

Tools for comparative connectomics: case studies from two sides of a larval *Drosophila* brain

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 [@bdpedigo](#) (Github)

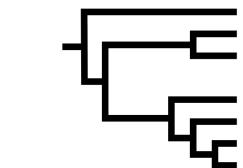
 [@bpedigod](#) (Twitter)

 bpedigo.github.io

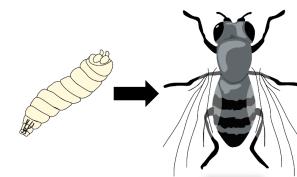
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These slides at:

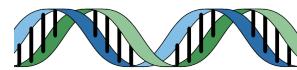
Many goals of connectomics involve linking connectome to other properties



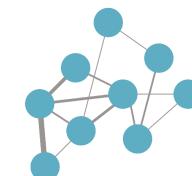
Evolution



Development



Genome



Connectome



Behavior



Activity

Comparative connectomics as a potential solution?

Connectome ↔ memory

...the acquisition of wiring diagrams across multiple individuals will yield insights into how experiences shape neural connections.

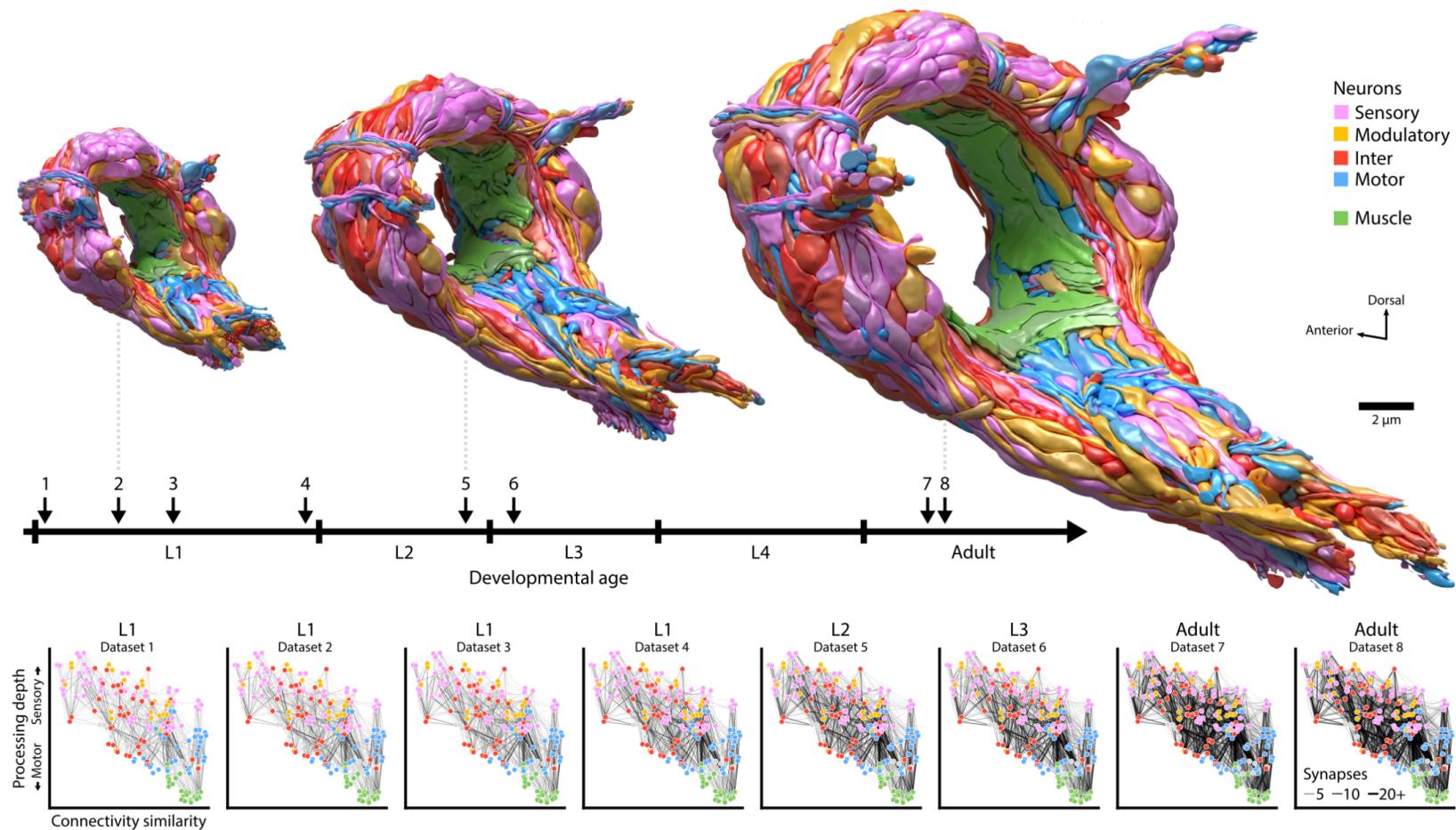
Emphasis added

Connectome \leftrightarrow {development, genetics}

... we selectively altered the location or activity of [...] neurons and generated new EM volumes of the manipulated samples **to investigate the effects on connectivity.**

Emphasis added

Connectome ↔ development



Why is comparative connectomics hard?

Collecting the data is still a large effort...

But how do we even compare connectomes?

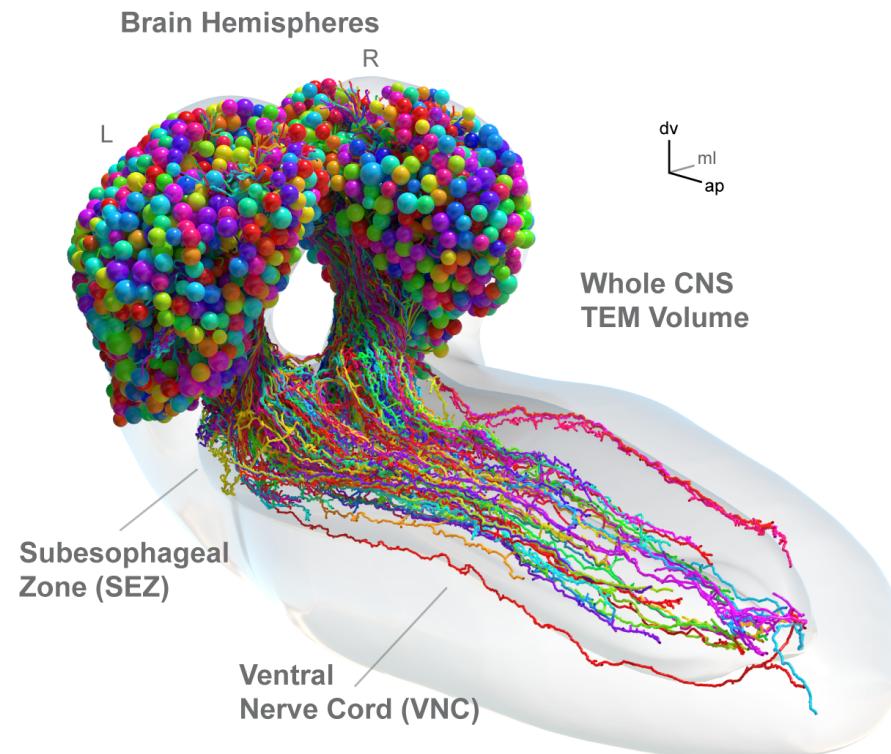
- Data are networks
 - Data are networks with rich attributes
- Data are noisy
 - "Experimental noise"
 - "Developmental noise"
- Data are big (and getting bigger)

Outline

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

Larval *Drosophila* brain connectome

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~3k neurons, ~550K synapses

Both hemispheres

Bilateral symmetry

"This brain is bilaterally symmetric"

-Neuroscientists

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

-Us

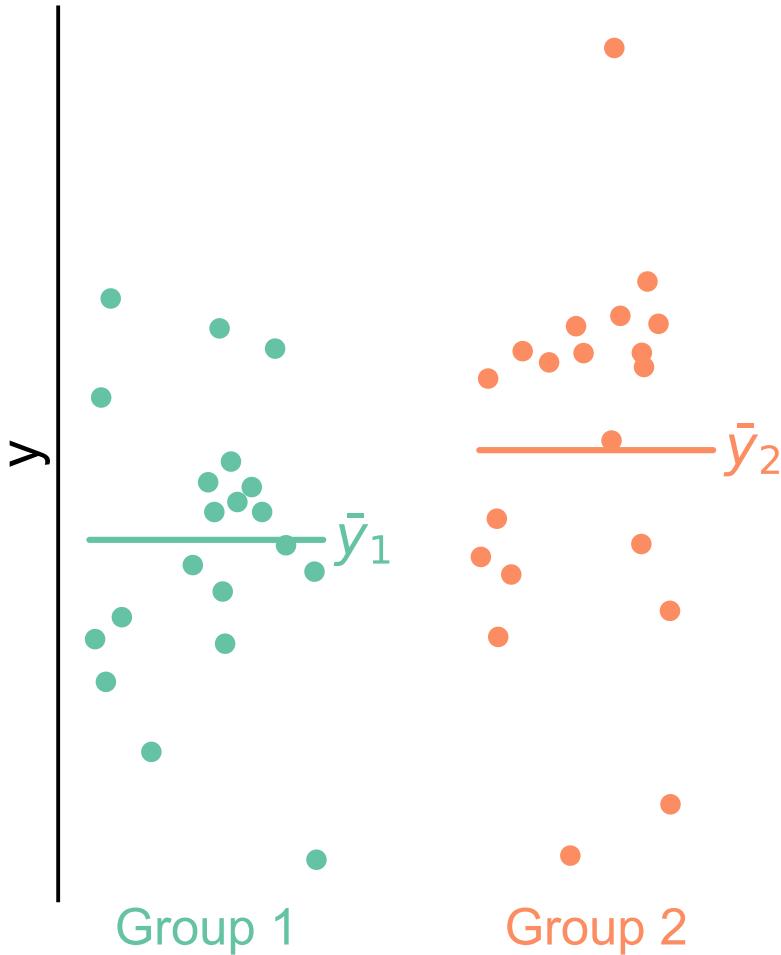
Are the <p> </p> different?

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Are these populations different?

