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

Benjamin D. Pedigo

(he/him)

NeuroData lab

Johns Hopkins University - Biomedical Engineering


bpedigo@jhu.edu


@bdpedigo (Github)

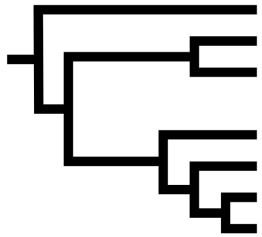

@bpedigod (Twitter)


bdpedigo.github.io

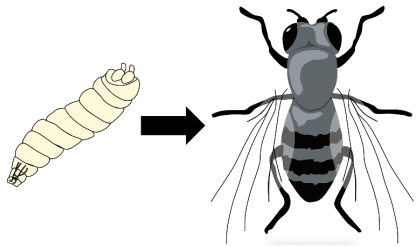
These slides at:

tinyurl.com/princeton-bilarva

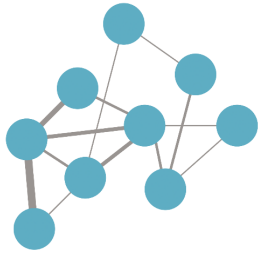
Many goals of connectomics involve linking connectome to other properties



Evolution

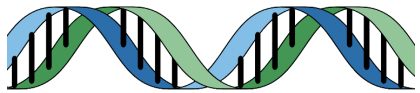


Development

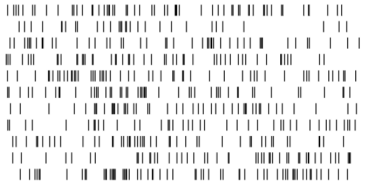


Connectome

Behavior



Genome



Activity

Comparative connectomics as a potential solution?

- Map connectomes from related individuals/organisms which may differ in feature X:
  - Genome
  - Behavioral patterns/habits
  - Life experience
  - Developmental stage
- Compare connectomes
- Understand how X {affects, is affected by, is associated with} connectome structure

Connectome ↔ memory

Commentary

The Mind of a Mouse

Larry F. Abbott,[1] Davi D. Bock,[2] Edward M. Callaway,[3] Winfried Denk,[4,25] Catherine Dulac,[5] Adrienne L. Fairhall,[6] Ila Fiete,[7] Kristen M. Harris,[8] Moritz Helmstaedter,[9] Viren Jain,[10,25,*] Narayanan Kasthuri,[11] Yann LeCun,[12] Jeff W. Lichtman,[13,25,*] Peter B. Littlewood,[14] Liqun Luo,[15] John H.R. Maunsell,[16] R. Clay Reid,[17,25] Bruce R. Rosen,[18] Gerald M. Rubin,[19] Terrence J. Sejnowski,[3,20] H. Sebastian Seung,[21,25] Karel Svoboda,[19] David W. Tank,[22,25] Doris Tsao,[23] and David C. Van Essen[24]

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

Abbott et al. Cell (2020)

Connectome ↔ disease

Commentary

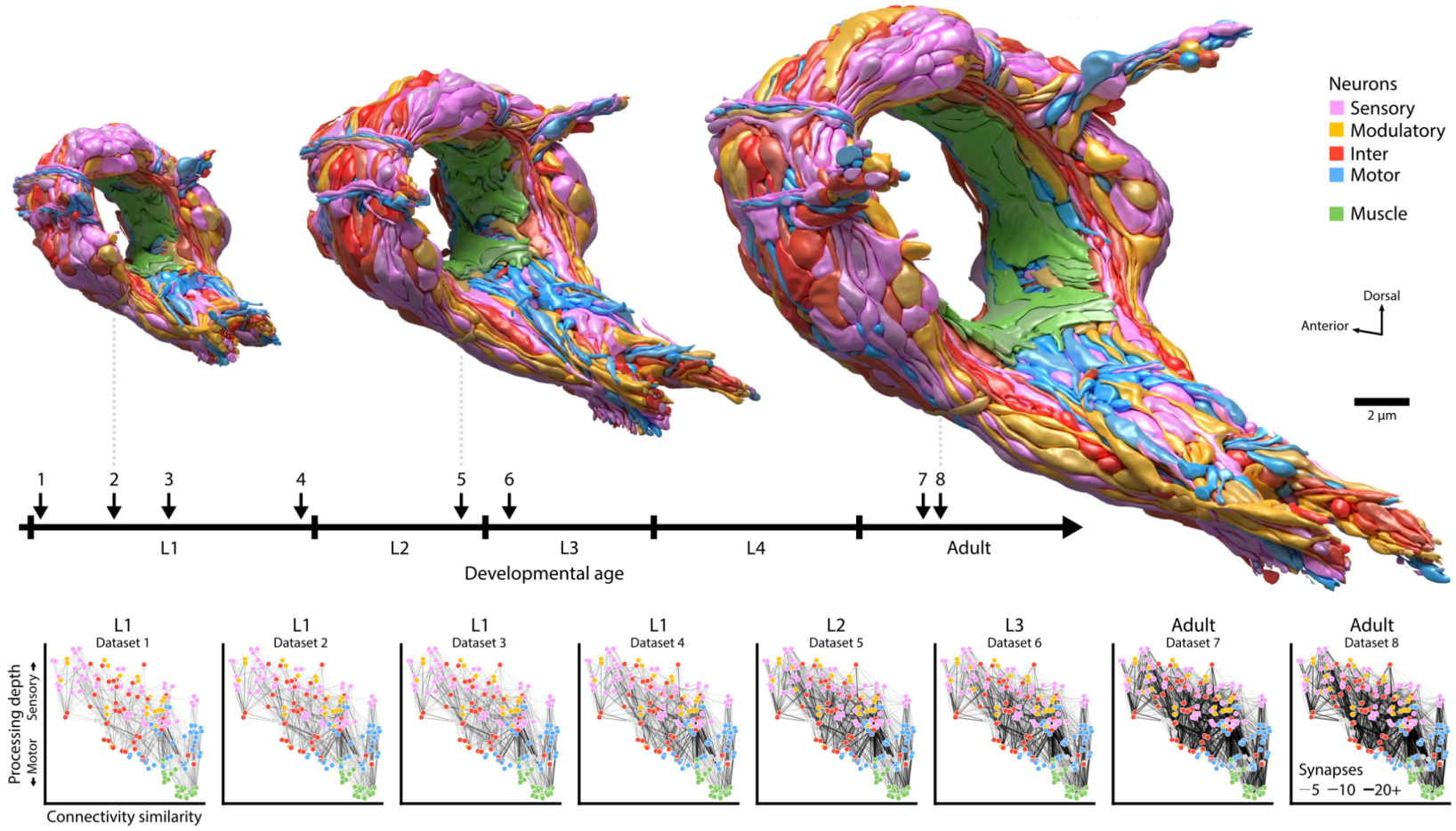
The Mind of a Mouse

Larry F. Abbott,[1] Davi D. Bock,[2] Edward M. Callaway,[3] Winfried Denk,[4,25] Catherine Dulac,[5] Adrienne L. Fairhall,[6] Ila Fiete,[7] Kristen M. Harris,[8] Moritz Helmstaedter,[9] Viren Jain,[10,25,*] Narayanan Kasthuri,[11] Yann LeCun,[12] Jeff W. Lichtman,[13,25,*] Peter B. Littlewood,[14] Liqun Luo,[15] John H.R. Maunsell,[16] R. Clay Reid,[17,25] Bruce R. Rosen,[18] Gerald M. Rubin,[19] Terrence J. Sejnowski,[3,20] H. Sebastian Seung,[21,25] Karel Svoboda,[19] David W. Tank,[22,25] Doris Tsao,[23] and David C. Van Essen[24]

> The first step would be to learn what the normal wiring diagram is [...] it should be feasible to do many additional connectomes [...] of animal models of brain disorders

Abbott et al. Cell (2020)

Connectome ↔ development



Witvliet et al. Nature (2021)

Why is comparative connectomics hard?

Collecting the data is still a large effort...

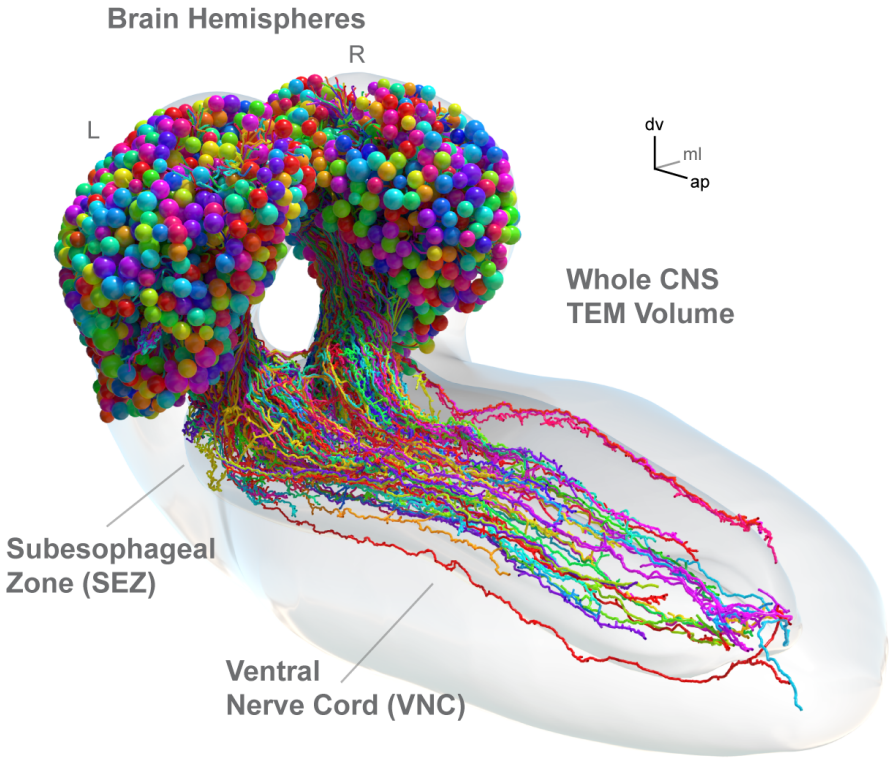
But how do we even compare connectomes once we have them?

