

Investigating the connectome of a larval *Drosophila* brain

Benjamin D. Pedigo

(he/him)

[NeuroData lab](#)

Johns Hopkins University - Biomedical Engineering

 bpedigo@jhu.edu

 [@bdpedigo](#) (Github)

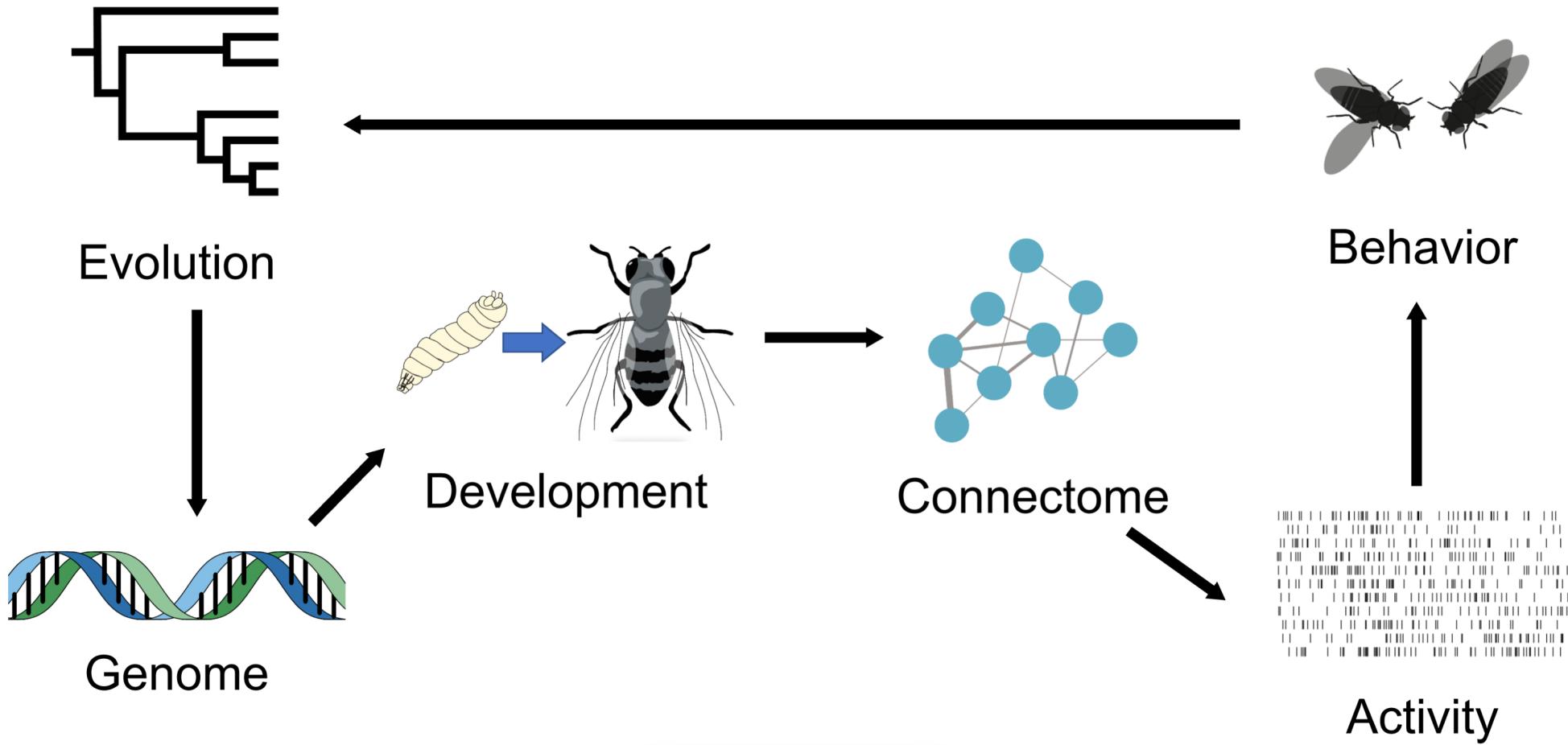
 [@bpedigod](#) (Twitter)

 bpedigo.github.io

These slides at:

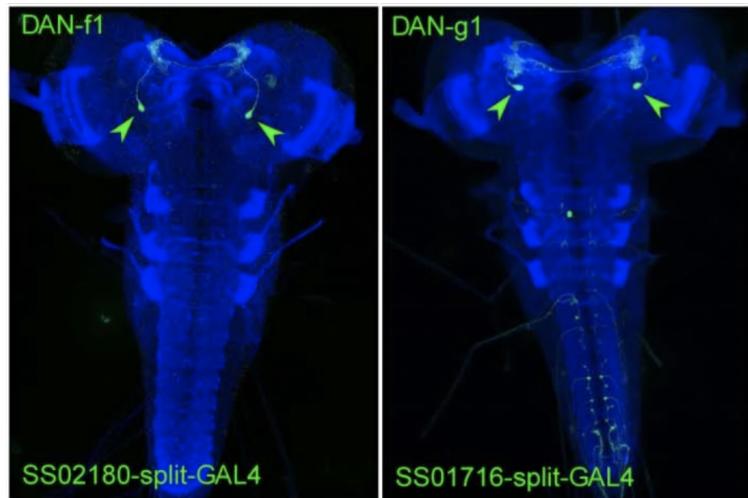


Many goals of connectomics involve linking the connectome to other properties



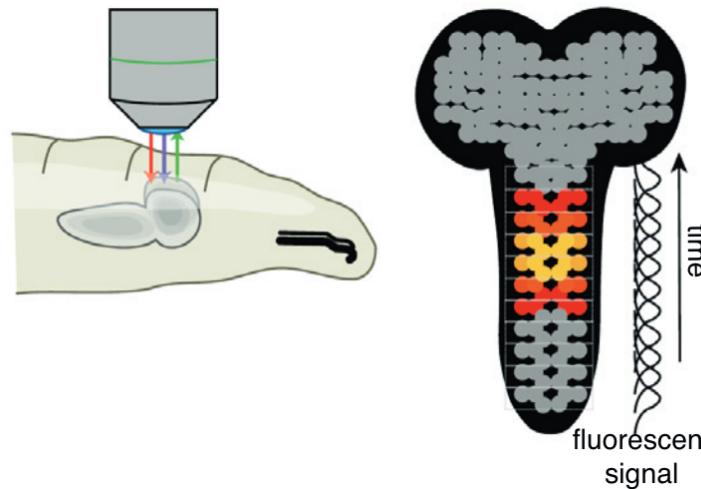
Larval *Drosophila* allows access to many properties, e.g.,

Genetics



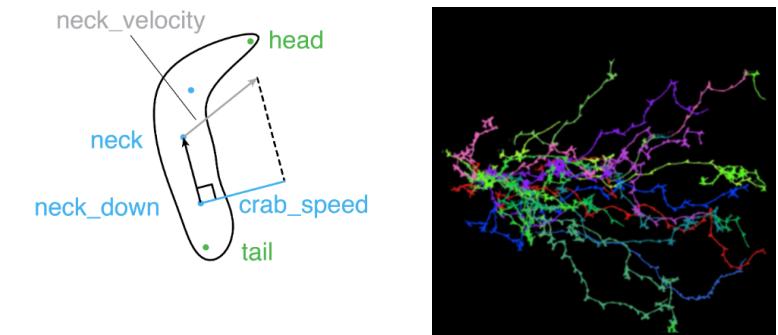
Eschbach et al. Nat. Neuro (2020)

Activity



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

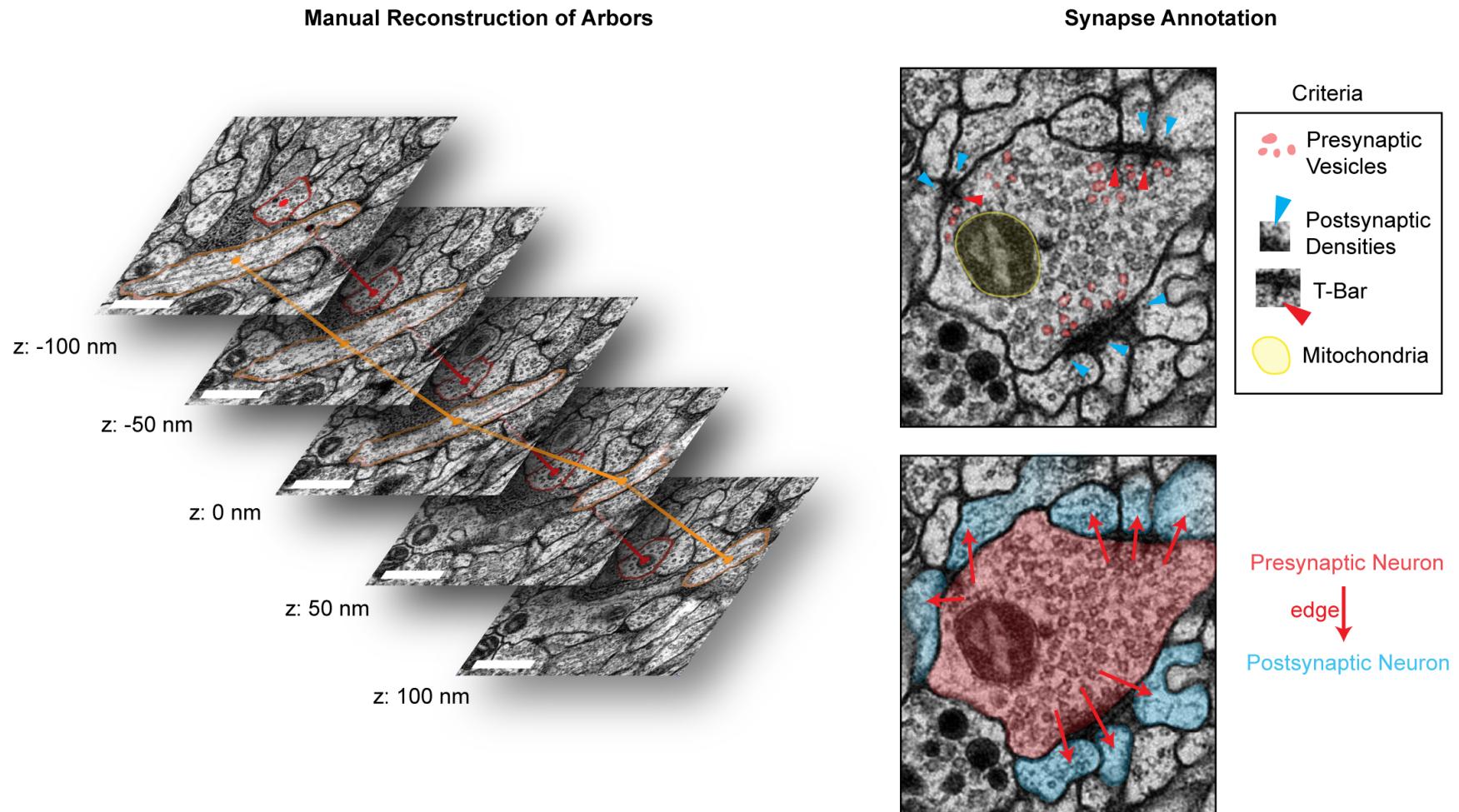
Behavior



Klein et al. bioRxiv (2021)

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

Mapping a larval *Drosophila* brain connectome



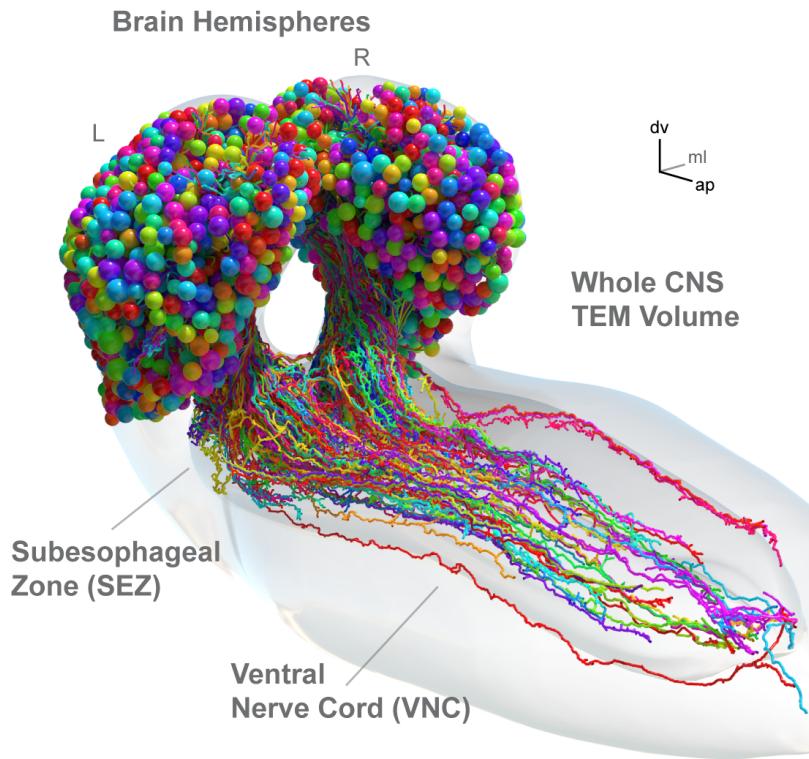
What do we do with these datasets once we have them?

- Characterizing network structure, e.g.,
 - How could signals travel on this network, from sensory inputs to motor outputs?
 - What cells have similar patterns of connectivity?
- Hypothesis testing
 - Connectome (network) as an object that we want to "do inference" on

Outline

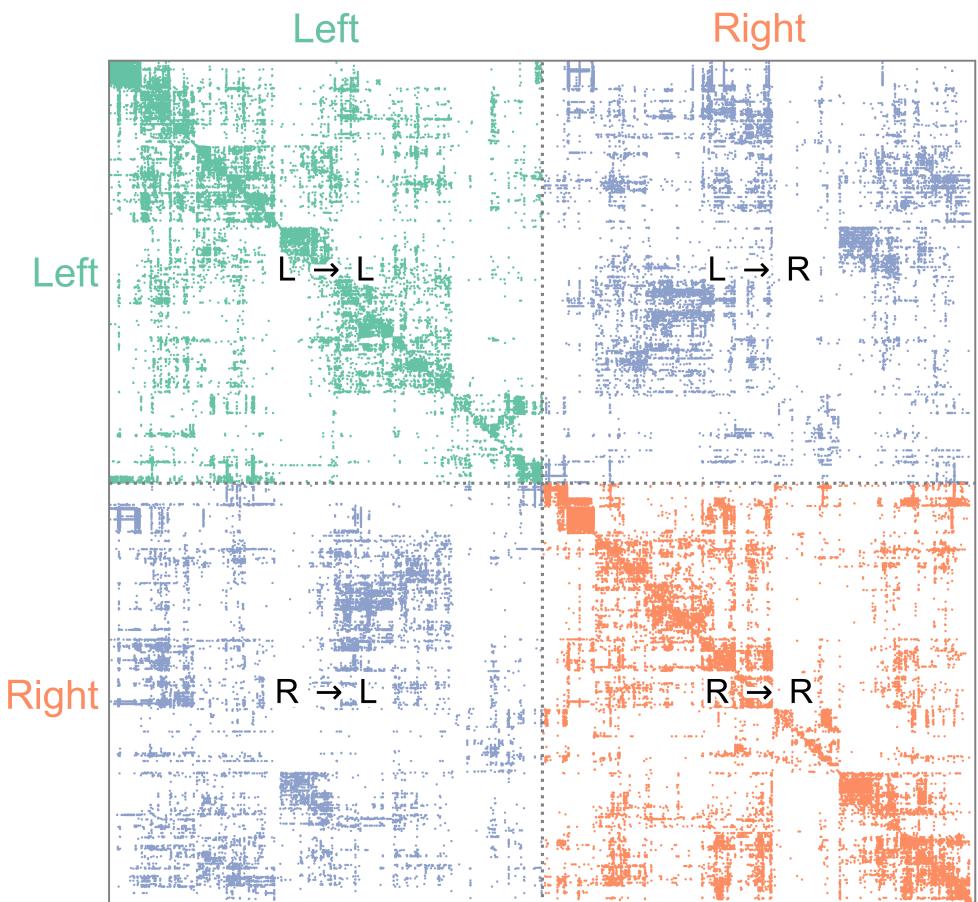
- **Larval connectome dataset**
 - Flow
 - Connectivity based cell types
- **Connectome comparison via network hypothesis testing**
- **Pairing neurons across connectomes via graph matching**
- **Ongoing extensions/applications**