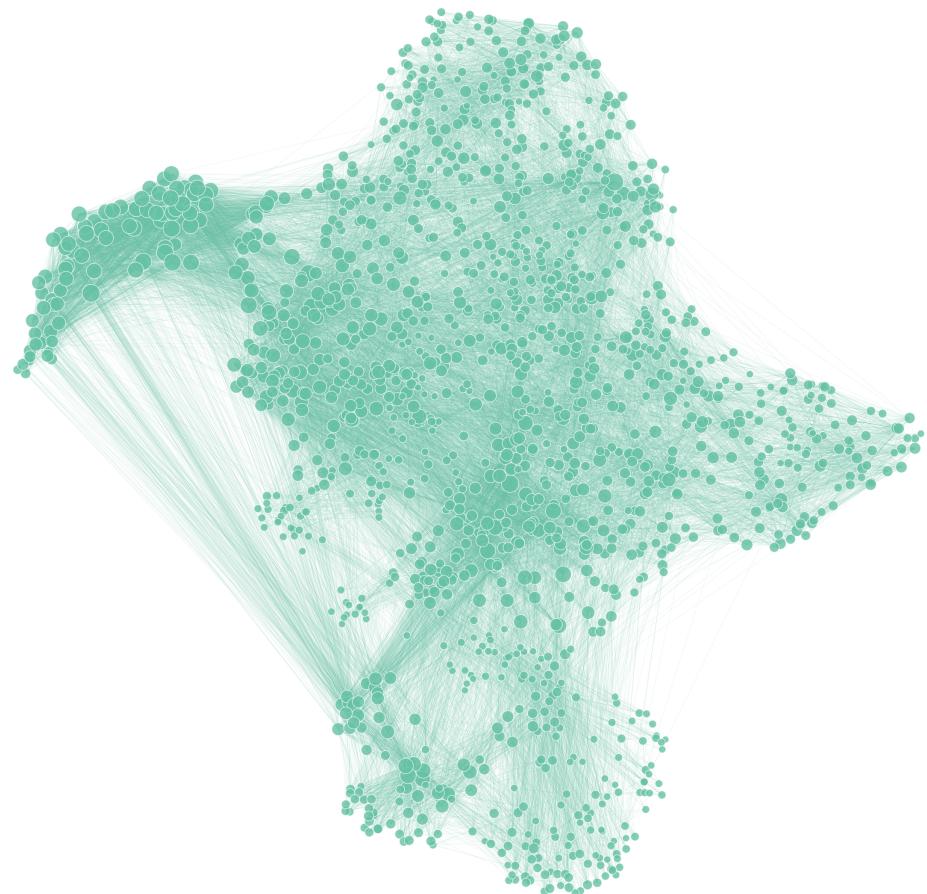
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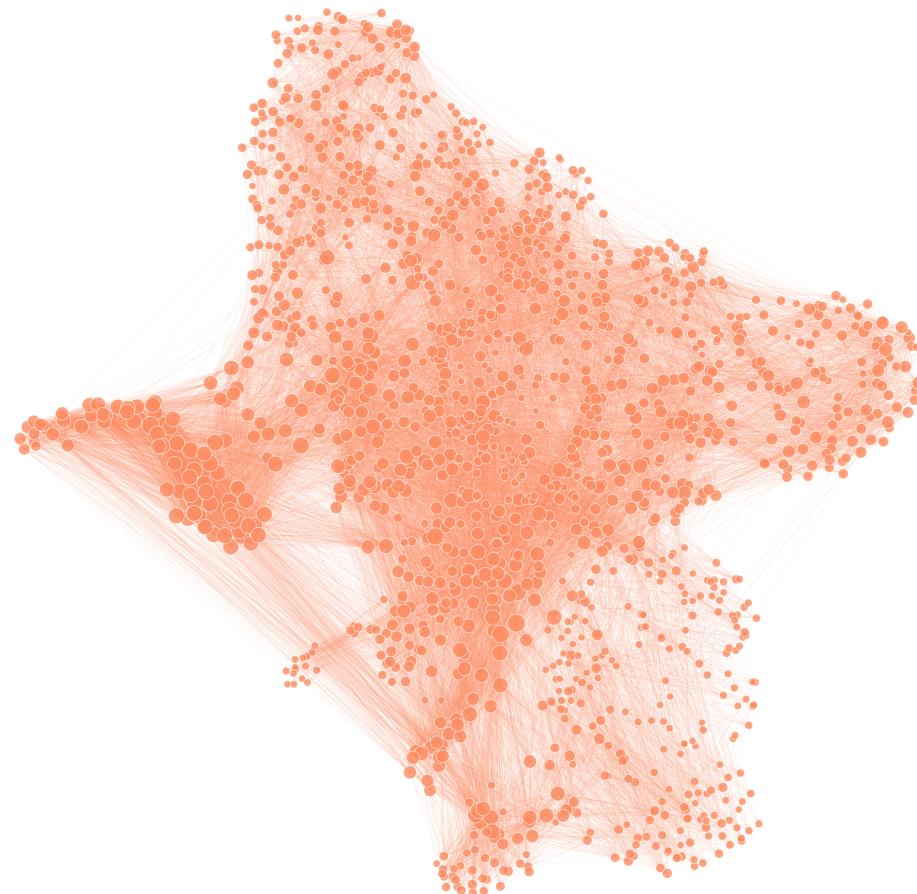
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Are these *networks* different?

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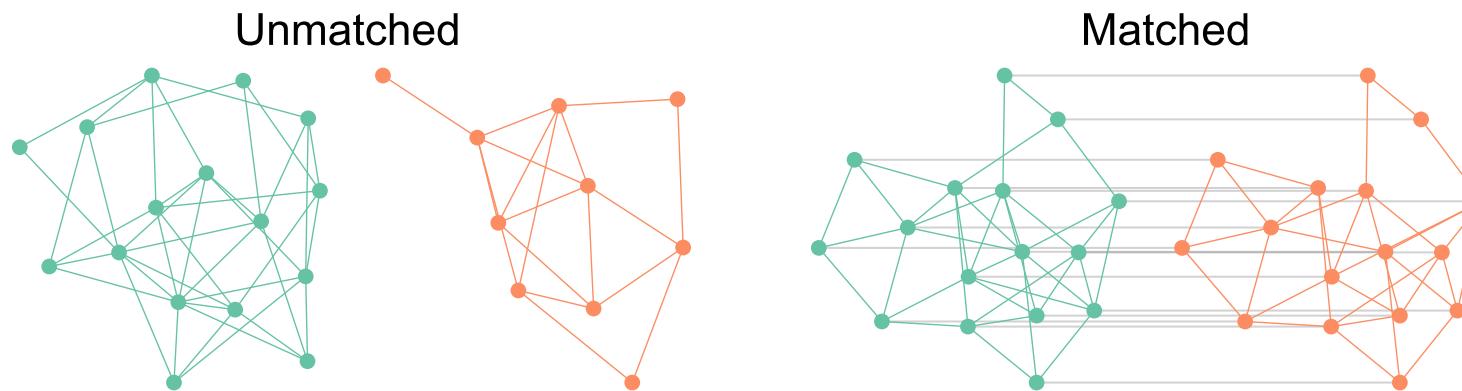
Left



Right

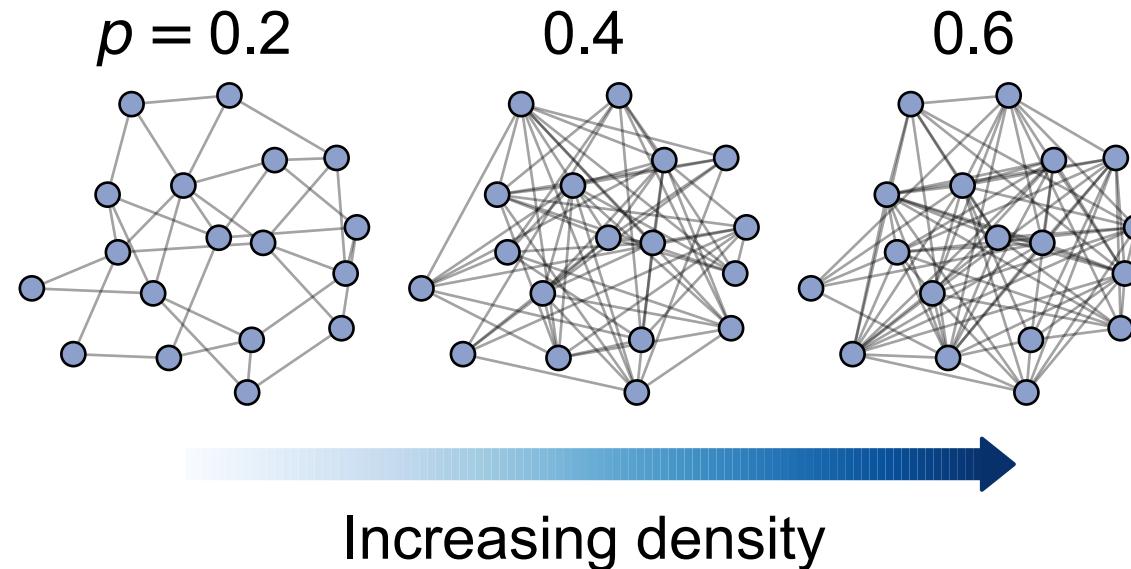
Assumptions

- We 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 → left ` and ` right → right ` (*ipsilateral*) connections only.
- Not going to assume any nodes are matched



Erdos-Renyi model

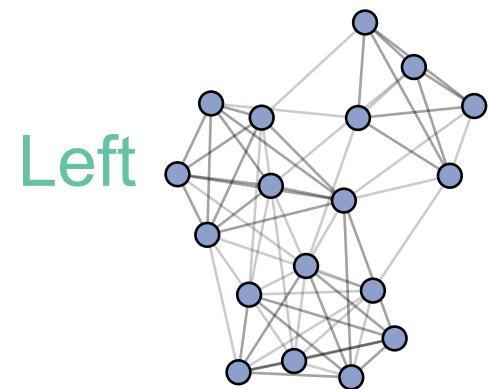
- All edges are independent
- All edges generated with the same probability, p



We detect a difference in density

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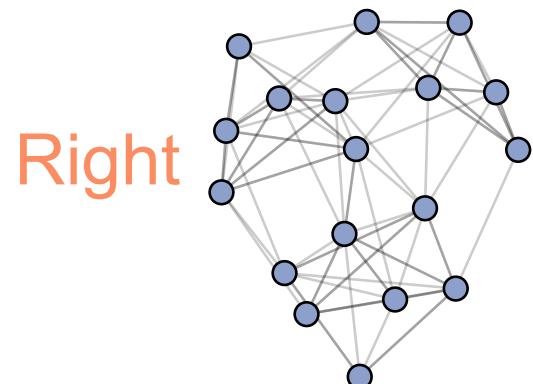
Compute global
connection density



Left

Compare ER
models

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



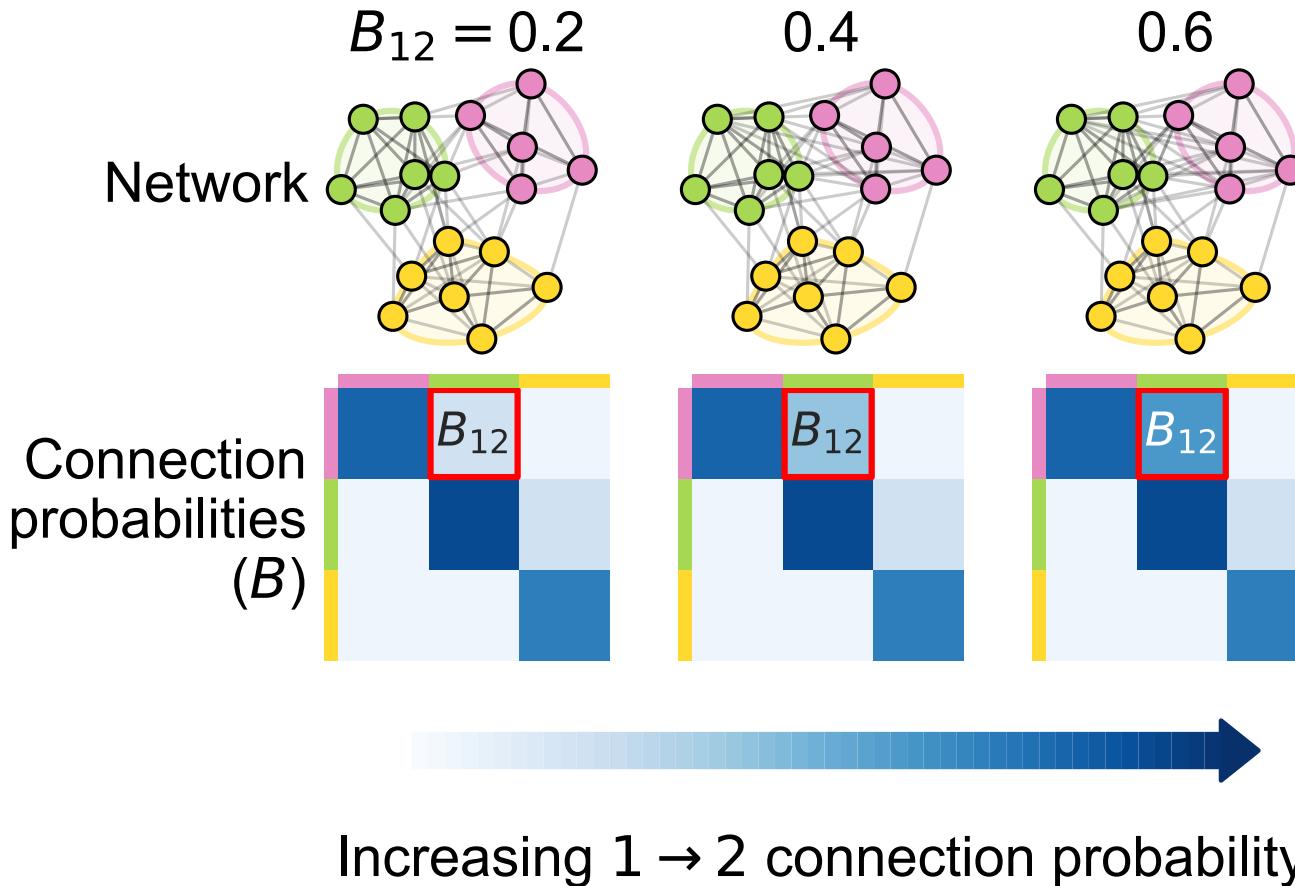
Right

$$\begin{aligned} H_0: p^{(L)} &= p^{(R)} \\ H_A: p^{(L)} &\neq p^{(R)} \end{aligned}$$