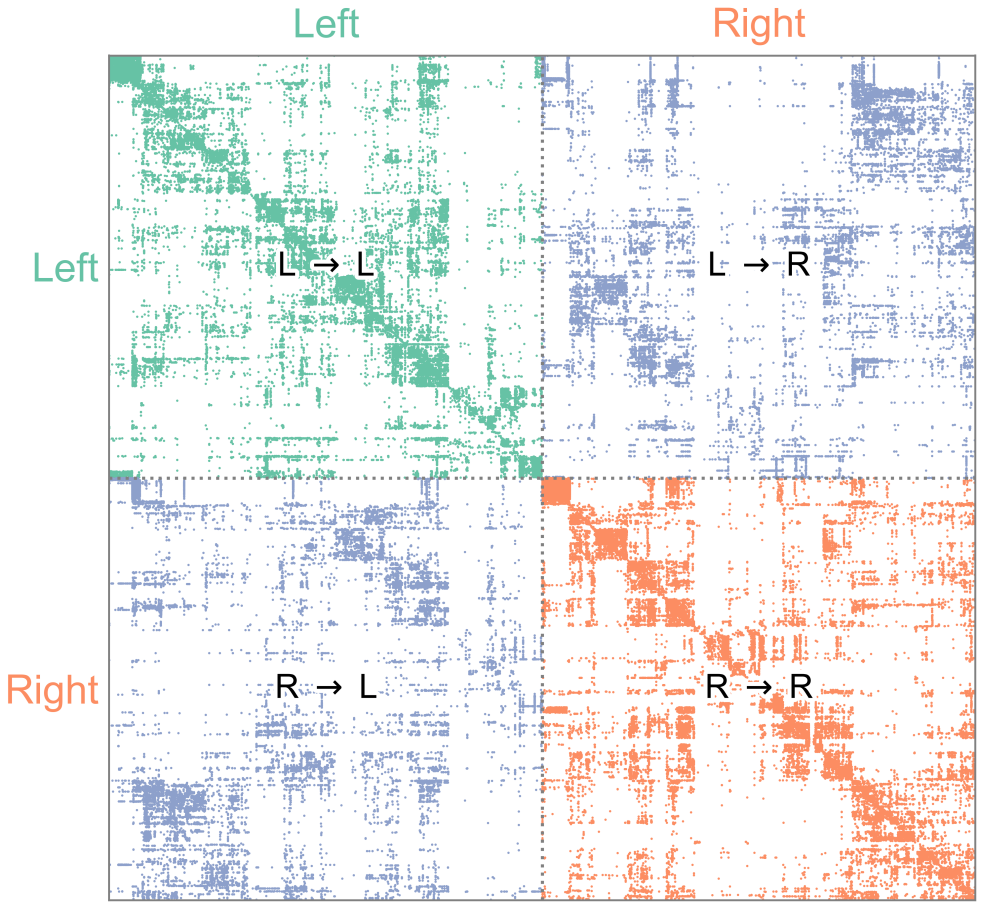
- ~~Data are networks~~
  - Data are networks with rich attributes
- Data will always have noise
  - "Experimental noise"
  - "Biological noise"
- Data are big (and getting bigger)

Outline

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

Larval *Drosophila* brain connectome





~3k neurons, ~550K synapses

Both hemispheres

Winding, Pedigo et al. Submitted (2022)

Bilateral symmetry

> "This brain is bilaterally symmetric."
>
> -Neuroscientists

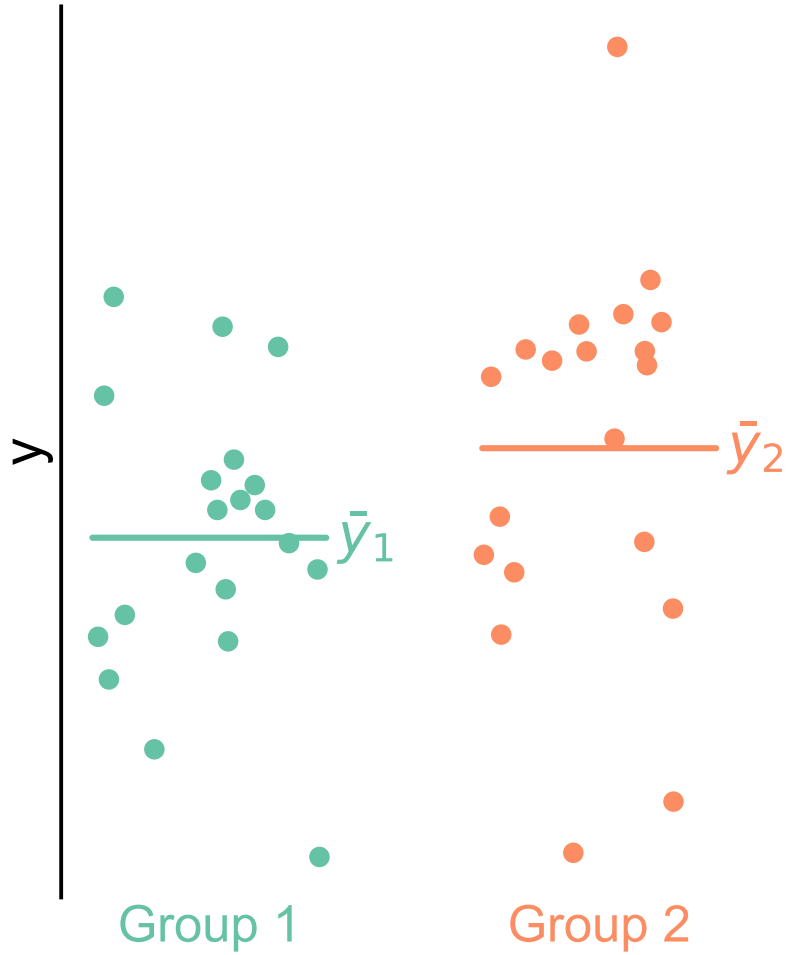
> "What does that even mean? And how would we know if it wasn't?"
>
> -Us

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

Outline

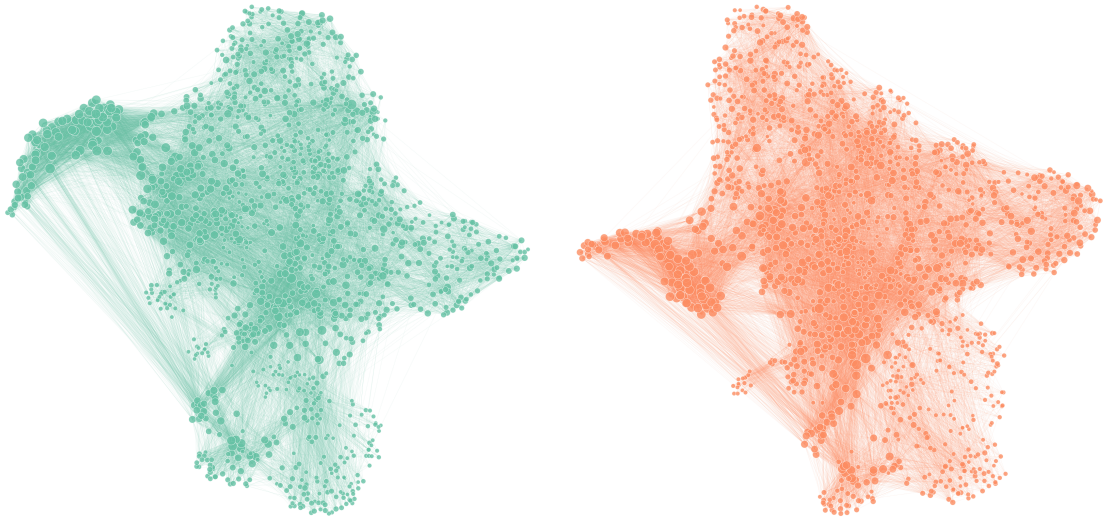
- Larval connectome dataset
- Connectome comparison via network hypothesis testing
- Pairing neurons across connectomes via graph matching
- Ongoing extensions/applications

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?

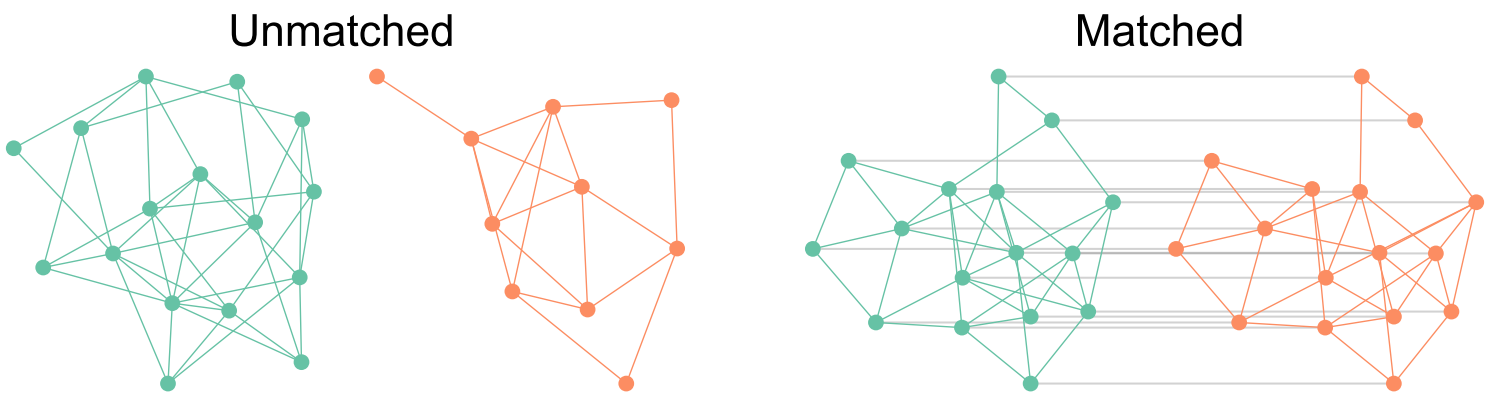


Left Right

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

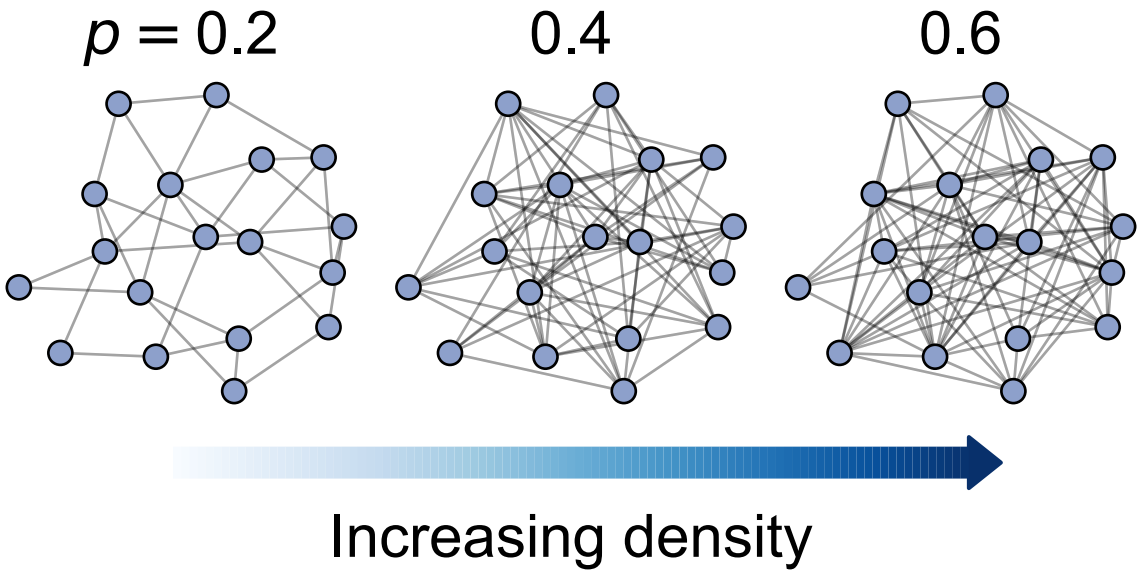
Assumptions

- 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 $\longrightarrow$ 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