Larval *Drosophila* brain connectome

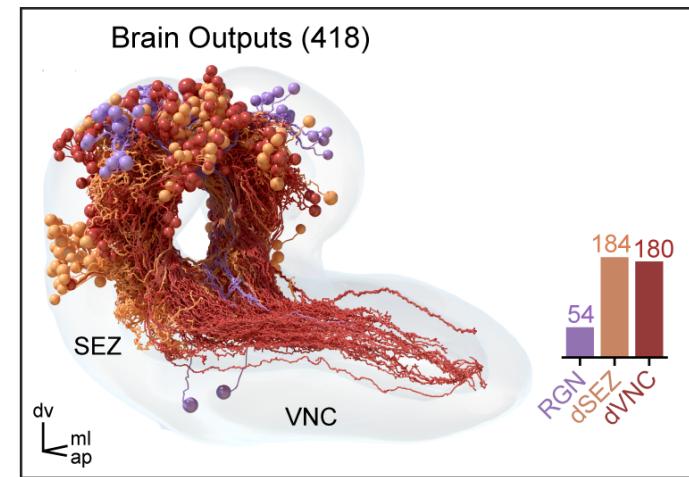
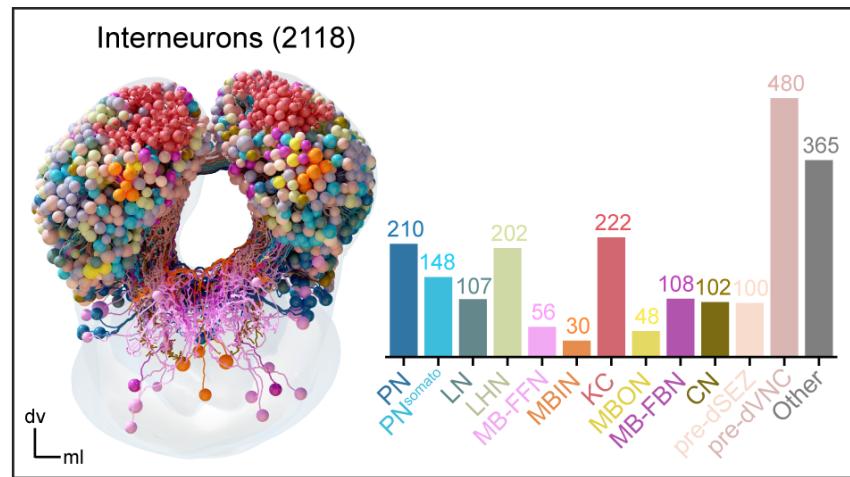
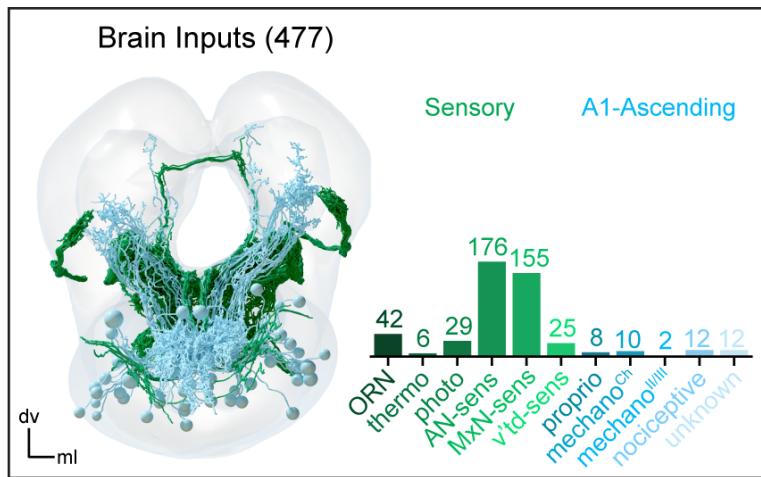


~3k neurons, ~550K synaptic sites

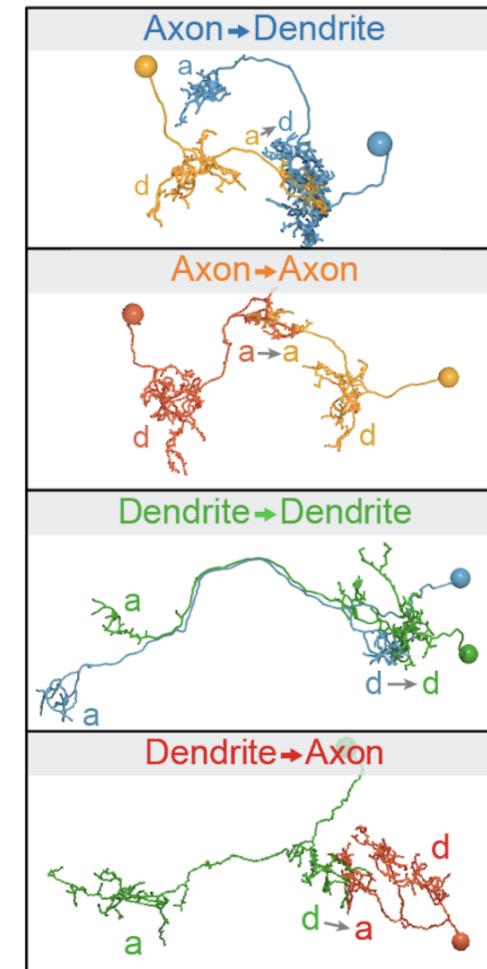
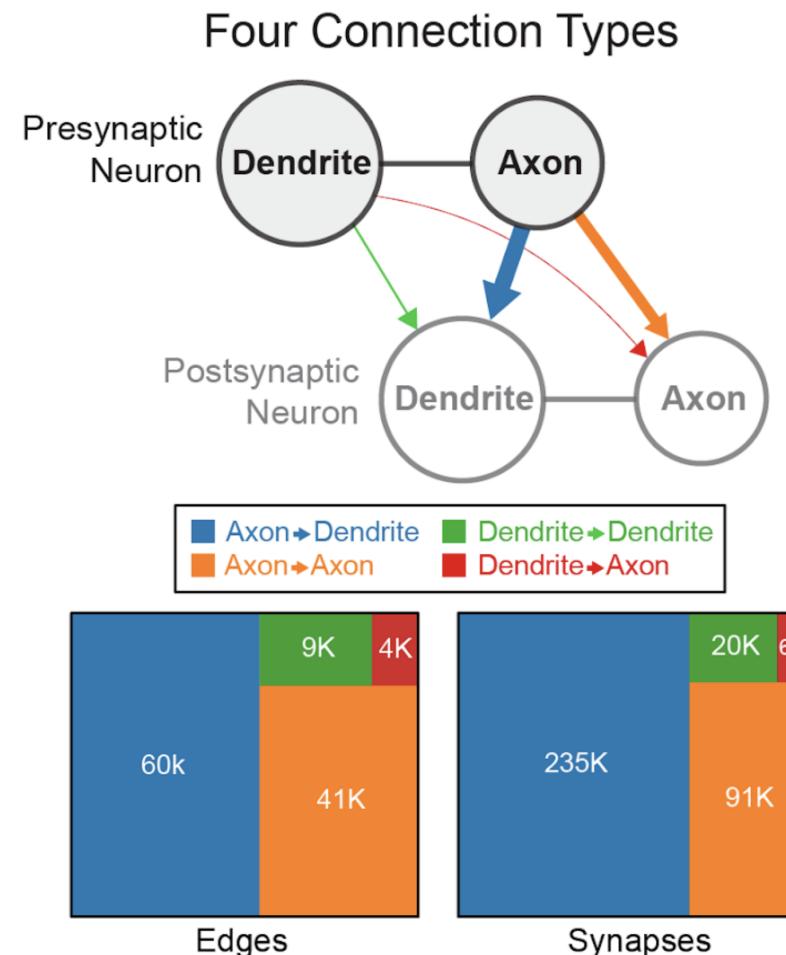
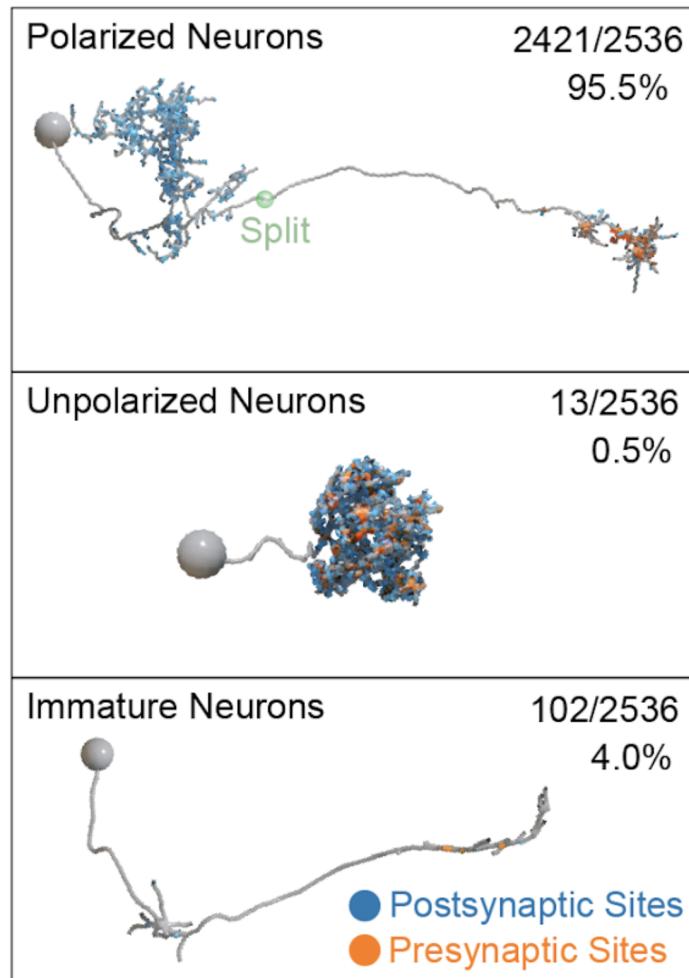
Both hemispheres



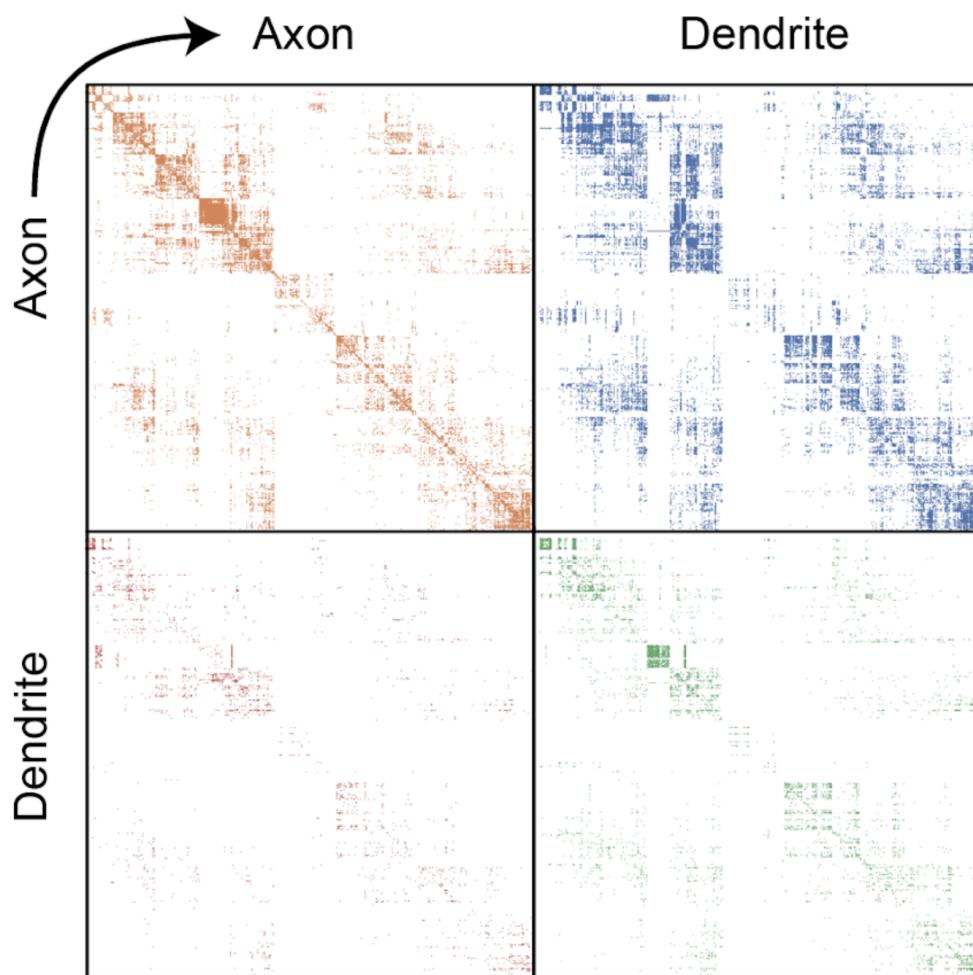
High level (mostly anatomical) cell types



Morphology enables splitting axons/dendrites



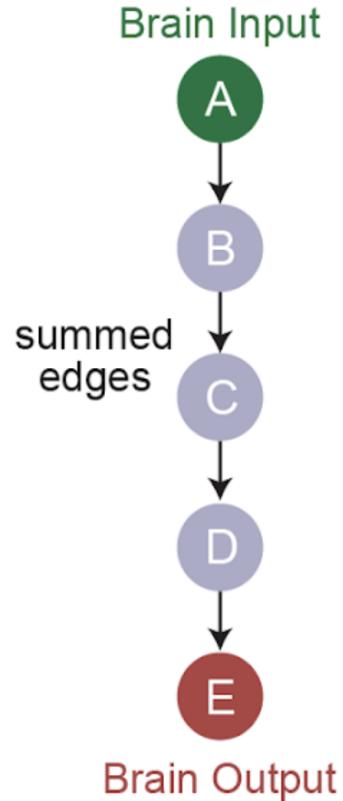
This split induces 4 graphs (or layers)



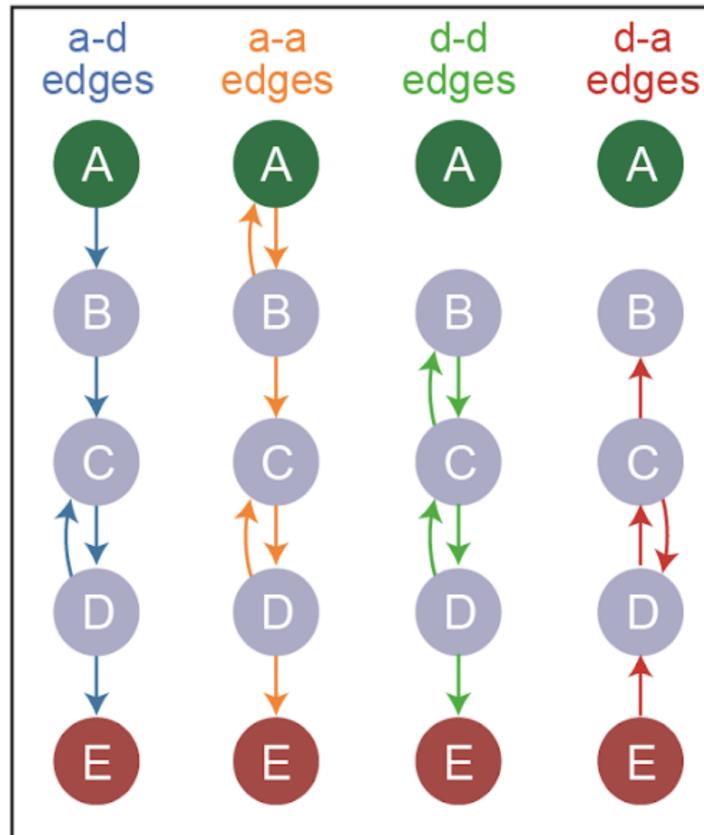
Edge	Nodes	Density	Max Degree
A→D	2880	0.77%	203
A→A	2887	0.49%	241
D→D	2204	0.19%	143
D→A	1907	0.10%	69

What are these different "channels" doing?

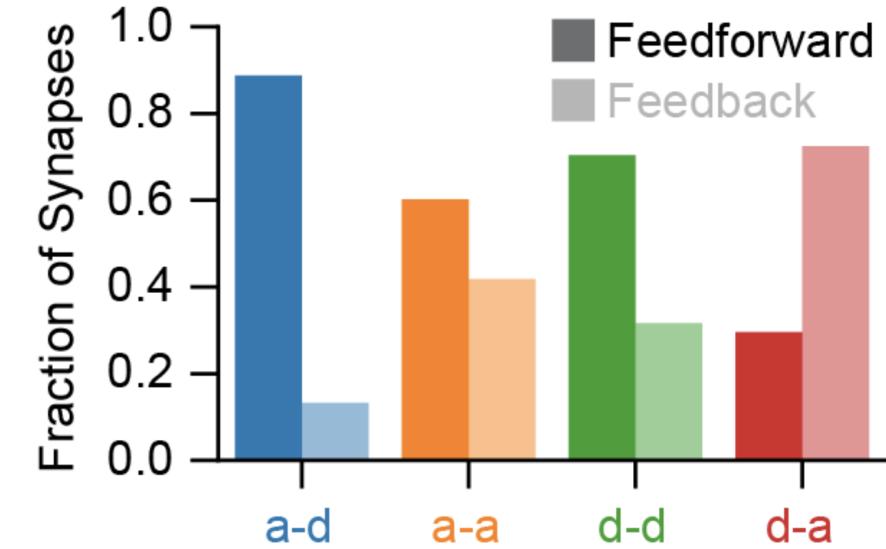
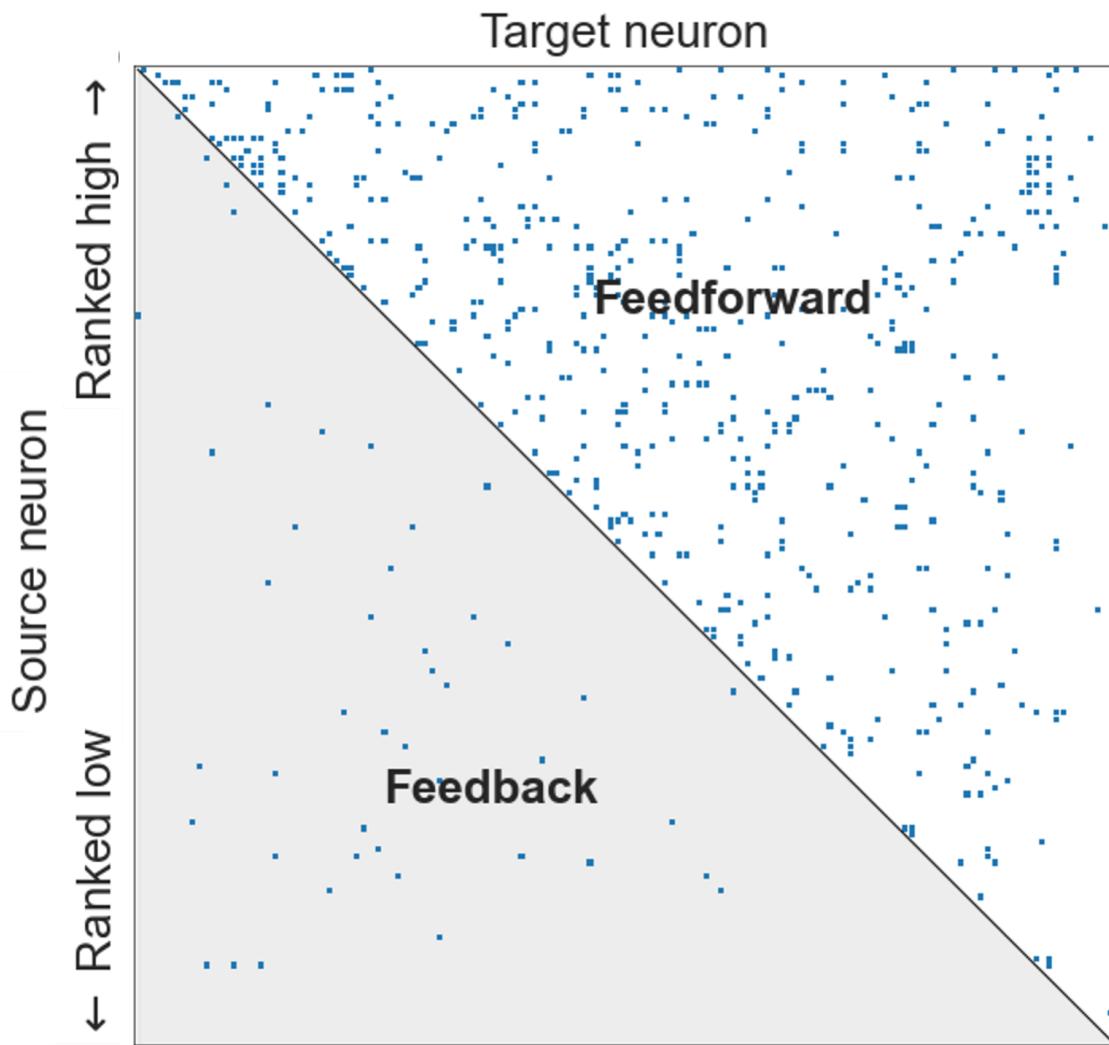
Sort based on
summed connectivity



