Investigating the connectome of a larval *Drosophila* brain

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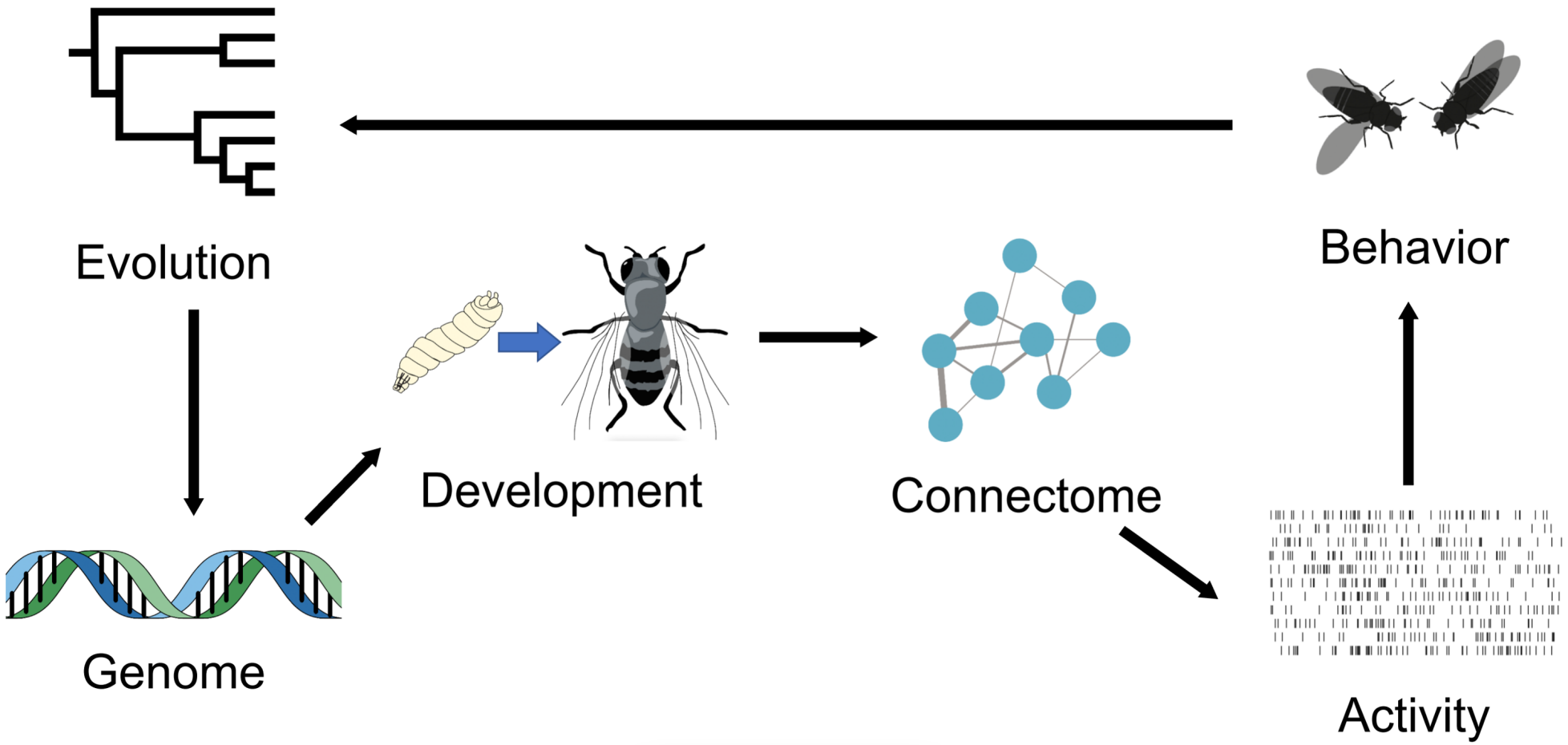
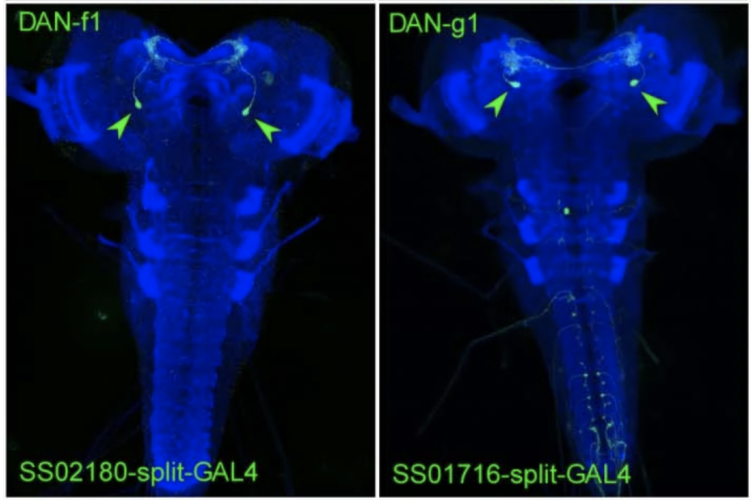
These slides at:

Many goals of connectomics involve linking the connectome to other properties



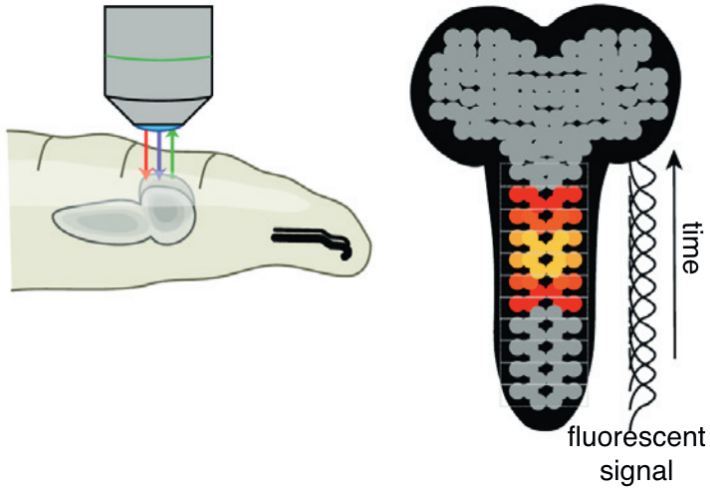
Larval *Drosophila* allows access to many properties, e.g.,

Genetics



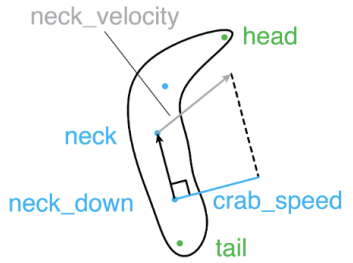
Eschbach et al. Nat. Neuro (2020)

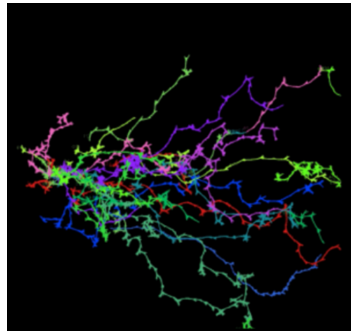
Activity



Eschbach & Zlatic Curr. Op. Neurobio. (2020)

Behavior



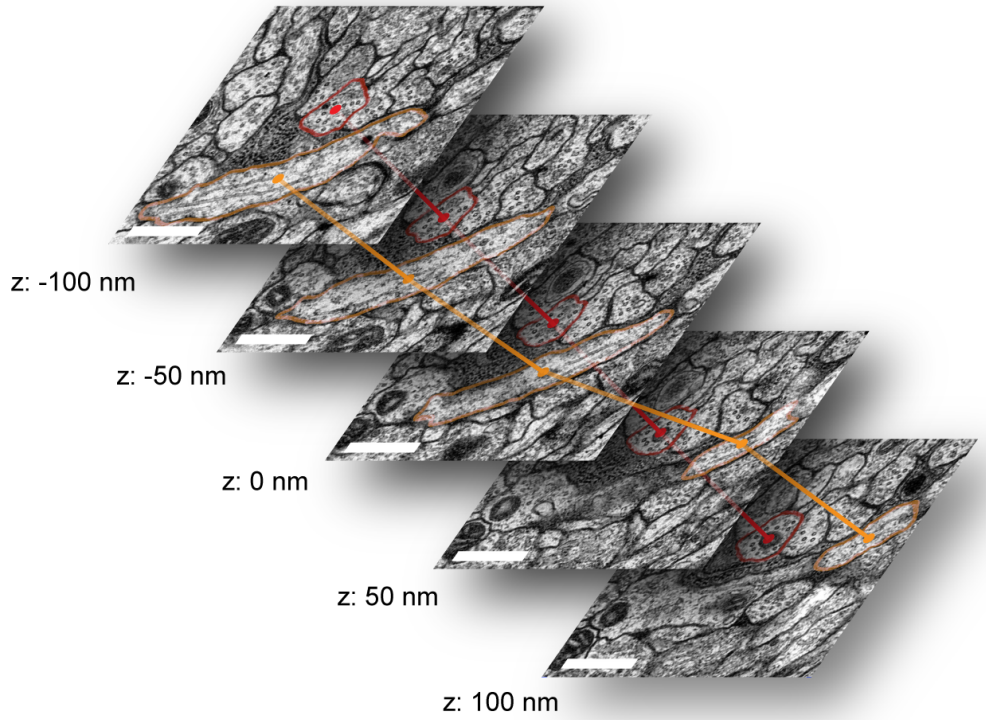


Klein et al. bioRxiv (2021)

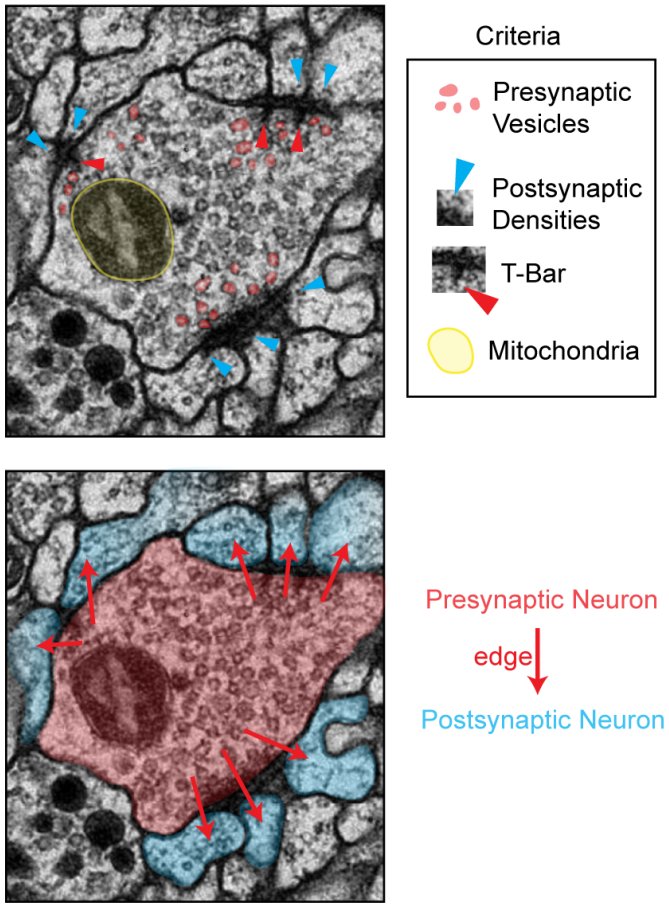
Almeida-Carvalho et al. J. Experimental Bio. (2017)

Mapping a larval *Drosophila* brain connectome

Manual Reconstruction of Arbors

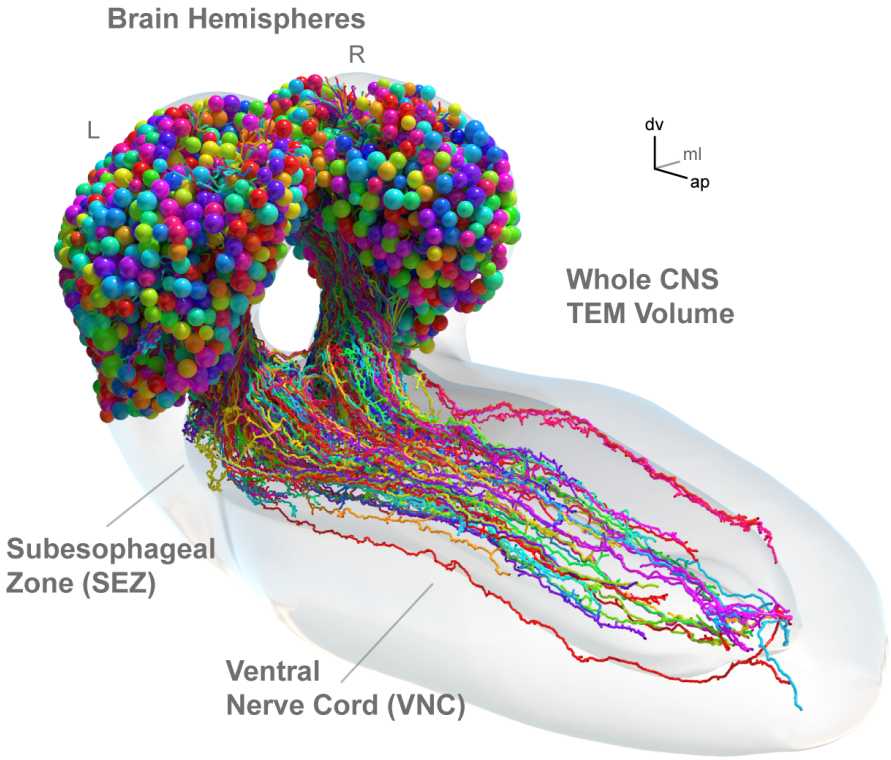


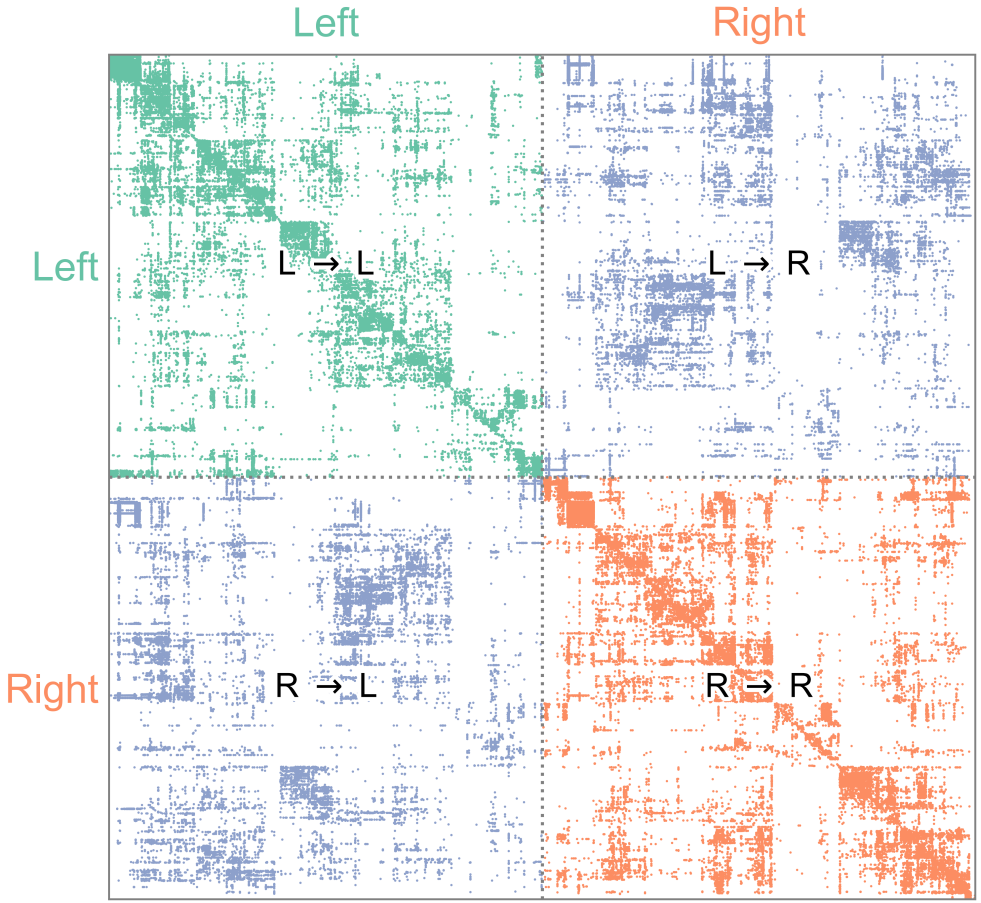
Synapse Annotation



Winding, Pedigo et al. bioRxiv (2022), Ohyama et al. Nature (2015)