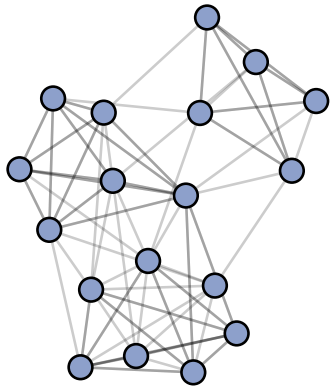


Detect a difference in density

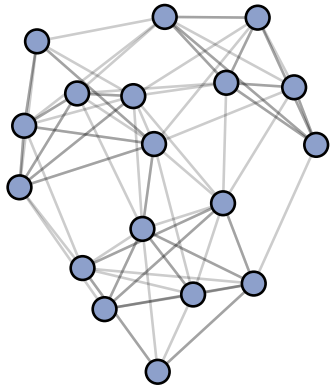
Compute global connection density

Compare ER models

Left

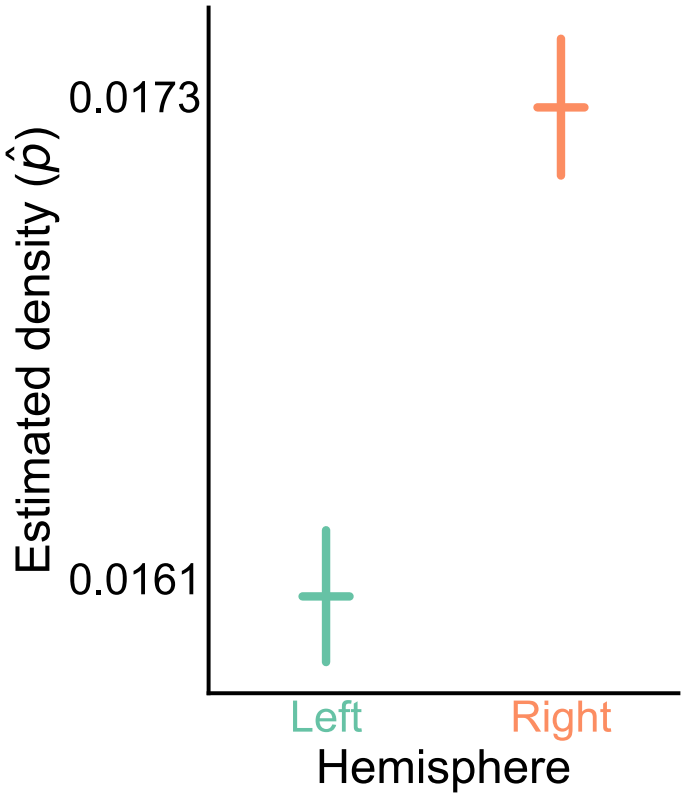


Right



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

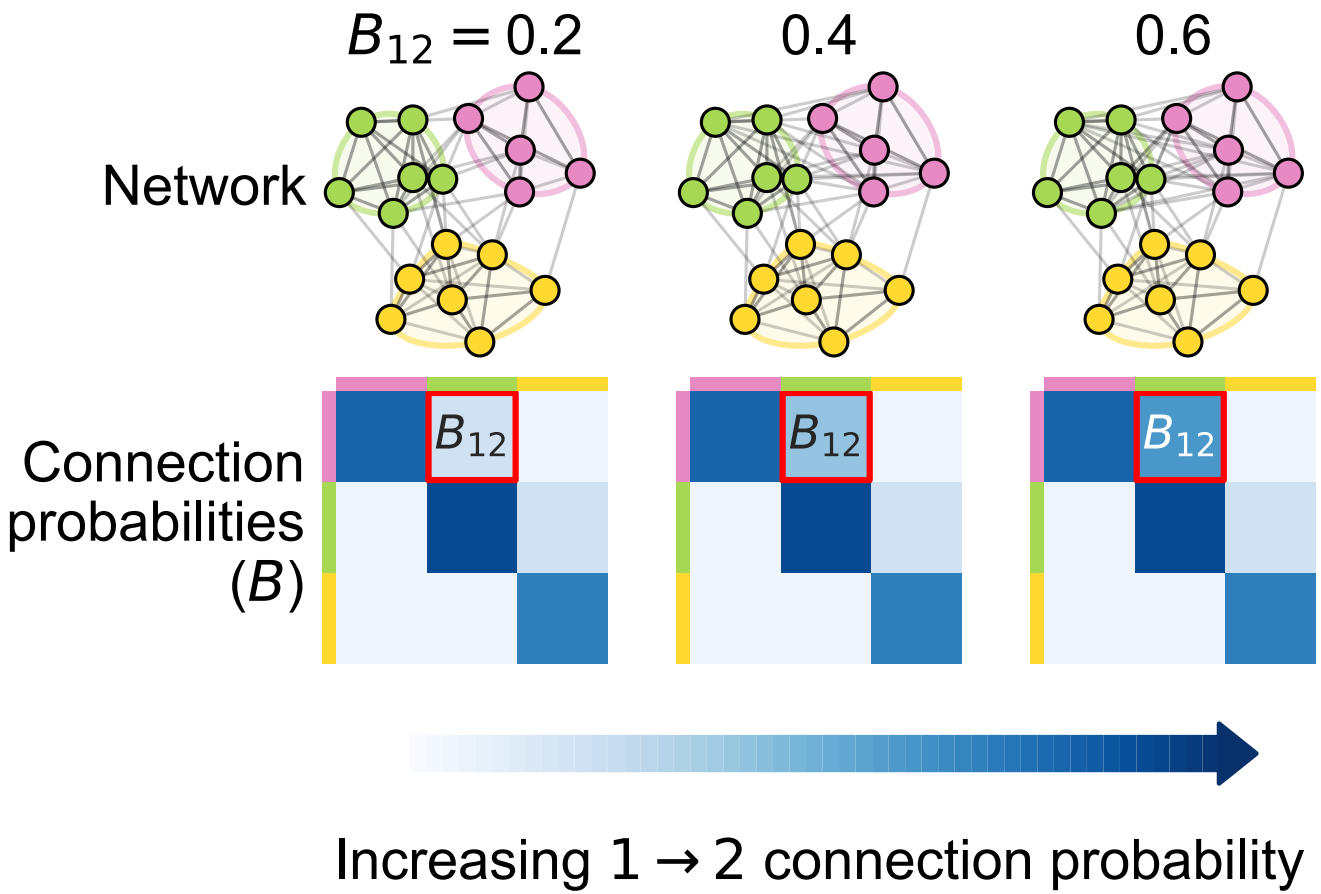
$$H_0: p^{(L)} = p^{(R)}$$
$$H_A: p^{(L)} \neq p^{(R)}$$