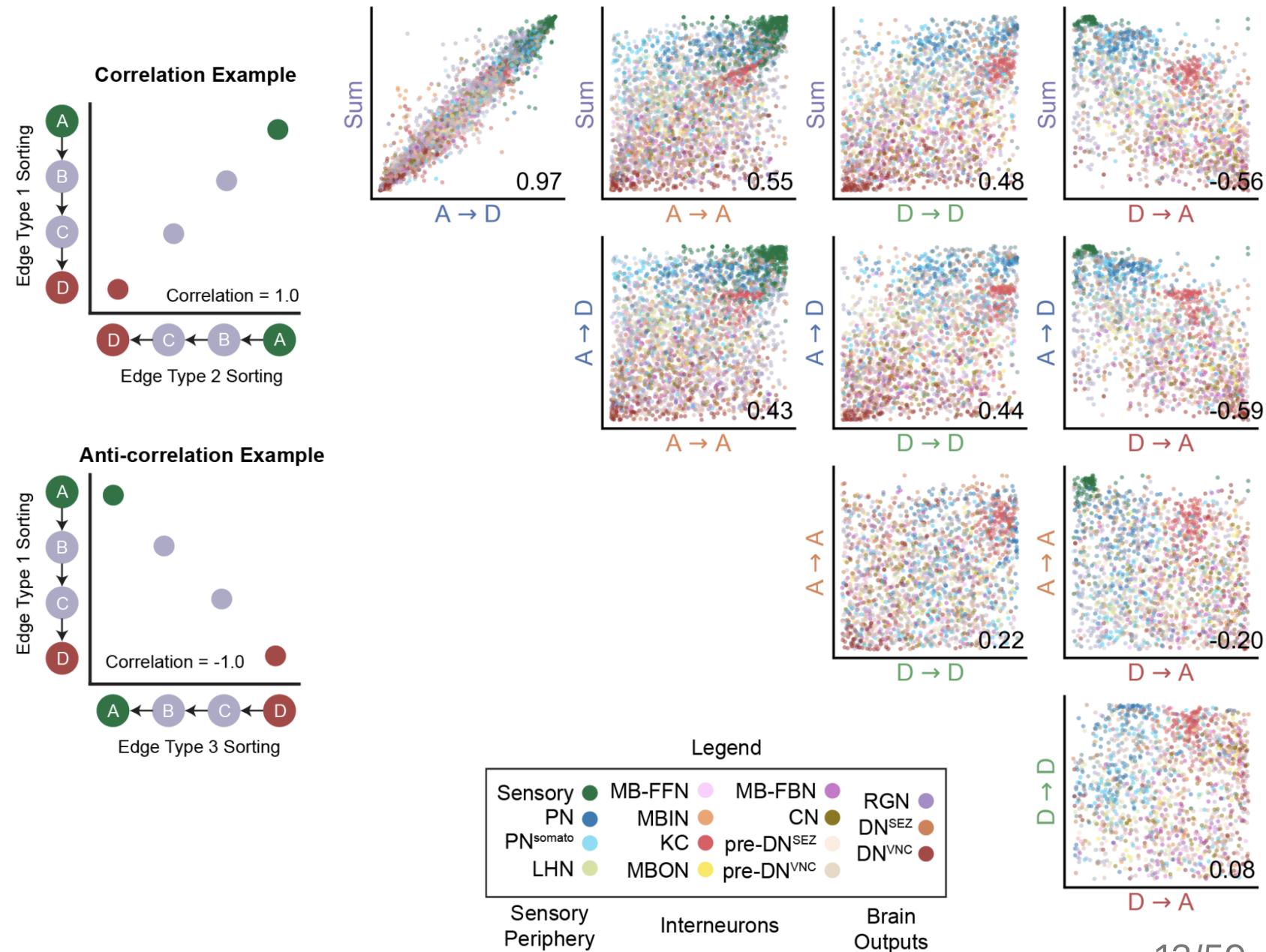
Analyze each edge type
based on this sorting



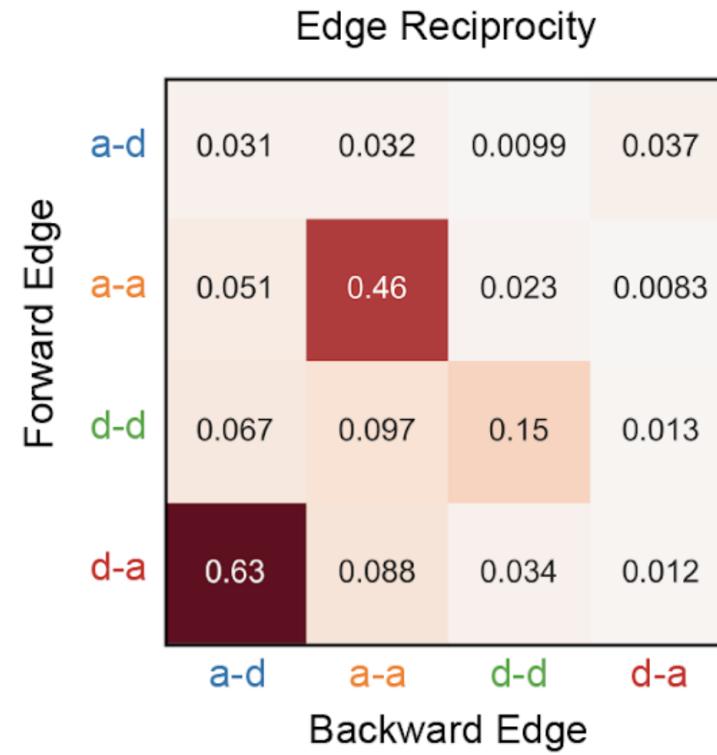
Quantifying high-level "feedforward/feedback"



Comparing independently sorted channels



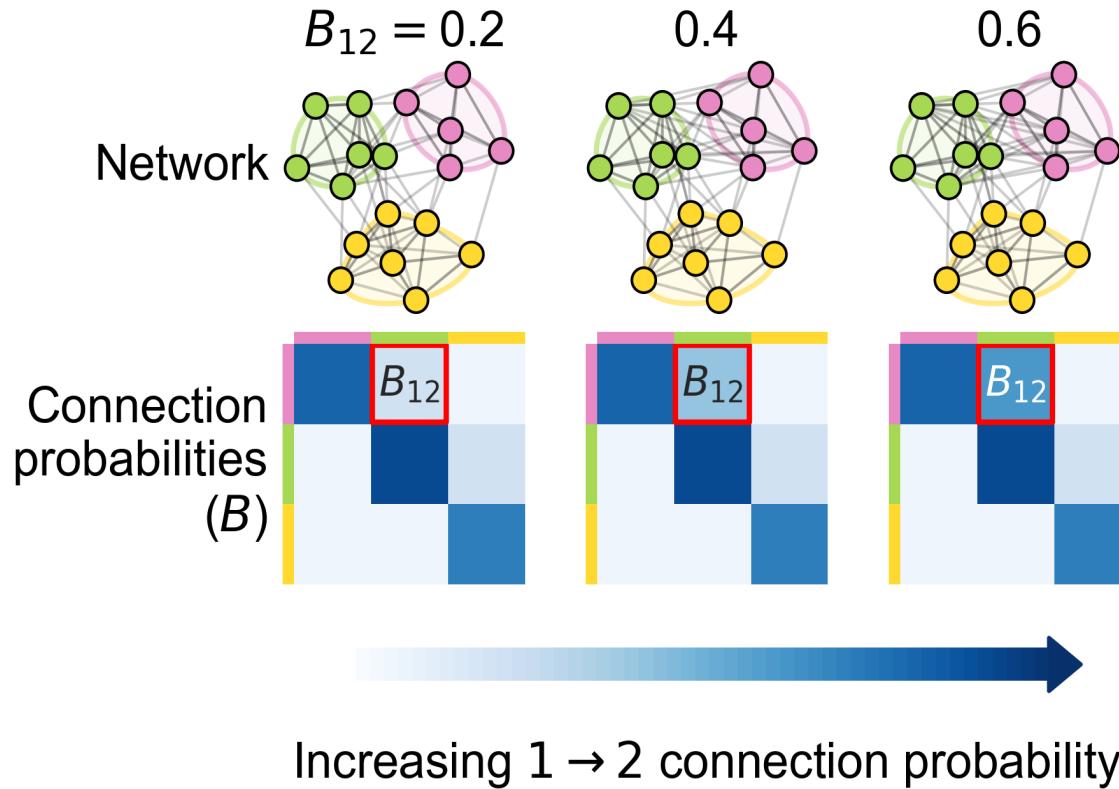
Edge reciprocity



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- **Ongoing extensions/applications**

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

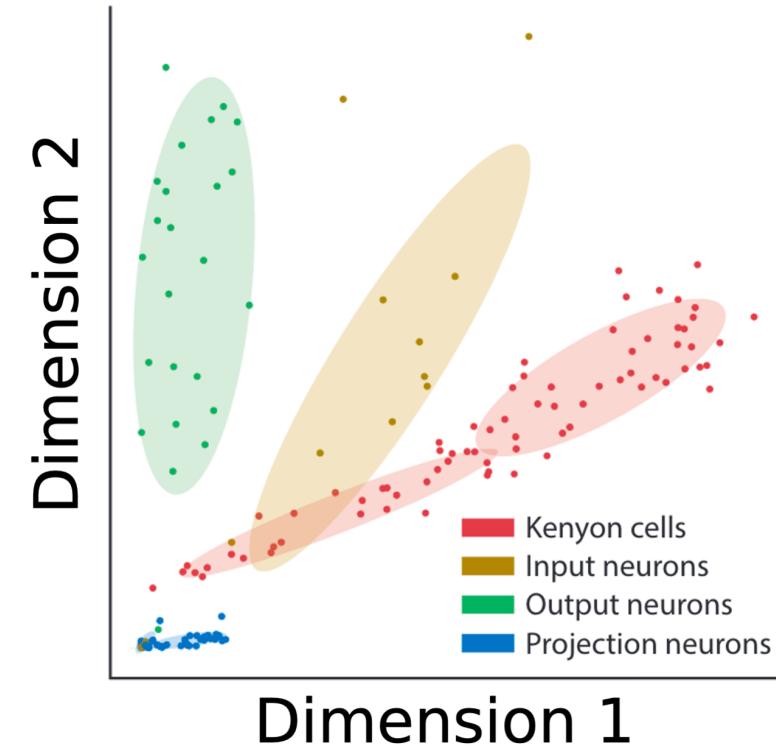
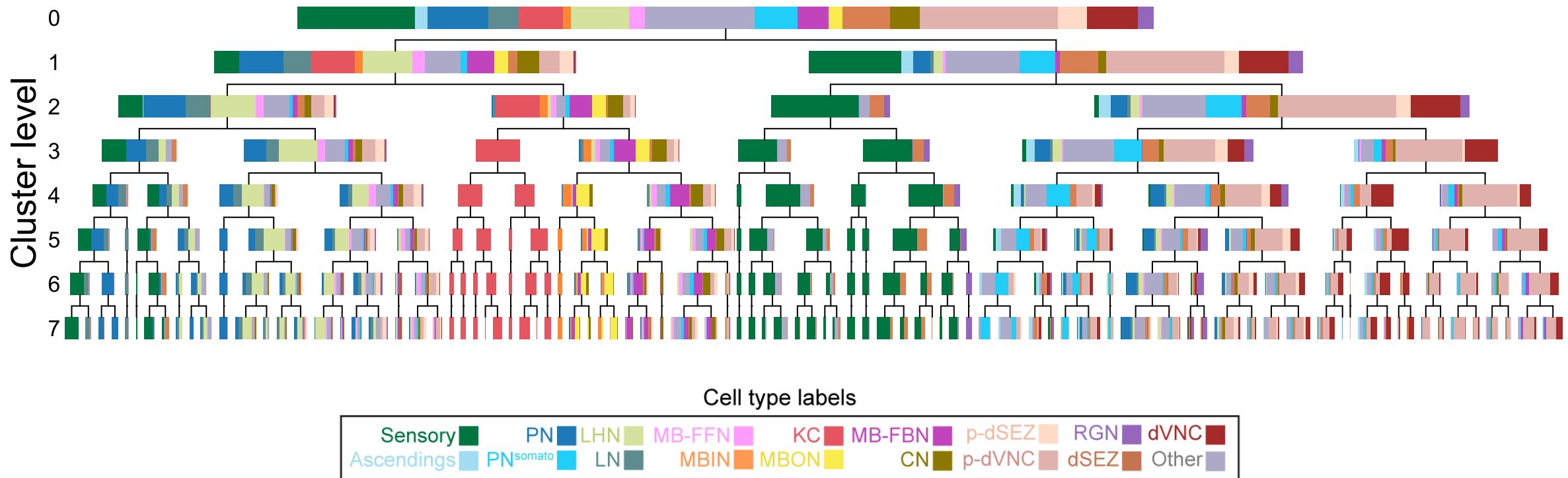
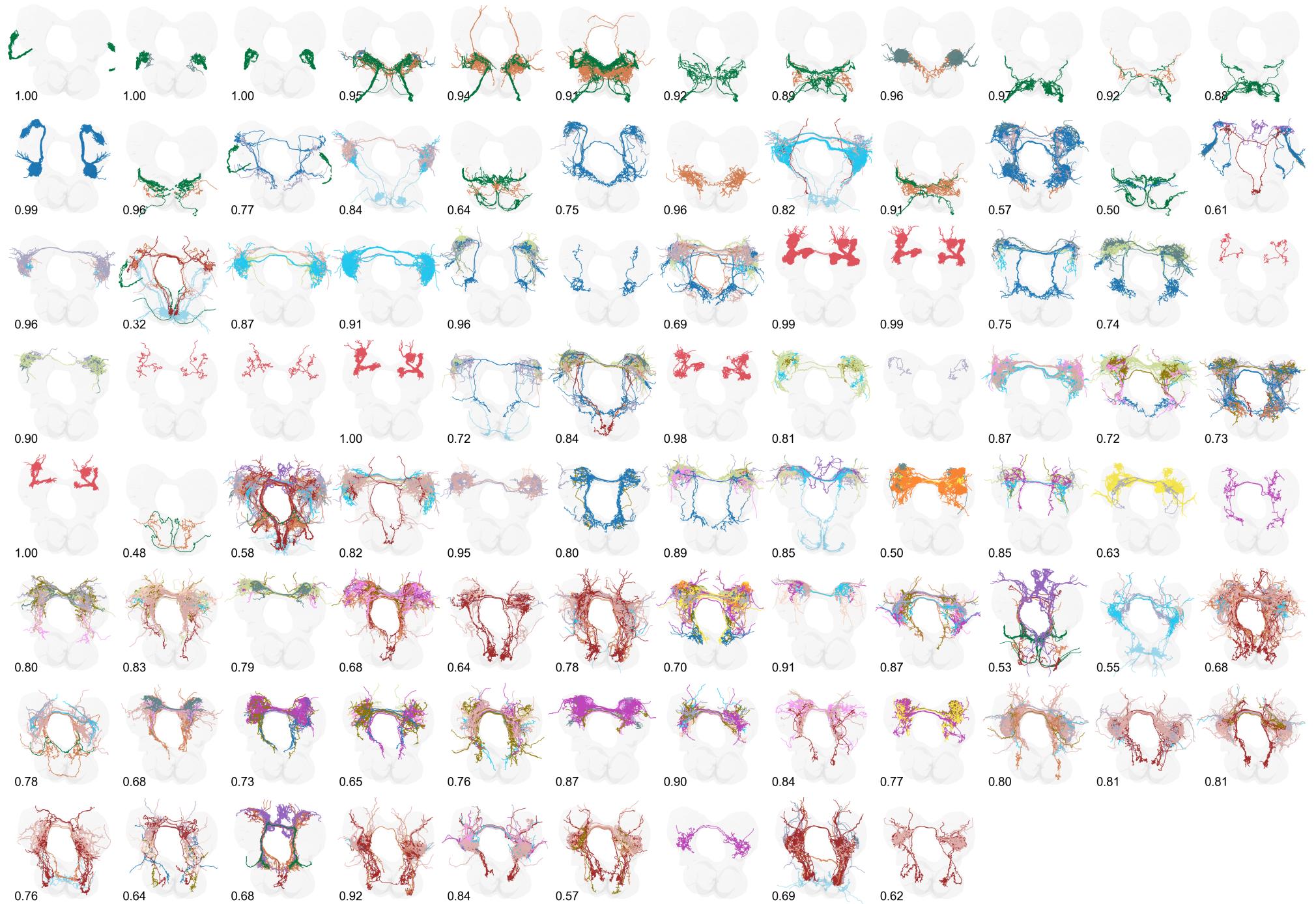