Larval *Drosophila* brain connectome





~3k neurons, ~550K synaptic sites

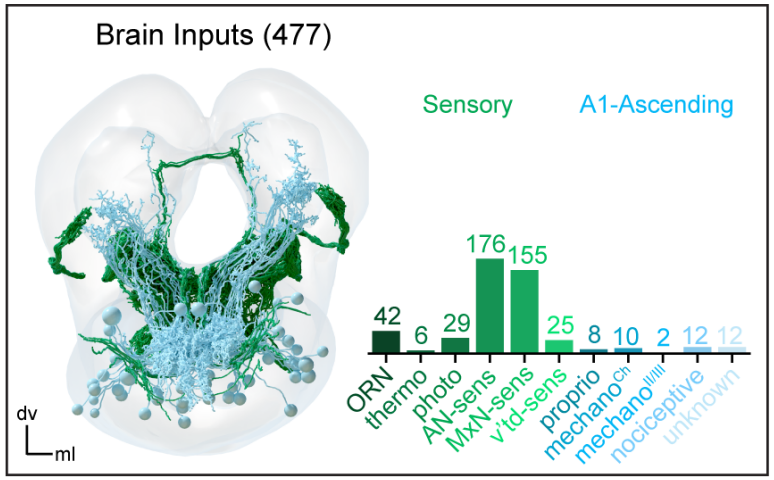
Both hemispheres

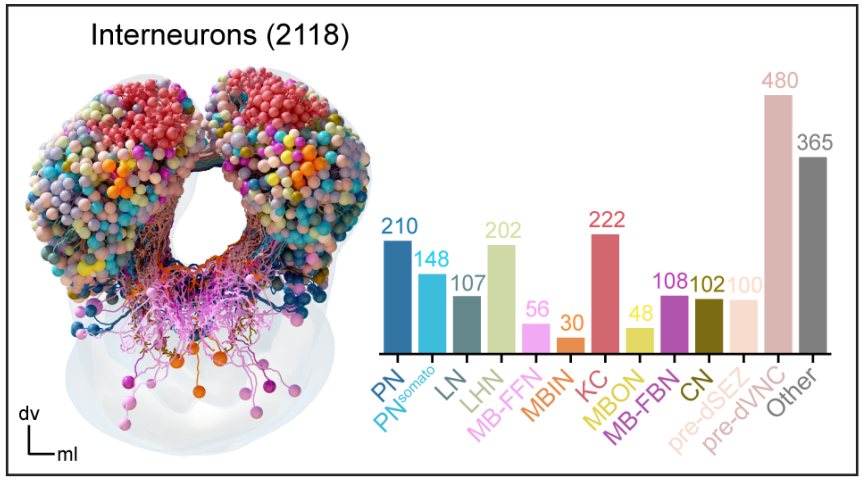
Winding, Pedigo et al. bioRxiv (2022)

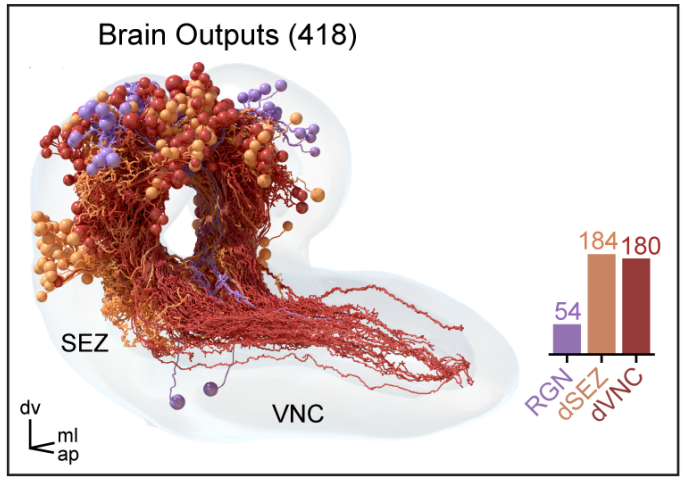
Outline

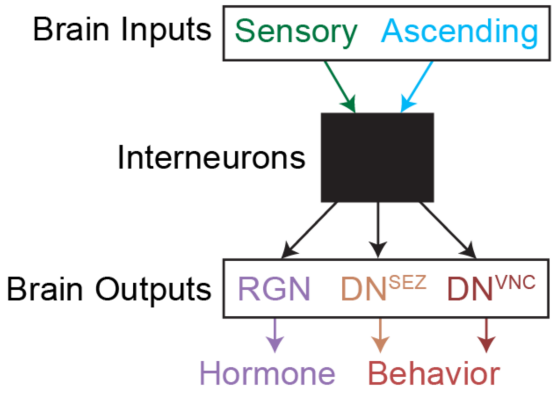
- Larval connectome dataset
  - Flow and edge types
  - Connectivity-based cell types
- **Connectome comparison via network hypothesis testing**
- **Pairing neurons across connectomes via graph matching**
- **Ongoing extensions/applications**

High level (mostly anatomical) cell types



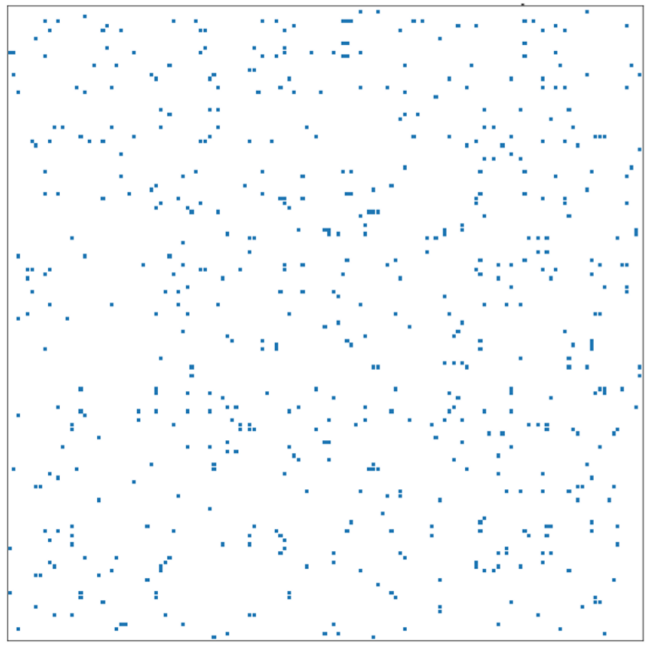






Sorting the network

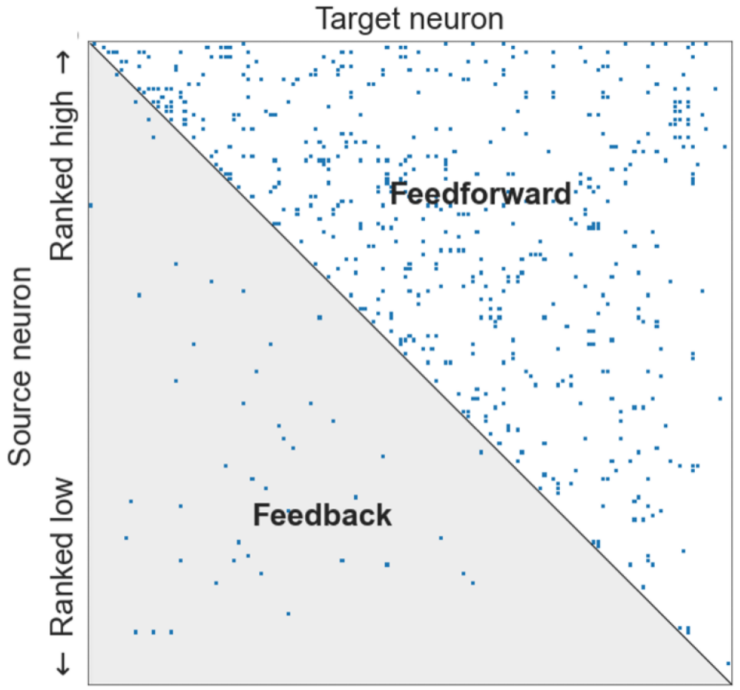
Adjacency matrix



Flow ordering

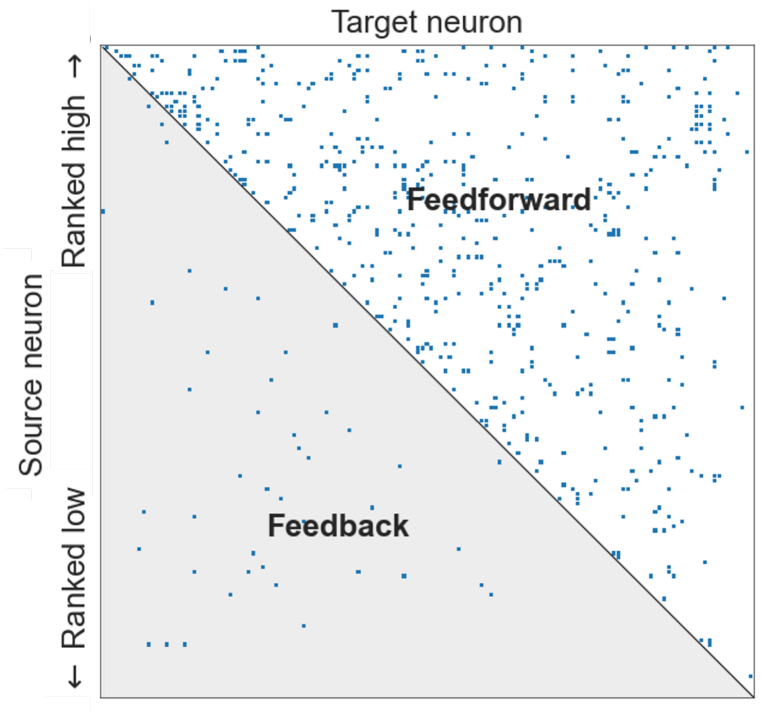
- “Feedback minimization”
- Signal flow
- Random-walk based

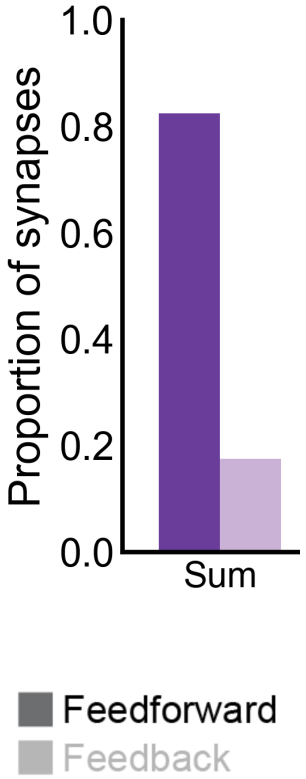
Sorted adjacency

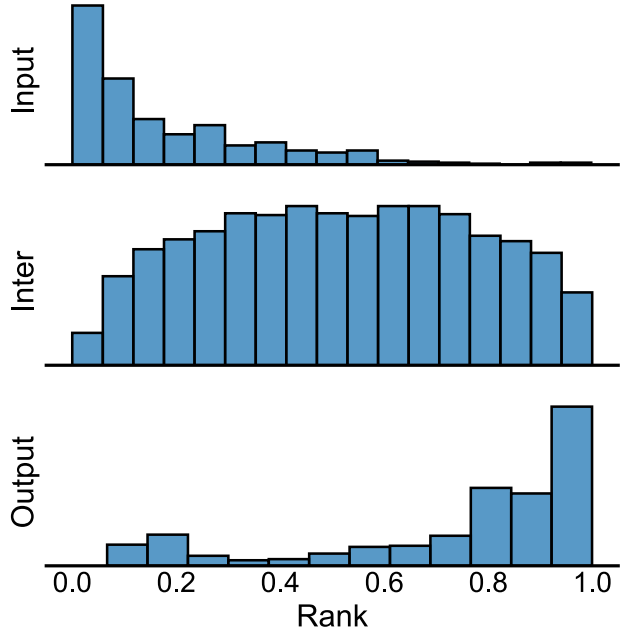


Carmel et al. IEEE Vis. and Comp. Graphics (2004), Burkard et al. Assignment Problems (2009)

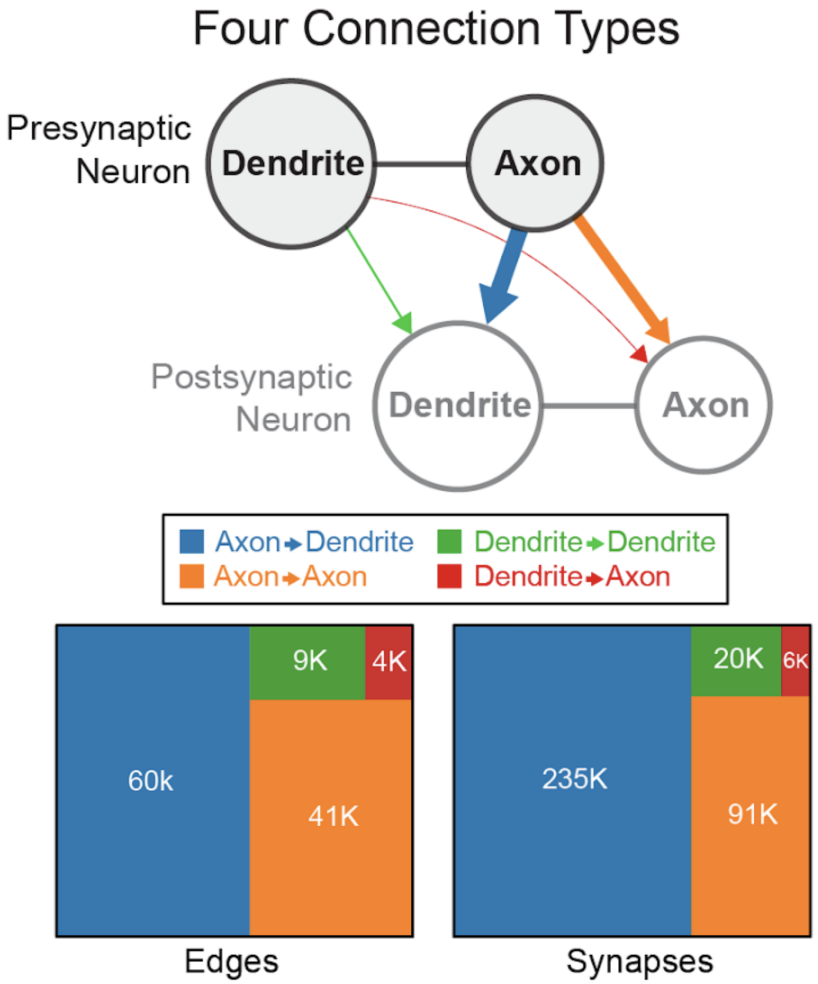
Quantifying high-level "feedforward/feedback"

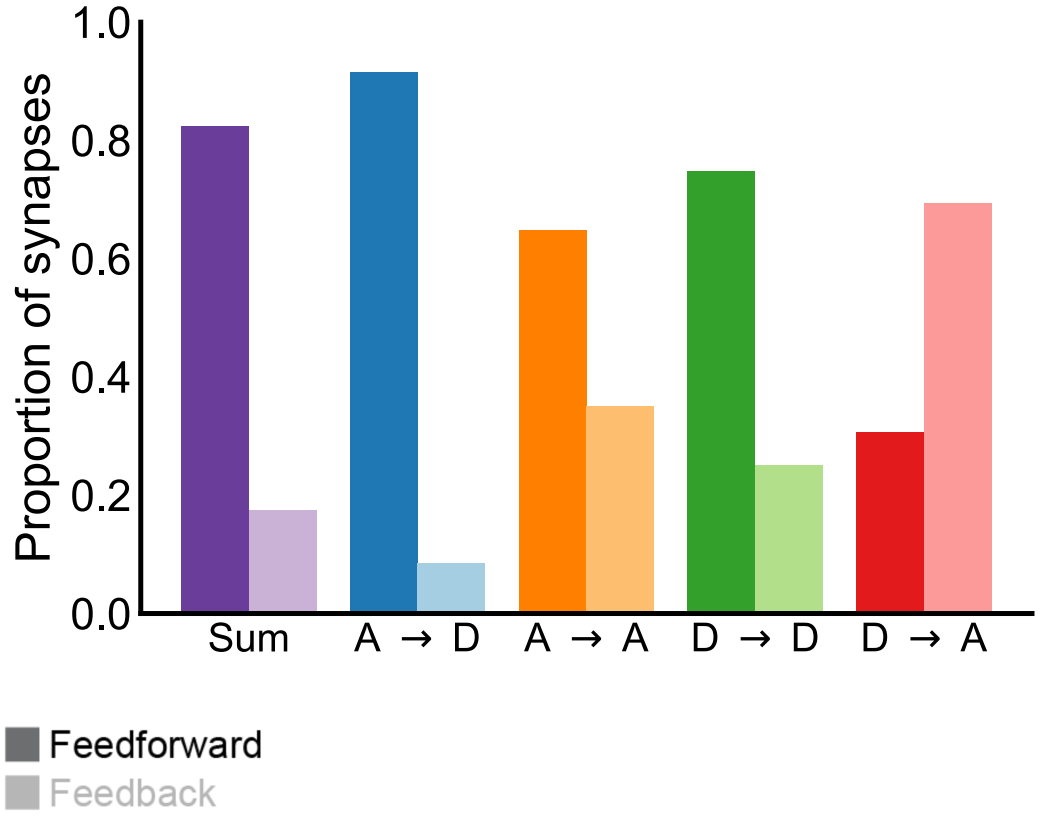






Morphology enables splitting axons/dendrites





Winding, Pedigo et al. bioRxiv (2022)