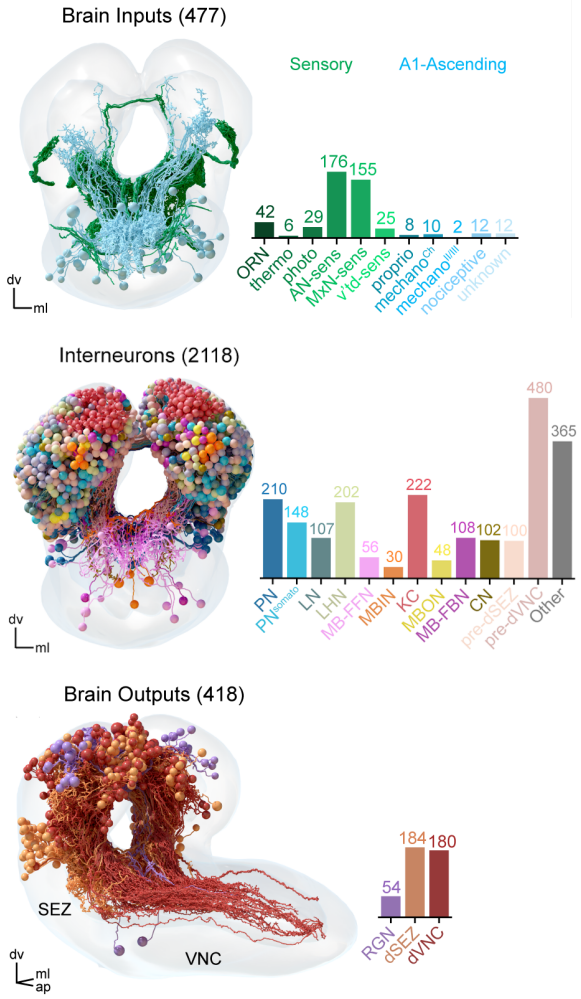
p-value < 10^{-22}

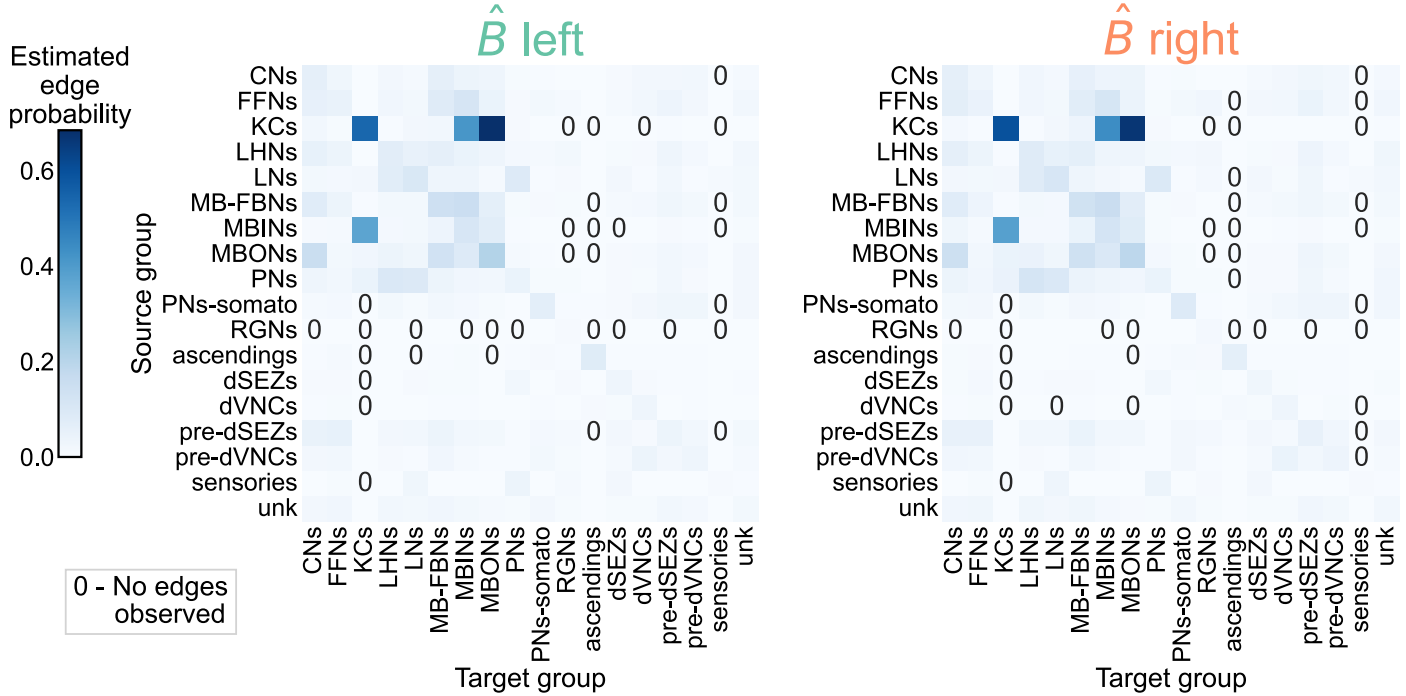
Stochastic block model

Edge probabilities are a function of a neuron's group



Connection probabilities between groups

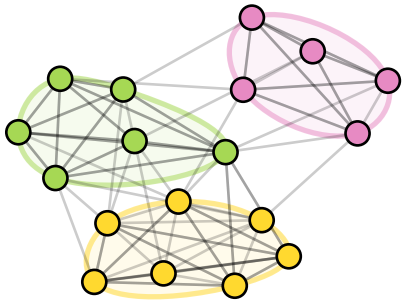




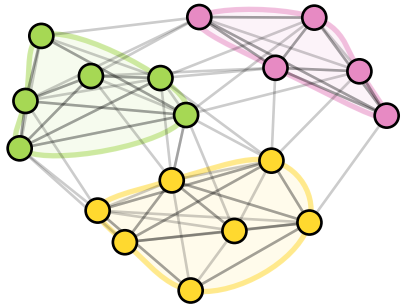
Group connection test

Group neurons

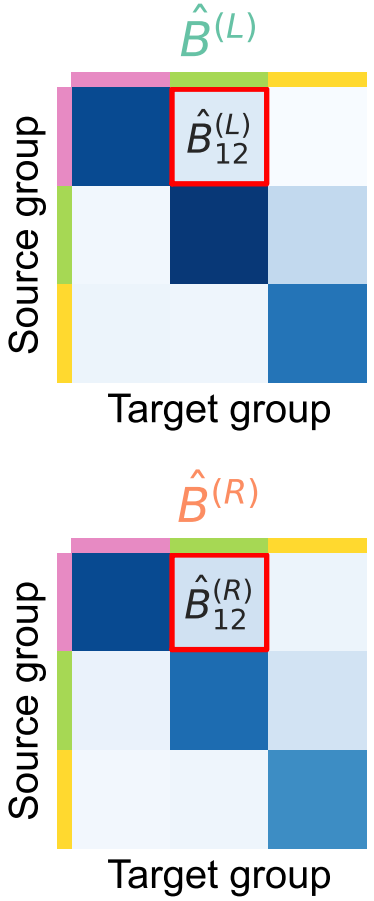
Left



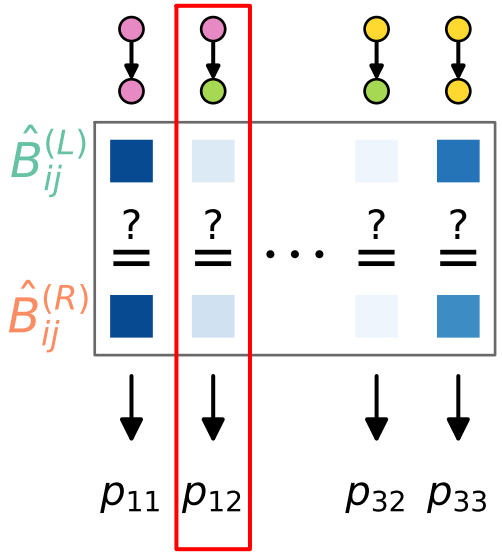
Right



Estimate group connection probabilities

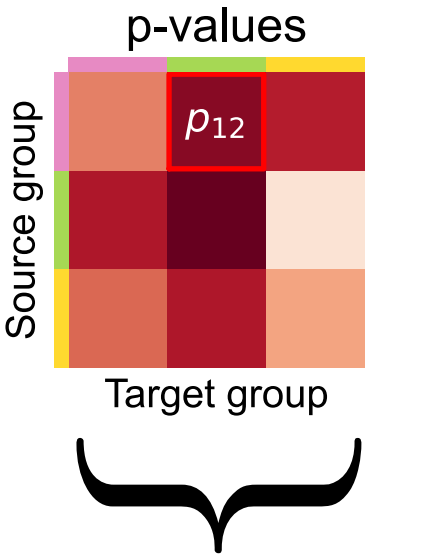


Compare probabilities, compute p-values



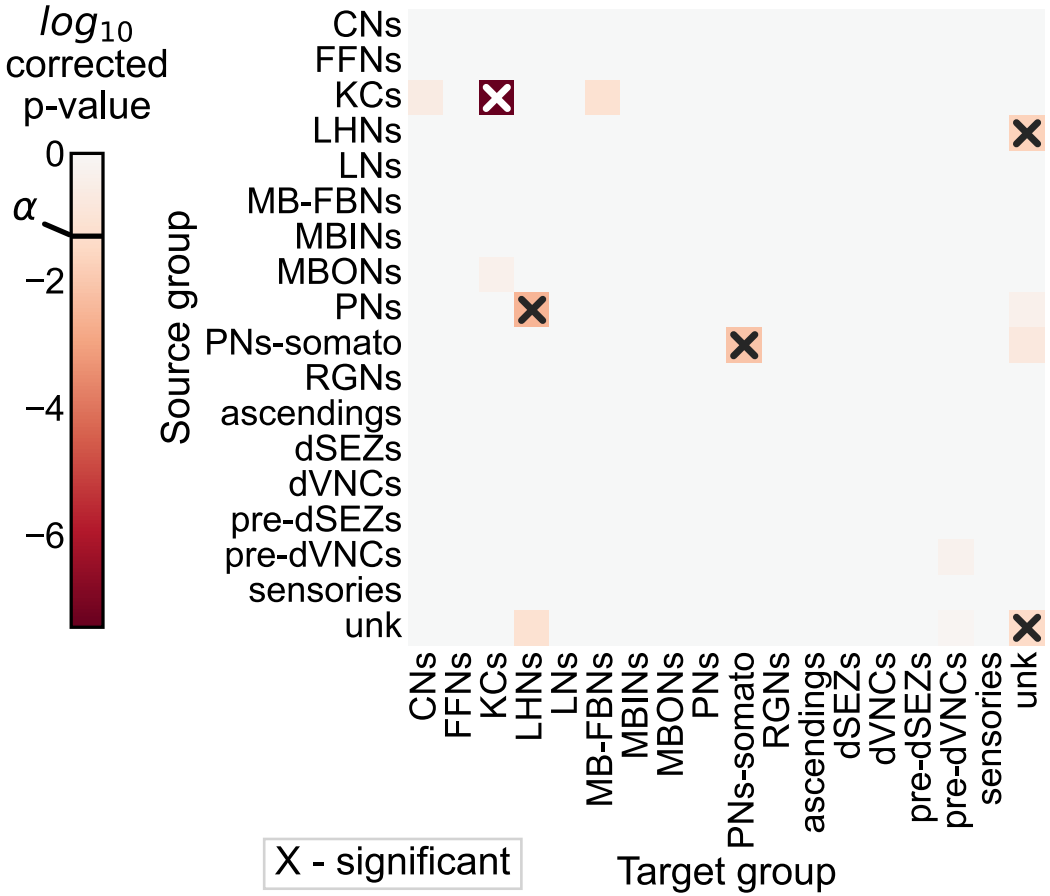
$$H_0: B^{(L)}_{ij} = B^{(R)}_{ij}$$
$$H_A: B^{(L)}_{ij} \neq B^{(R)}_{ij}$$

Combine p-values for overall test



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

Detect differences in group connection probabilities

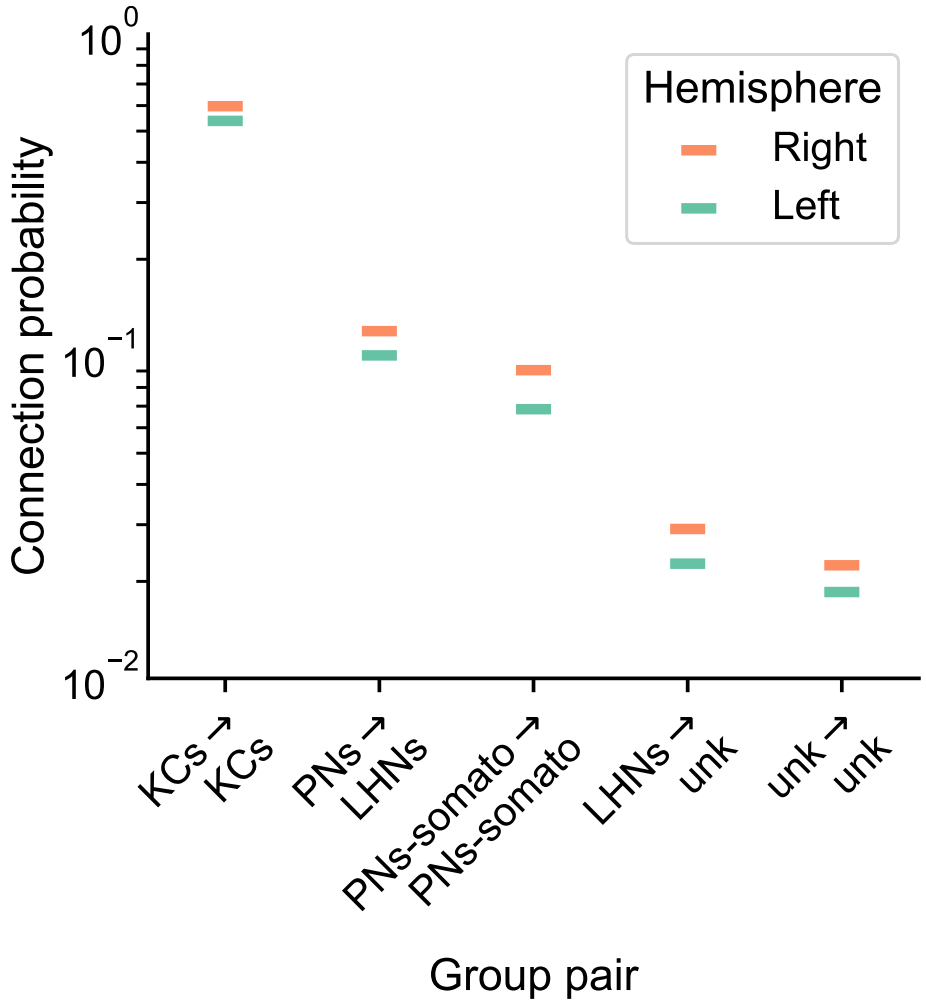


- 5 group-to-group connections are significantly different (after multiple comparisons correction)
- Overall test (comparing all blocks): p-value $< 10^{-7}$

Should we be surprised?

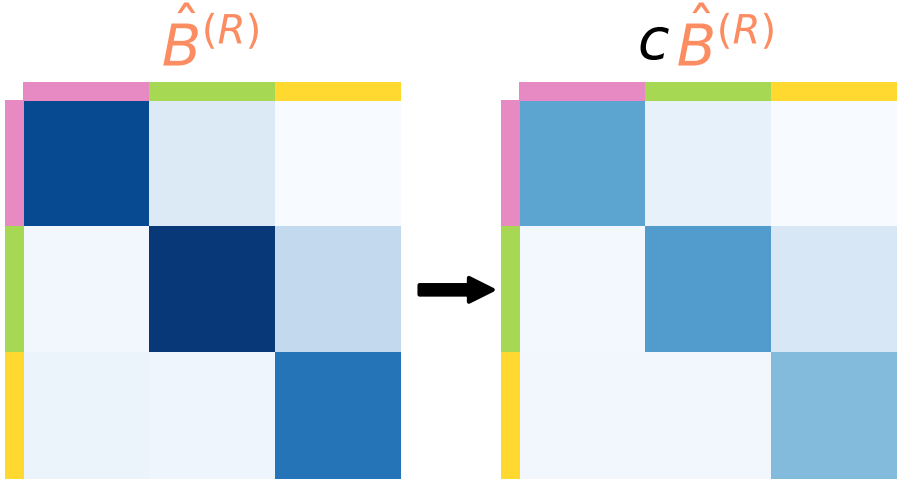
- Already saw that even the overall densities were different
- For all significant comparisons, probabilities on the right hemisphere were higher
- Maybe the right is just a "scaled up" version of the left?
  - $H_0 : B^{(L)} = cB^{(R)}$