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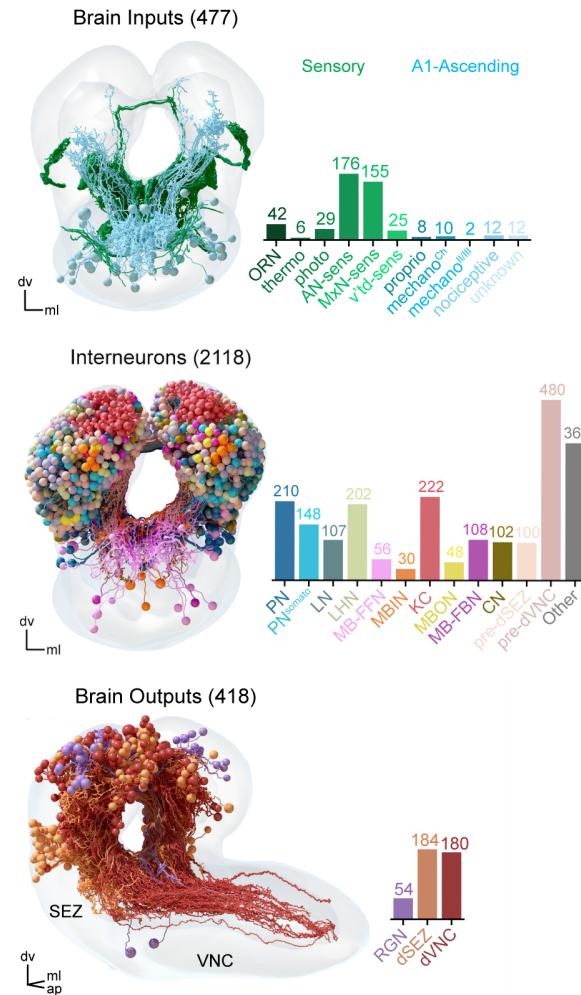
Stochastic block model

Edge probabilities are a function of a neuron's group



Connection probabilities between groups

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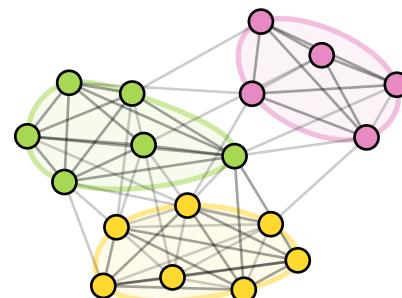


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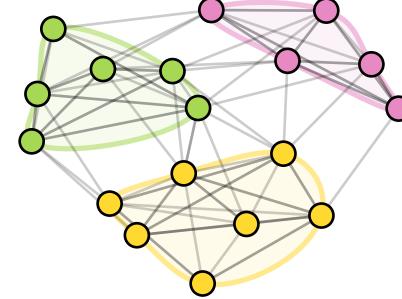
Group-based testing

Group neurons

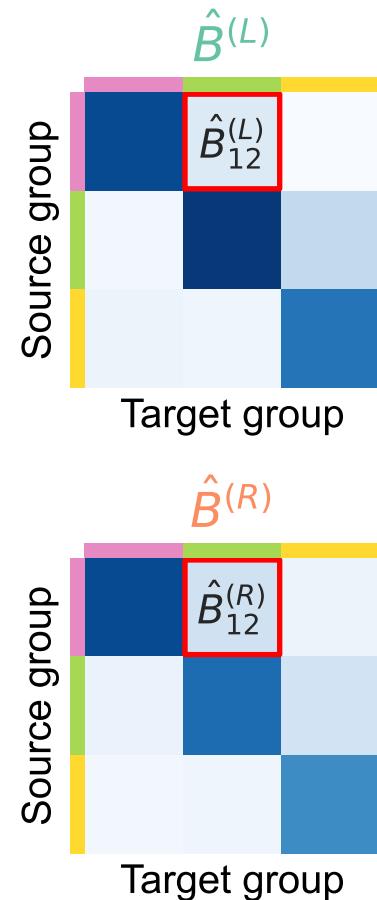
Left



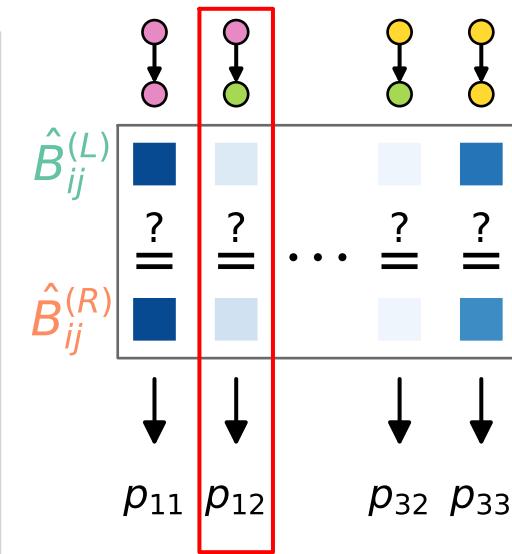
Right



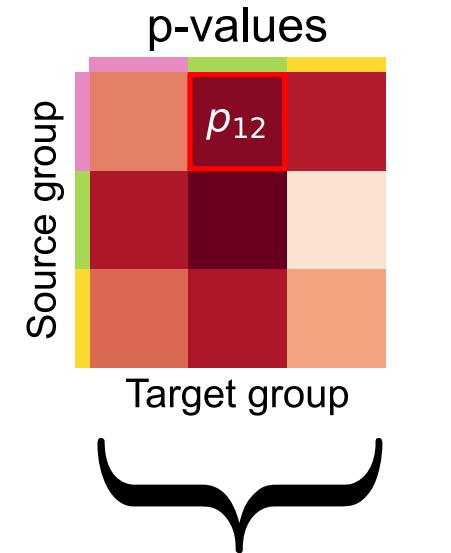
Estimate group connection probabilities



Compare probabilities, compute p-values



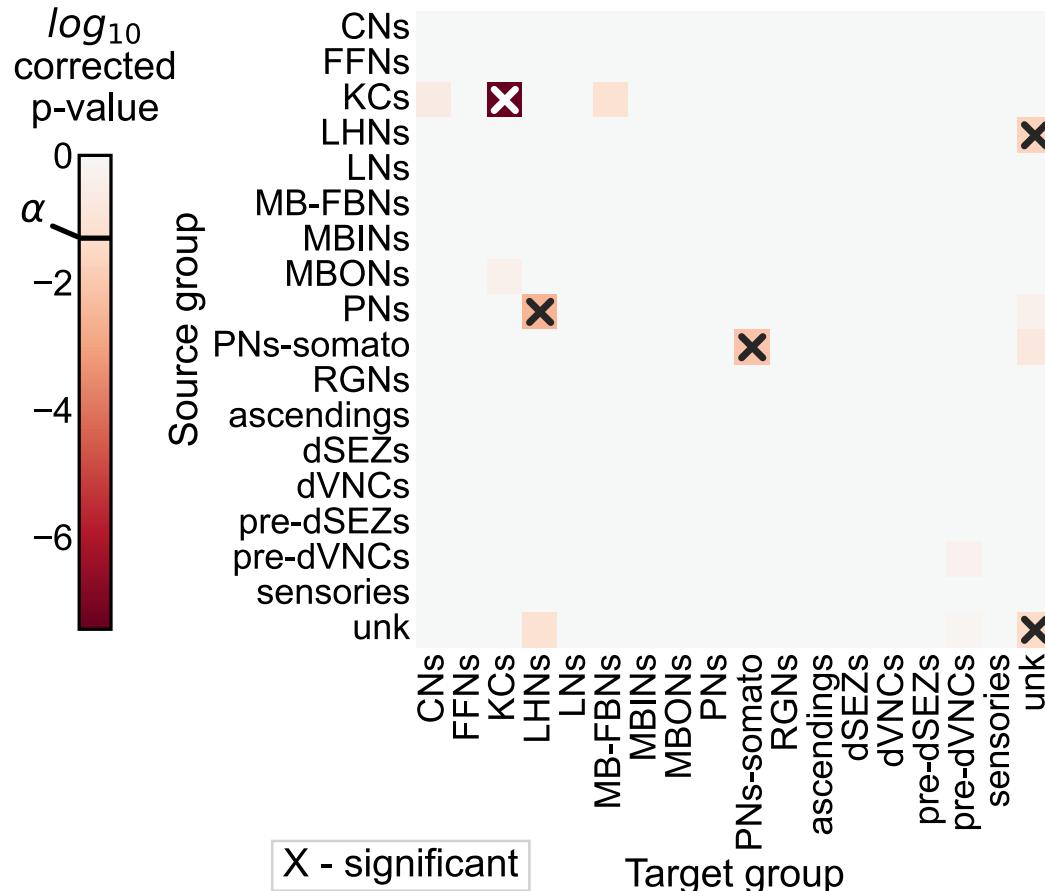
Combine p-values for overall test



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

Detect a difference in group connection probabilities

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- After multiple comparison, find 5 group-to-group connections which are significantly

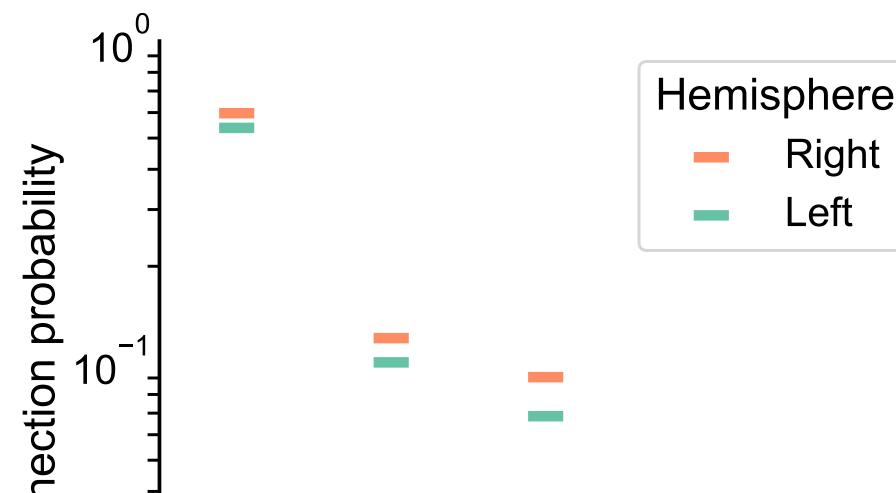
Should we be surprised?