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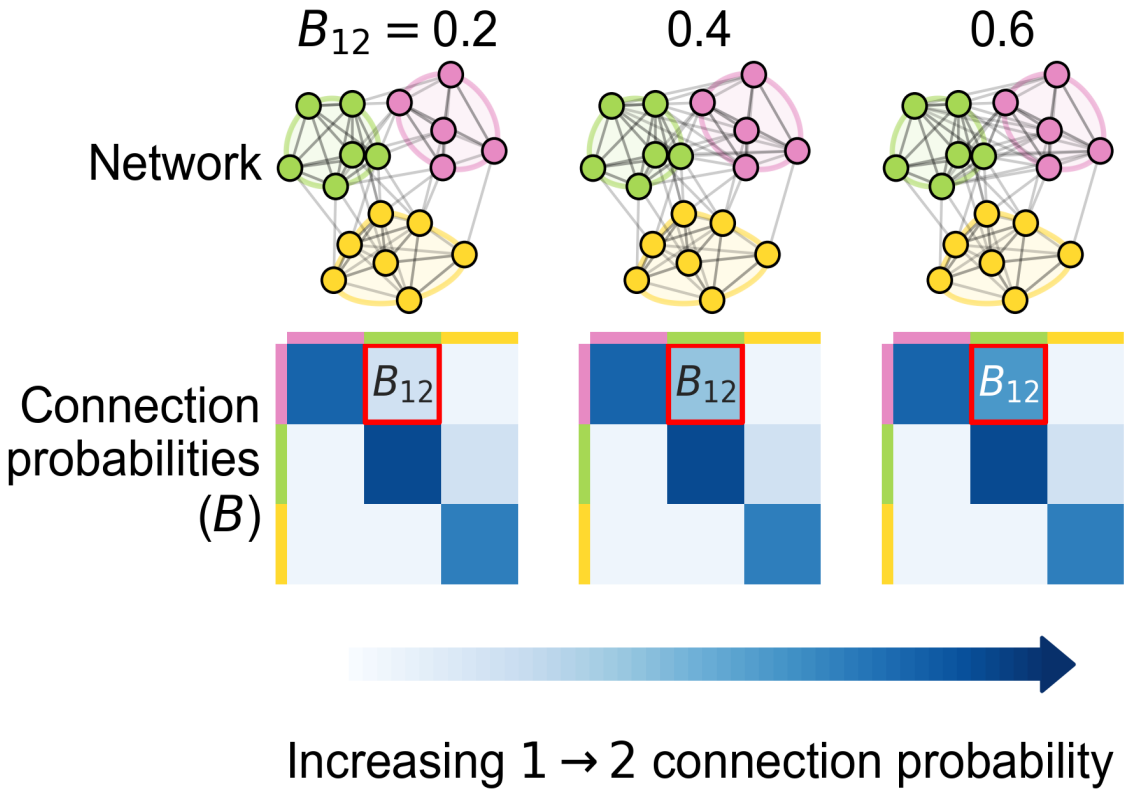
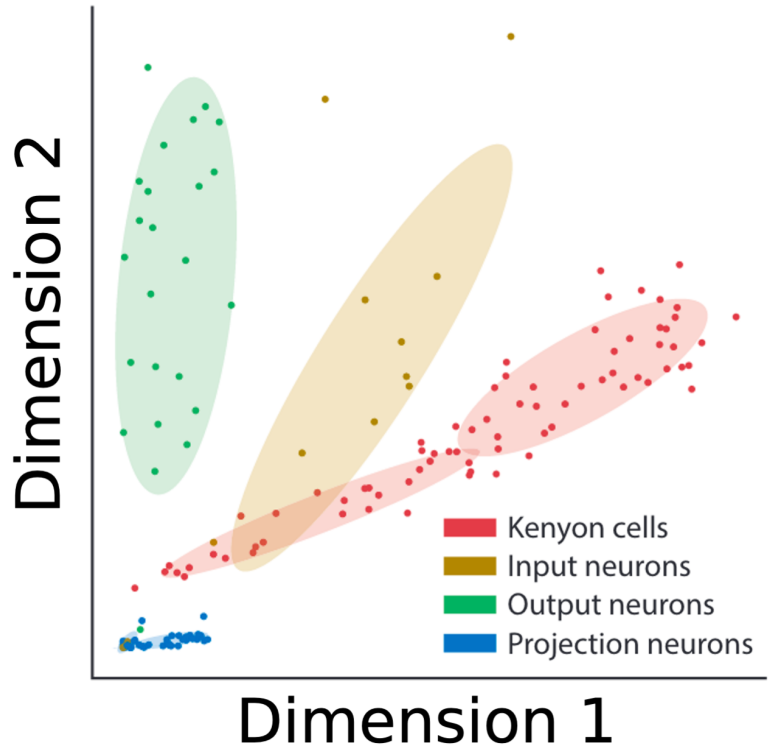
Stochastic block model



- Each node is assigned to a group
- B is a matrix of connection probabilities between groups
- Edges generated independently according to these probabilities

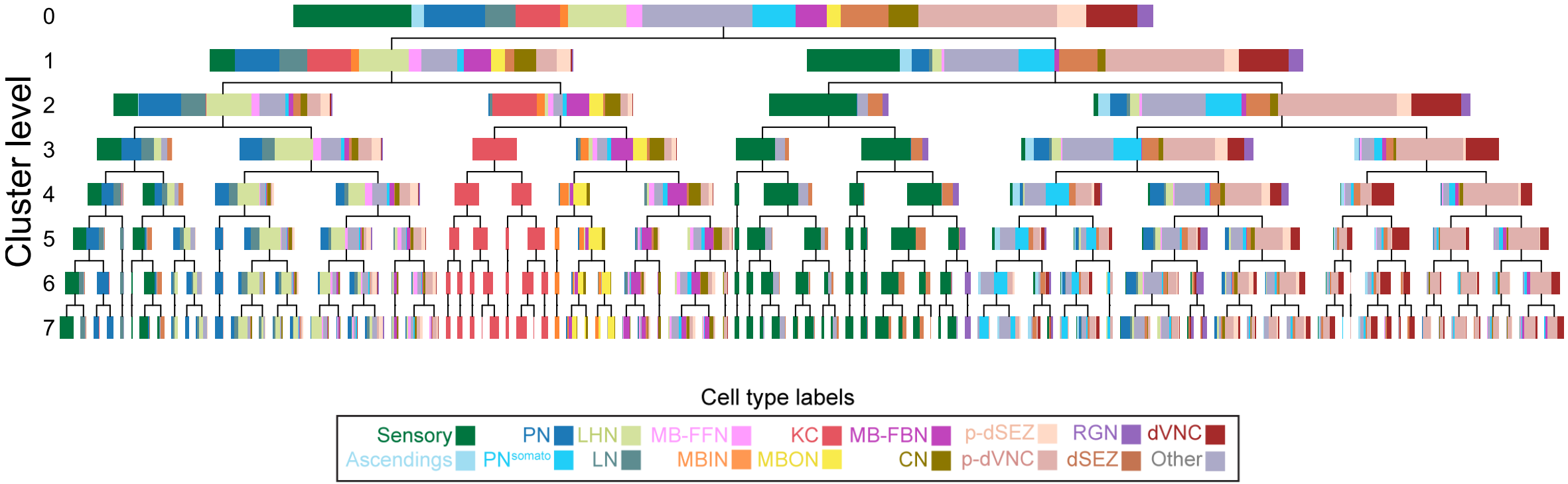
Spectral embedding

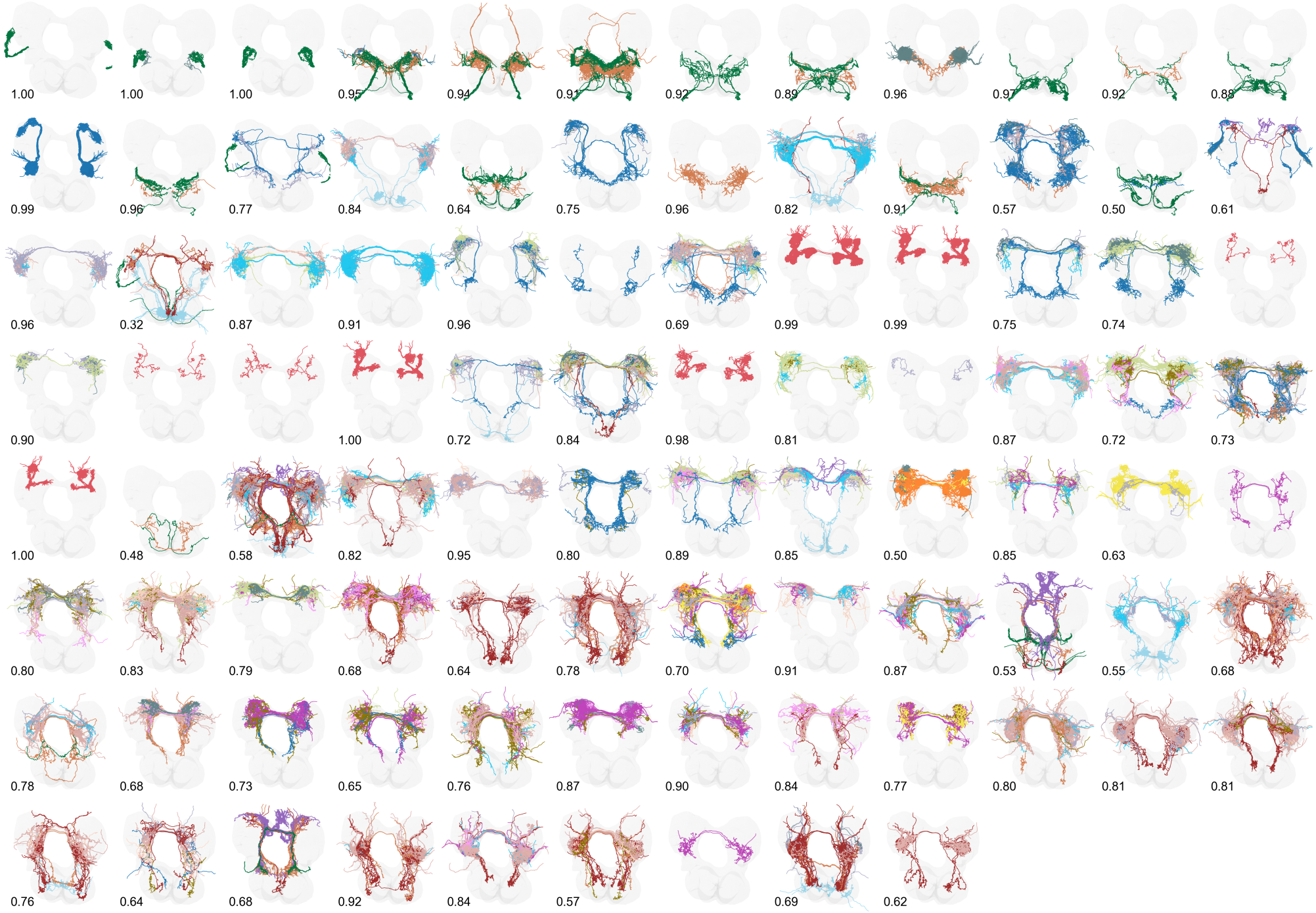
- Spectral decomposition of the adjacency matrix (or Laplacian)
- Clustering on this representation is a consistent estimator of block model labels



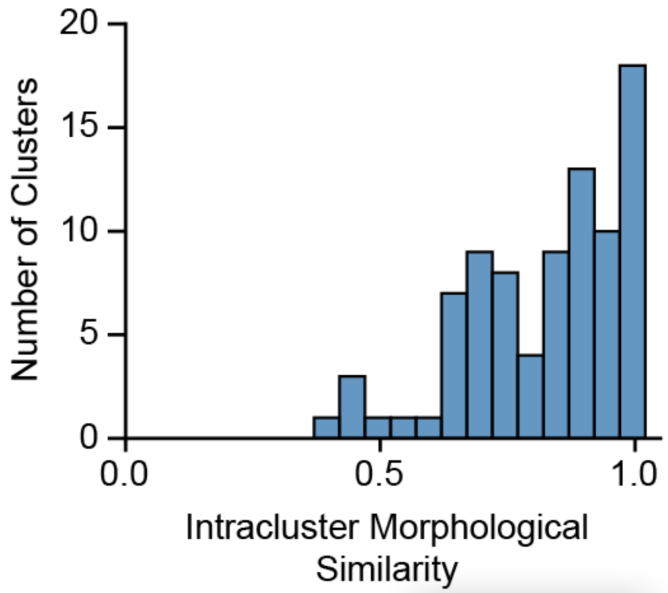
Sussman et al. JASA (2012), Chung et al. Annual Review of Statistics (2021)

Neurons clustered by connectivity using recursive spectral clustering




1.00 1.00 1.00 0.95 0.94 0.91 0.92 0.89 0.96 0.97 0.92 0.88
0.99 0.96 0.77 0.84 0.64 0.75 0.96 0.82 0.91 0.57 0.50 0.61
0.96 0.32 0.87 0.91 0.96 0.69 0.99 0.99 0.75 0.74
0.90 1.00 0.72 0.84 0.98 0.81 0.87 0.72 0.73
1.00 0.48 0.58 0.82 0.95 0.80 0.89 0.85 0.50 0.85 0.63
0.80 0.83 0.79 0.68 0.64 0.78 0.70 0.91 0.87 0.53 0.55 0.68
0.78 0.68 0.73 0.65 0.76 0.87 0.90 0.84 0.77 0.80 0.81 0.81
0.76 0.64 0.68 0.92 0.84 0.57 0.69 0.62

Cluster morphology



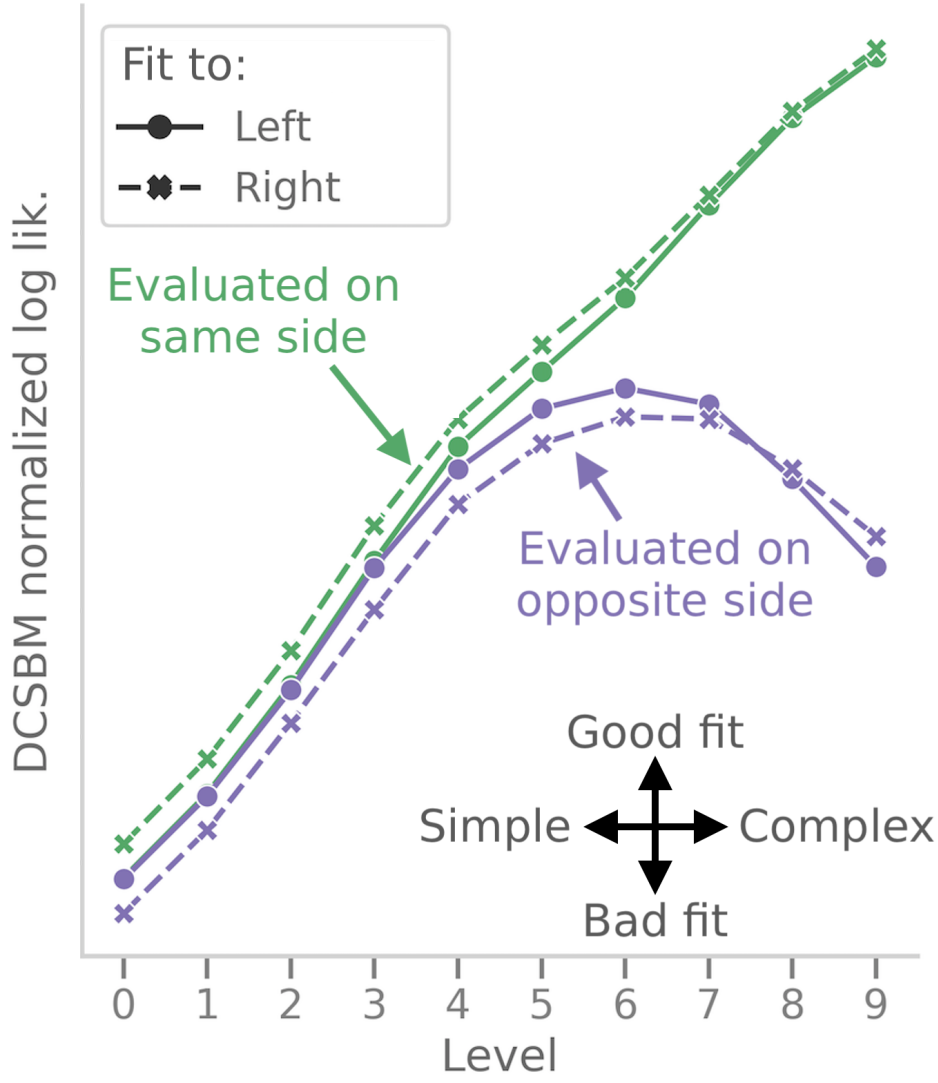
Discriminability:

$$P[\text{ within cluster NBLAST sim.} > \text{between cluster NBLAST sim.}] \approx 0.81$$

Costa et al. Neuron (2016), Bridgeford et al. PLOS Comp. Bio. (2021)

Using models to evaluate cell type groupings

- How well do these models generalize to the other side of the brain (let alone the next maggot)?