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

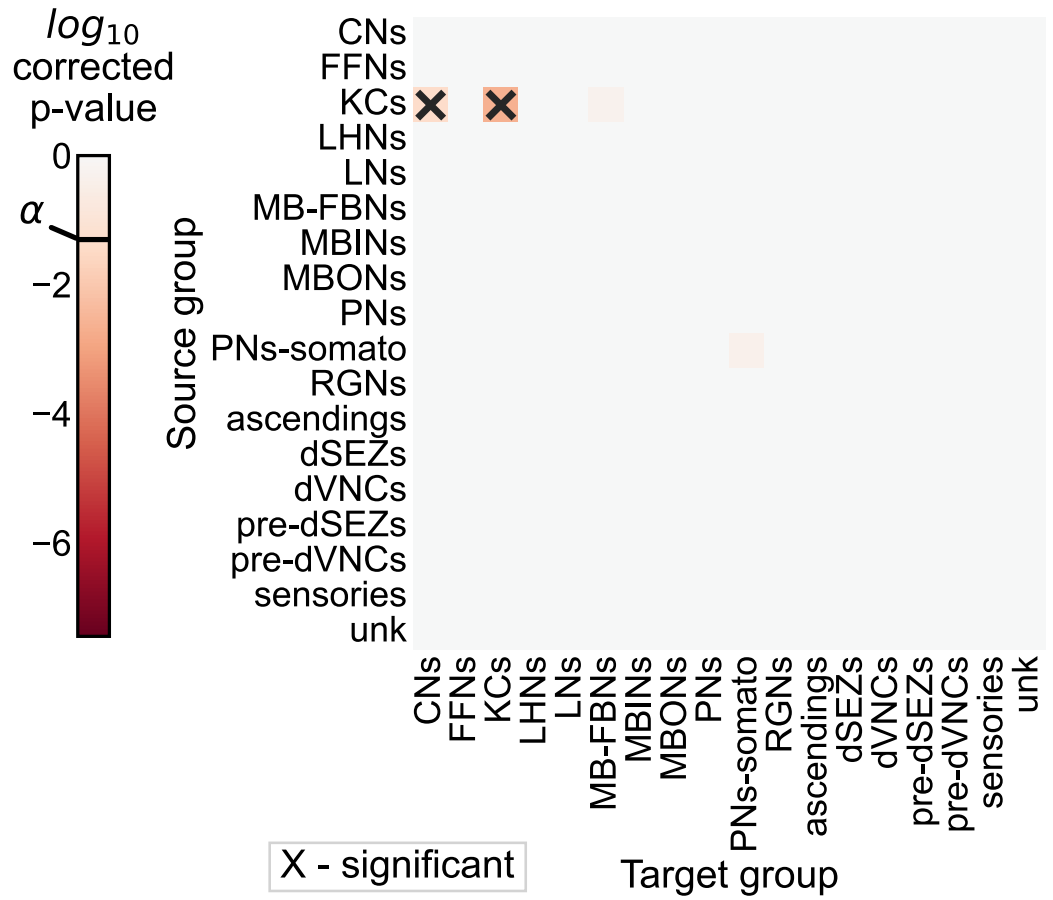


After adjusting for density, differences are in KCs

Scale connection probabilities
to match densities



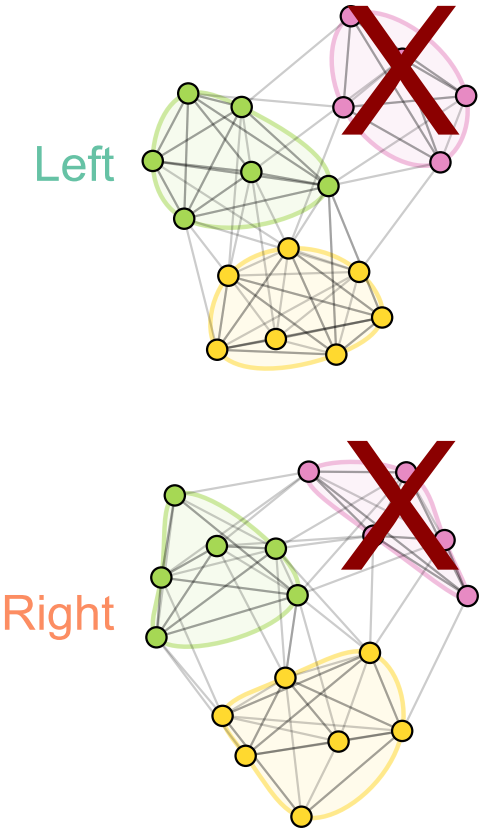
$$H_0: B^{(L)} = cB^{(R)}$$
$$H_A: B^{(L)} \neq cB^{(R)}$$



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

When we remove KCs...

Remove Kenyon cells



Re-run all tests

Density

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

Group connection

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

Density-adjusted group connection

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

- Density test:
  $p < 10^{-26}$
- Group connection test:
  $p < 10^{-2}$
- **Density-adjusted group connection test:**
  $p \approx 0.51$

To sum up...

> "This brain is bilaterally symmetric."
>
> -Neuroscientists

Depends on what you mean...