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- 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, $\frac{p^{(L)}}{p^{(R)}}$

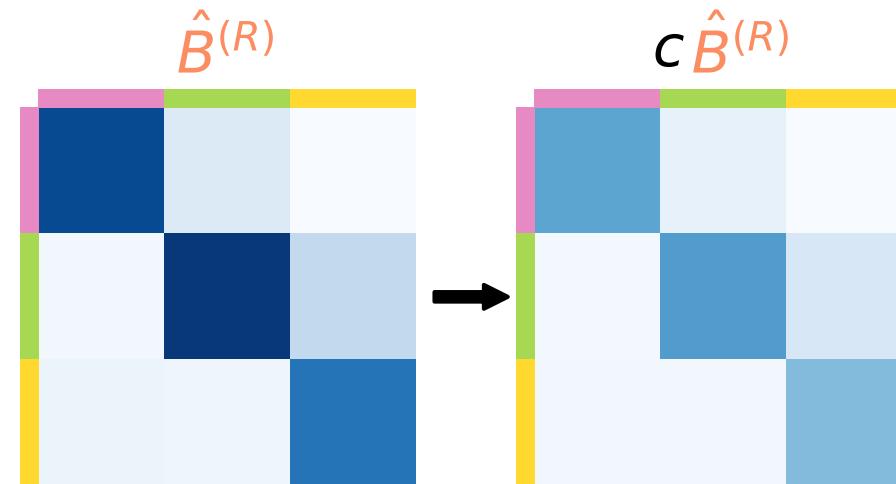
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After adjusting for density, differences are in KCs

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Scale connection probabilities
to match densities



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

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\log_{10}
corrected
p-value

CNs

FFNs

KCs

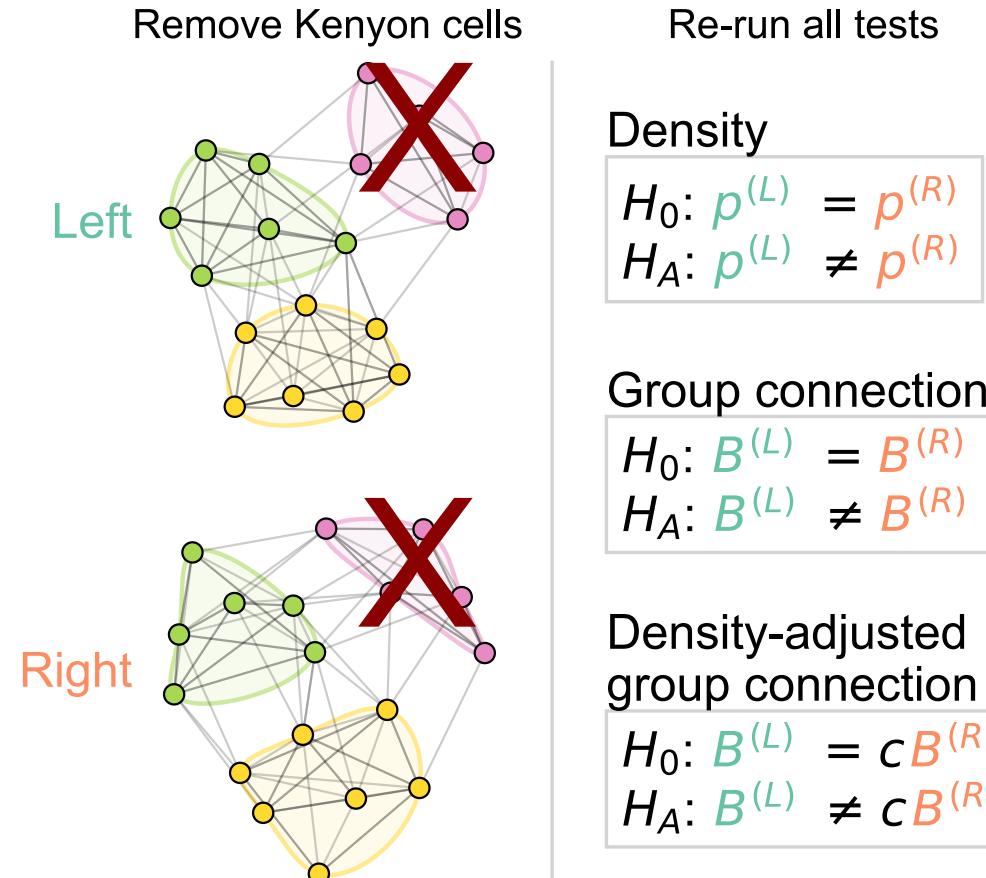
X

X

●

When we remove KCs...

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- Density test:

To sum up...

"This brain is bilaterally symmetric"

-Neuroscientists

Depends on what you mean...

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With Kenyon cells

Model	H_0 (vs. $H_A \neq$)	p-value

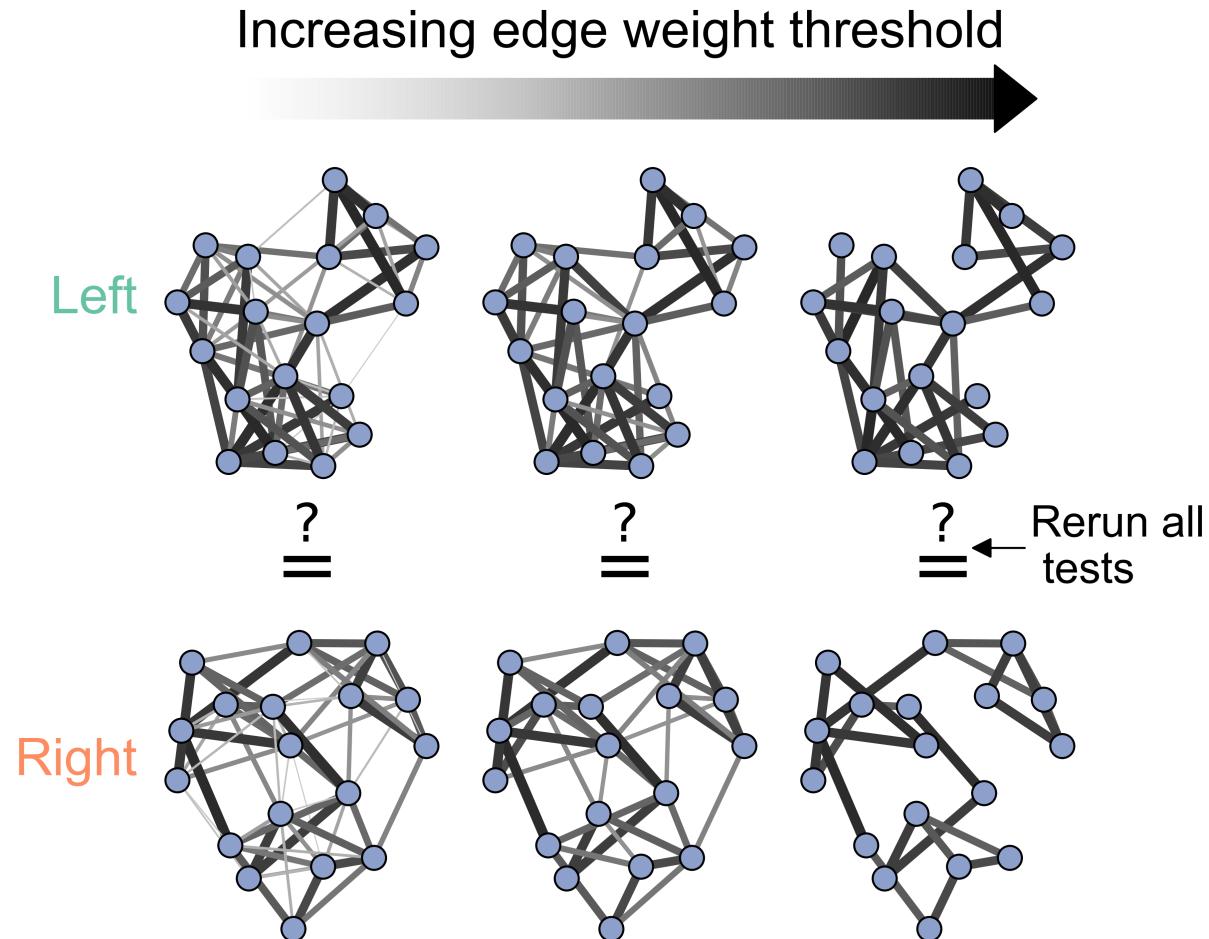
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Without Kenyon cells

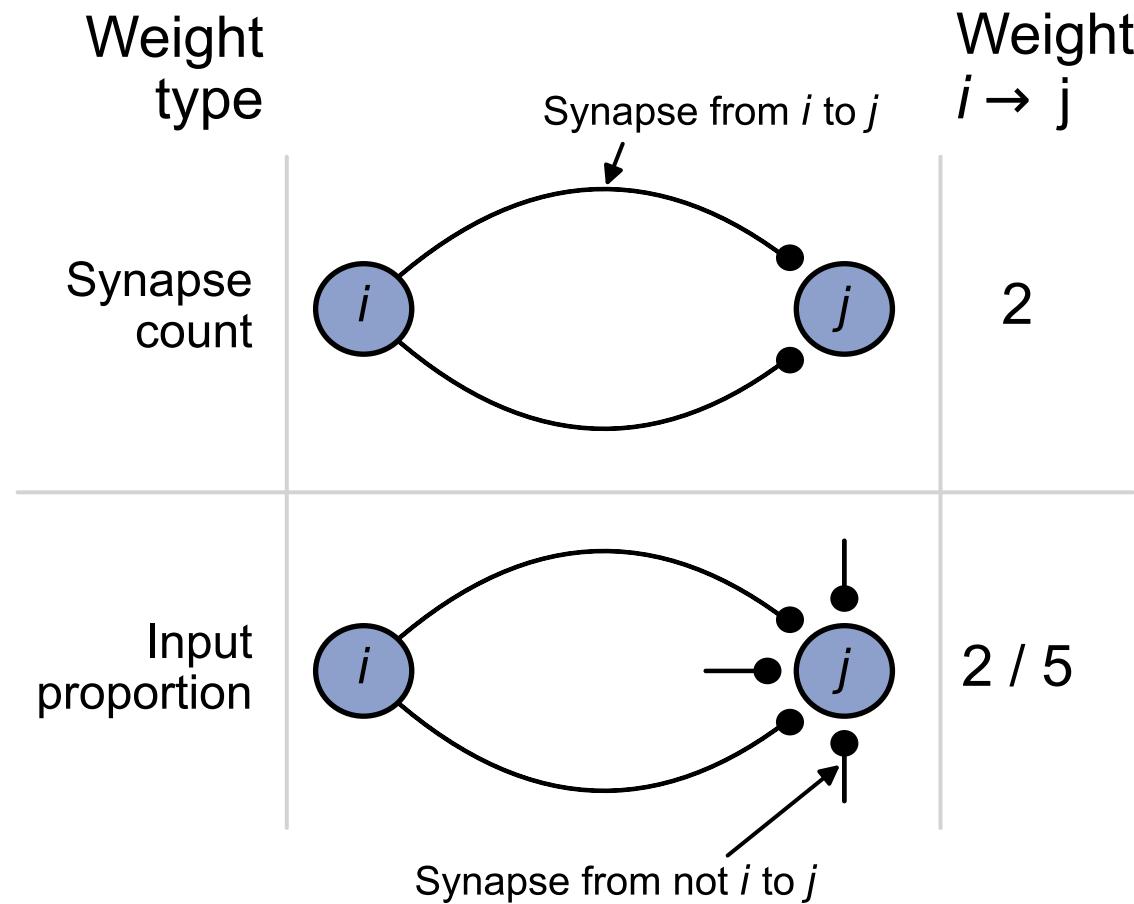
Model	H_0 (vs. $H_A \neq$)	p-value

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Examining the effect of edge weights



