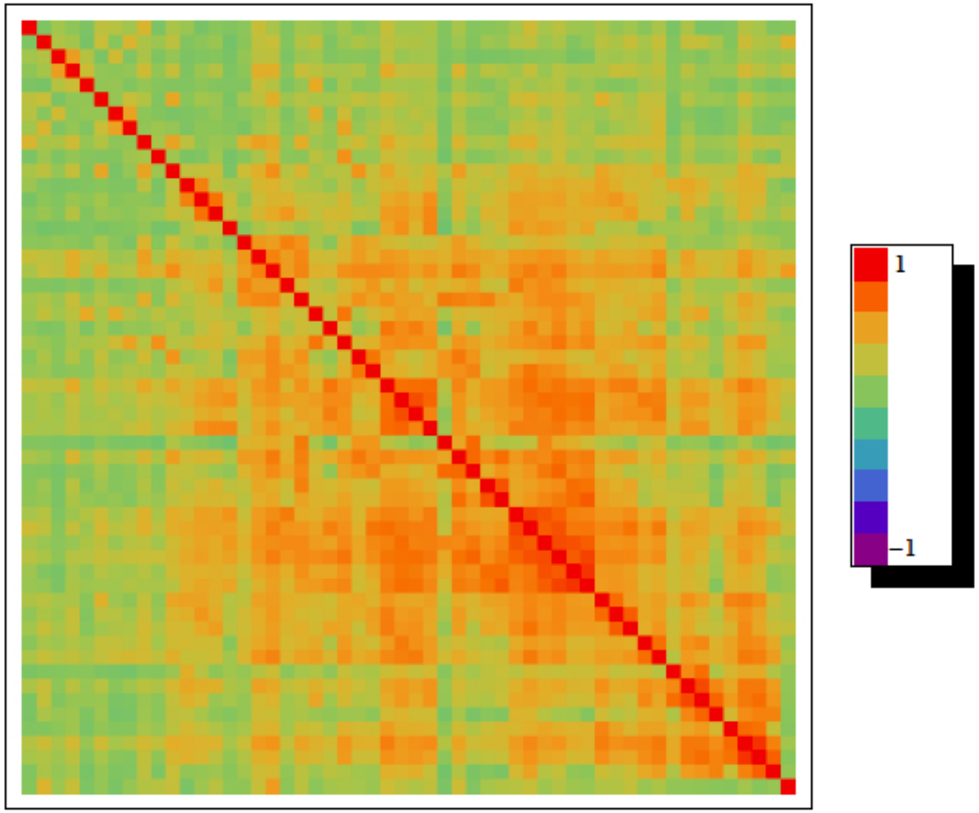
# Validated MSF clusters

Link-specific statistical significance? One-tailed test (95% CL)