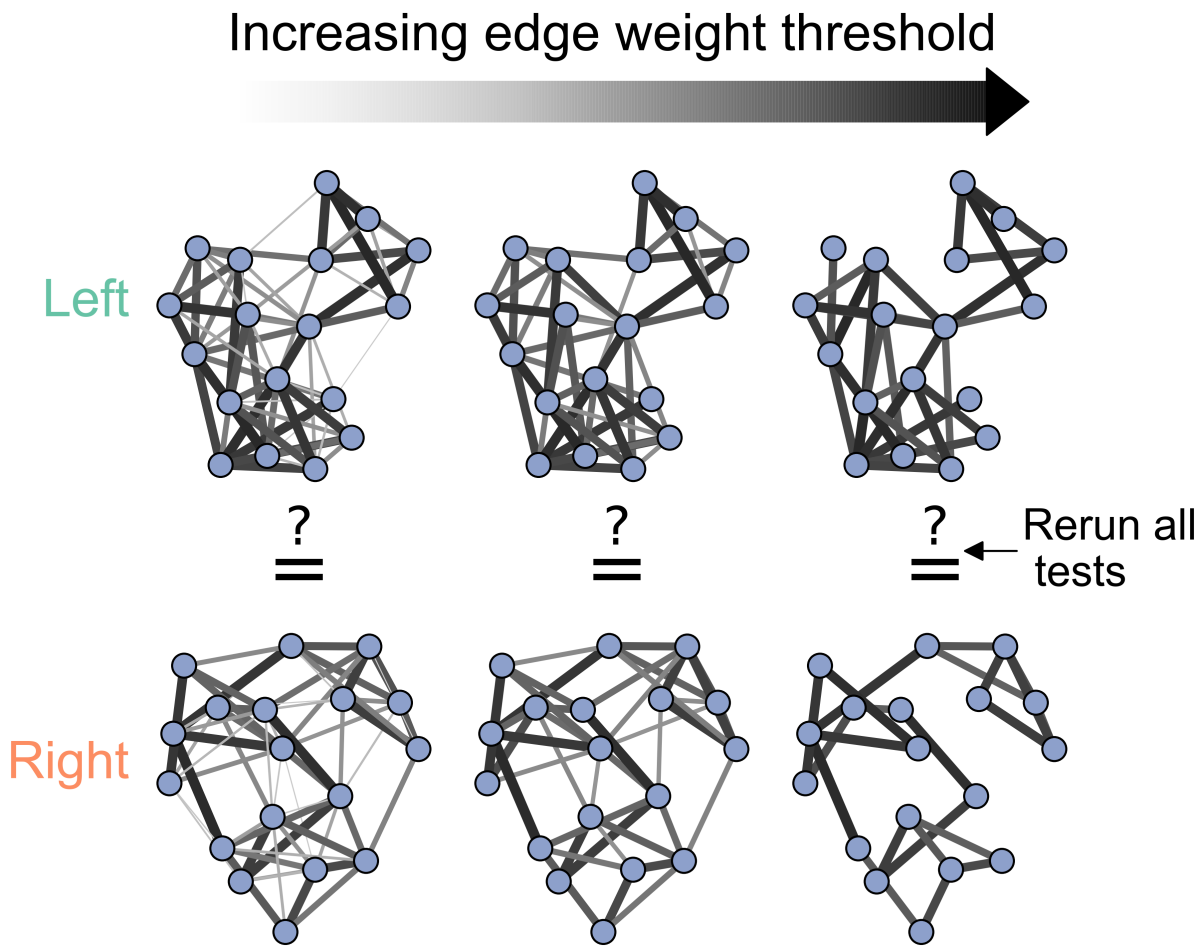
With Kenyon cells

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

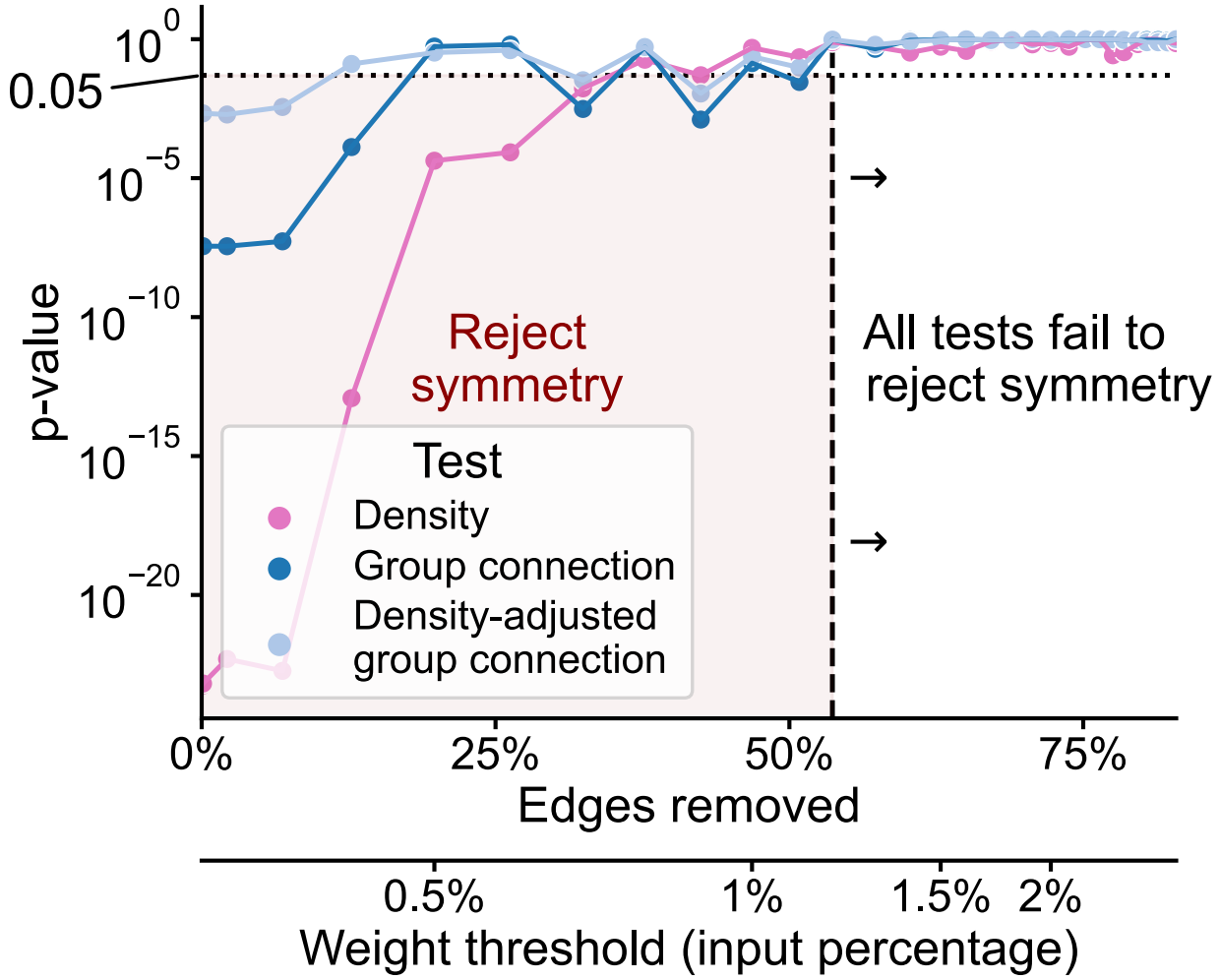
Without Kenyon cells

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



Highest edge weight networks show no asymmetry

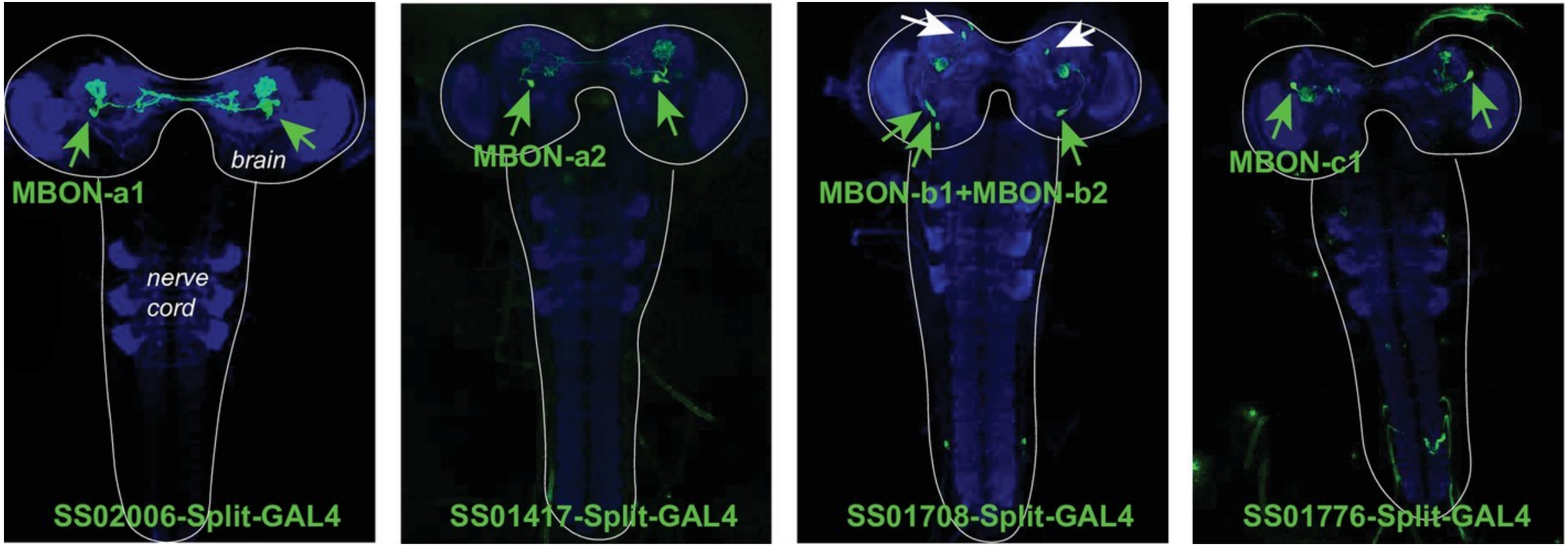


Outline

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

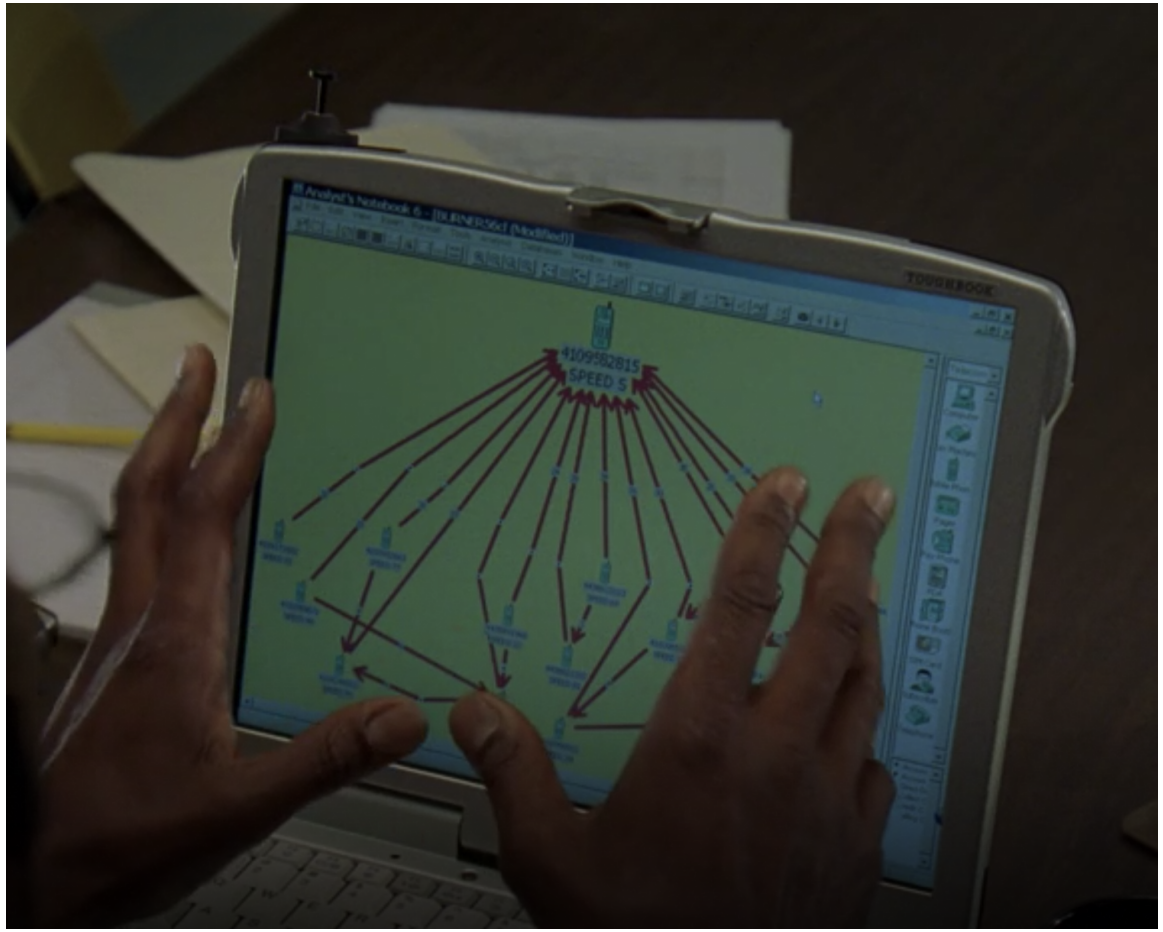
Bilaterally homologous neuron pairs

We believe a matching exists!



Eschbach et al. eLife (2021)

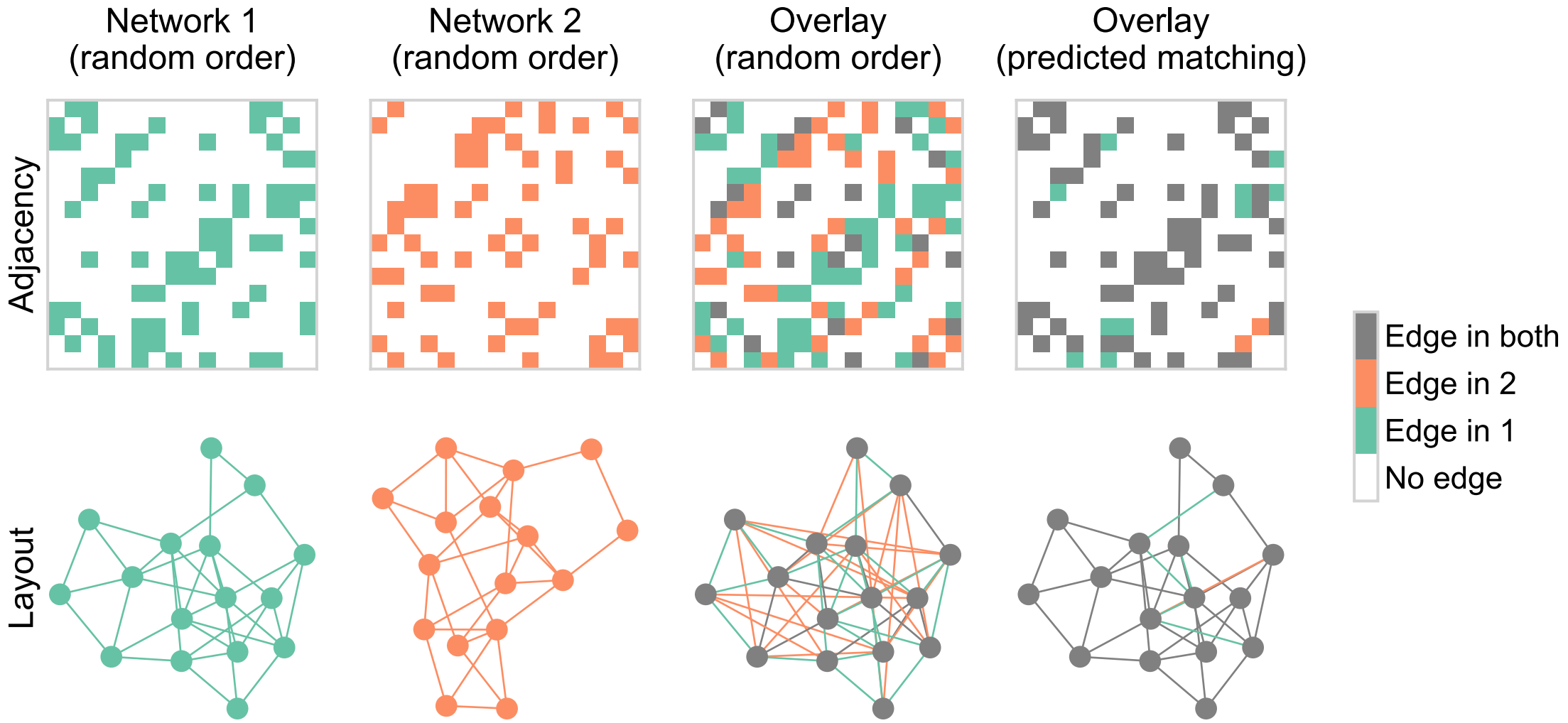
Can we use network structure to predict this pairing?



- Week 1: observe a network (A) of phone #s and the calls they make to each other
- Week 2: all of the #s change! But a (noisy) version of that network still exists, with different labels... (B)
- How to map nodes of network A to those of network B?

The Wire Season 3 Episode 7, HBO

What is graph matching?



How do we measure network overlap?