Diagram of procedure

- mention embedding
- cluster - when to stop

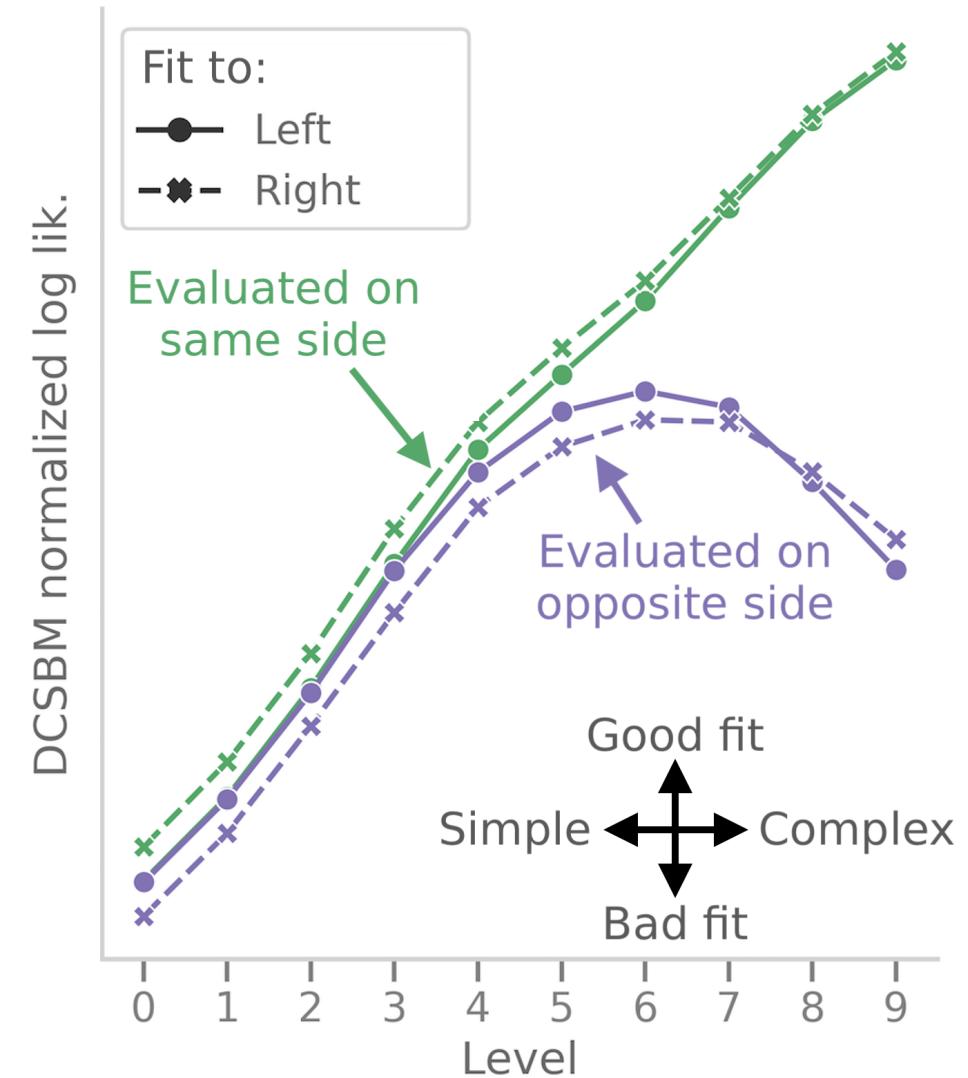
Neurons clustered by connectivity using recursive spectral clustering





Using models to evaluate cell type groupings

- 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)?



Bilateral symmetry

"This brain is bilaterally symmetric."

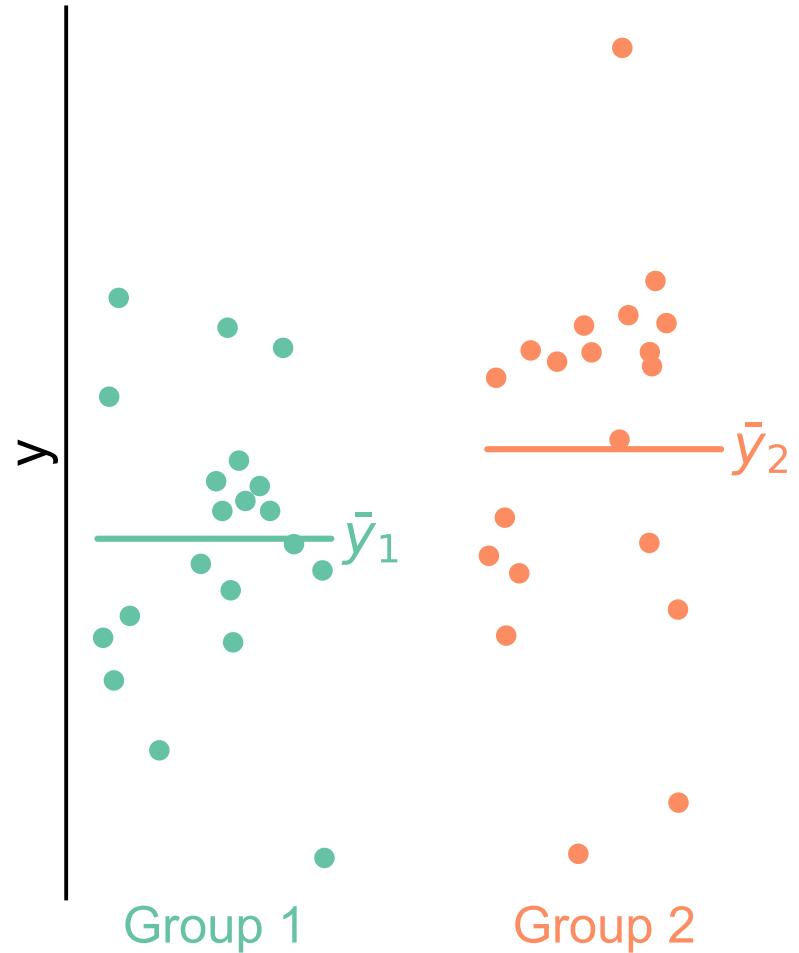
"What does that even mean? And how would we know if it wasn't?"

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

Outline

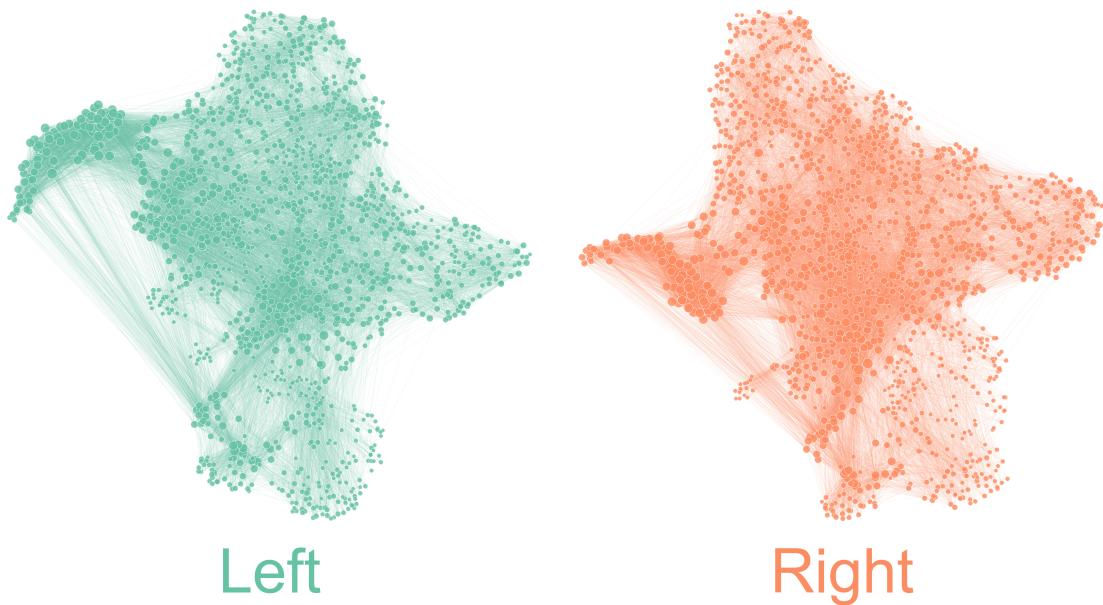
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Are these populations different?



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

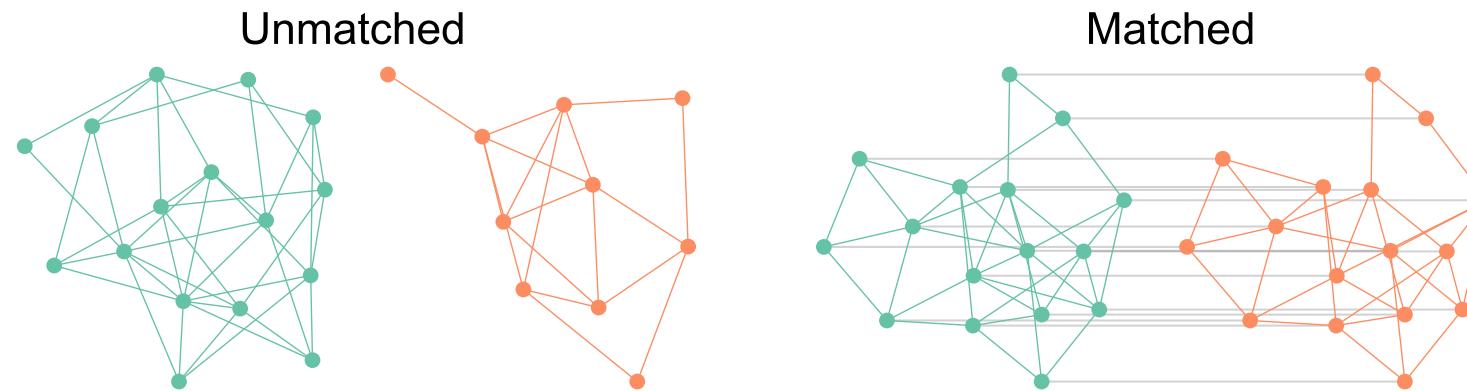
Are these *networks* different?