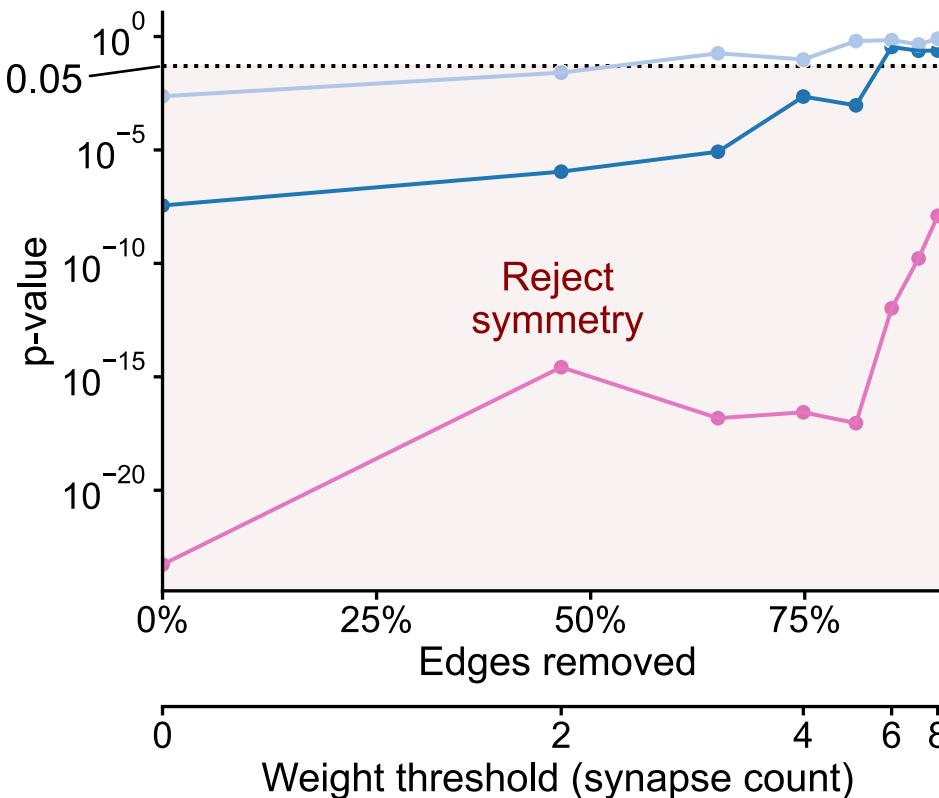
What is an edge weight anyway?



Some high-edge weight networks show no asymmetry

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Synapse count thresholding



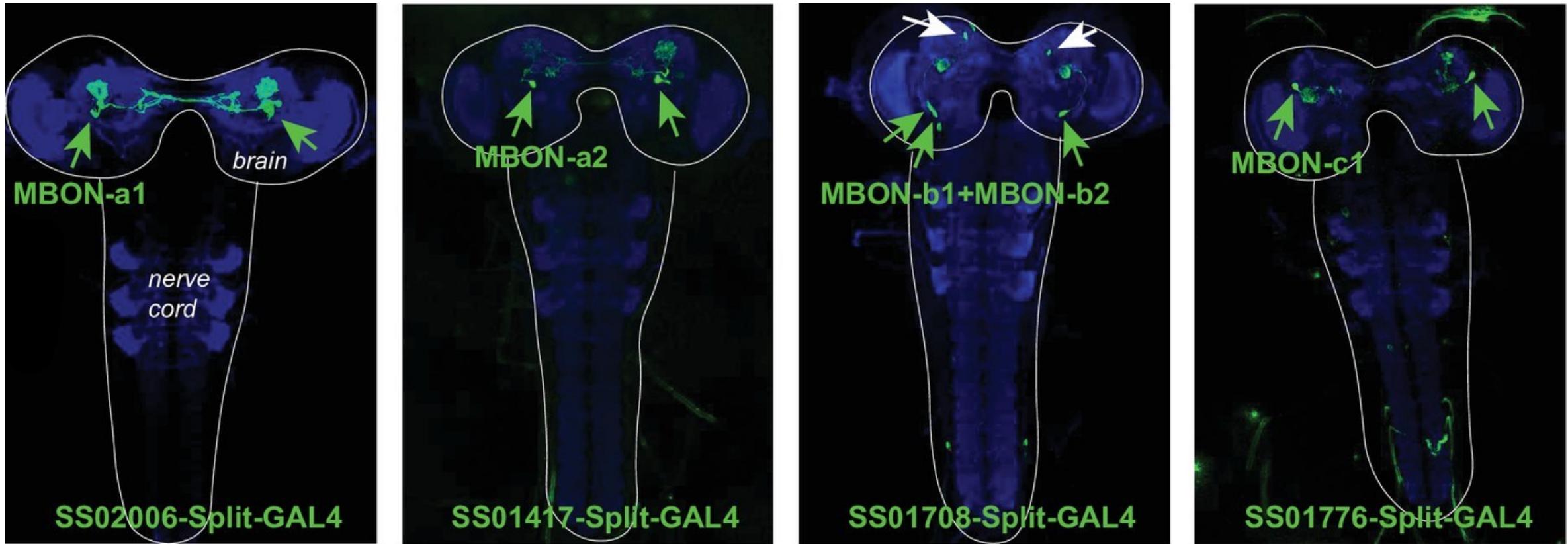
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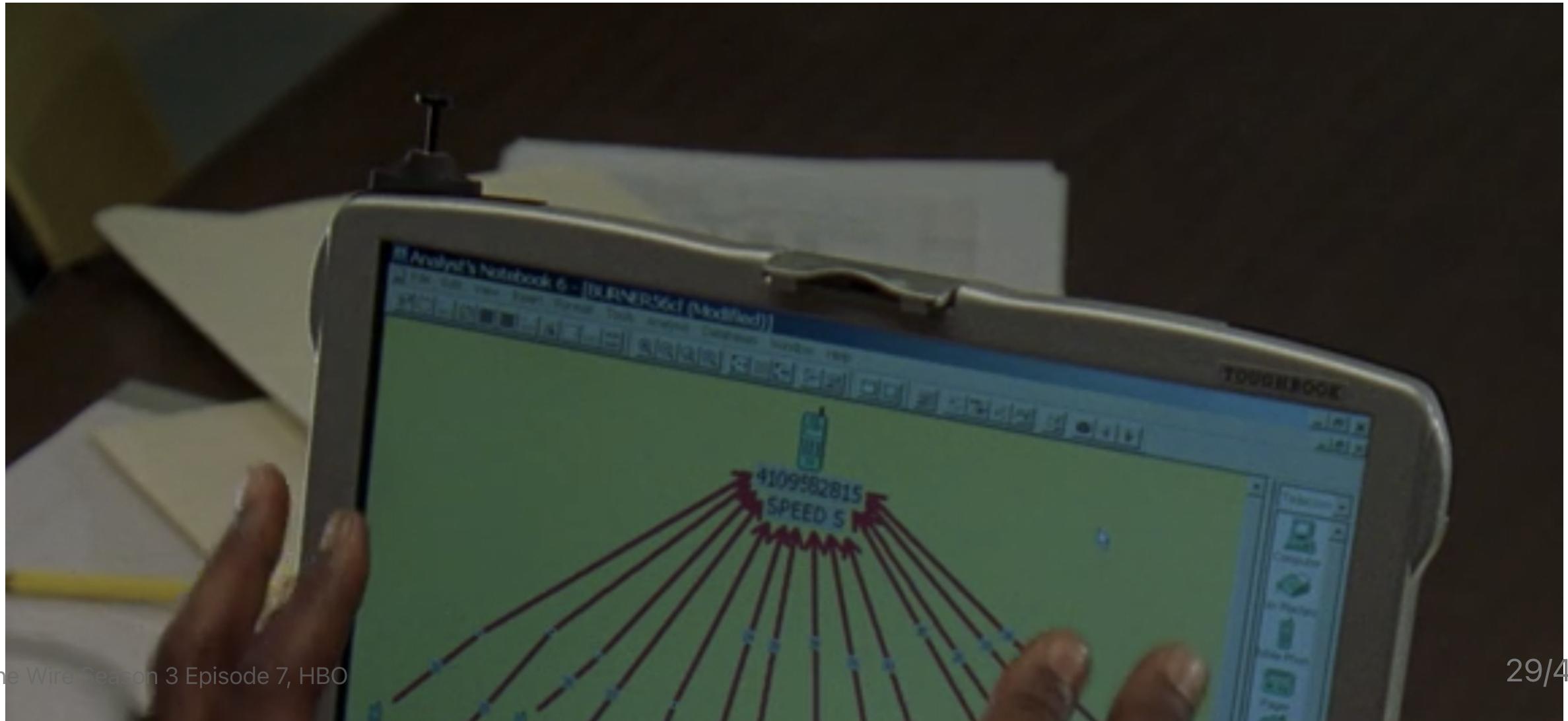
Bilaterally homologous neuron pairs

We believe a matching exists!

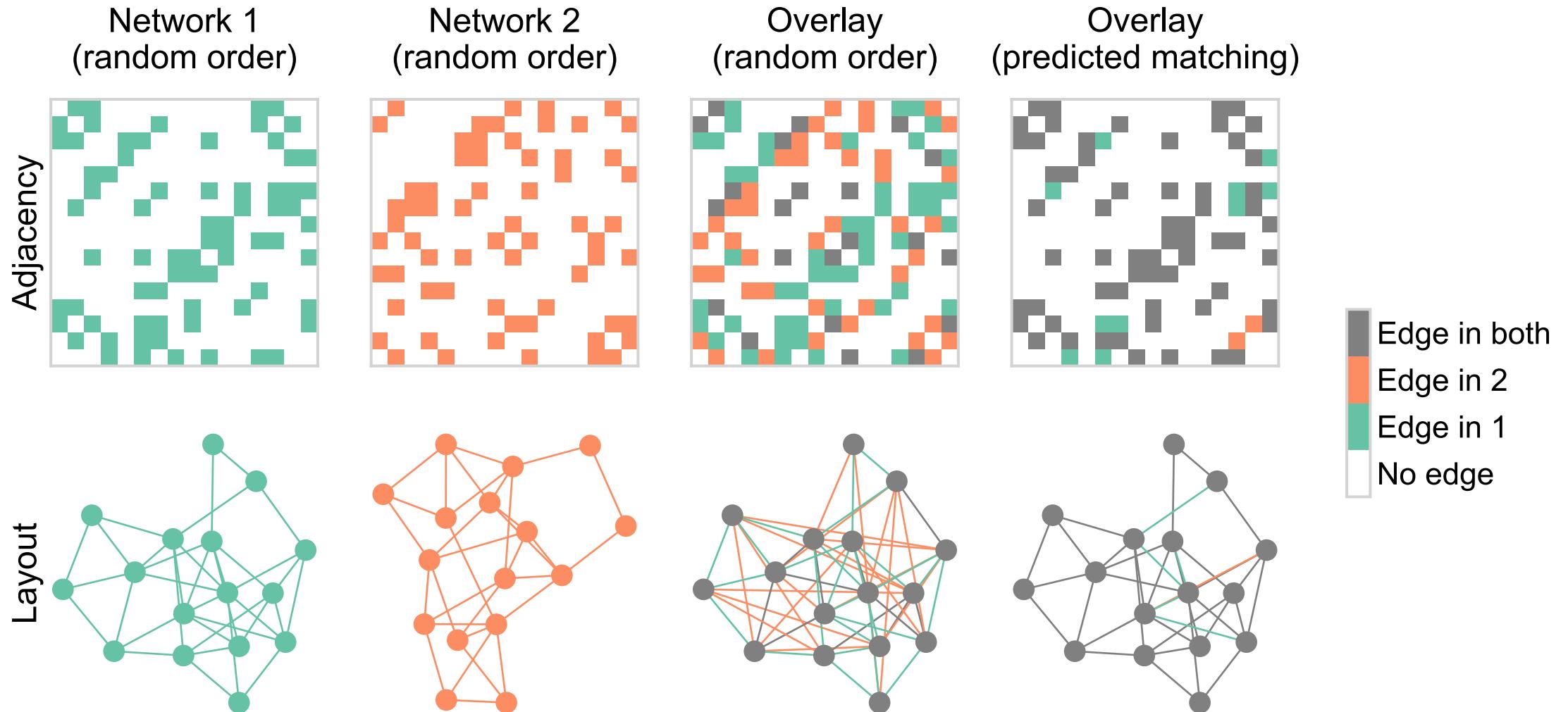


Can we use network structure to predict this pairing?