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

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

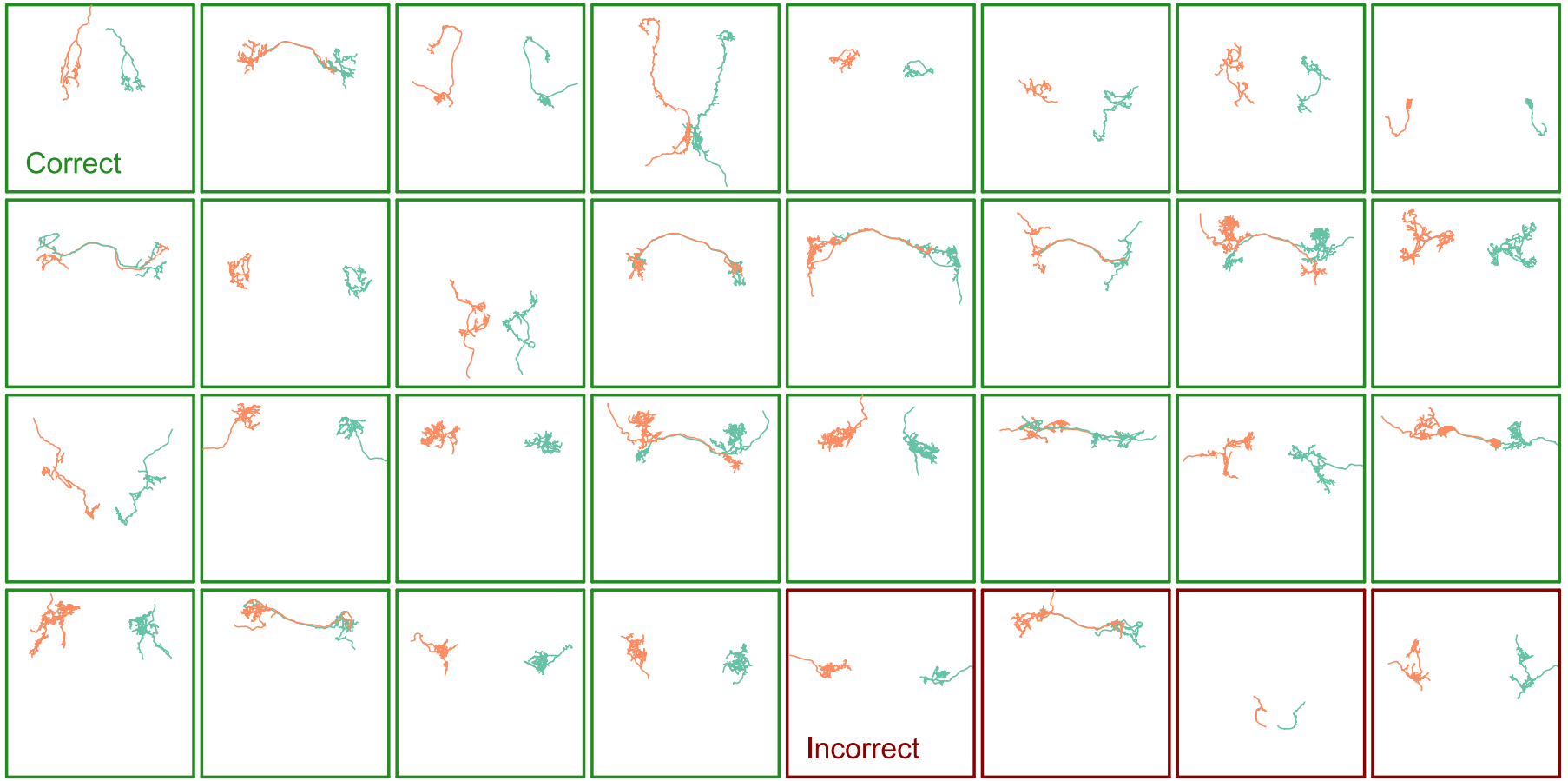
- Measures the number of edge disagreements for unweighted networks,
- Norm of edge disagreements for weighted networks

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)

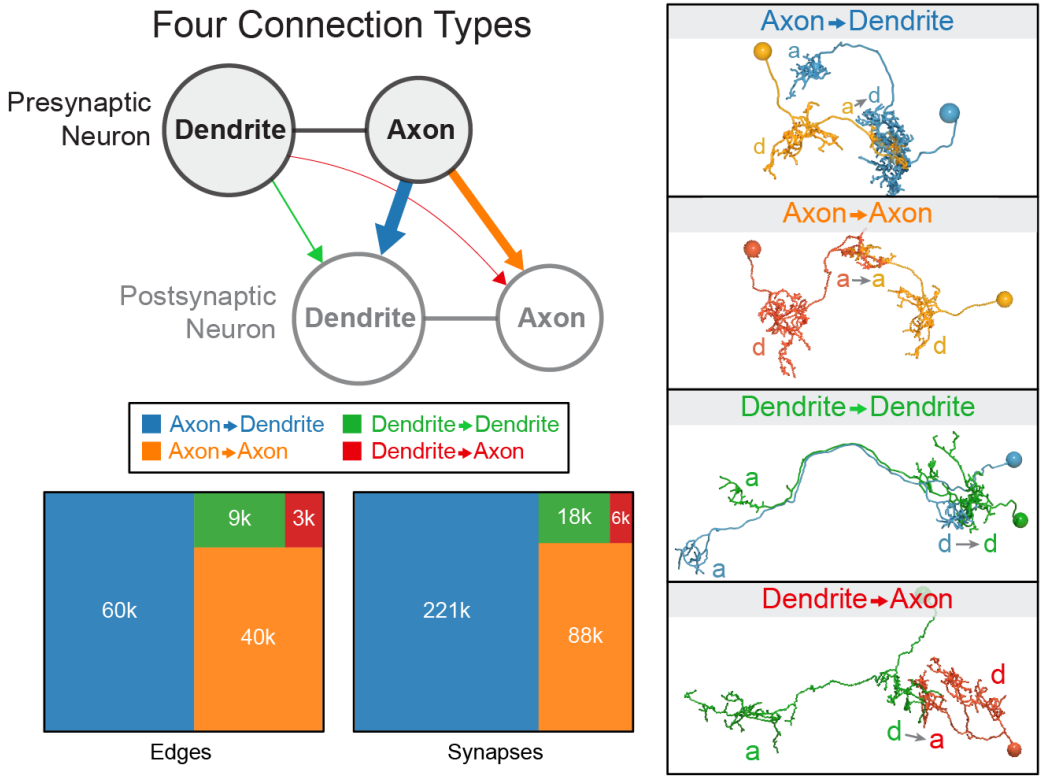
Matching (by connectivity only) performs fairly well



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)



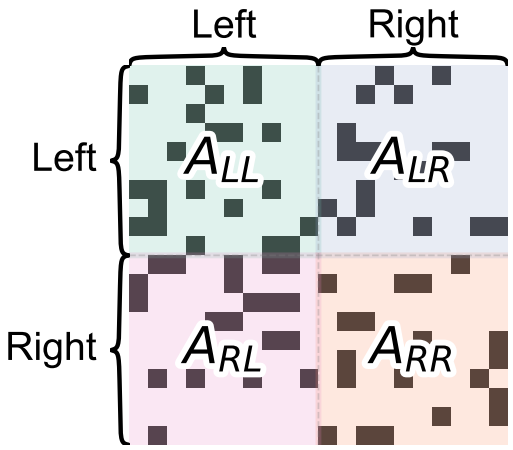
Summary of "edge types" based on neuron compartments

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

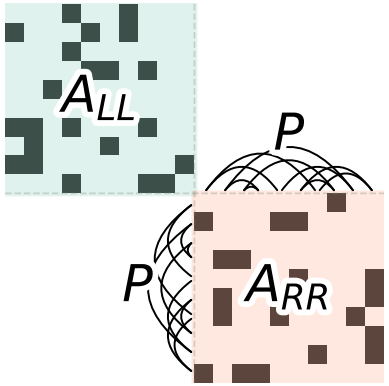
Adjacency matrix



Ipsilateral → A_{LL}, A_{RR}

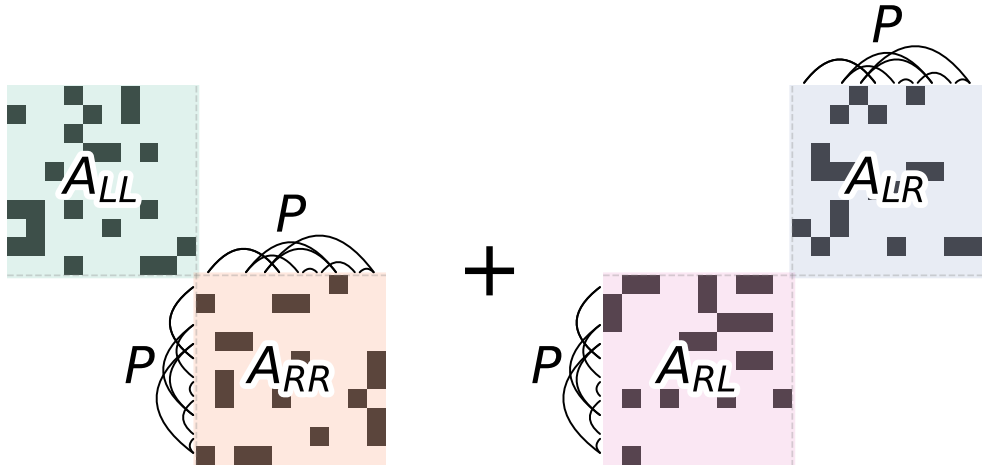
Contralateral → A_{LR}, A_{RL}

Graph matching (GM)



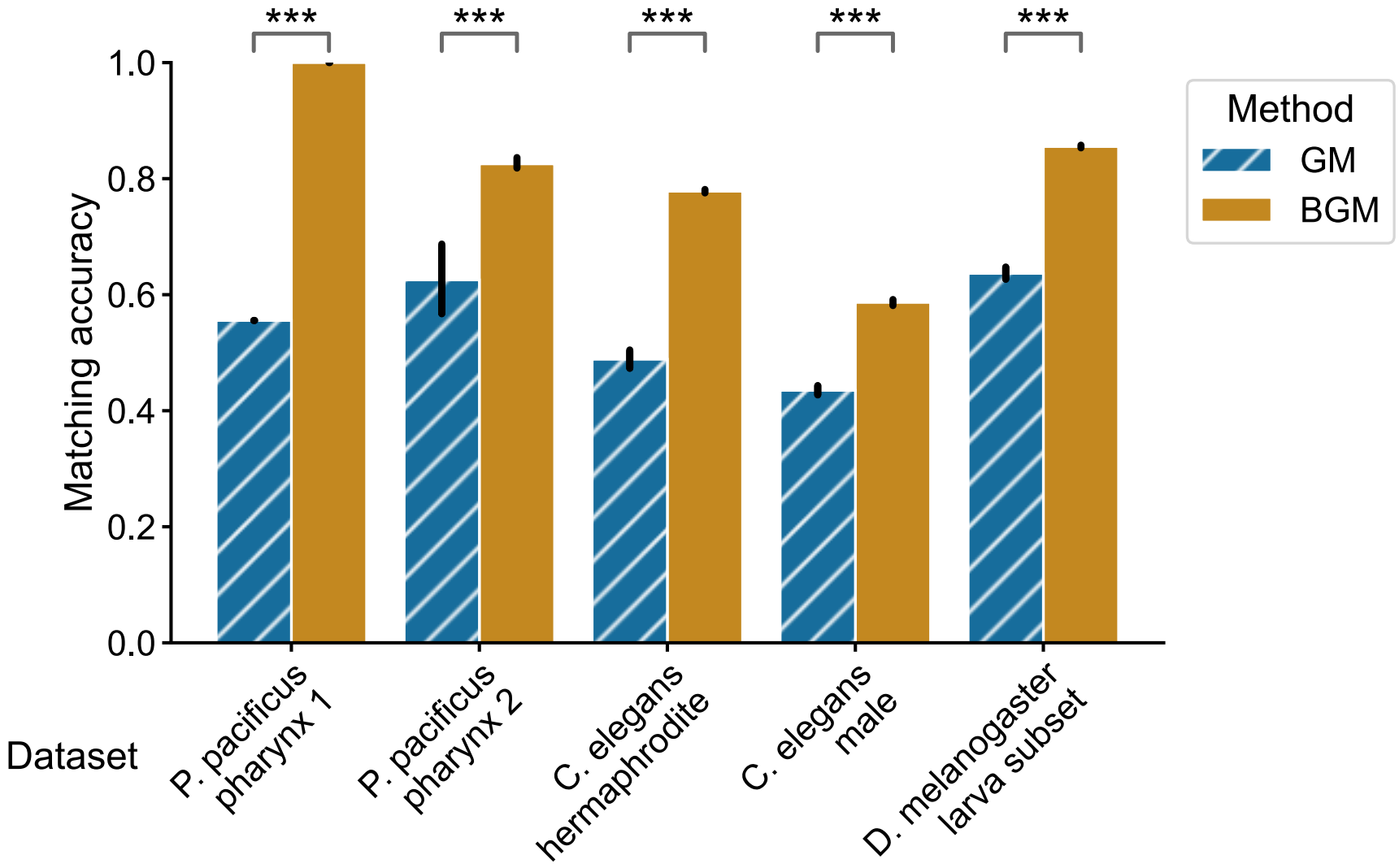
$$\min_P \| A_{LL} - P\, A_{RR}\, P^T \|_F^2$$