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

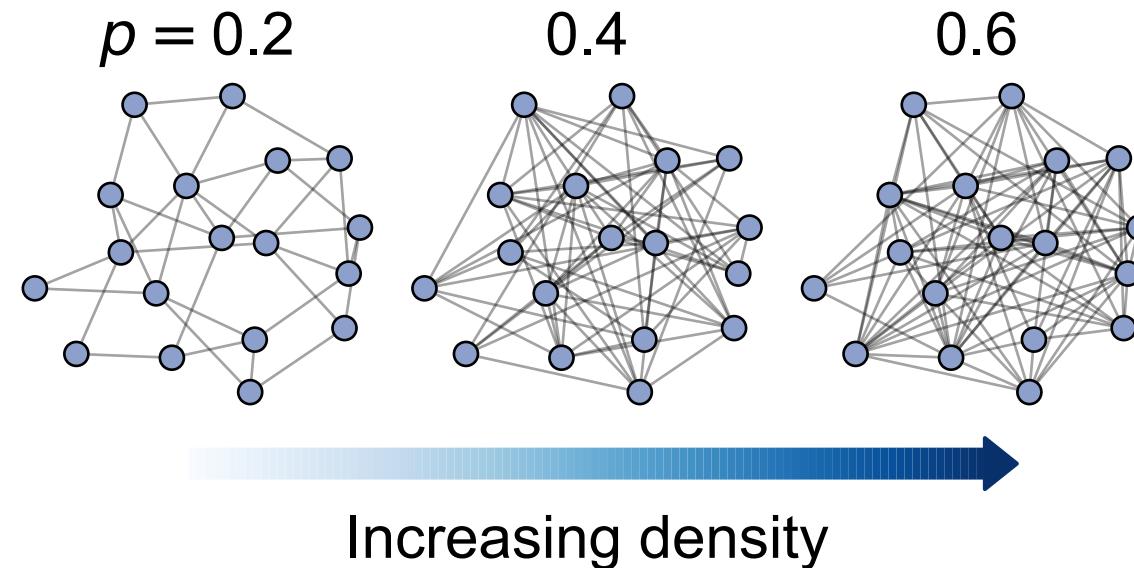
Assumptions

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



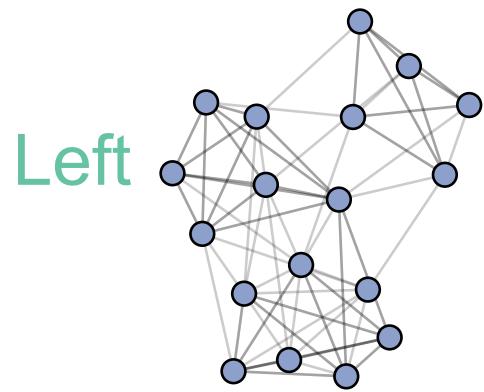
Erdos-Renyi model

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

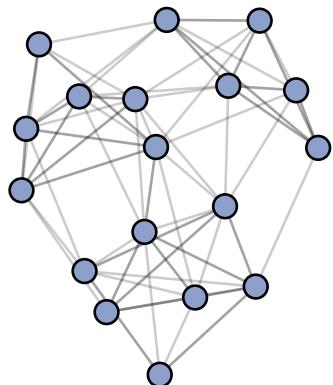


Detect a difference in density

Compute global connection density



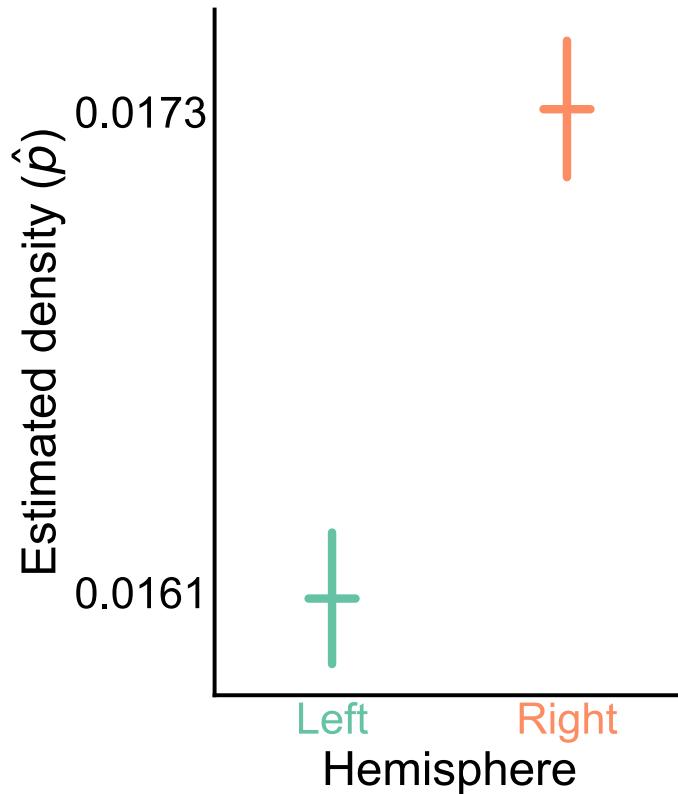
Right



Compare ER models

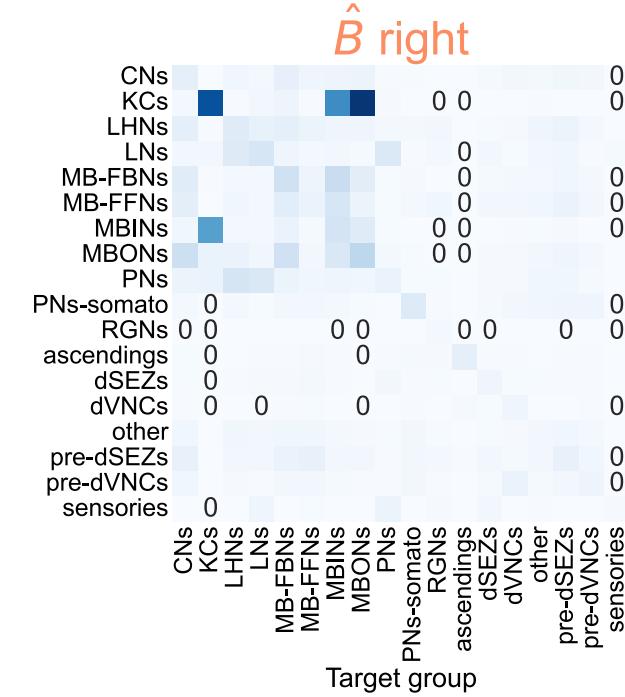
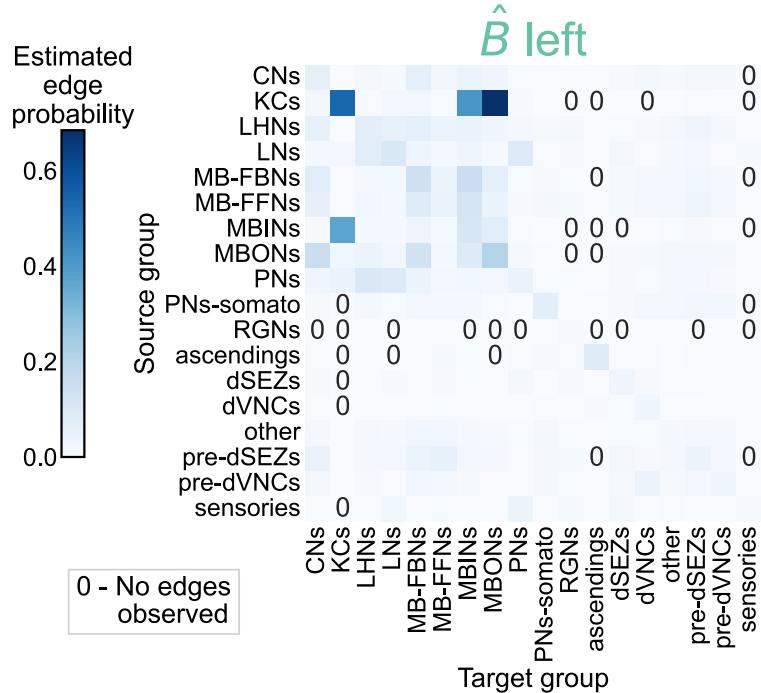
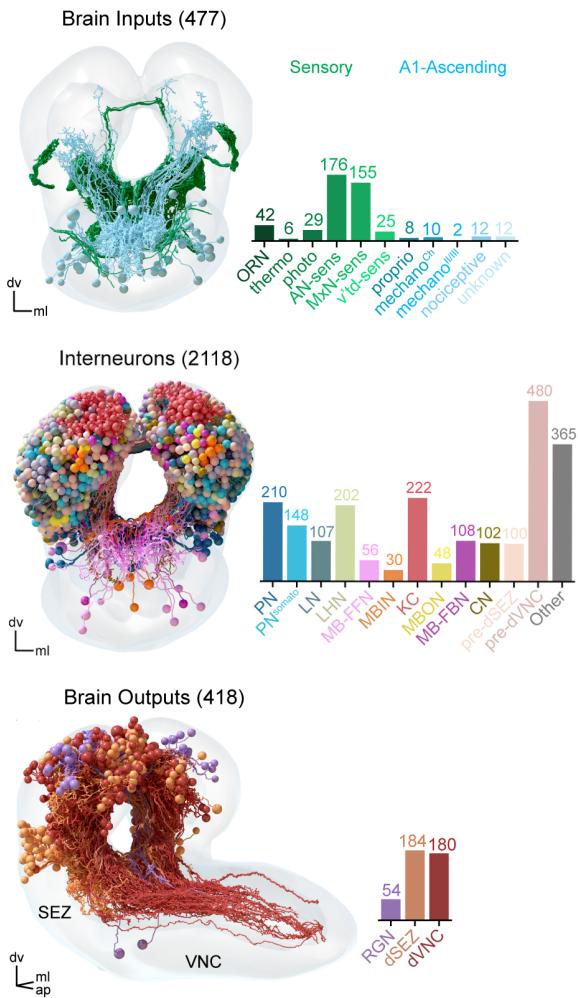
$$p = \frac{\text{\# edges}}{\text{\# potential edges}}$$

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



p-value $< 10^{-22}$

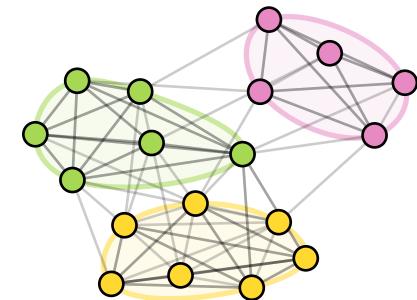
Connection probabilities between groups



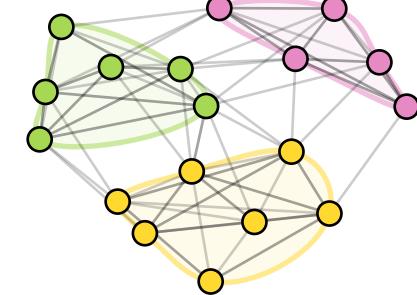
Group connection test

Group neurons

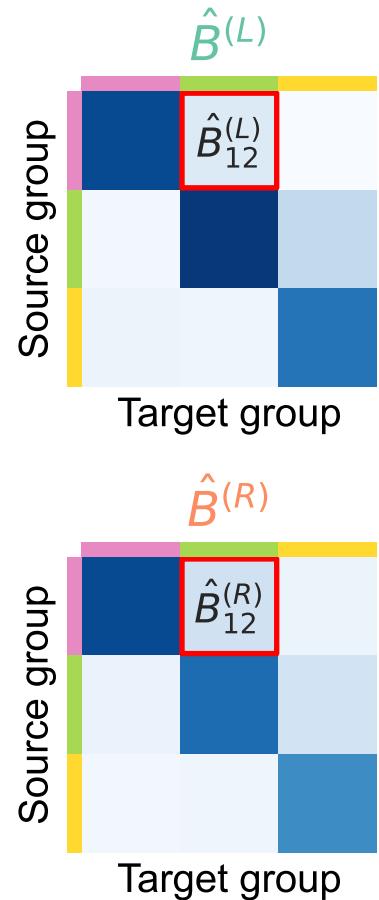
Left



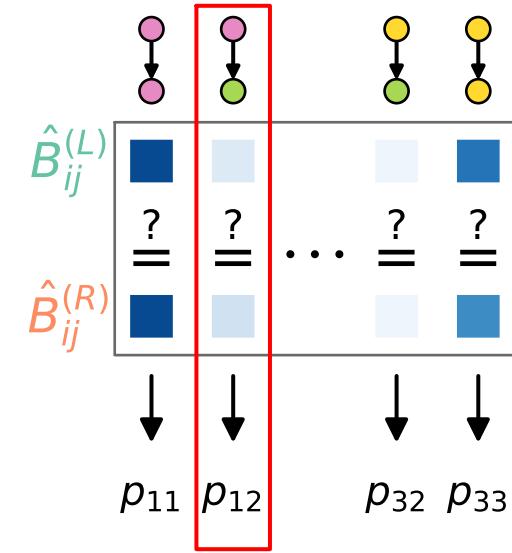
Right



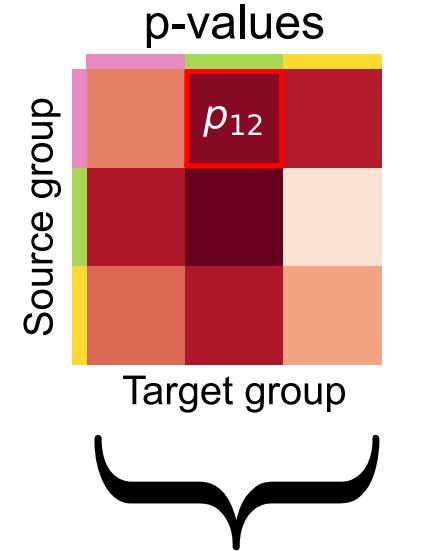
Estimate group connection probabilities



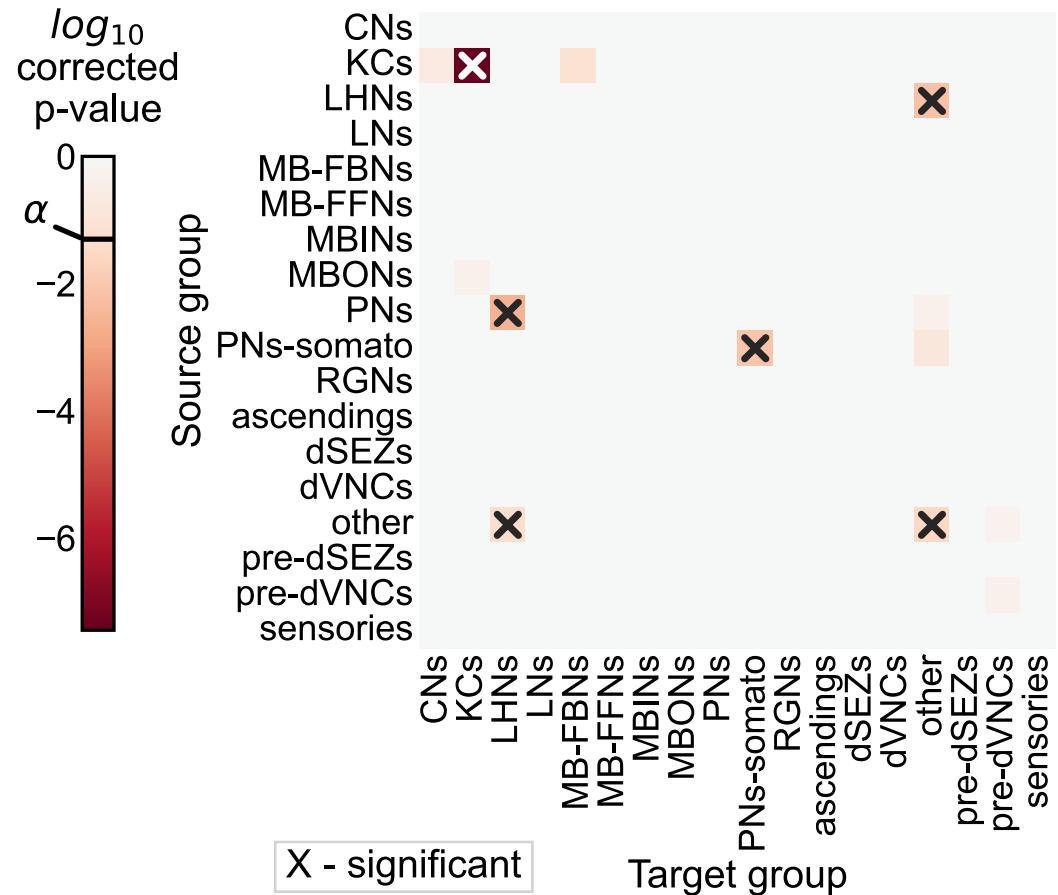
Compare probabilities, compute p-values



Combine p-values for overall test



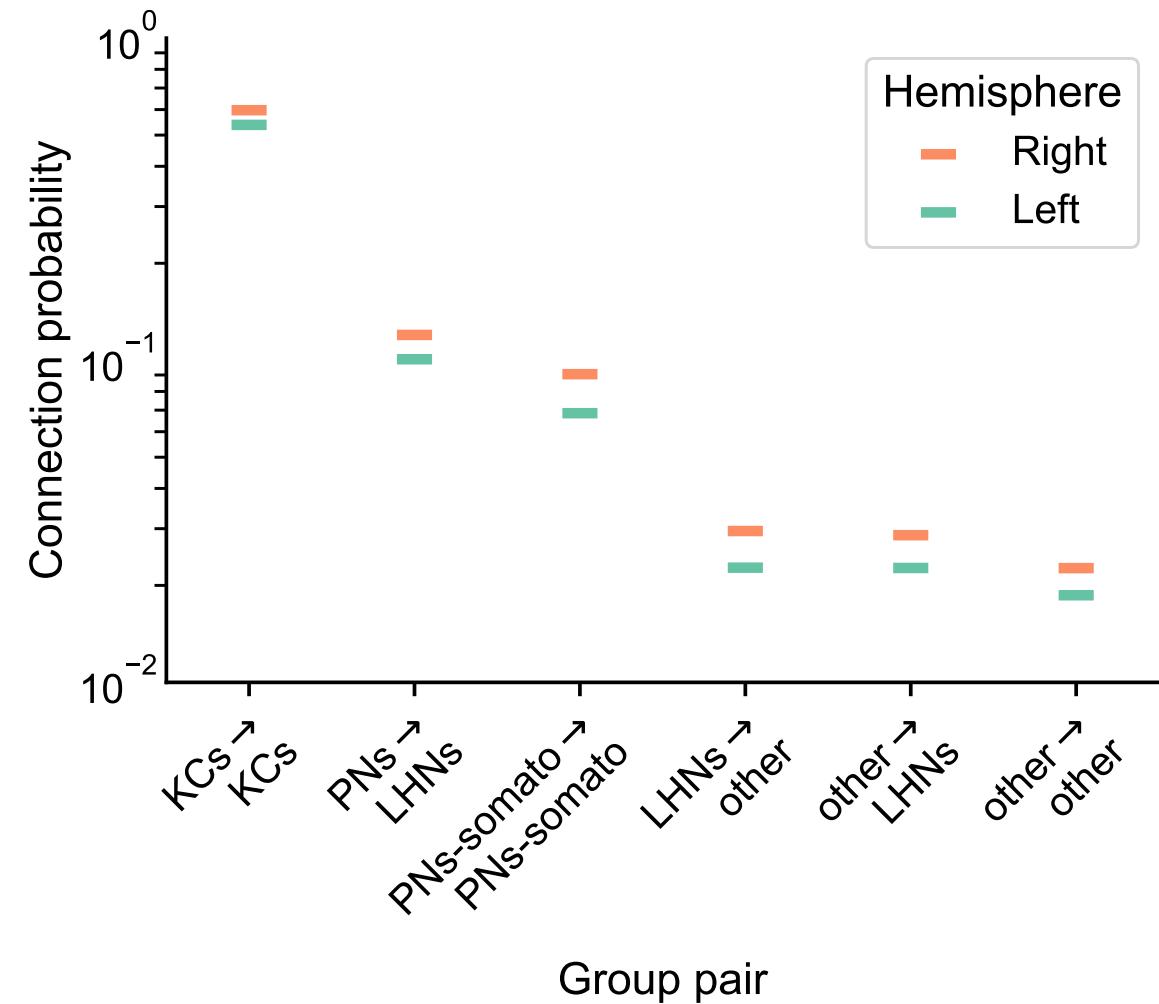
Detect differences in group connection probabilities



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

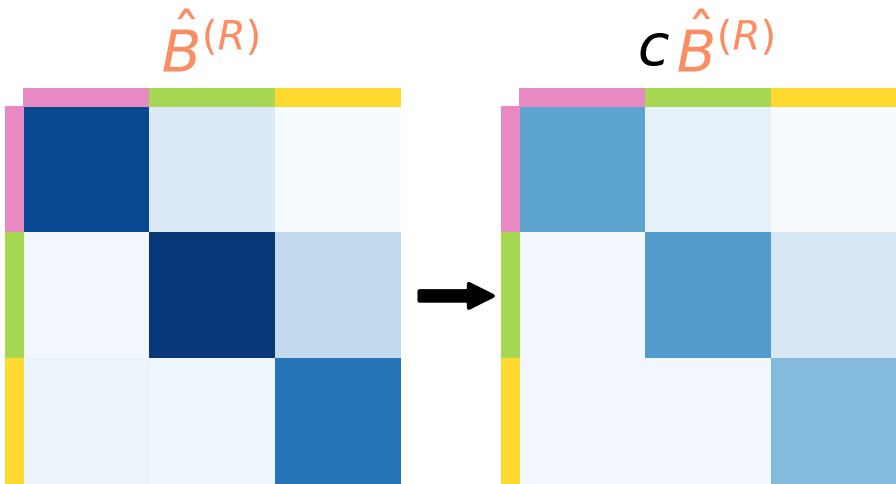
Should we be surprised?

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

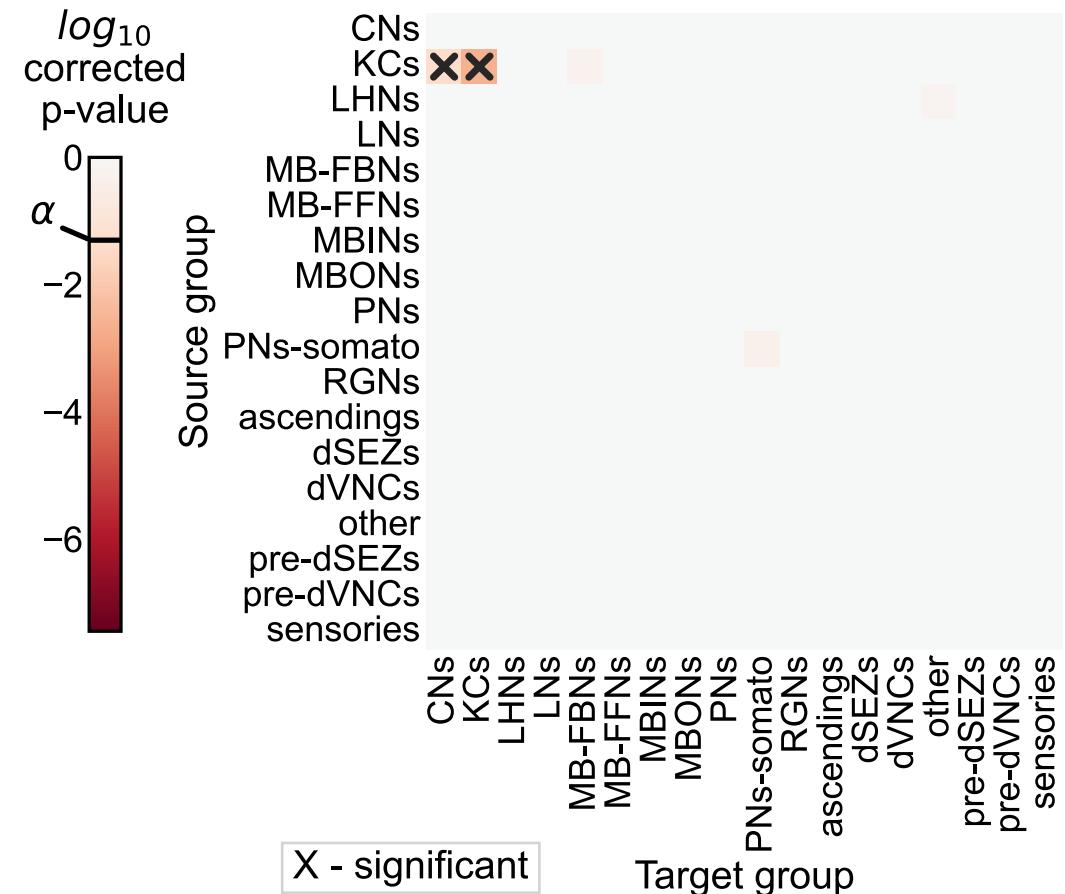


After adjusting for density, differences are in KCs

Scale connection probabilities
to match densities



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



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

To sum up...