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What is graph matching?



How do we measure network overlap?

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$$\min_{P \in \mathcal{P}} \|A - \underbrace{PBP^T}_{\text{distance between adj. mats.}}\|_F^2$$

reordered B

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

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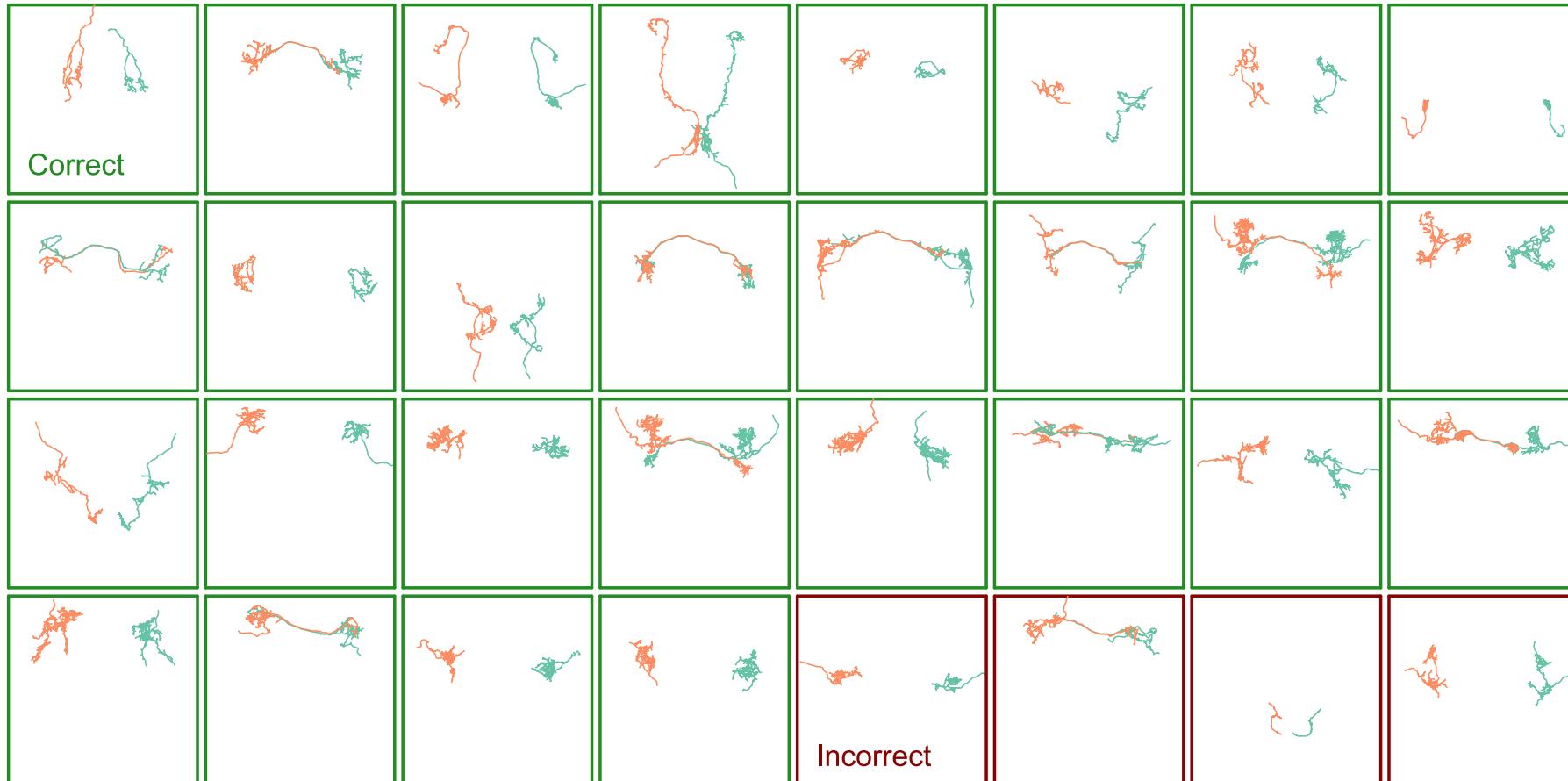
- Measures the number of edge disagreements for unweighted networks,
- Norm of edge disagreements for weighted networks

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How do we do graph matching?

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

Matching (by connectivity only) performs fairly well



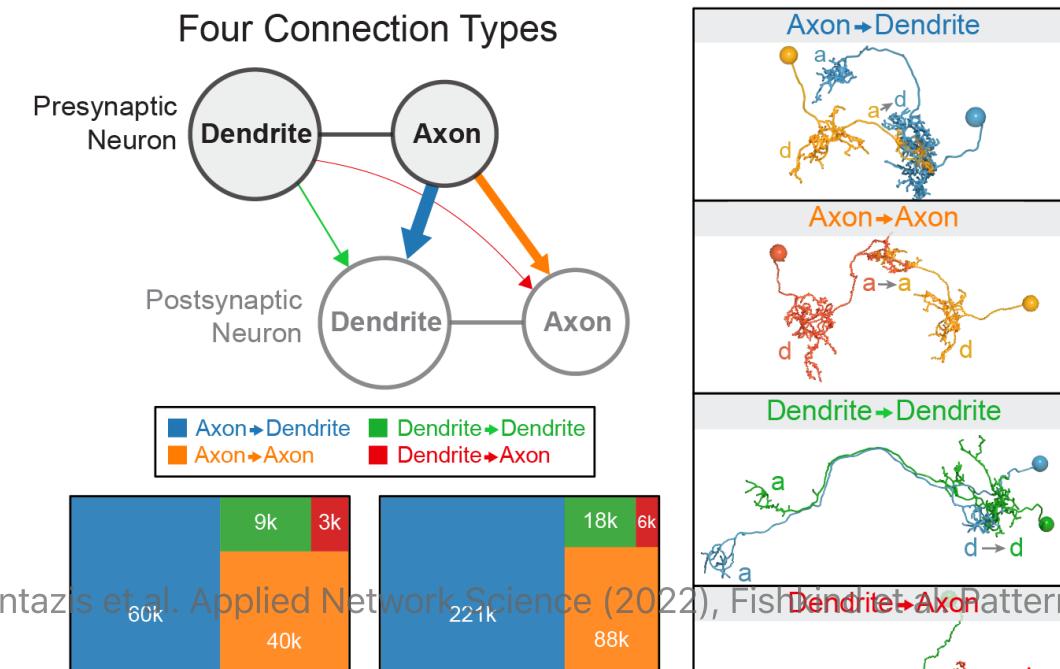
With "vanilla" graph matching: ~80% correct (according to expert annotator)

Many ways to try to improve on this...

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

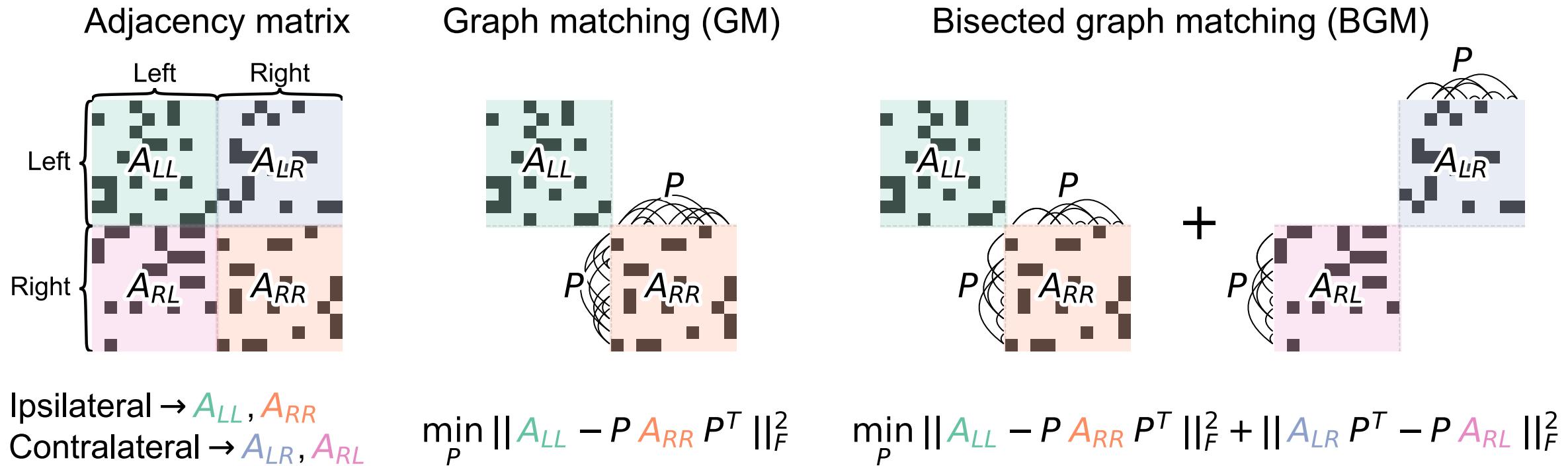
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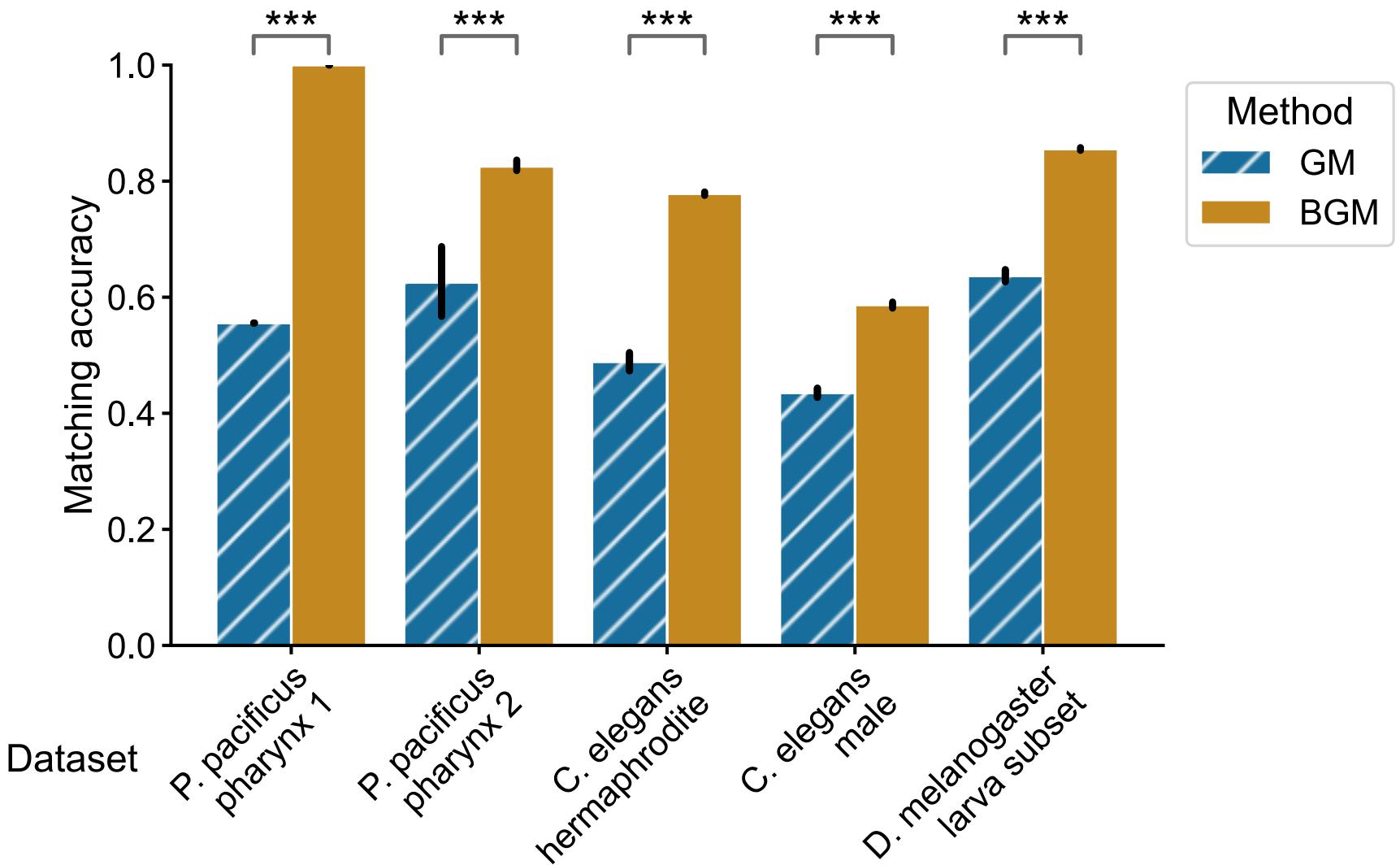
Thus far, we've not used the contralateral connections