Bilateral symmetry

> "This brain is bilaterally symmetric."

> "What does that even mean? And how would we know if it wasn't?"

Are the left and right sides of this connectome *different*?

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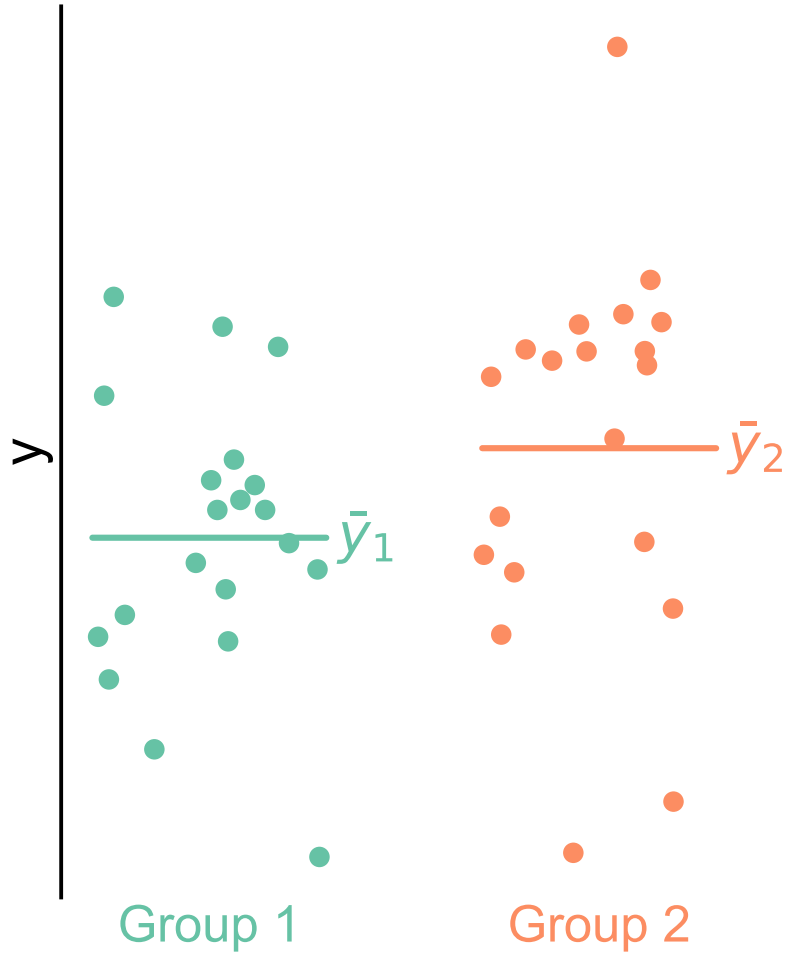
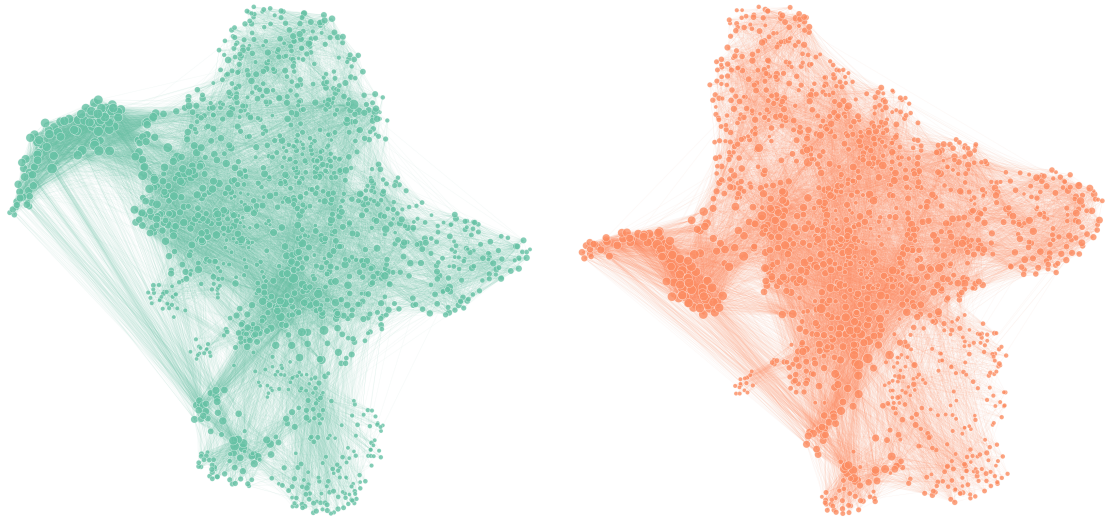
Are these populations different?



- Known as two-sample testing
- $Y^{(1)} \sim F^{(1)}, Y^{(2)} \sim F^{(2)}$
- $H_0 : F^{(1)} = F^{(2)}$
  $H_A : F^{(1)} \neq F^{(2)}$

Are these *networks* different?

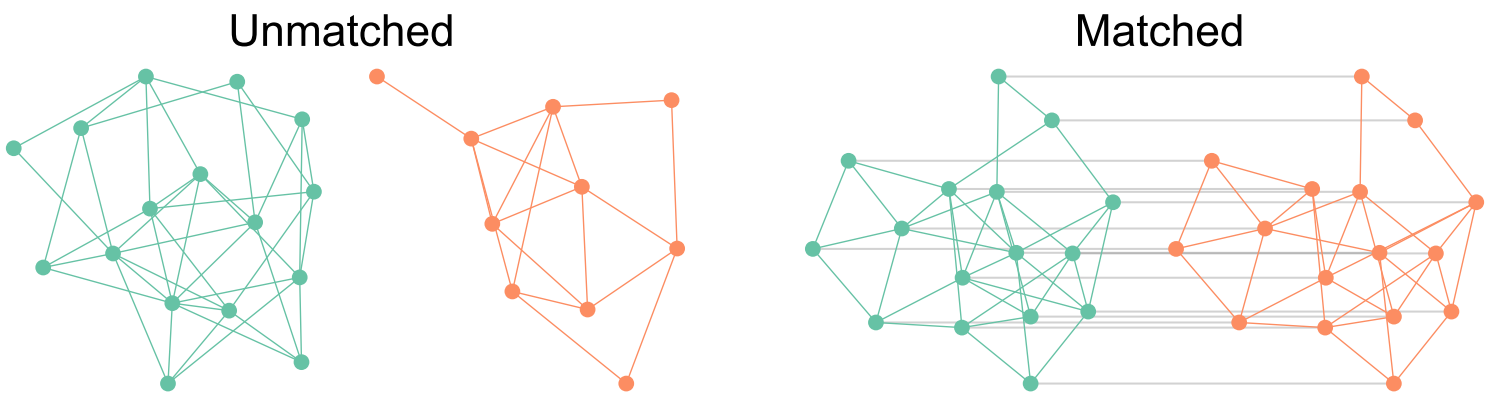


- Want a two-network-sample test!
- $A^{(L)} \sim F^{(L)}$, $A^{(R)} \sim F^{(R)}$
- $H_0 : F^{(L)} = F^{(R)}$
  $H_A : F^{(L)} \neq F^{(R)}$

Pedigo et al. bioRxiv (2022)

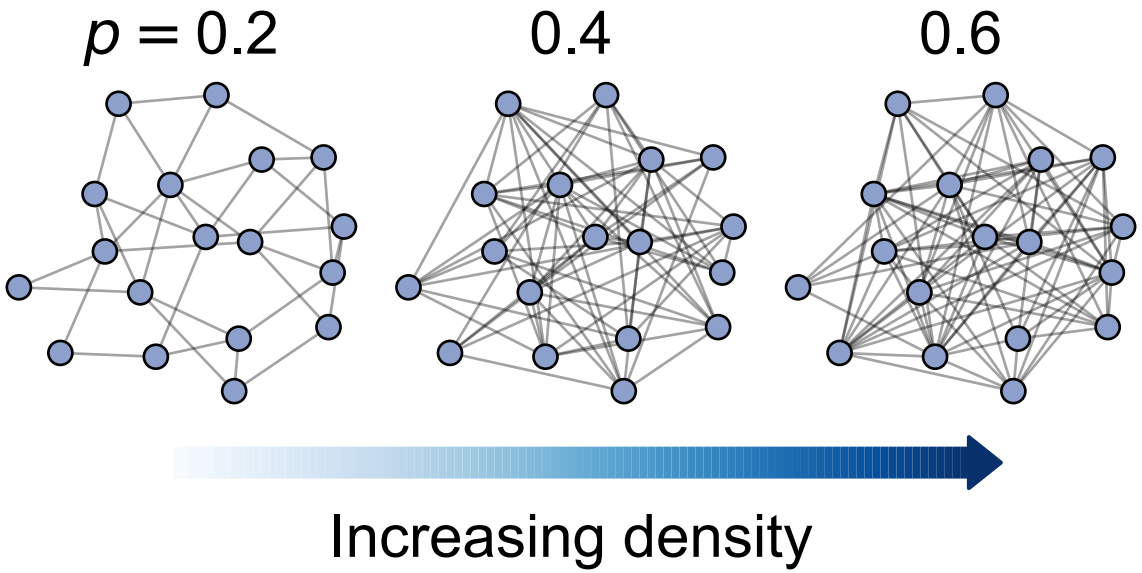
Assumptions

- Know the direction of synapses, so network is *directed*
- For simplicity (for now), consider networks to be *unweighted*
- For simplicity (for now), consider the left $\longrightarrow$ left and right $\longrightarrow$ right (*ipsilateral*) connections
- Not going to assume any nodes are matched



Erdos-Renyi model

- All edges are independent
- All edges generated with the same probability, p

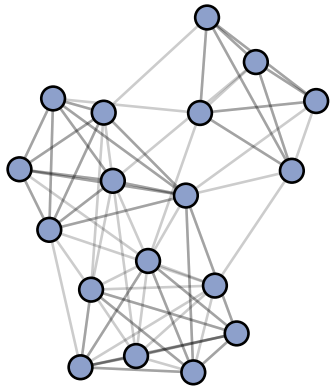


Detect a difference in density

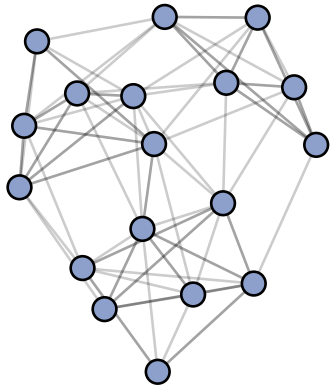
Compute global connection density

Compare ER models

Left

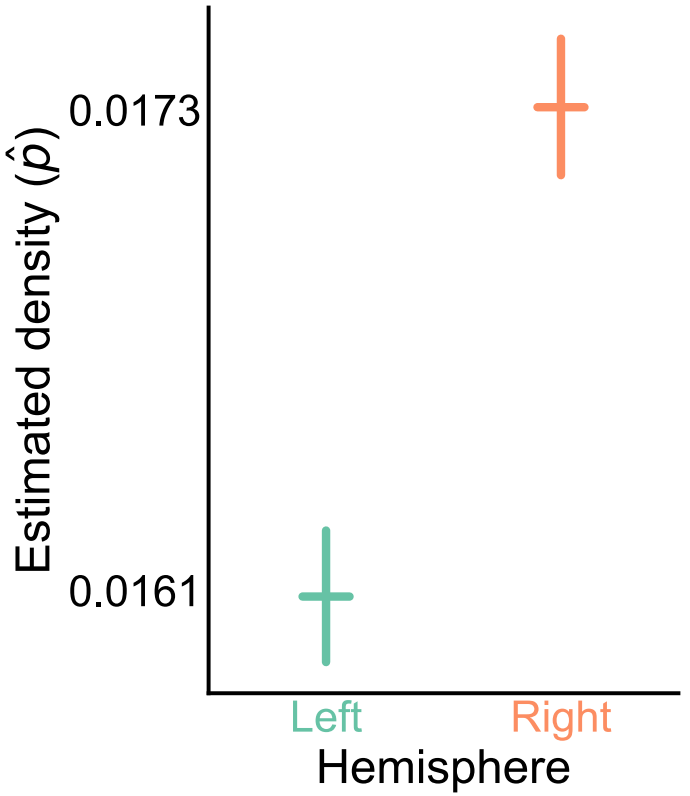


Right



$$p = \frac{\#\ edges}{\#\ potential\ edges}$$

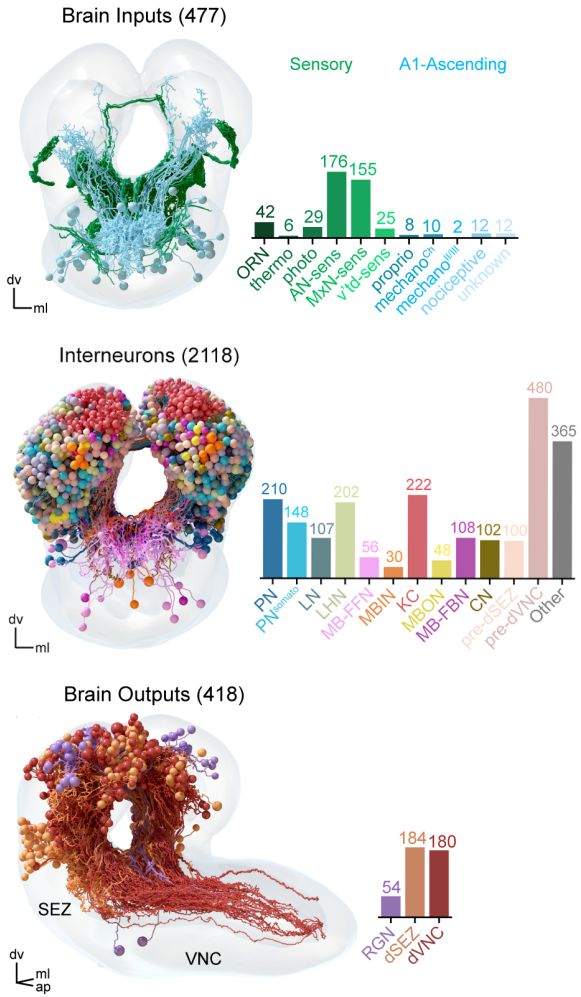
$$H_0: p^{(L)} = p^{(R)}$$
$$H_A: p^{(L)} \neq p^{(R)}$$