"This brain is bilaterally symmetric."

Depends on what you mean...

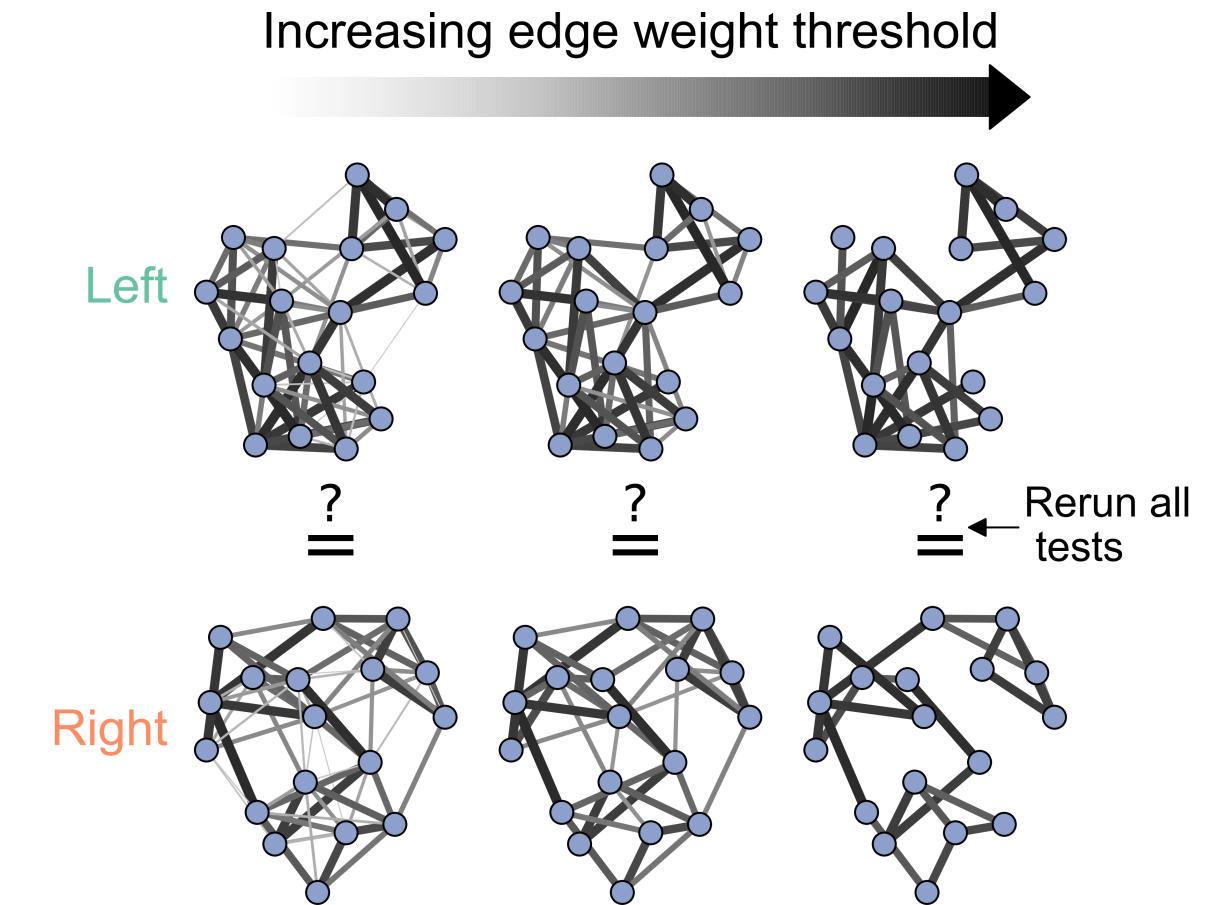
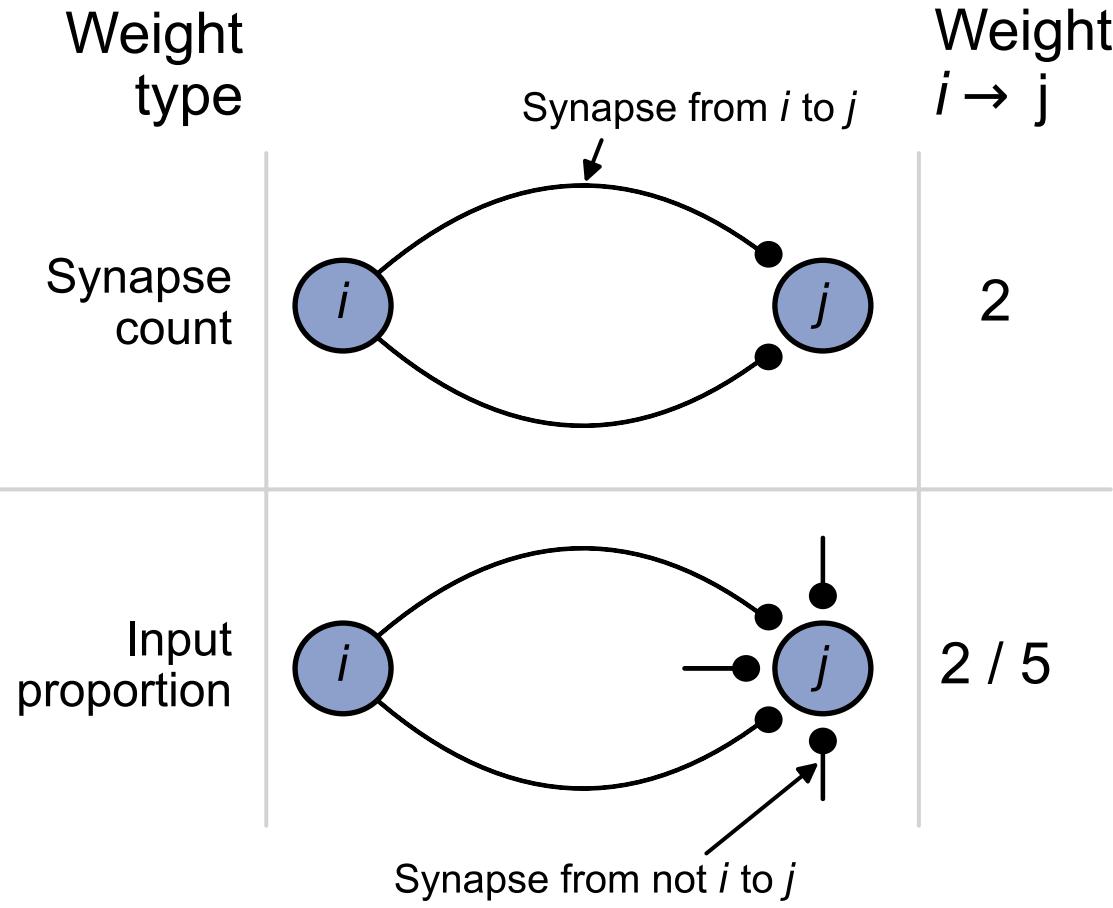
With Kenyon cells

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

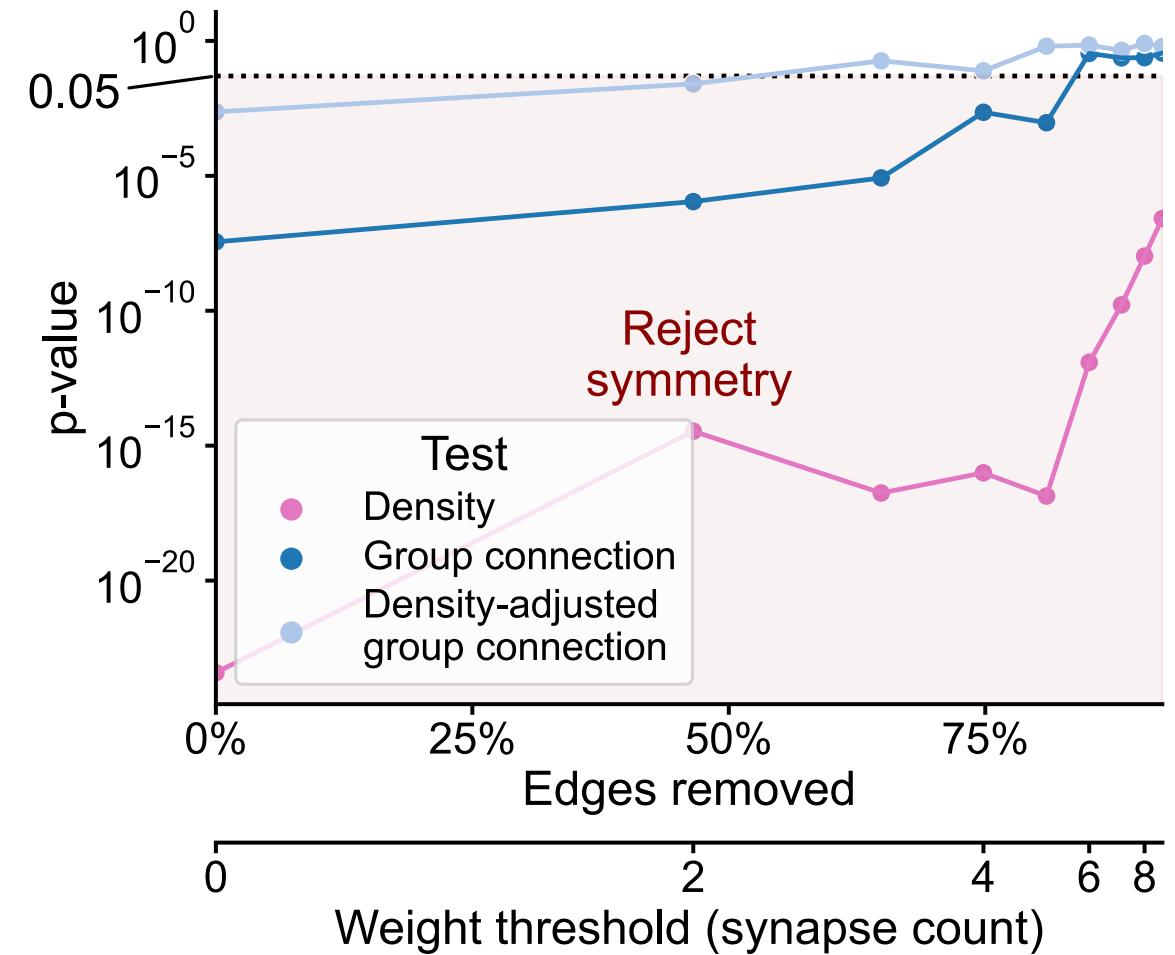
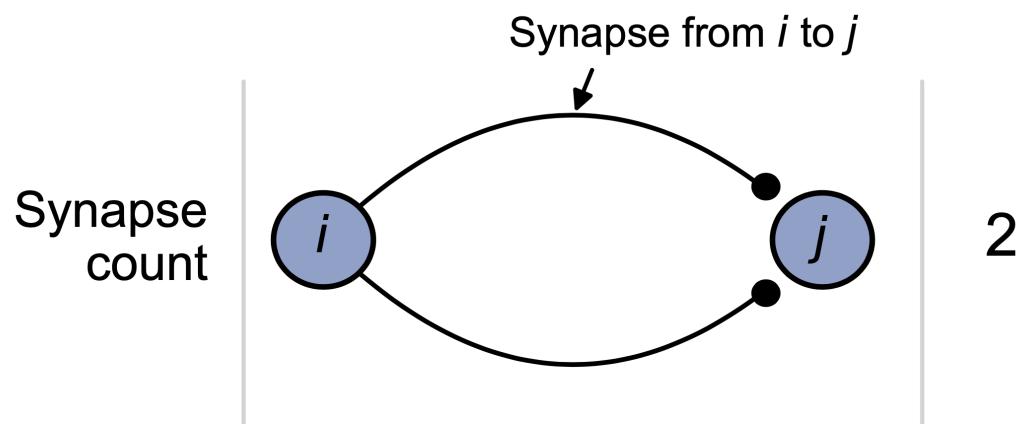
Without Kenyon cells

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

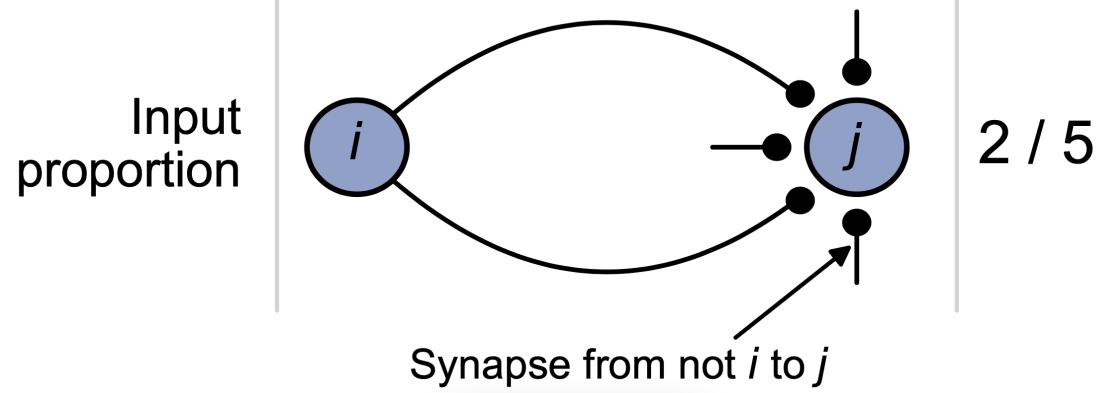
Examining the effect of edge weights



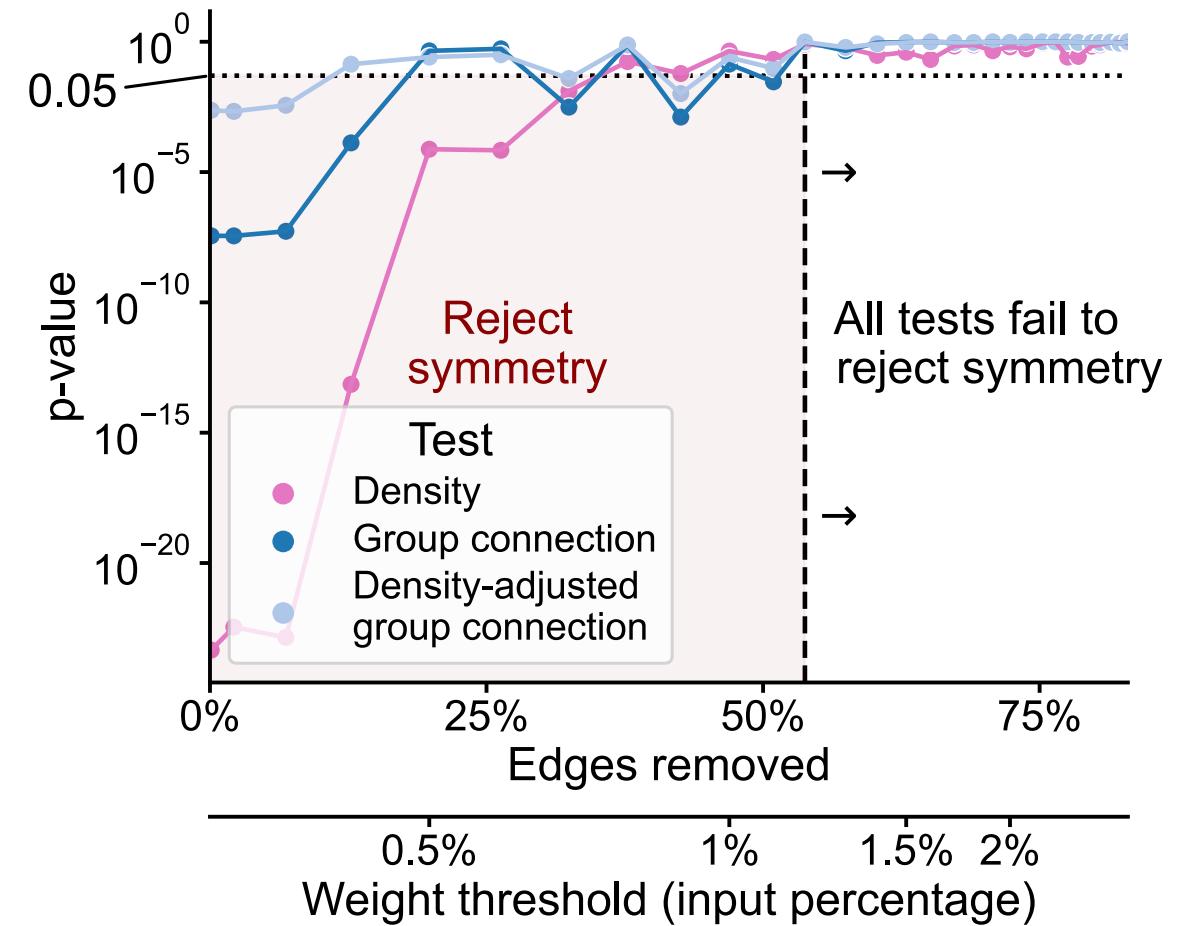
Even high synapse count networks show asymmetry