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

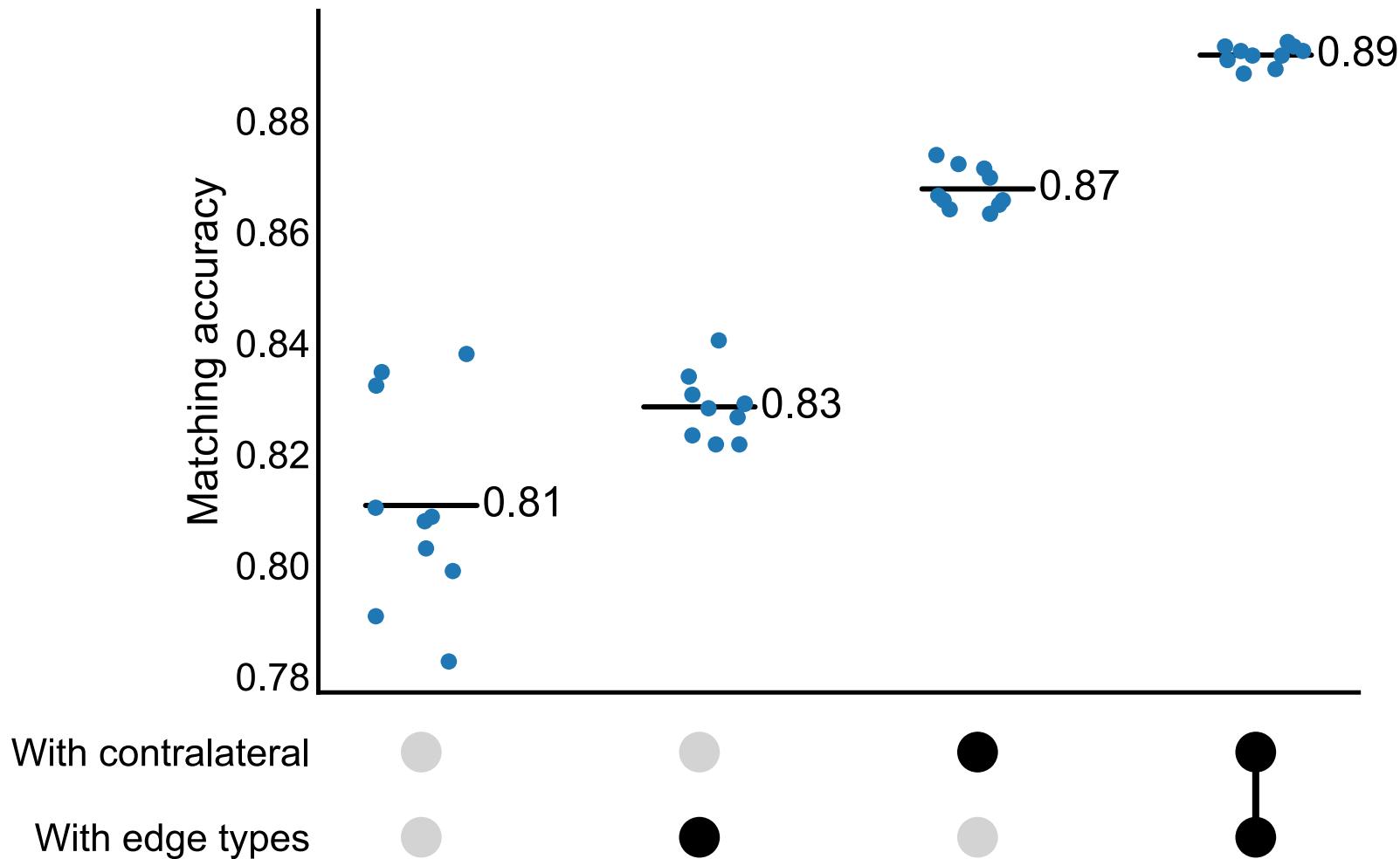
From graph matching to bisected graph matching



Contralateral connections are helpful!



Performance improvement on the full brain



Outline

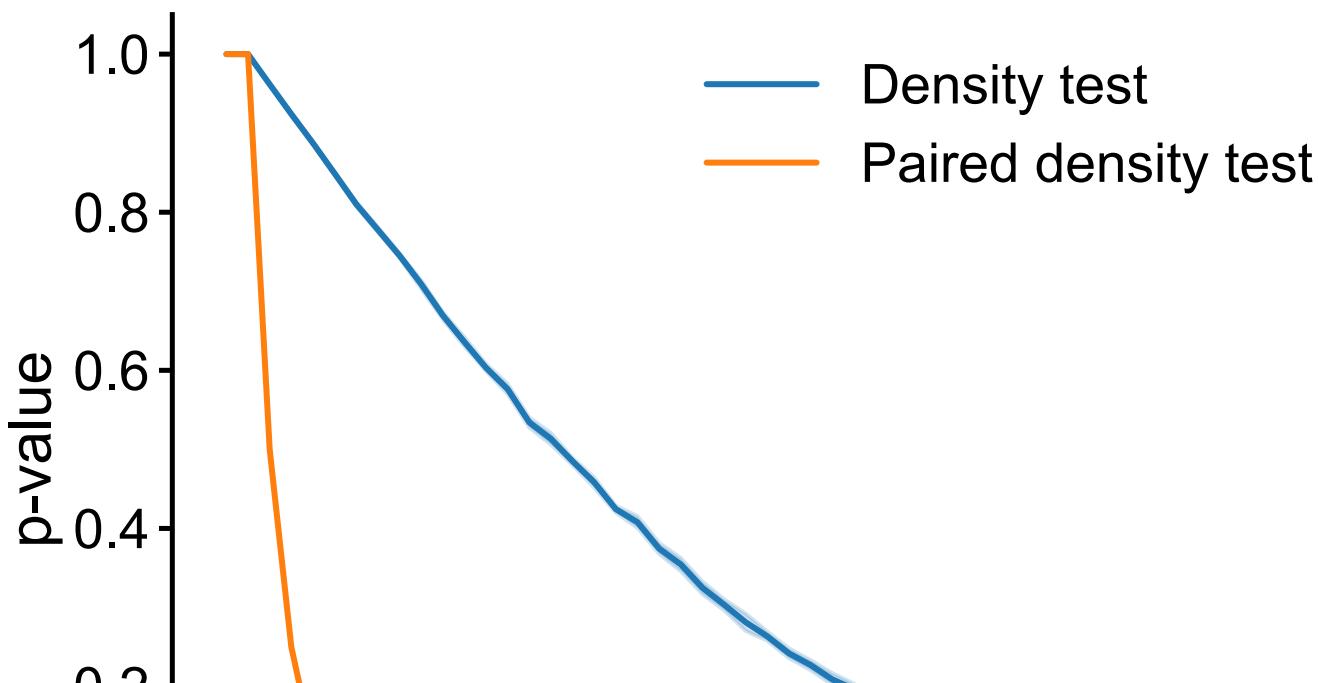
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Pairs facilitate more powerful tests

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- Generate an Erdos-Renyi network (A)
- Perturb a copy of it (B)
- Test for differences between A and B

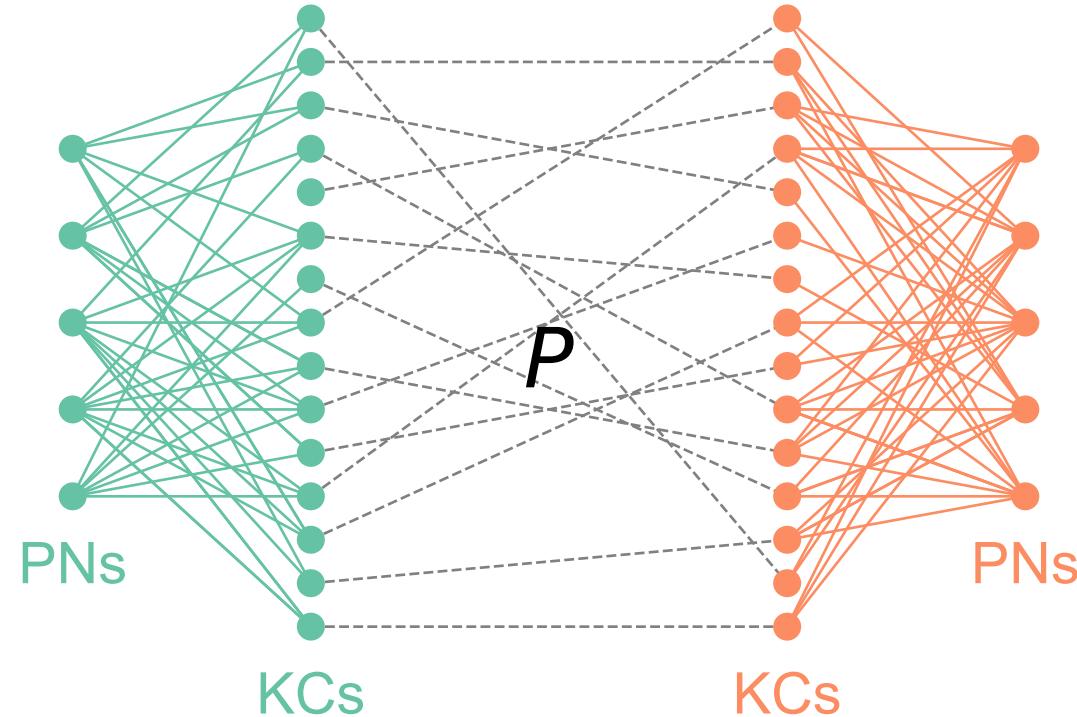
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Testing for "stereotypy" at the edge level

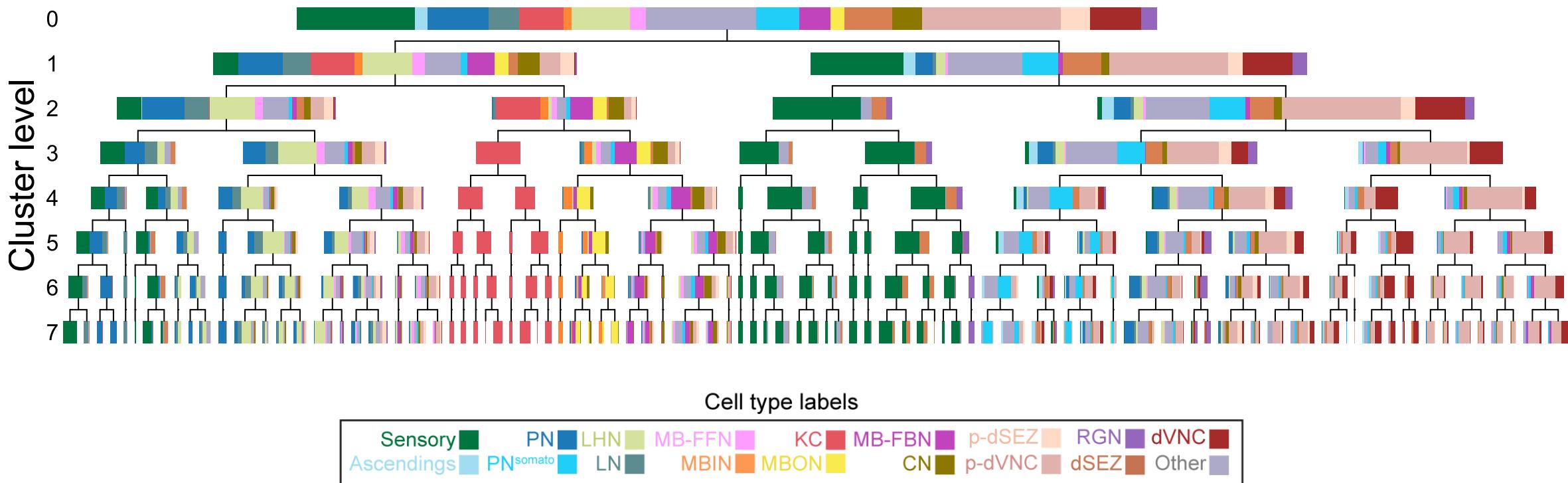
Is matching stronger than expected under some model of independent networks?