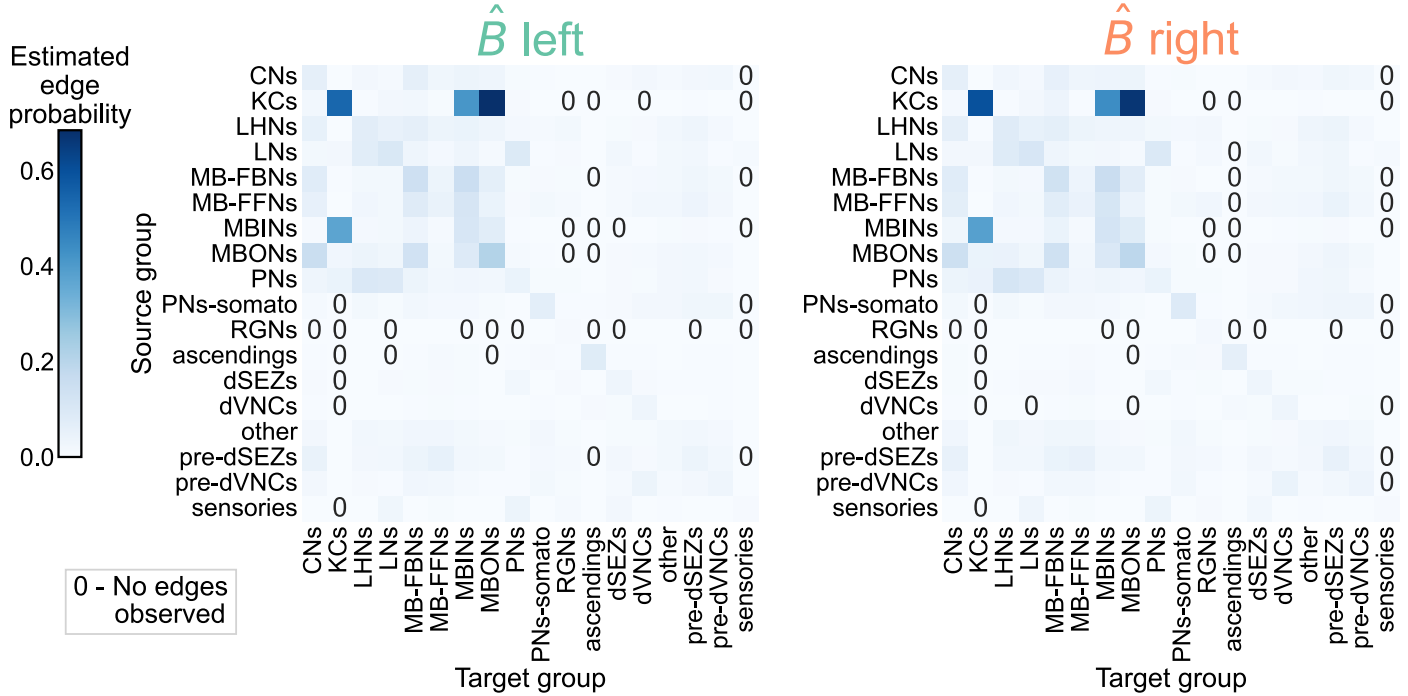


p-value < 10^{-22}

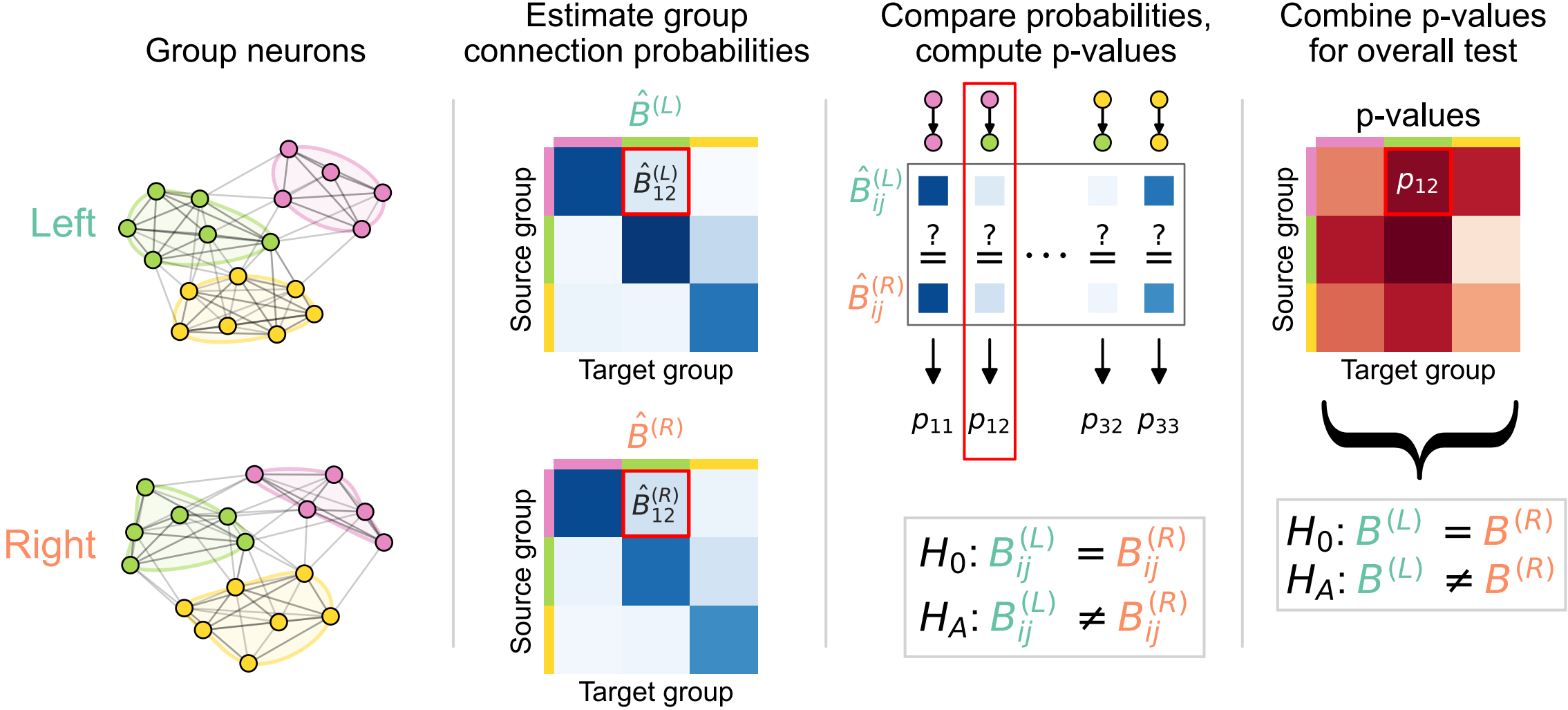
Pedigo et al. bioRxiv (2022)

Connection probabilities between groups

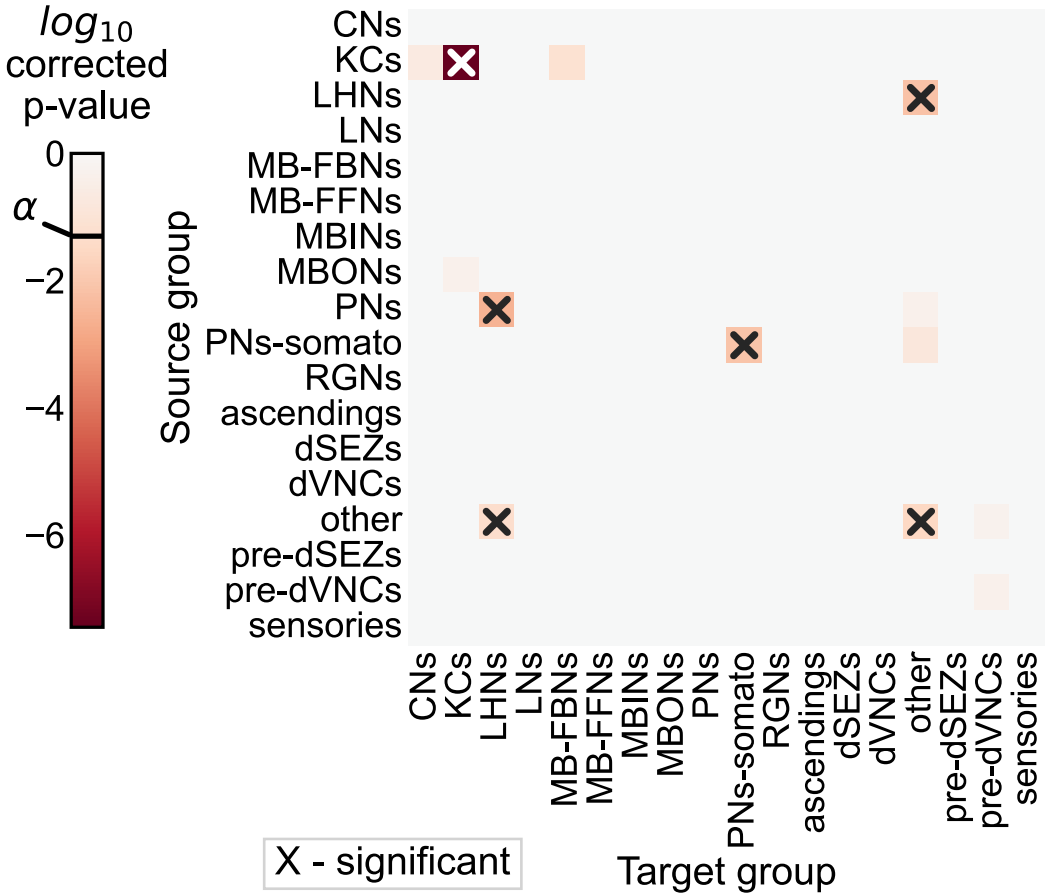




Group connection test



Detect differences in group connection probabilities

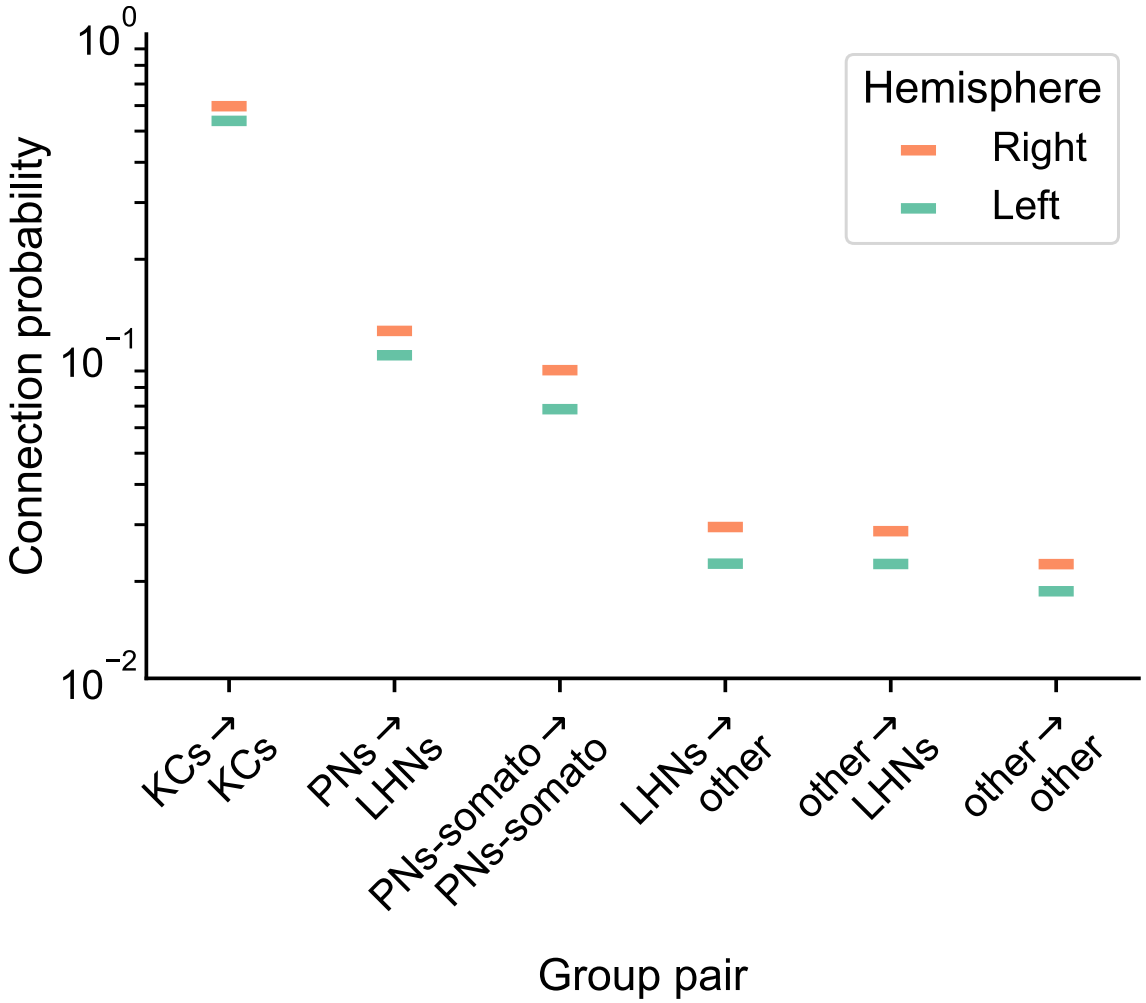


- 6 group-to-group connections are significantly different (after multiple comparisons correction)
- Overall test (comparing all blocks): p-value $< 10^{-7}$

Should we be surprised?

- Already saw that even the overall densities were different
- For all significant comparisons, probabilities on the right hemisphere were higher
- Maybe the right is just a "scaled up" version of the left?
  - $H_0 : B^{(L)} = cB^{(R)}$

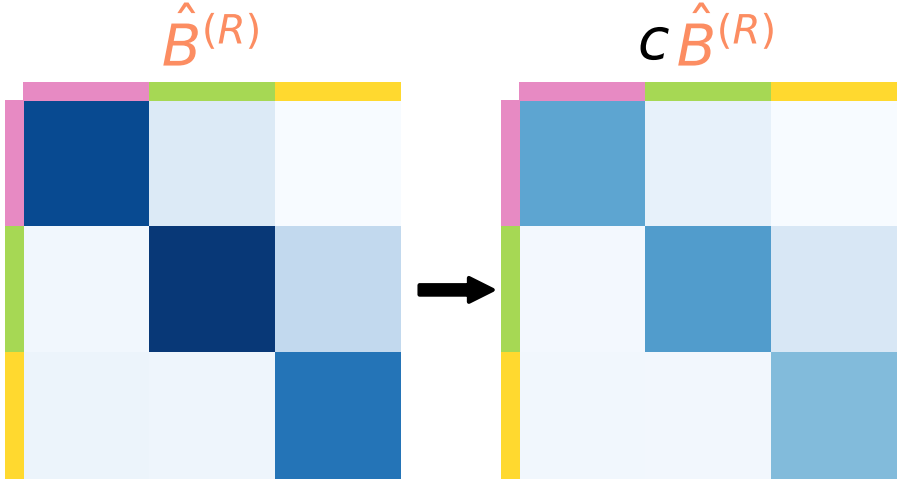
    where c is a density-adjusting constant, $\frac{p^{(L)}}{p^{(R)}}$



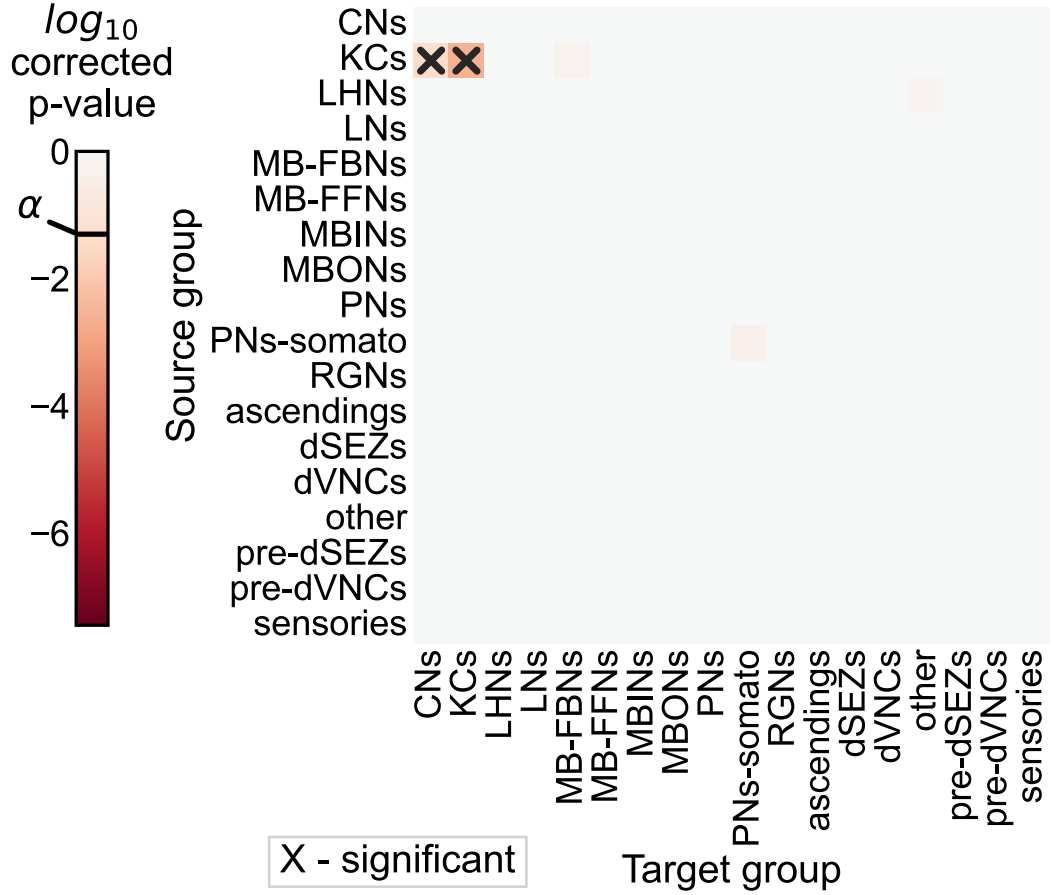
Pedigo et al. bioRxiv (2022)

After adjusting for density, differences are in KCs

Scale connection probabilities to match densities



$$H_0: B^{(L)} = cB^{(R)}$$
$$H_A: B^{(L)} \neq cB^{(R)}$$



Overall p-value: $< 10^{-2}$

To sum up...

> "This brain is bilaterally symmetric."

Depends on what you mean...