High input percentage networks show no asymmetry



2 / 5

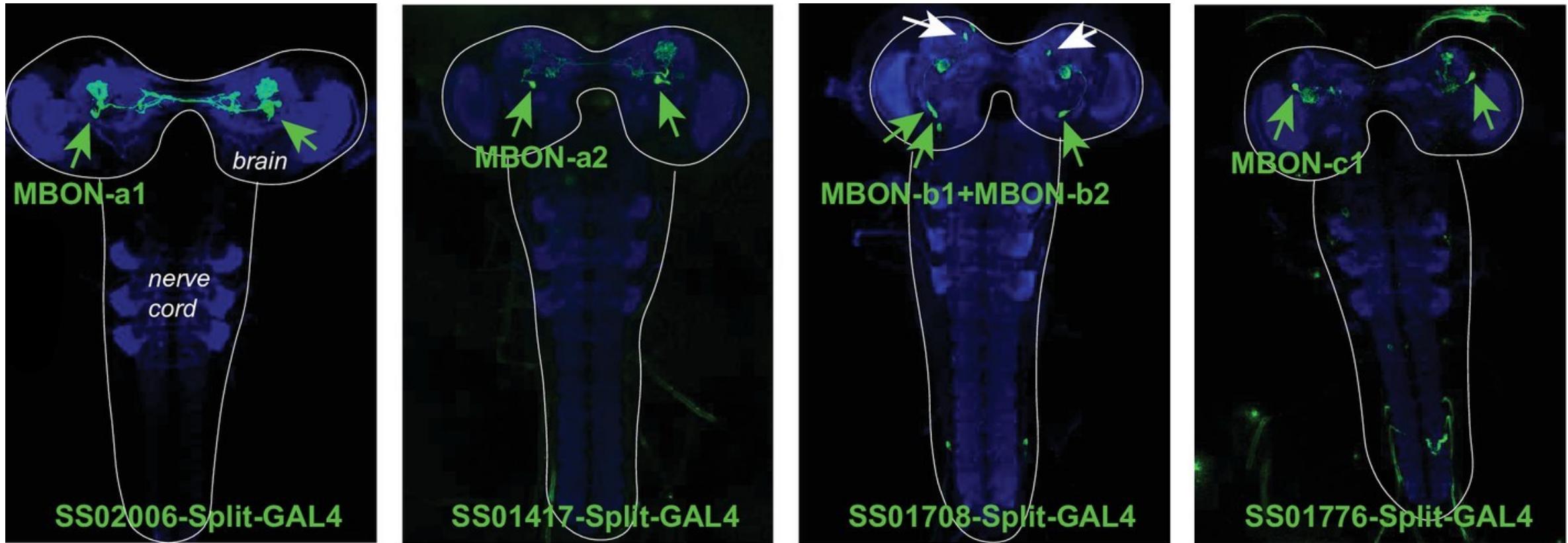


Outline

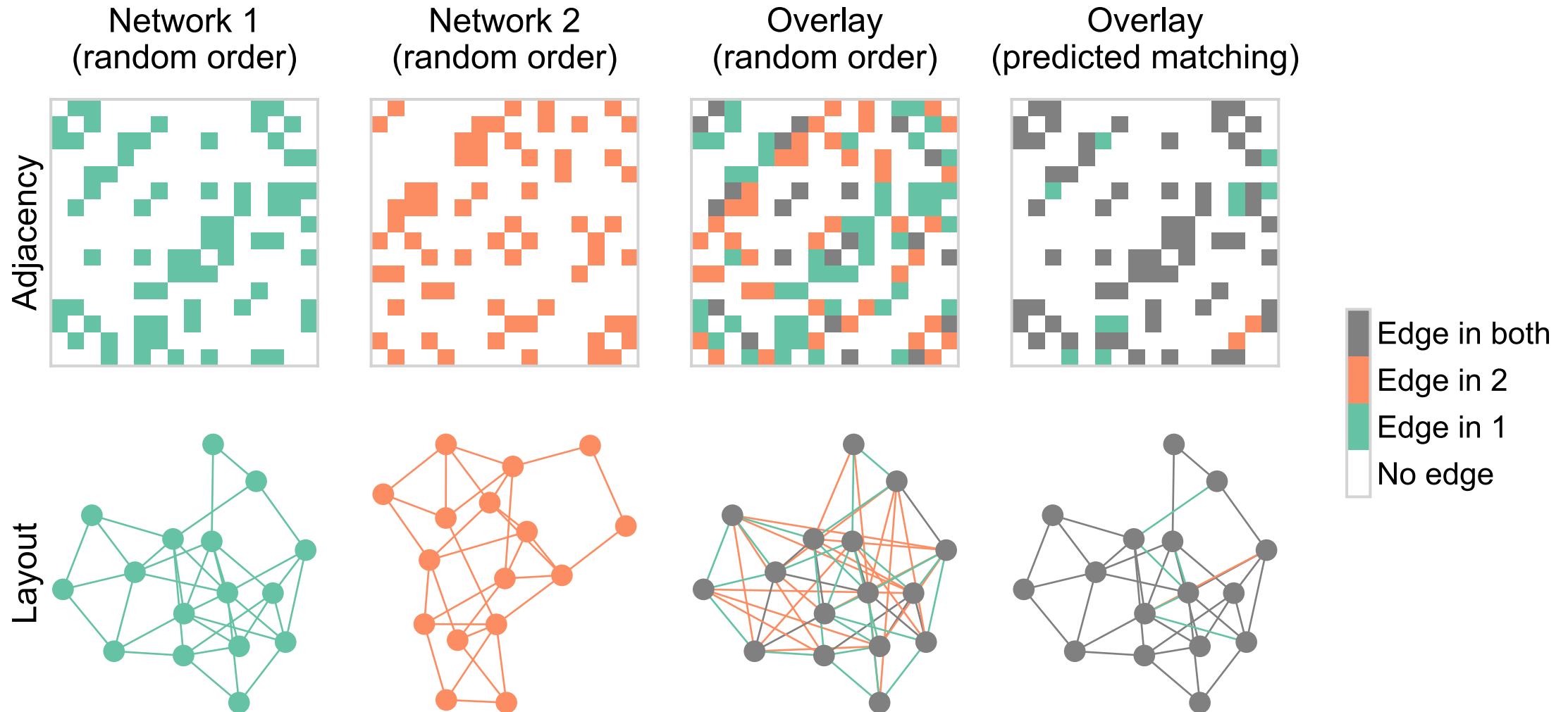
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Bilaterally homologous neuron pairs

We believe a matching exists!



What is graph matching?



How do we measure network overlap?

$$\min_{P \in \mathcal{P}} \underbrace{\|A_1 - \underbrace{PA_2P^T}_{\text{distance between adj. mats.}}\|_F^2}_{\text{reordered } A_2}$$

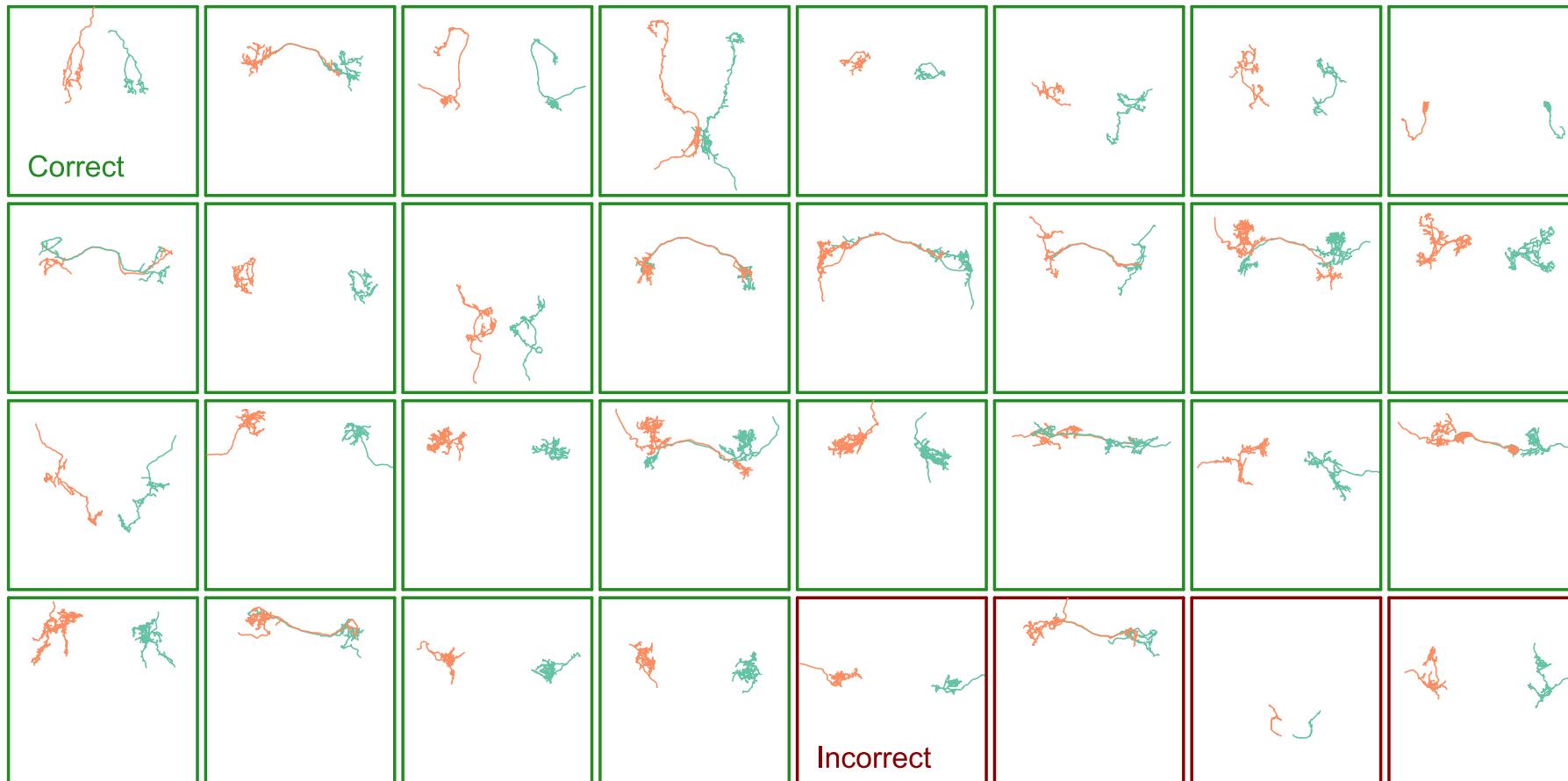
where \mathcal{P} is the set of permutation matrices

- 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 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)

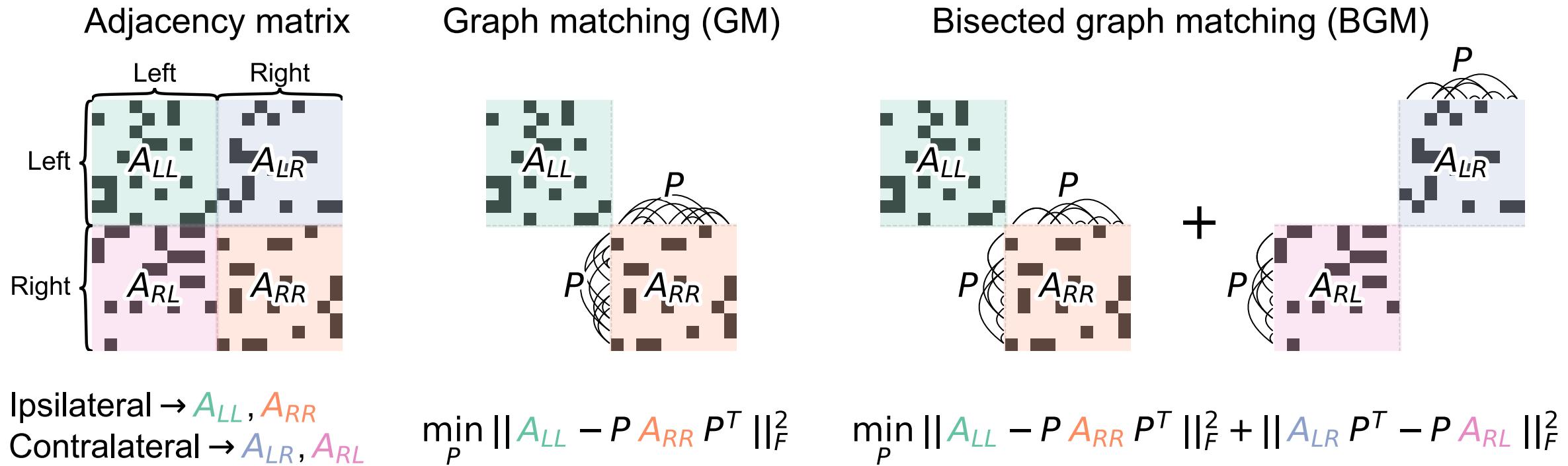
Many ways to try to improve on this...

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

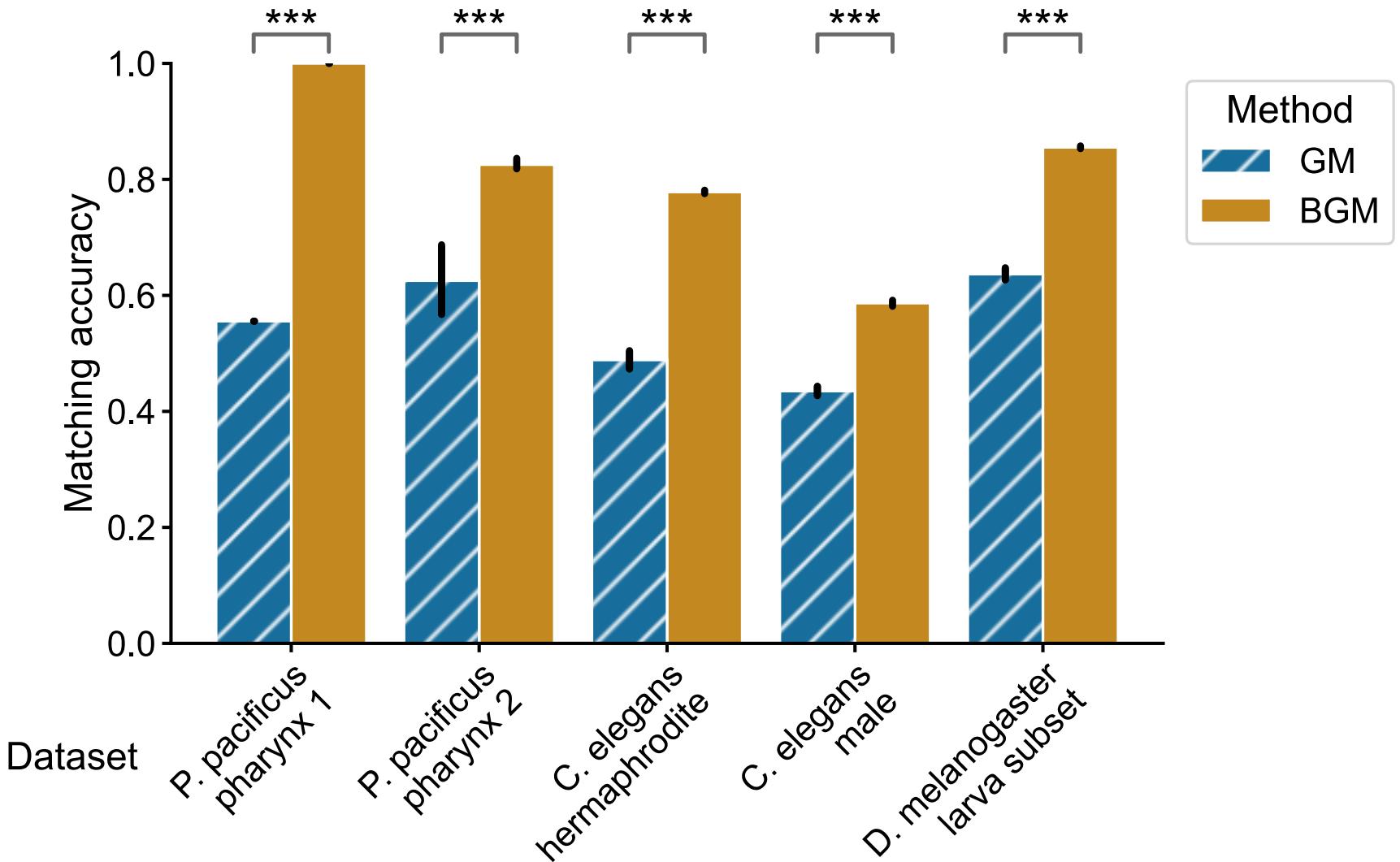
Thus far, we've not used the contralateral connections