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Neurons clustered by connectivity using recursive spectral clustering

Where to stop splitting?

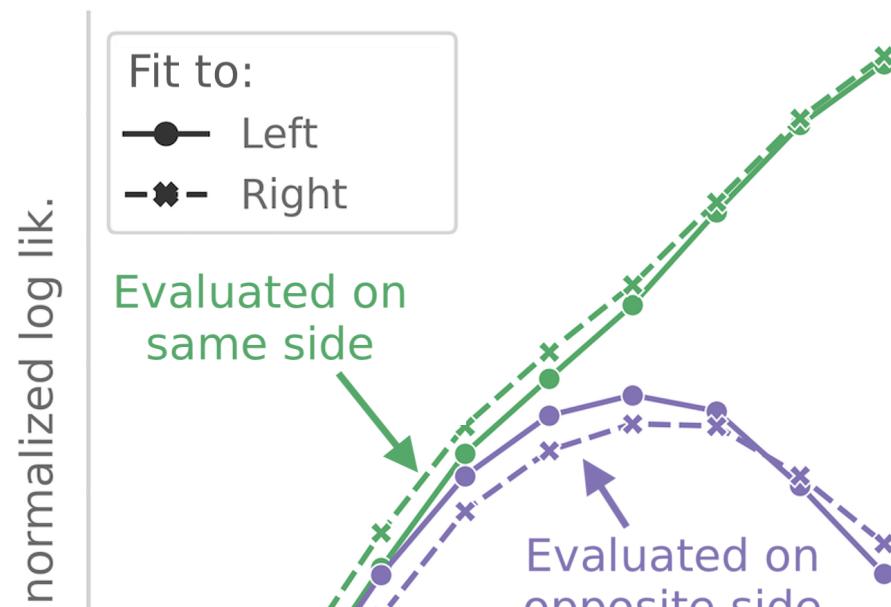


Using pairs and models to evaluate cell type groupings

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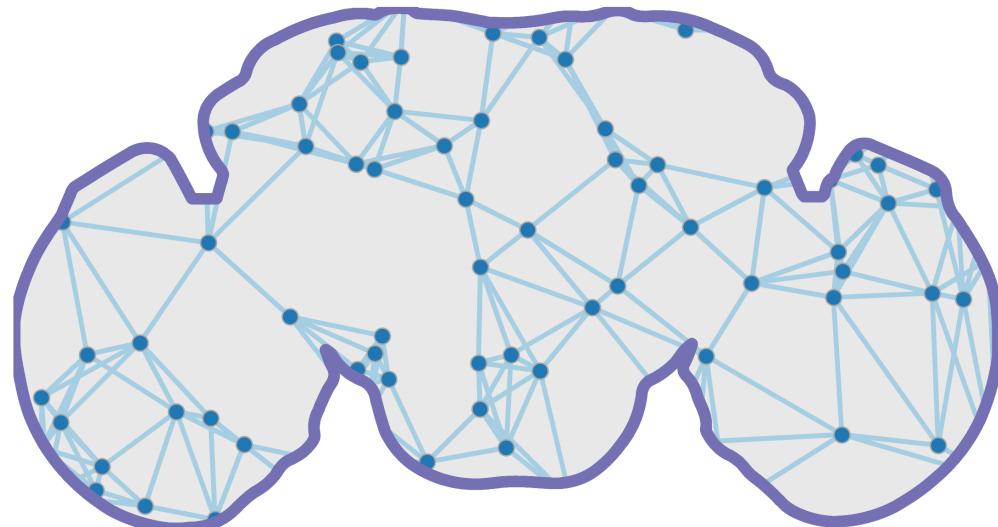
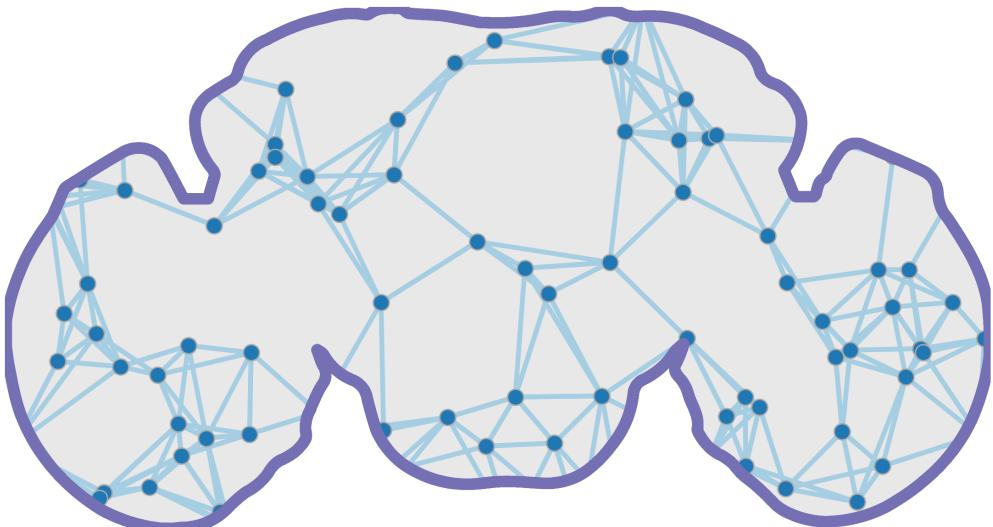
- Clustering nodes corresponds with inferring groups in a stochastic block model (DCSBM)...
- How well do these models generalize to the other side of the brain (let alone the next maggot)?

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Summary

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- Model-based network comparison enables testing (and refining) hypotheses about connectomes
 - We proposed a few tests, but just the beginning!

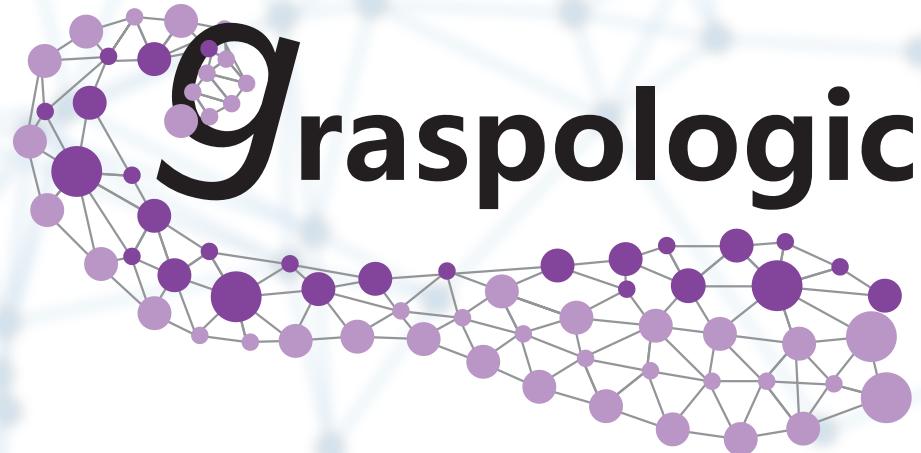
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How to use these (and other) tools?

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graspologic

github.com/microsoft/graspologic



downloads 139k

Stars 260

contributors 49

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Model-based testing

Acknowledgements

Team

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Michael Winding

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Mike Powell

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Questions?

Slides:

tinyurl.com/princeton-bilarva

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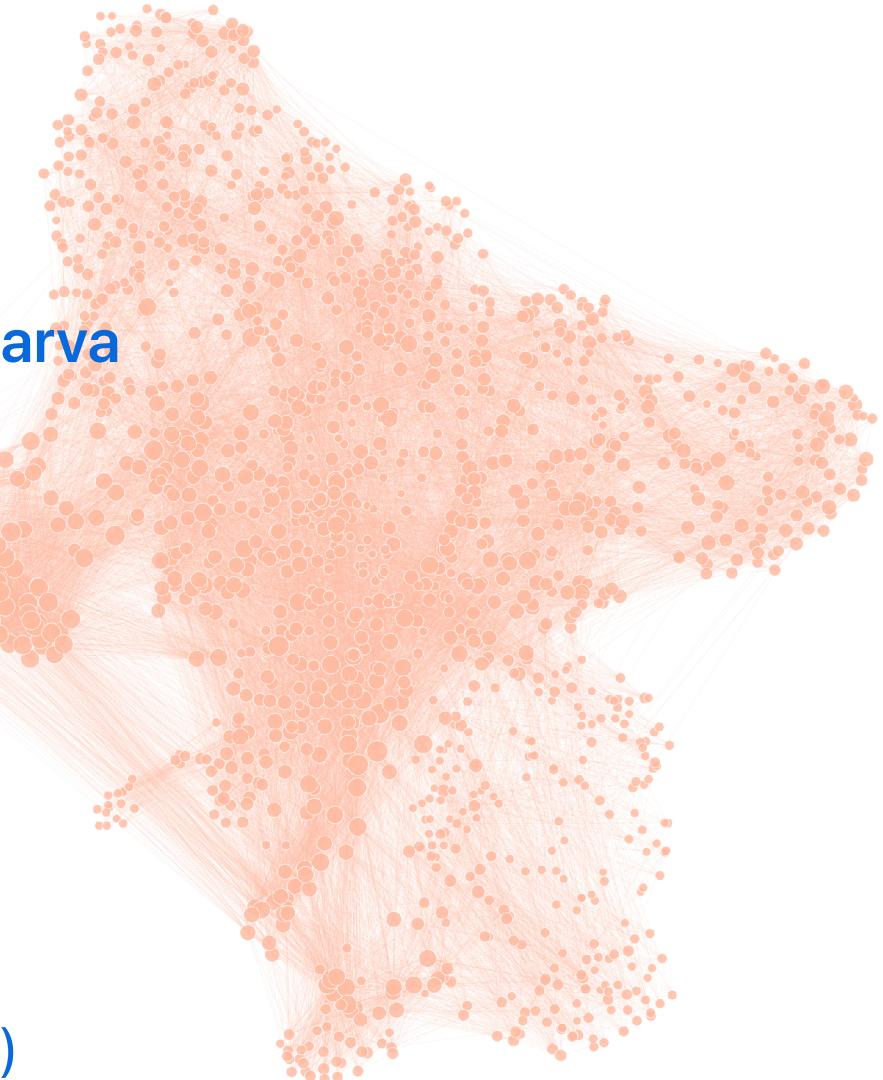
 [@bdpedigo](https://github.com/bdpedigo) (Github)

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Left



Right