With Kenyon cells

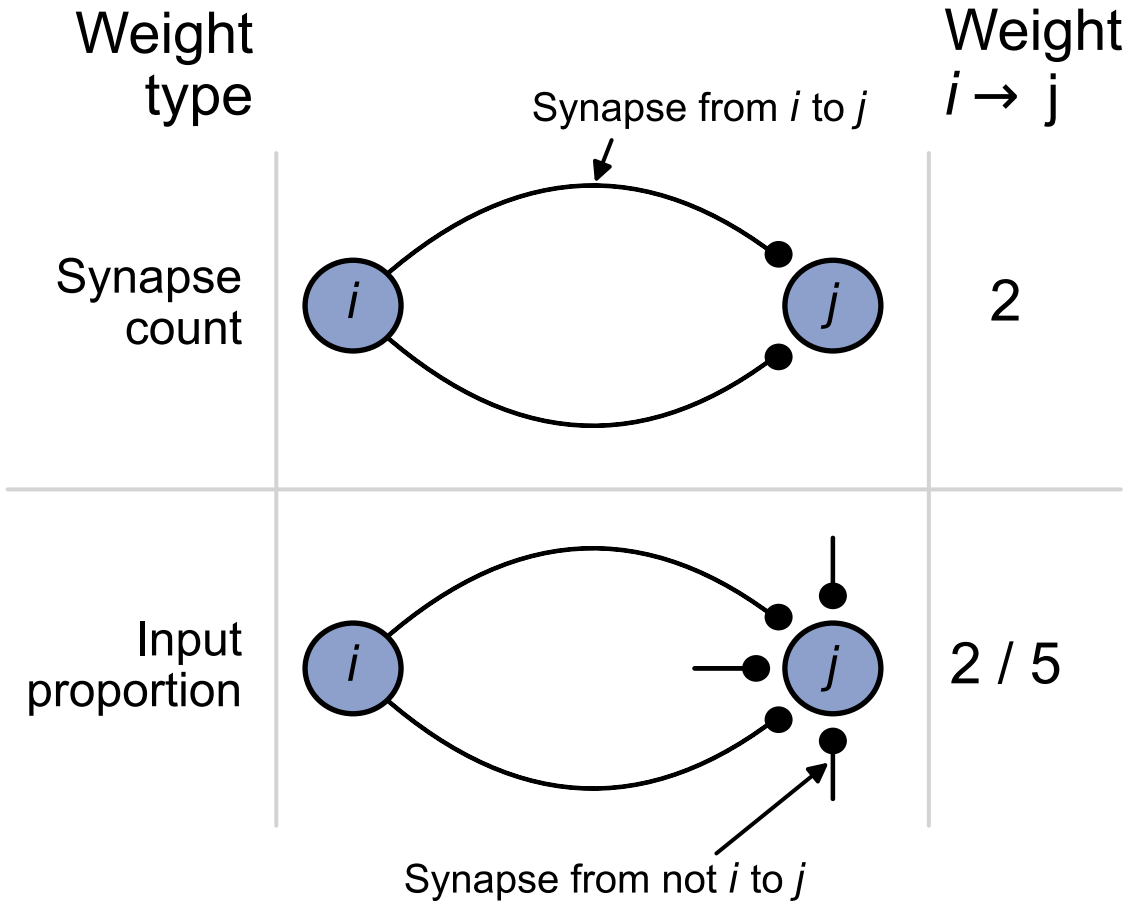
Model	H_0 (vs. $H_A \neq$)	p-value
ER	$p^{(L)} = p^{(R)}$	$<10^{-23}$
SBM	$B^{(L)} = B^{(R)}$	$<10^{-7}$
daSBM	$B^{(L)} = cB^{(R)}$	$<10^{-2}$

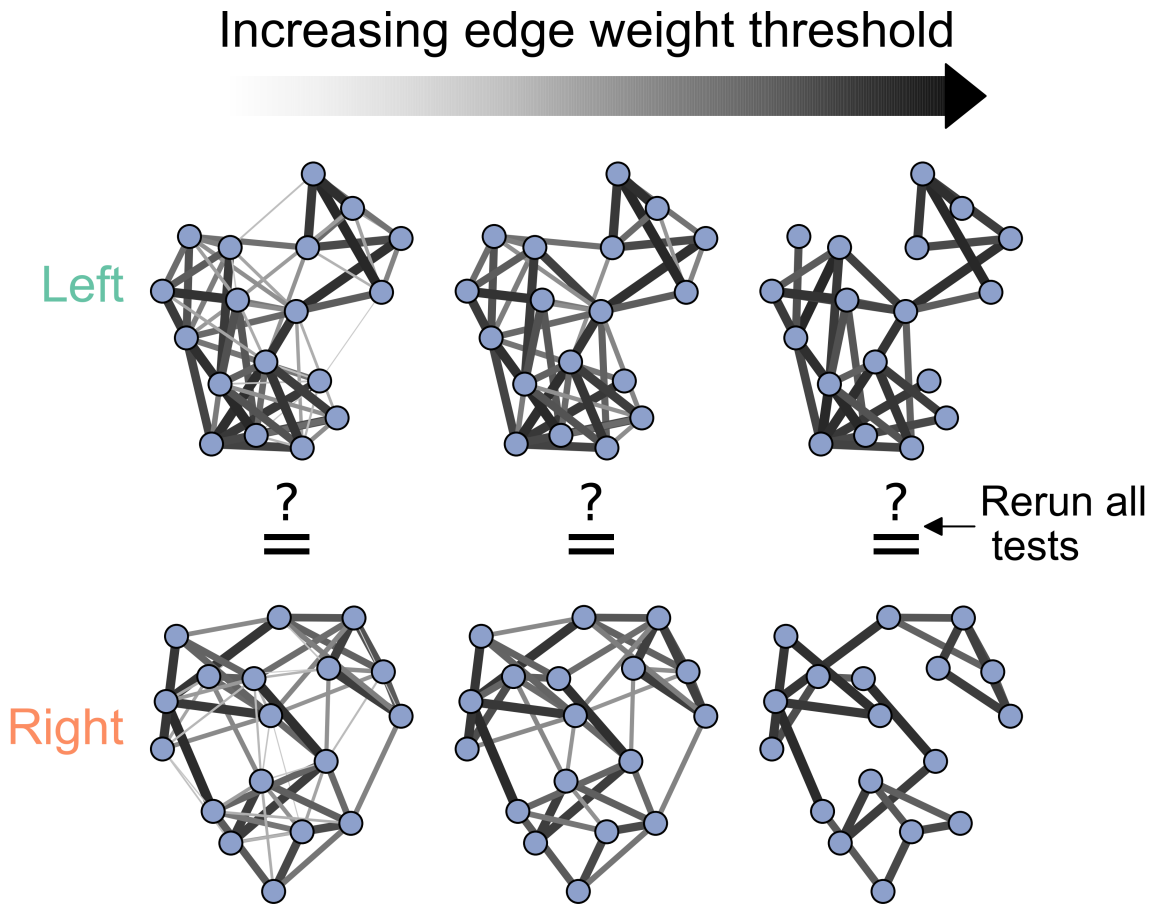
Without Kenyon cells

Model	H_0 (vs. $H_A \neq$)	p-value
ER	$p^{(L)} = p^{(R)}$	$<10^{-26}$
SBM	$B^{(L)} = B^{(R)}$	$<10^{-2}$
daSBM	$B^{(L)} = cB^{(R)}$	≈ 0.51

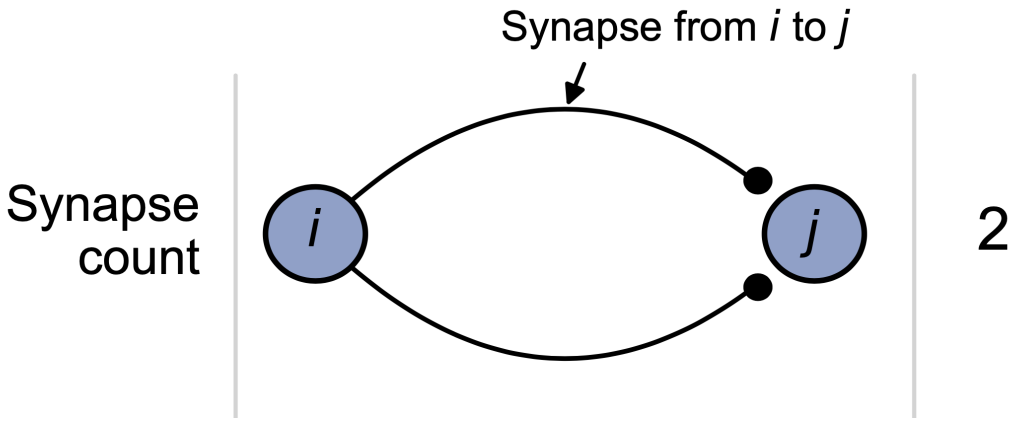
Pedigo et al. bioRxiv (2022)

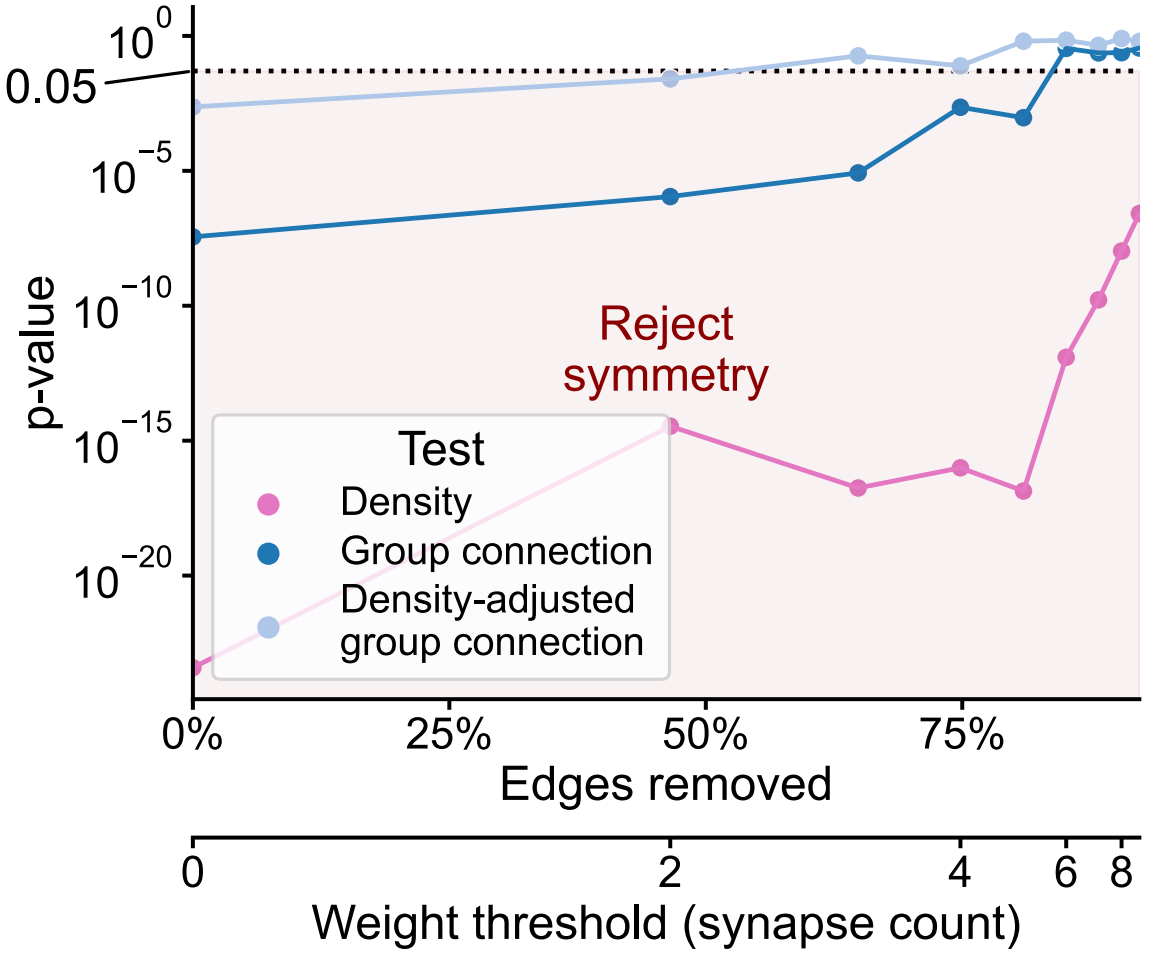
Examining the effect of edge weights



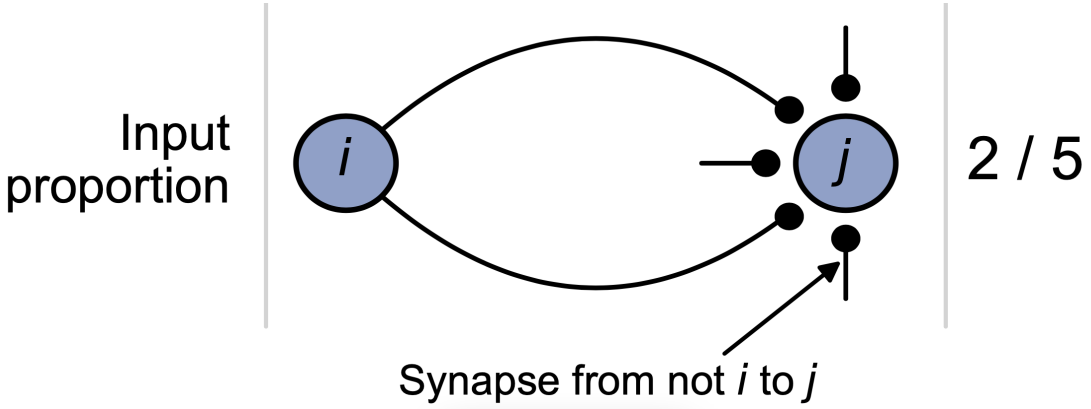


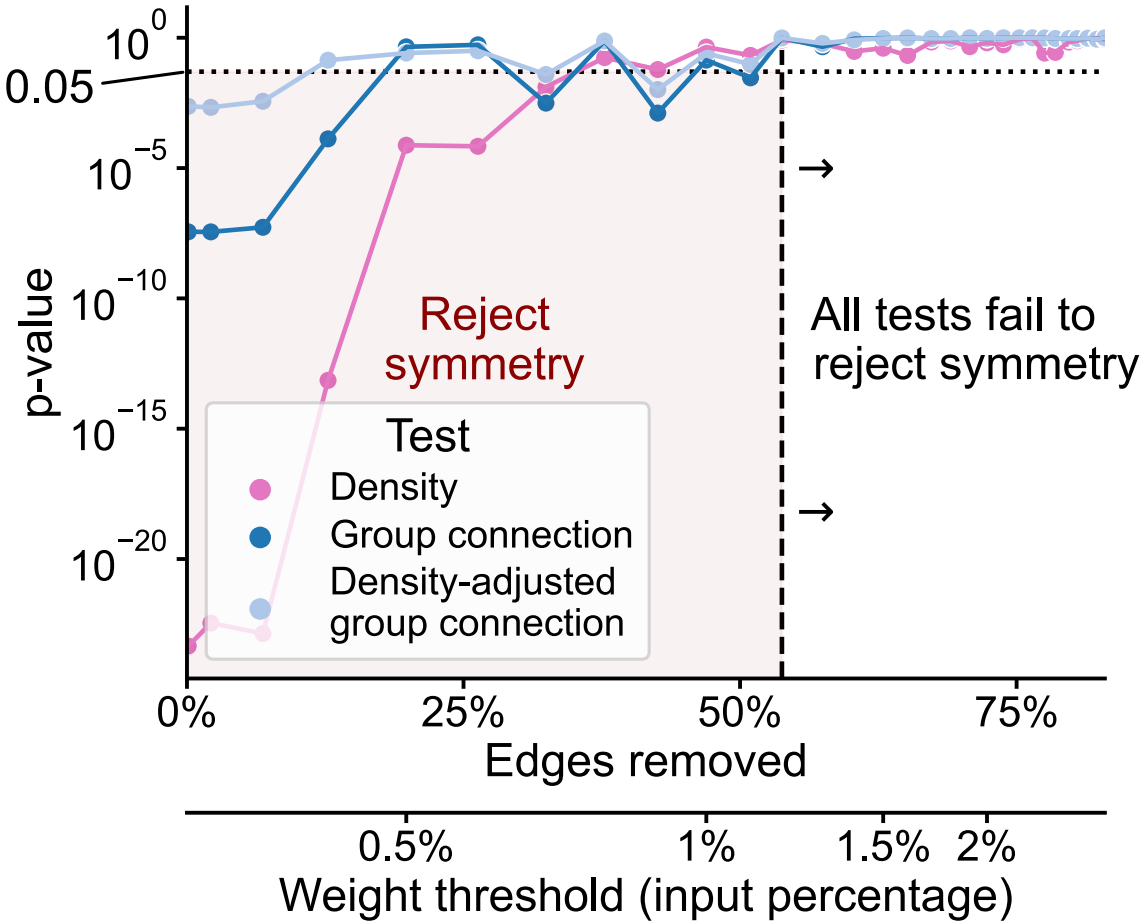
Even high synapse count networks show asymmetry





High input percentage networks show no asymmetry





Pedigo et al. bioRxiv (2022)