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

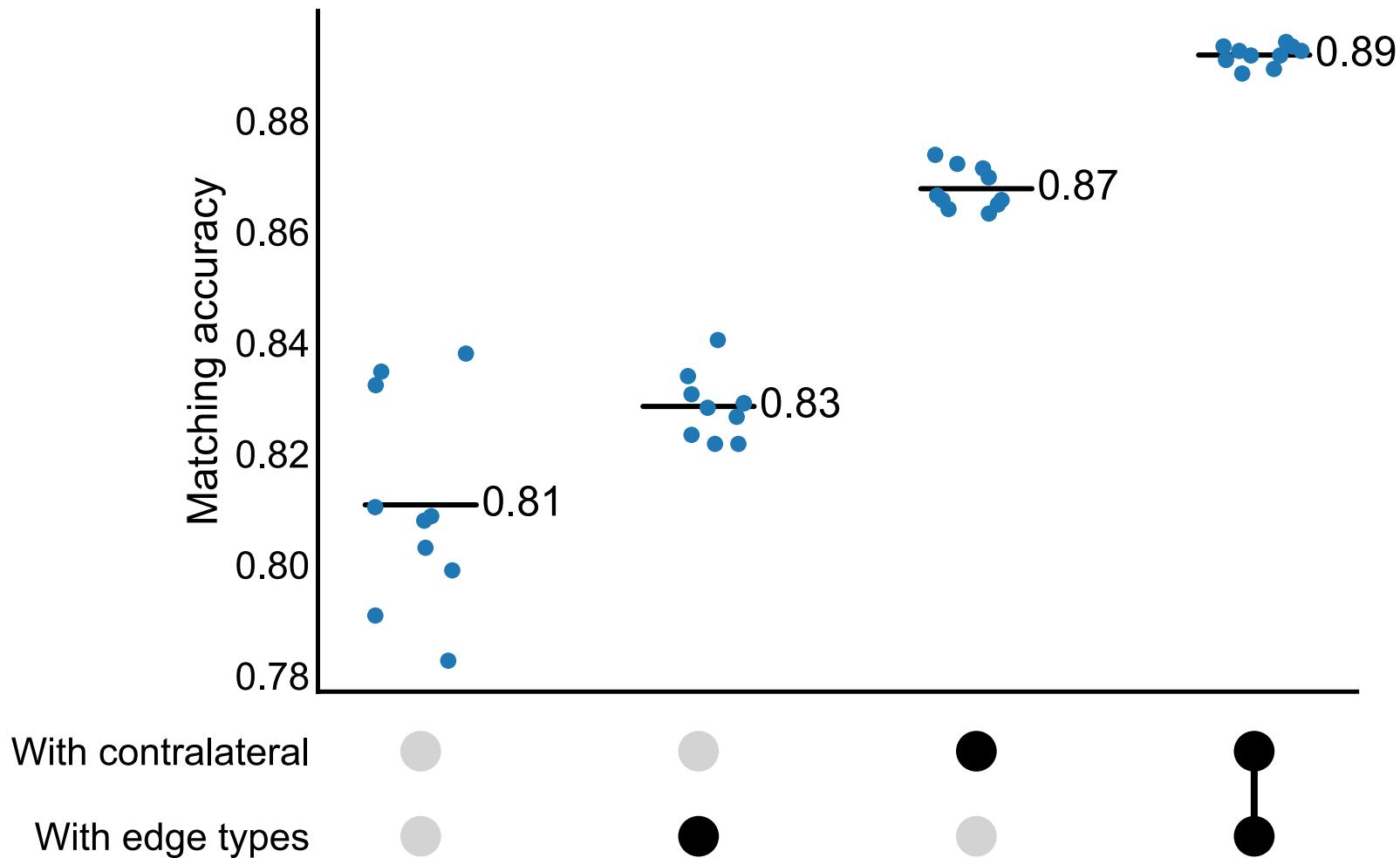
From graph matching to bisected graph matching



Contralateral connections are helpful!



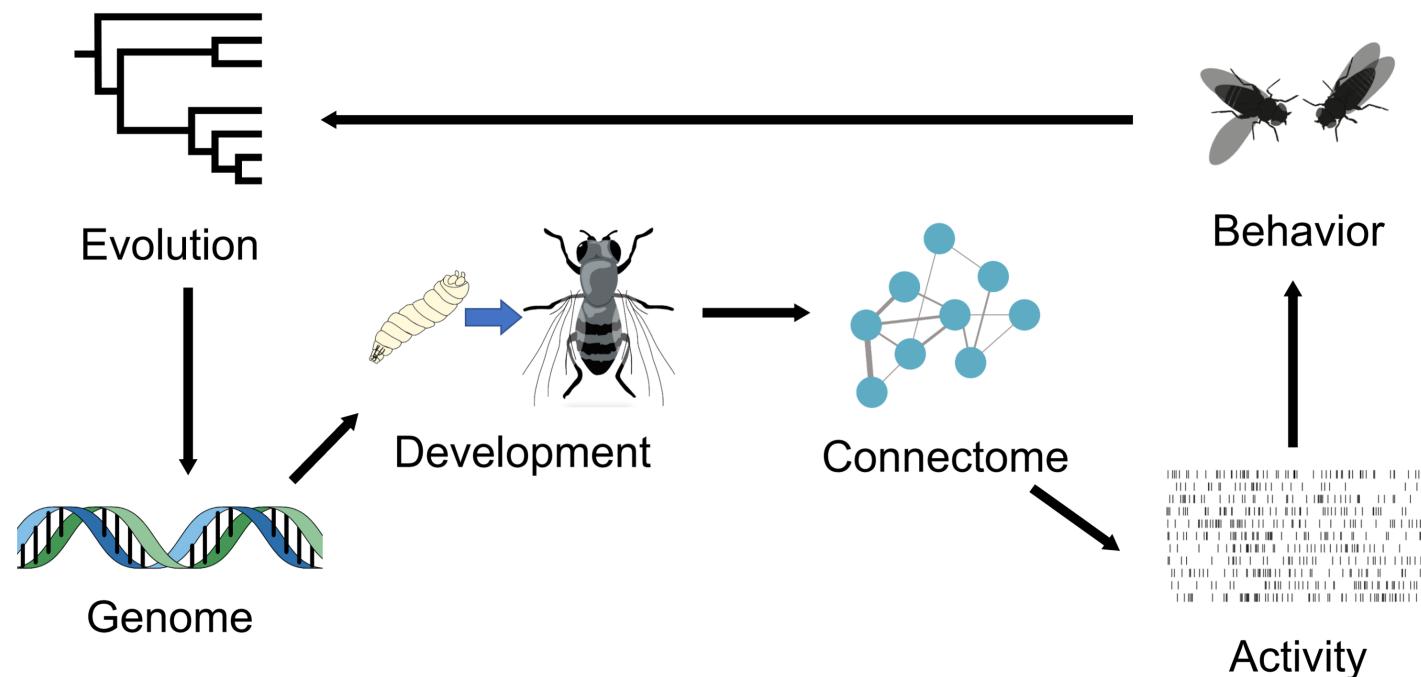
Performance improvement on the full brain



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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



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.



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?

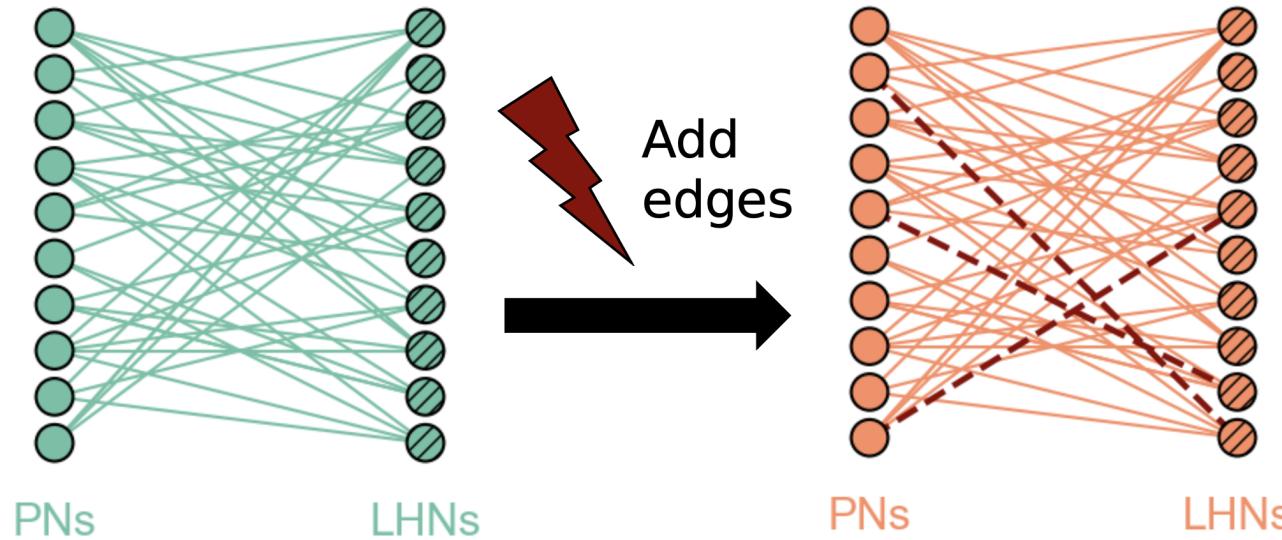
Collecting the data is still a large effort...

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?

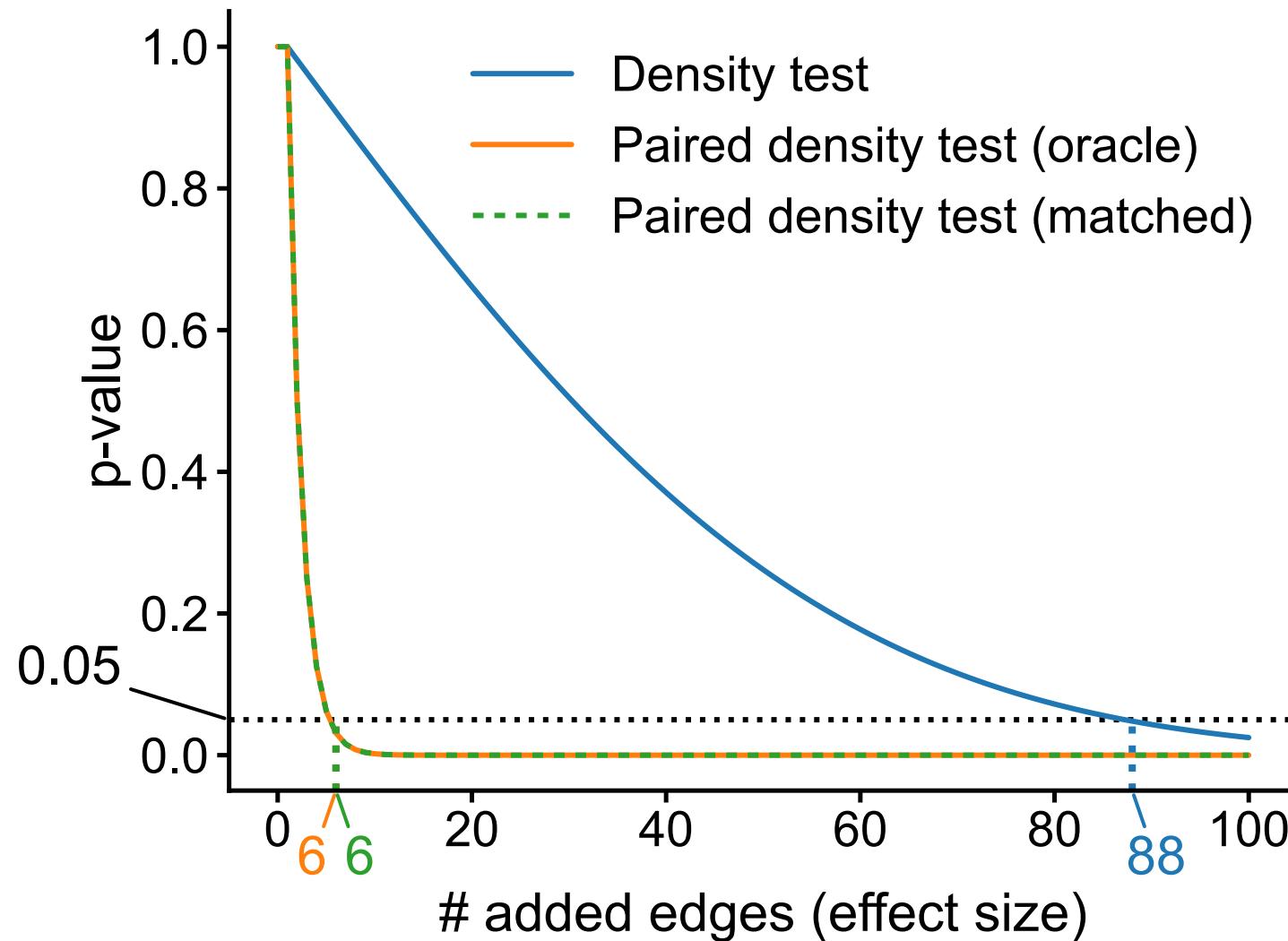
How powerful is comparative connectomics?

A hypothetical difference we want to detect...

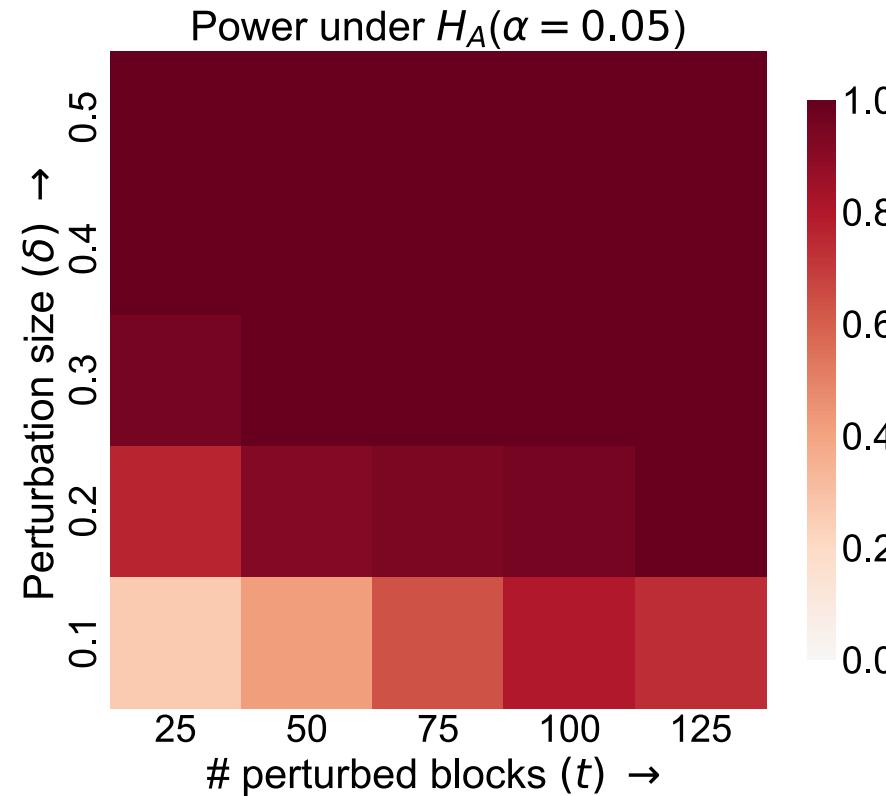


- Start from some subgraph in the connectome, A
- Perturb a copy of it, B (add edges)
- Test for differences between A and B

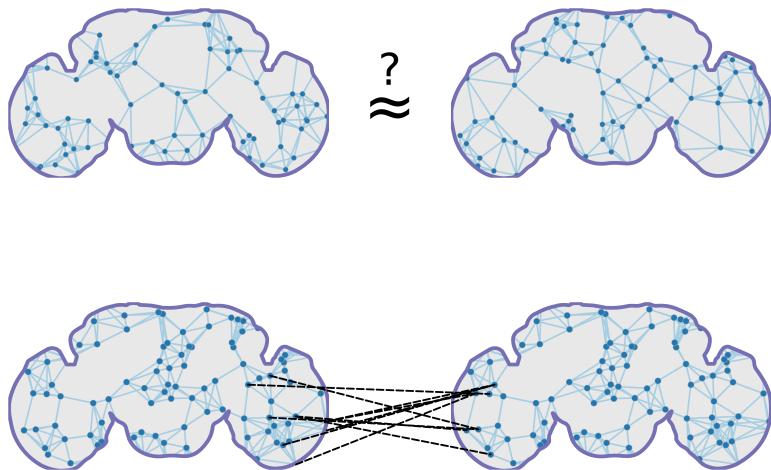
Pairs facilitate more powerful tests



Ensuring robustness to different alternatives



Summary



- Model-based network comparison enables testing (and refining) hypotheses about connectomes
 - We proposed a few tests, but just the beginning!
- Graph matching can pair neurons across datasets
 - Helpful to adapt off-the-shelf algos. to use biological info (e.g contralaterals, edge types)

Aim to apply these (and other) tools to make inferences from connectome comparisons!

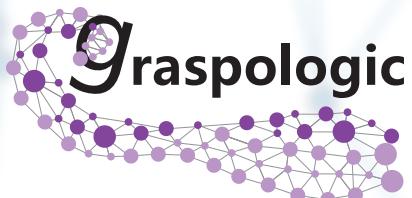
References

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

downloads 167k
 Stars 273

github.com/neurodata/maggot_models
github.com/neurodata/bilateral-connectome
github.com/neurodata/bgm

Acknowledgements

Team



Michael
Winding



Mike Powell



Eric
Bridgeford



Ali
Saad-Eldin



Marta Zlatic



Albert
Cardona



Carey Priebe



Joshua
Vogelstein

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Questions?

Slides:



Benjamin D. Pedigo

 bpedigo@jhu.edu

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

 [@bpedigod](https://twitter.com/bpedigod)

 bpedigo.github.io