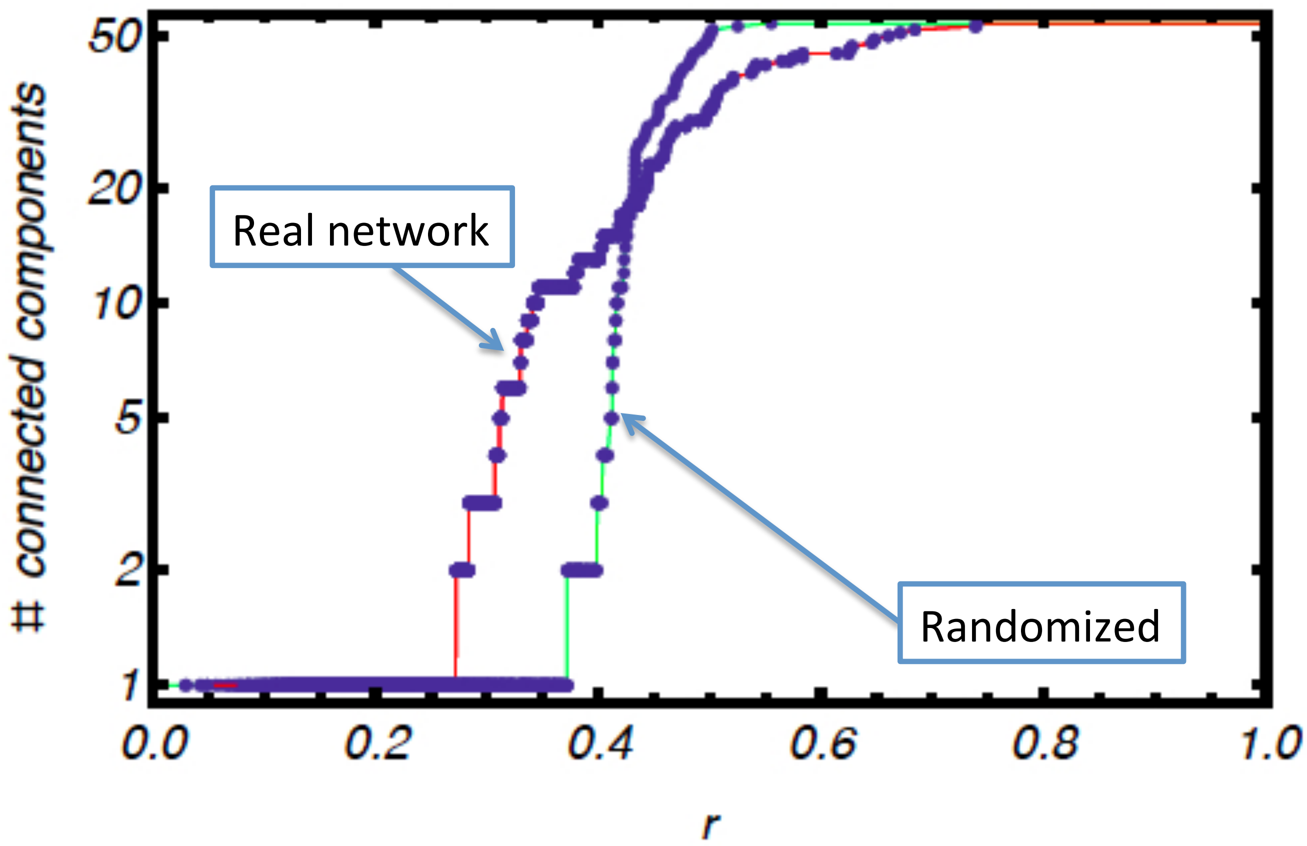


# What about the "average mouse"?

Average correlation matrix $\overline{C}_{ij}$: mean of the correlation matrices



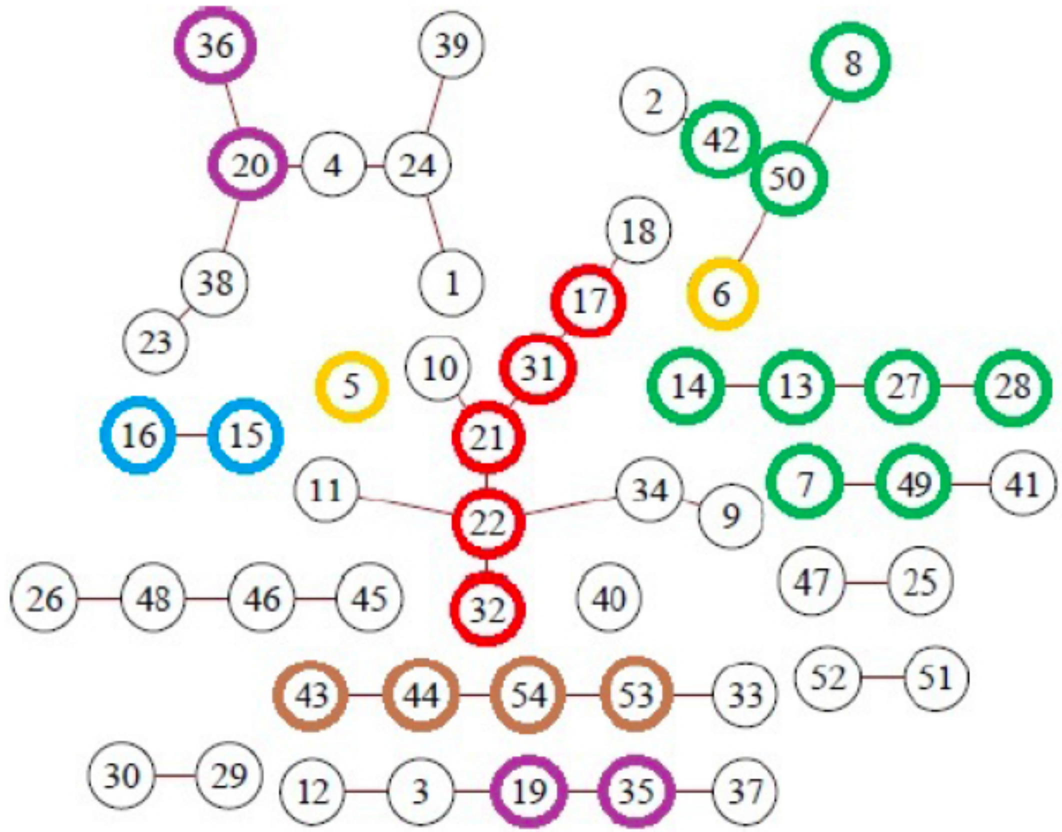
# Percolation on the "average mouse"




Stepwise trend: species-specific genuine sign of self-organization!

# Clusters of the "average mouse"

Link-specific statistical significance? One-tailed test (95% CL)



- Group of areas previously identified with (part of) the limbic system (i.e. areas 19, 20, 35 and 36 - the right dentate gyrus and the right posterior gyrus) is observed again and, further rising the threshold, the “core pairs” 19-35 and 20-36 are recovered.

- Moreover, areas like the auditory (i.e. 7, 8) and the temporal association cortex (i.e. 49, 50), are found to be linked via the double pair 7-49 and 8-50 (i.e. the left parts and the right parts separately)

# Conclusions

- network theory-based analysis (percolation, modules detection);
- definition of null models to detect statistically significant signals of self-organization (modular structure);
- correlations are normally distributed at different scales;
- constraining the whole correlations distribution is not enough to explain the nested structure of real mice brains;
- the block-model \philosophy" can be exported to analyse correlations matrices, given the normal nested structure of correlations matrices;
- much better results are obtained when constraining the blocks-specific normal distributions.

Thank you!!

Villa (Curzio) Malaparte: Masterpiece of Adalberto Libera