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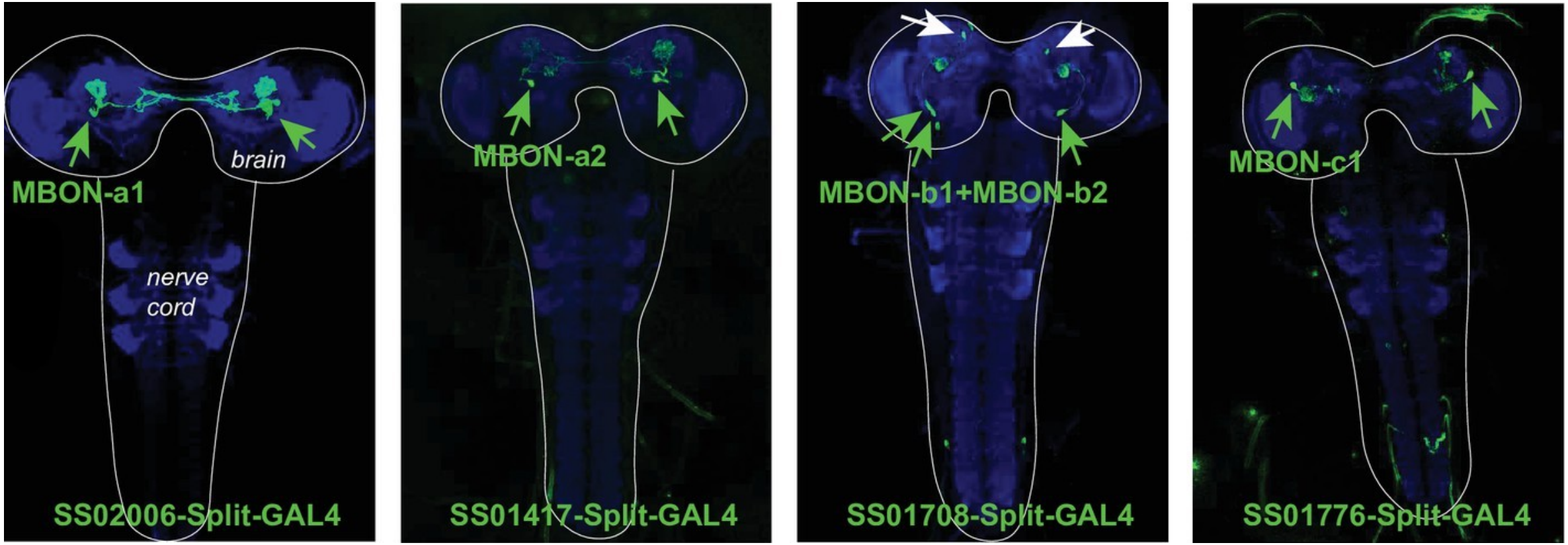
- Ongoing extensions/applications

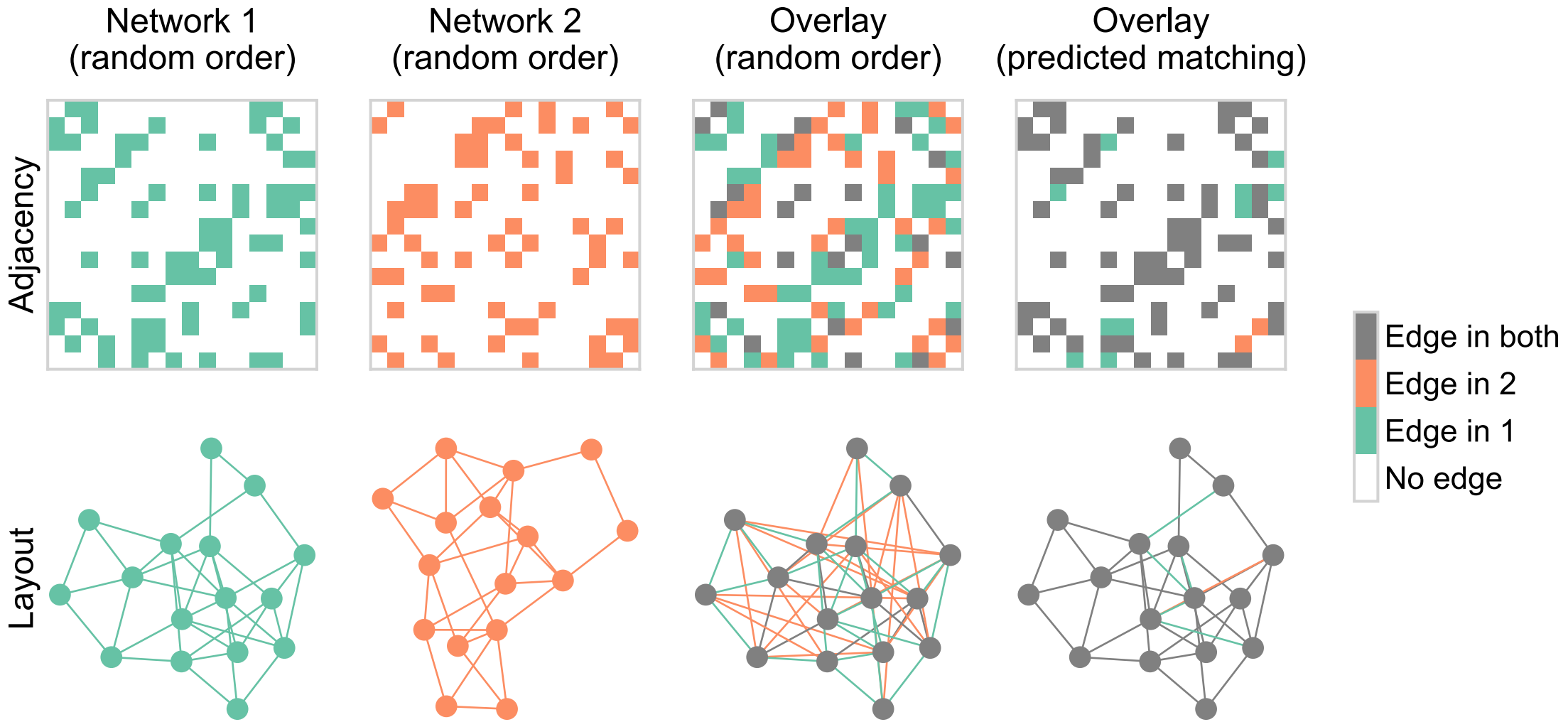
Bilaterally homologous neuron pairs

We believe a matching exists!



Eschbach et al. eLife (2021)

What is graph matching?



How do we measure network overlap?

$$\min_{P \in \mathcal{P}} \underbrace{\| A_1 - \overbrace{P A_2 P^T}^{\text{reordered } A_2} \|_F^2}_{\text{distance between adj. mats.}}$$

where $\mathcal{P}$ is the set of permutation matrices

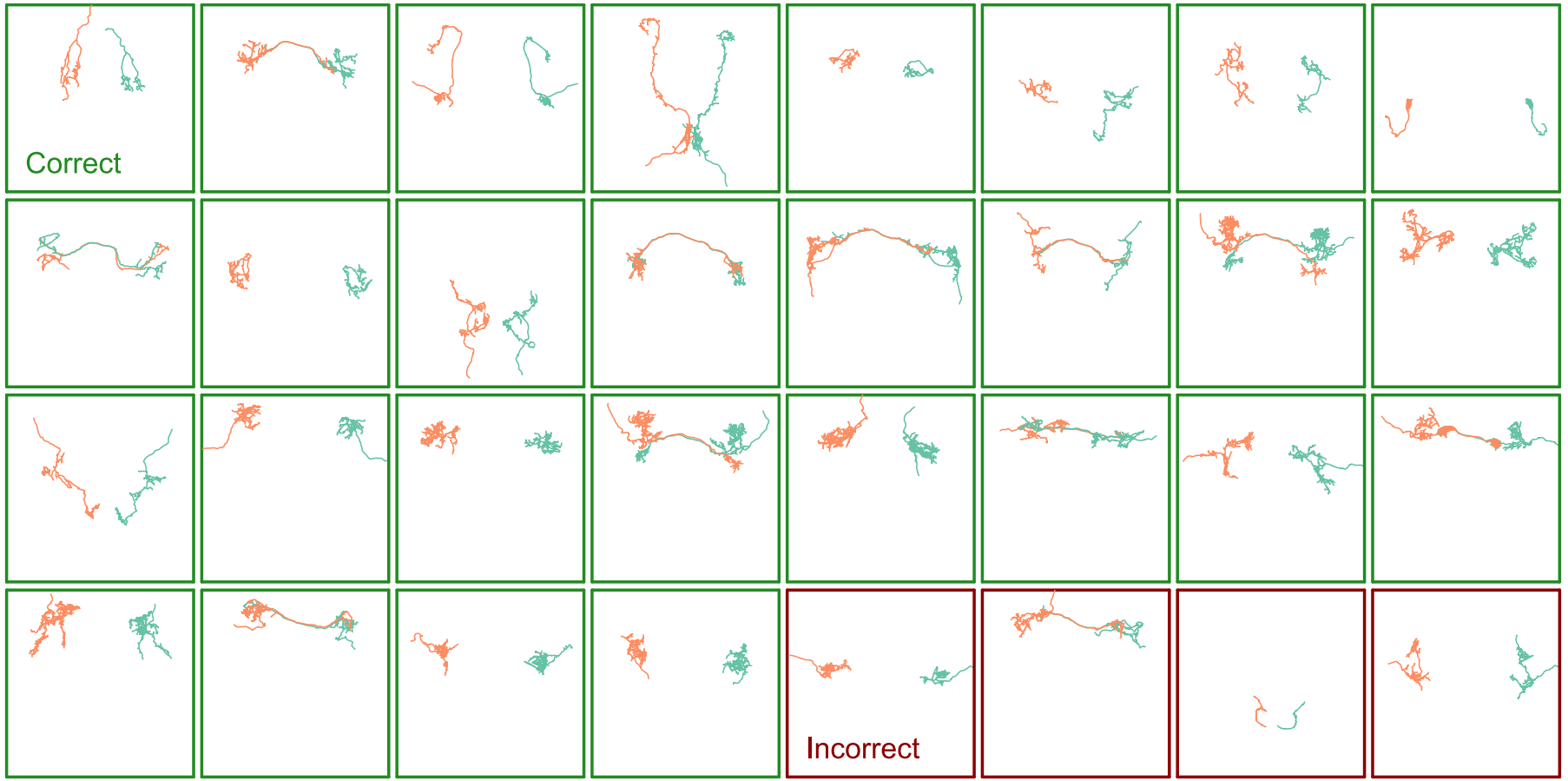
- Measures the number of edge disagreements for unweighted networks,
- Norm of edge disagreements for weighted networks

How do we do graph matching?

- Relax the problem to a continuos space
  - Convex hull of permutation matrices
- Minimize a linear approximation of objective function (repeat)
- Project back to the closest permutation matrix

Vogelstein et al. PLOS One (2015)

Matching (by connectivity only) performs fairly well



With "vanilla" graph matching: ~80% correct (according to expert annotator)

Many ways to try to improve on this...

- Edge types allow for "multilayer" graph matching
- Partial knowledge of the matching (seeds)
- Morphology (e.g. NBLAST)

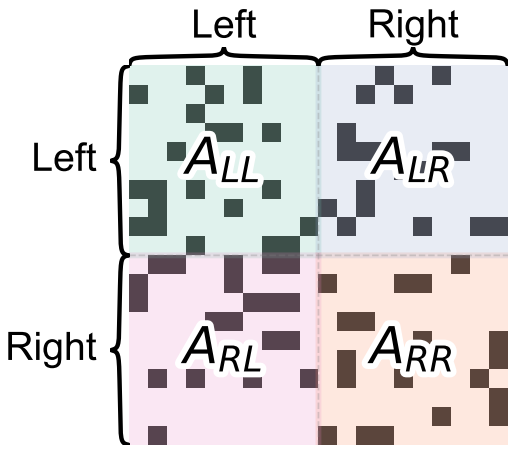
Thus far, we've not used the contralateral connections

These are about 1/3 of the edges in the brain!

Pantazis et al. Applied Network Science (2022), Fishkind et al. Pattern Recognition (2019)

From graph matching to bisected graph matching

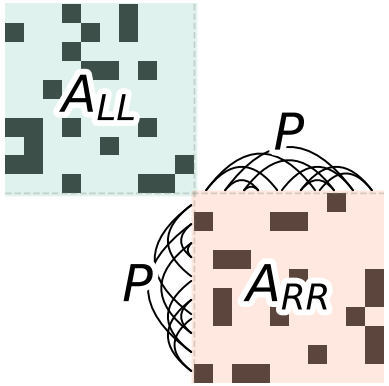
Adjacency matrix



Ipsilateral → A_{LL}, A_{RR}

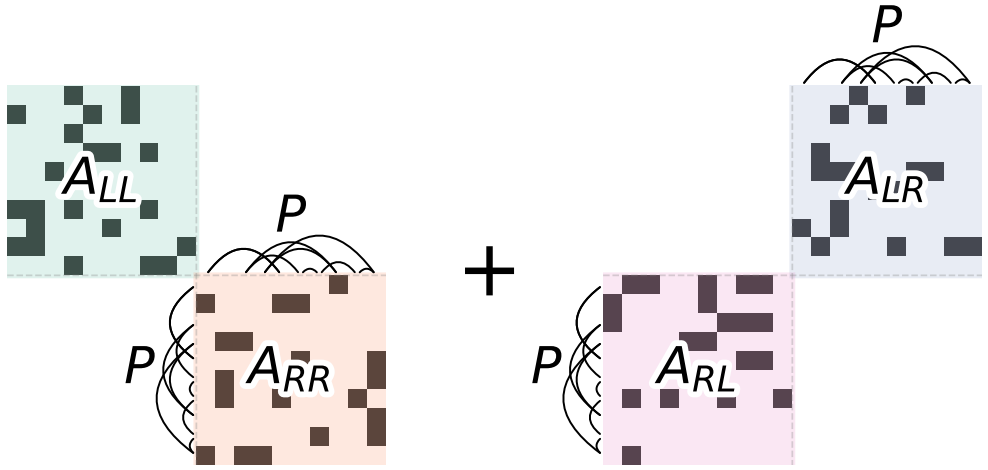
Contralateral → A_{LR}, A_{RL}

Graph matching (GM)



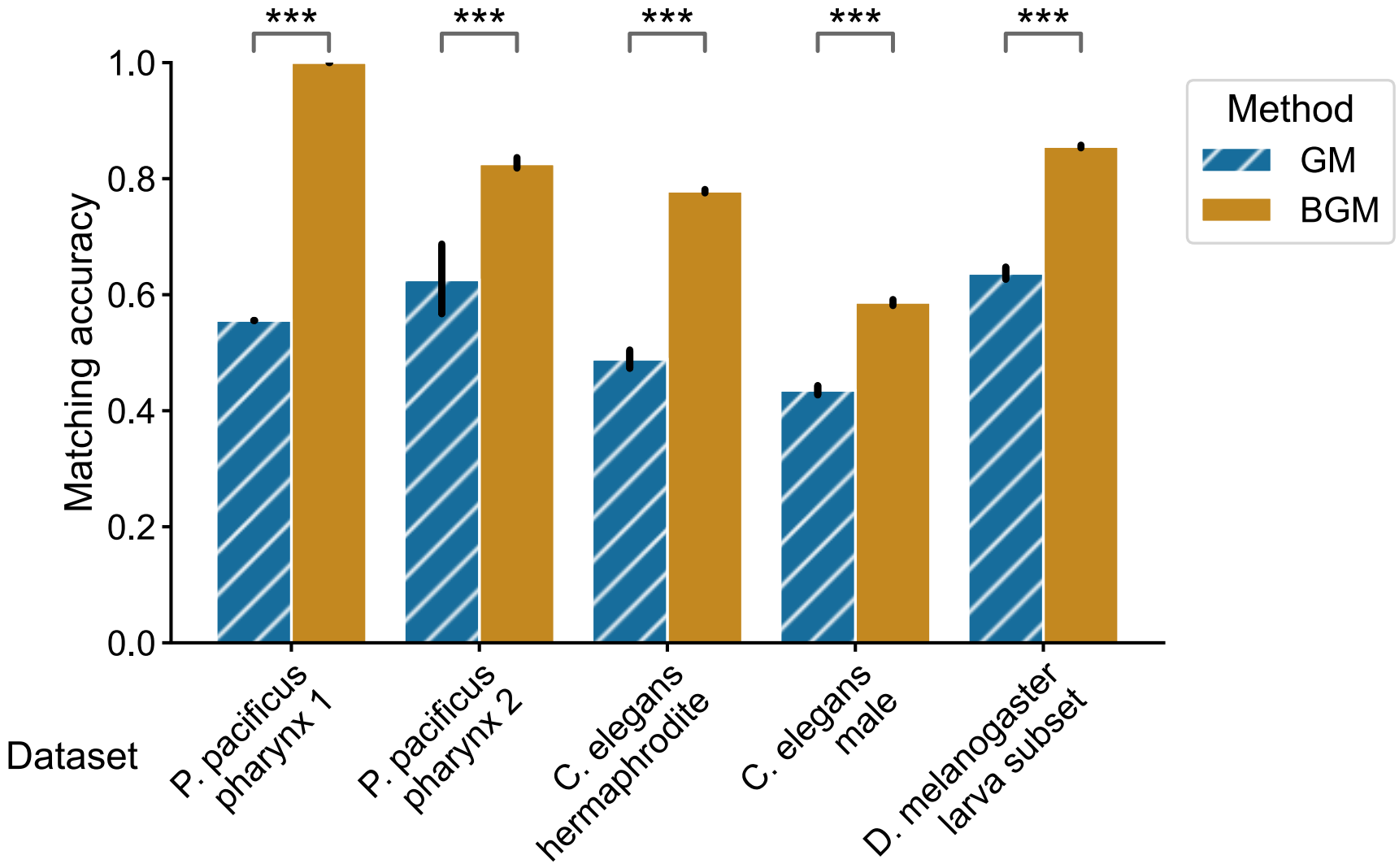
$$\min_P \| A_{LL} - P A_{RR} P^T \|_F^2$$

Bisected graph matching (BGM)



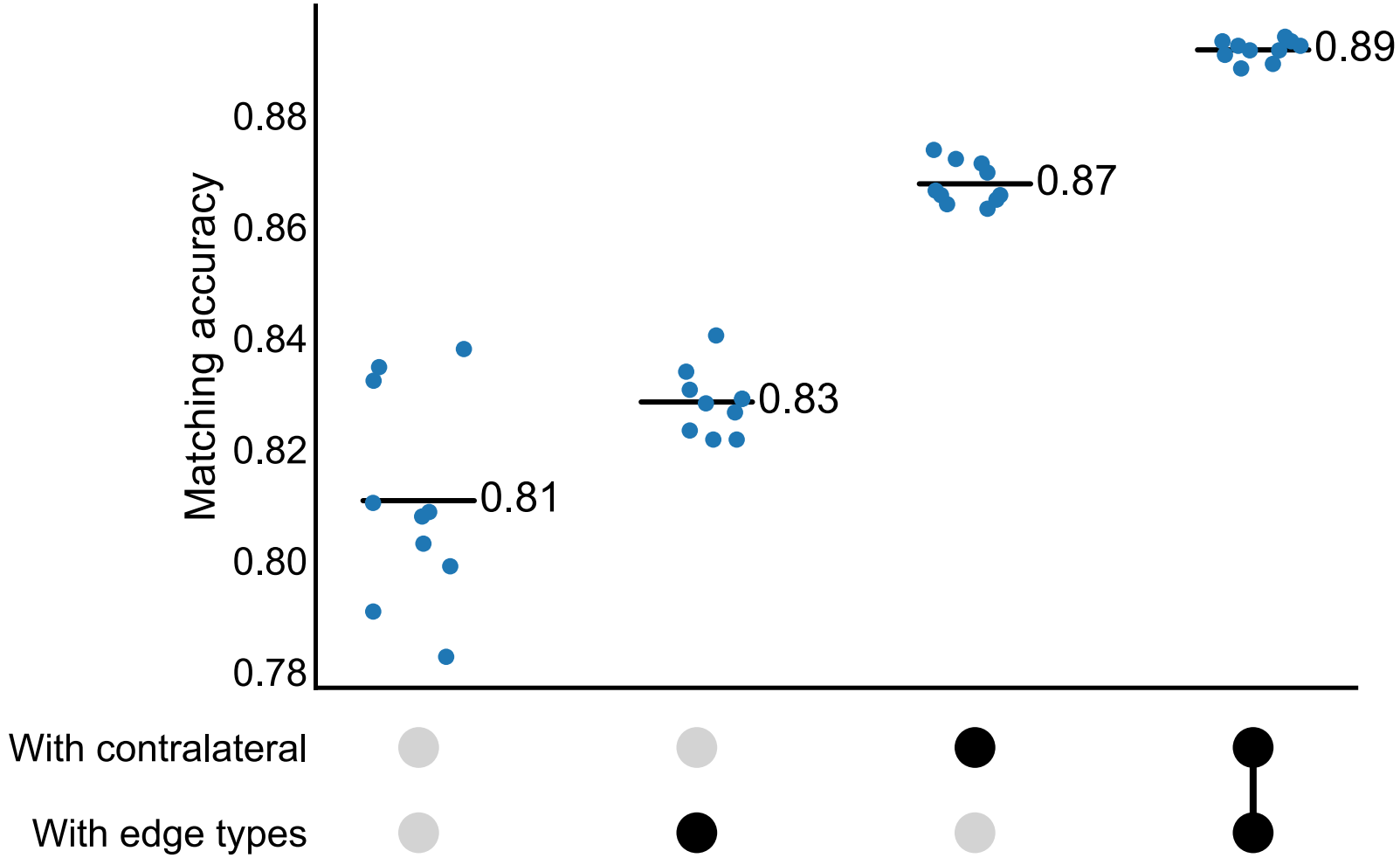
$$\min_P \| A_{LL} - P A_{RR} P^T \|_F^2 + \| A_{LR} P^T - P A_{RL} \|_F^2$$

Contralateral connections are helpful!



Pedigo et al. bioRxiv (2022)

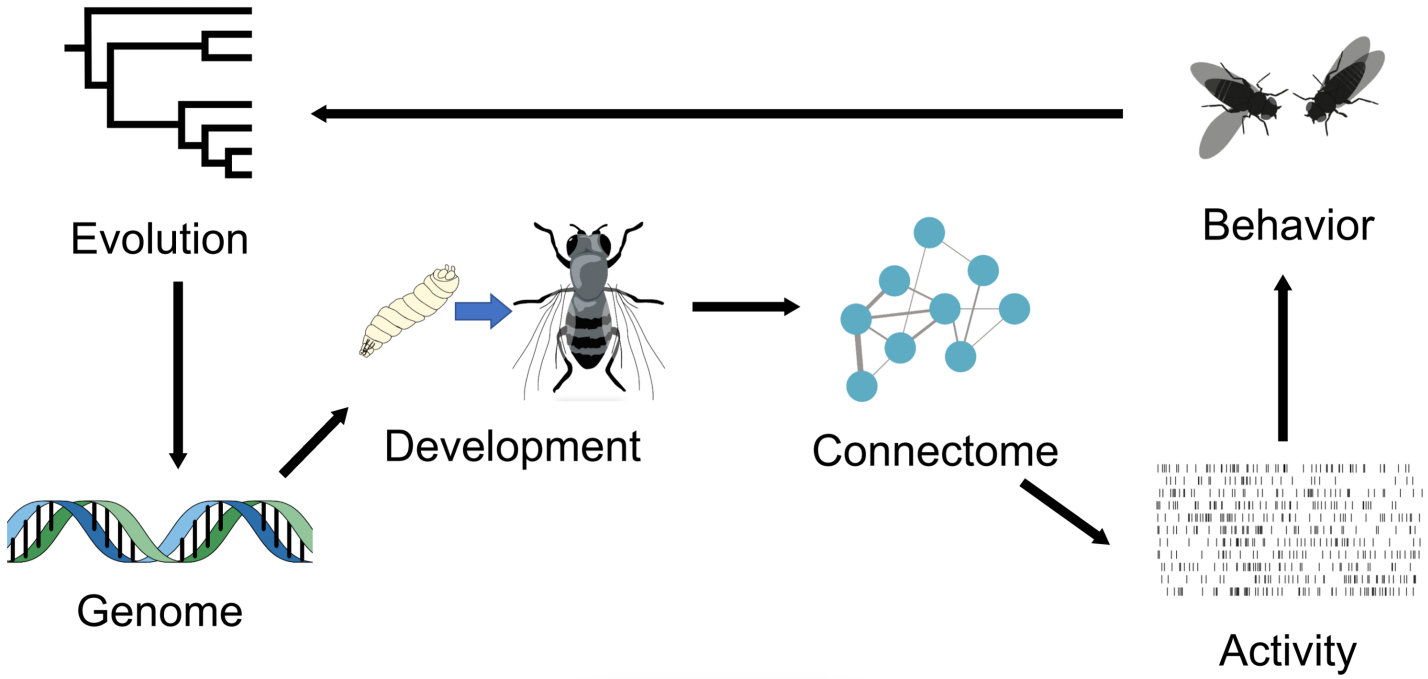
Performance improvement on the full brain



Outline

- Larval connectome dataset
- Connectome comparison via network hypothesis testing
- Pairing neurons across connectomes via graph matching
- Ongoing extensions/applications

Comparative connectomics



- Map connectomes from related individuals/organisms which may differ in feature X
- Compare connectomes
- Understand how X {affects, is affected by, is associated with} connectome structure

Current Opinion in Insect Science
Volume 54, December 2022, 100968

Connectomics and the neural basis of behaviour

Dana S Galili [1], Gregory SXE Jefferis [1, 2], Marta Costa [2] ✉

> Comparative connectomics across experience, sex and species is a key next step.

Current Opinion in Neurobiology
Volume 71, December 2021, Pages 139-149

Neural architectures in the light of comparative connectomics

Elizabeth Barsotti [1, 2, a], Ana Correia [1, 2, a], Albert Cardona [1, 2] ✉

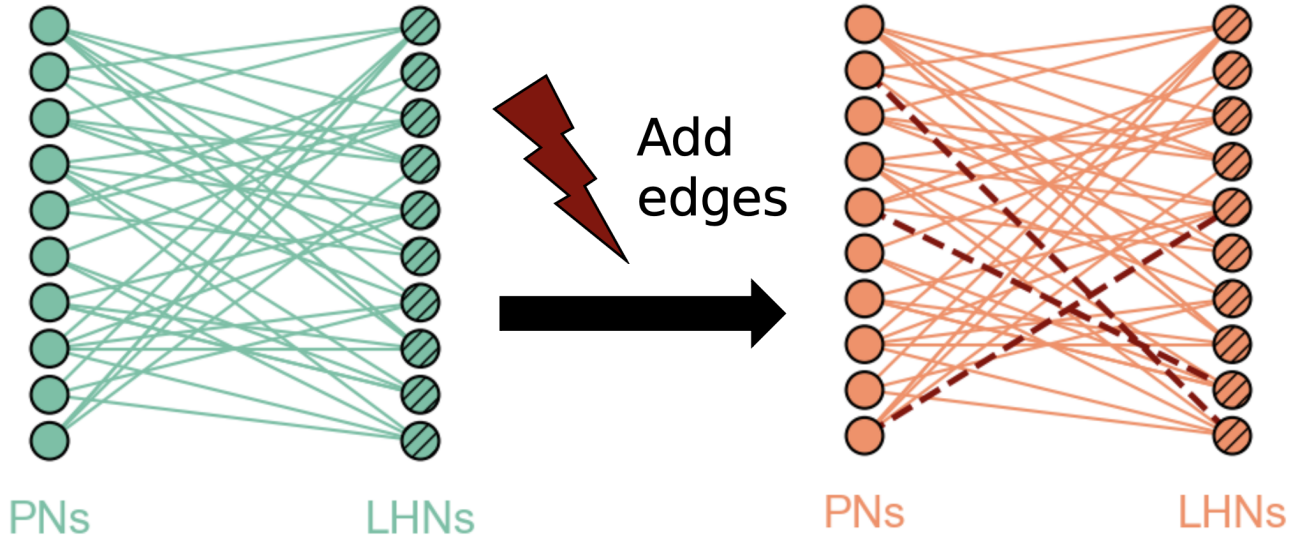
> With comparative connectomics, the search for neural circuit architectures common across species or independently converged into an optimal layout is now possible.

Why is comparative connectomics hard?

- Collecting the data is still a large effort...
- But how do we even compare connectomes once we have them?

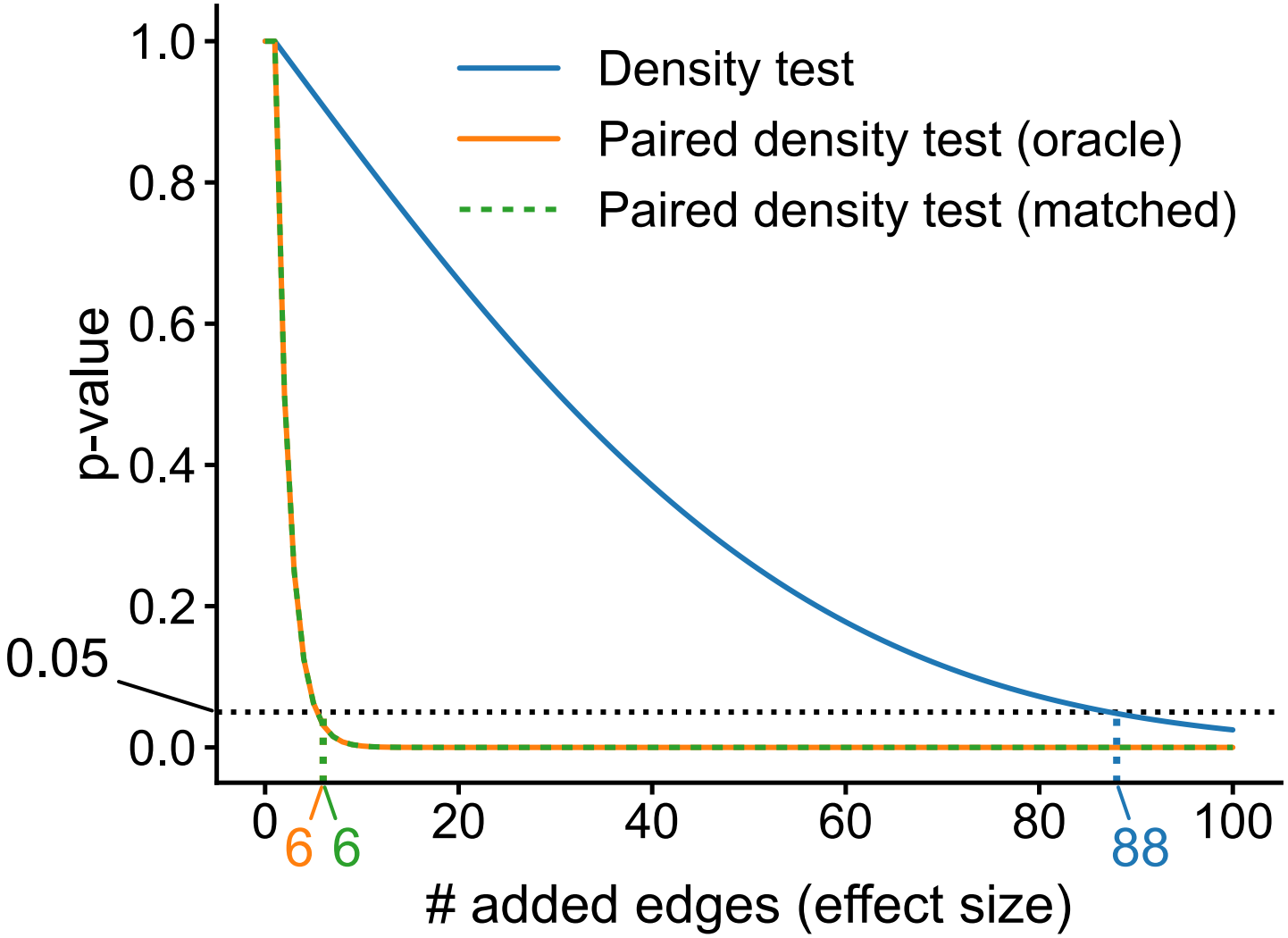
How do we know whether a proposed experiment could even *hope* to answer our questions? How powerful is comparative connectomics?

A hypothetical difference we want to detect...



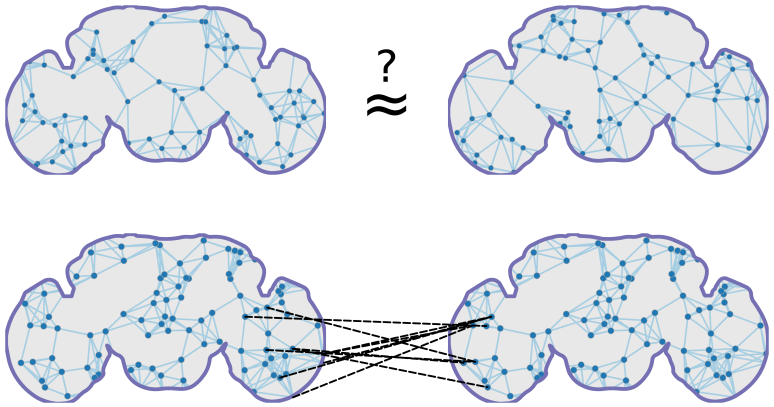
- Start from some subgraph in the connectome, A
- Perturb a copy of it, B (add edges)
- Test for differences between A and B

Pairs facilitate more powerful tests



Summary

- Characterized "feedforwardness" of this connectome
- Estimated cell types by connectivity



- Model-based network comparison enables testing (and refining) hypotheses about connectomes
- Graph matching can pair neurons across datasets

Aim to apply these (and other) tools to:

- Inform the design of future comparative experiments,

- Make inferences from connectome comparisons!

References

Winding, M. & Pedigo, B.D. et al. The connectome of an insect brain. bioRxiv 2022.11.28.516756 (2022).

Pedigo, B. D. et al. Generative network modeling reveals quantitative definitions of bilateral symmetry exhibited by a whole insect brain connectome. bioRxiv 2022.11.28.518219 (2022).

Pedigo, B. D. et al. Bisected graph matching improves automated pairing of bilaterally homologous neurons from connectomes. Network Neuroscience (2022).

Code

github.com/microsoft/graspologic

github.com/neurodata/maggot_models

github.com/neurodata/bilateral-connectome

github.com/neurodata/bgm

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Team


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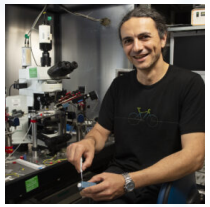
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