Bisected graph matching (BGM)



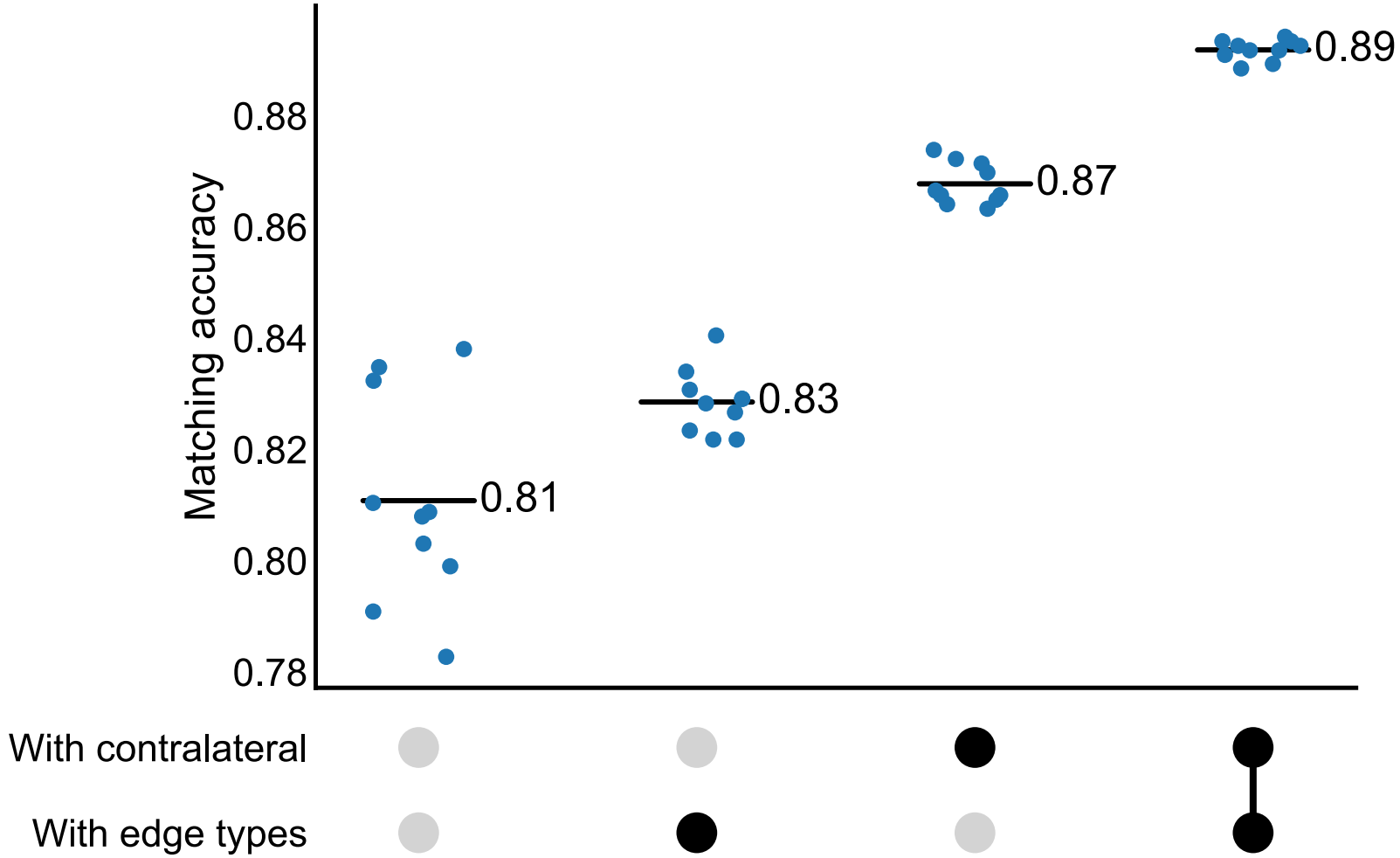
$$\min_P \| A_{LL} - P\, A_{RR}\, P^T \|_F^2 + \| A_{LR}\, P^T - P\, A_{RL} \|_F^2$$

Contralateral connections are helpful!



Pedigo et al. bioRxiv (2022)

Performance improvement on the full brain

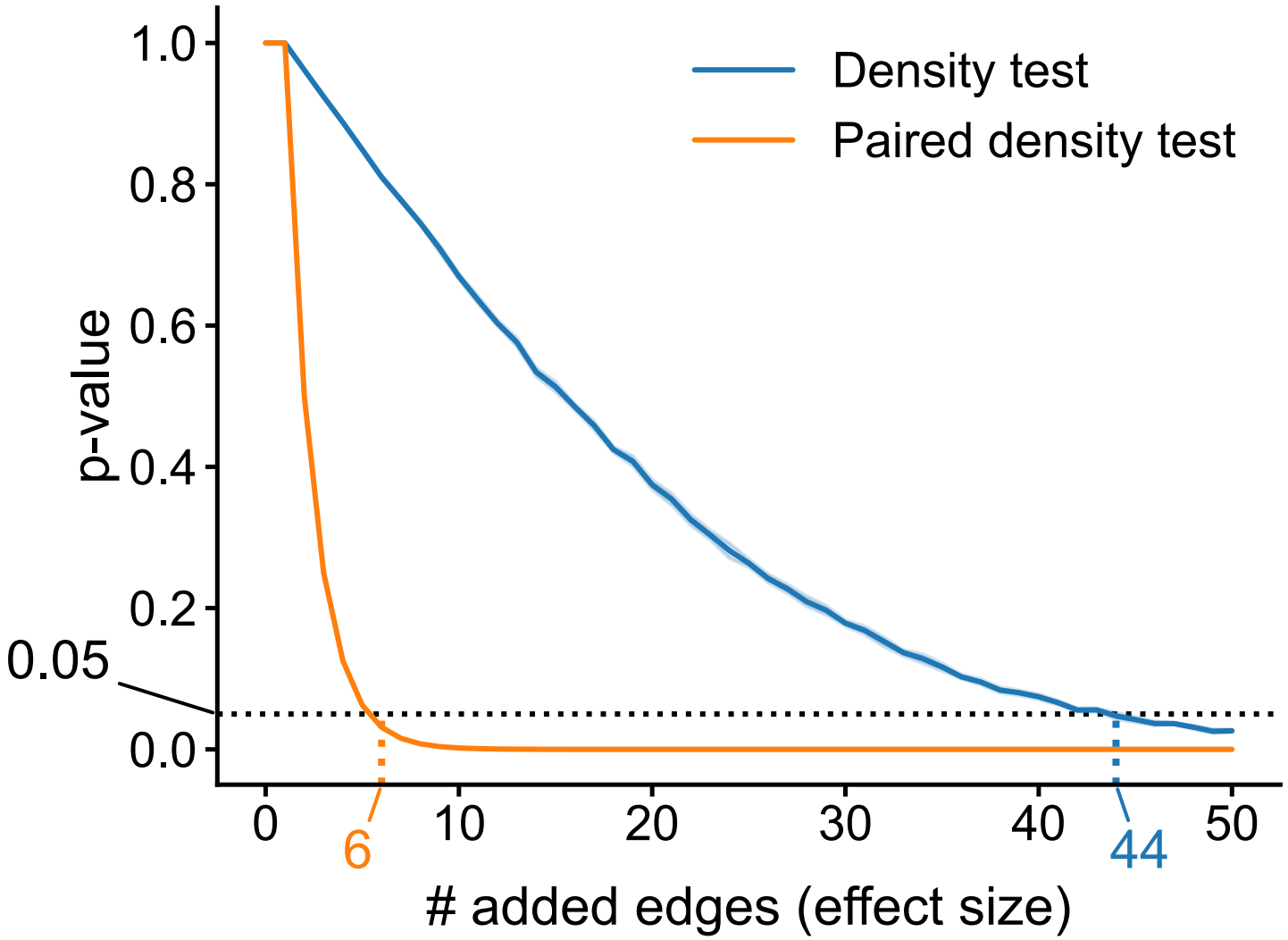


Outline

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

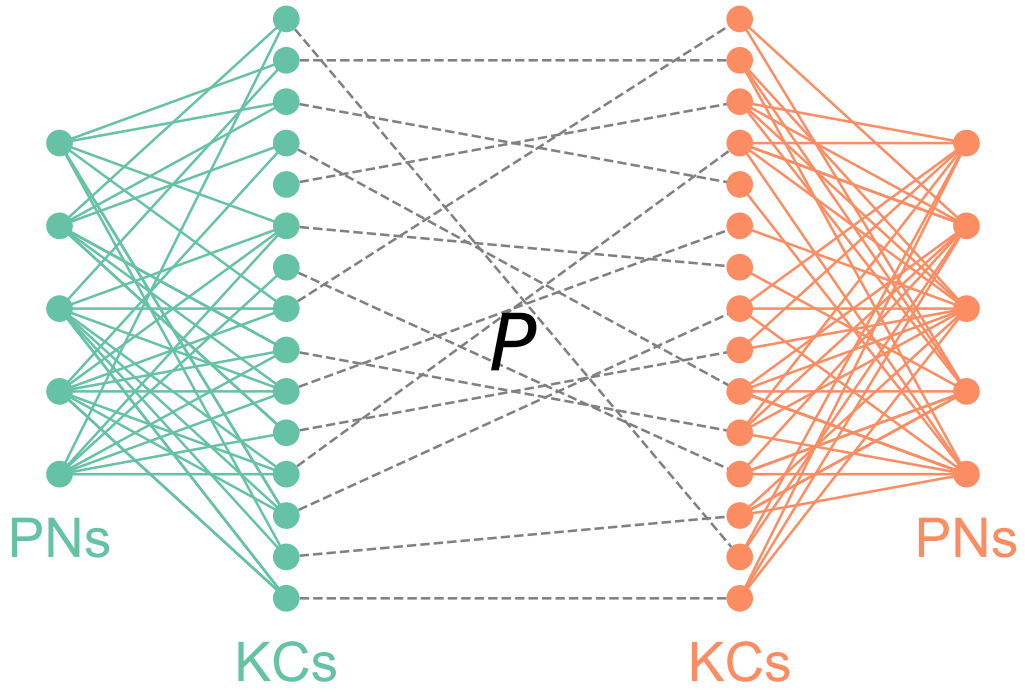
Pairs facilitate more powerful tests

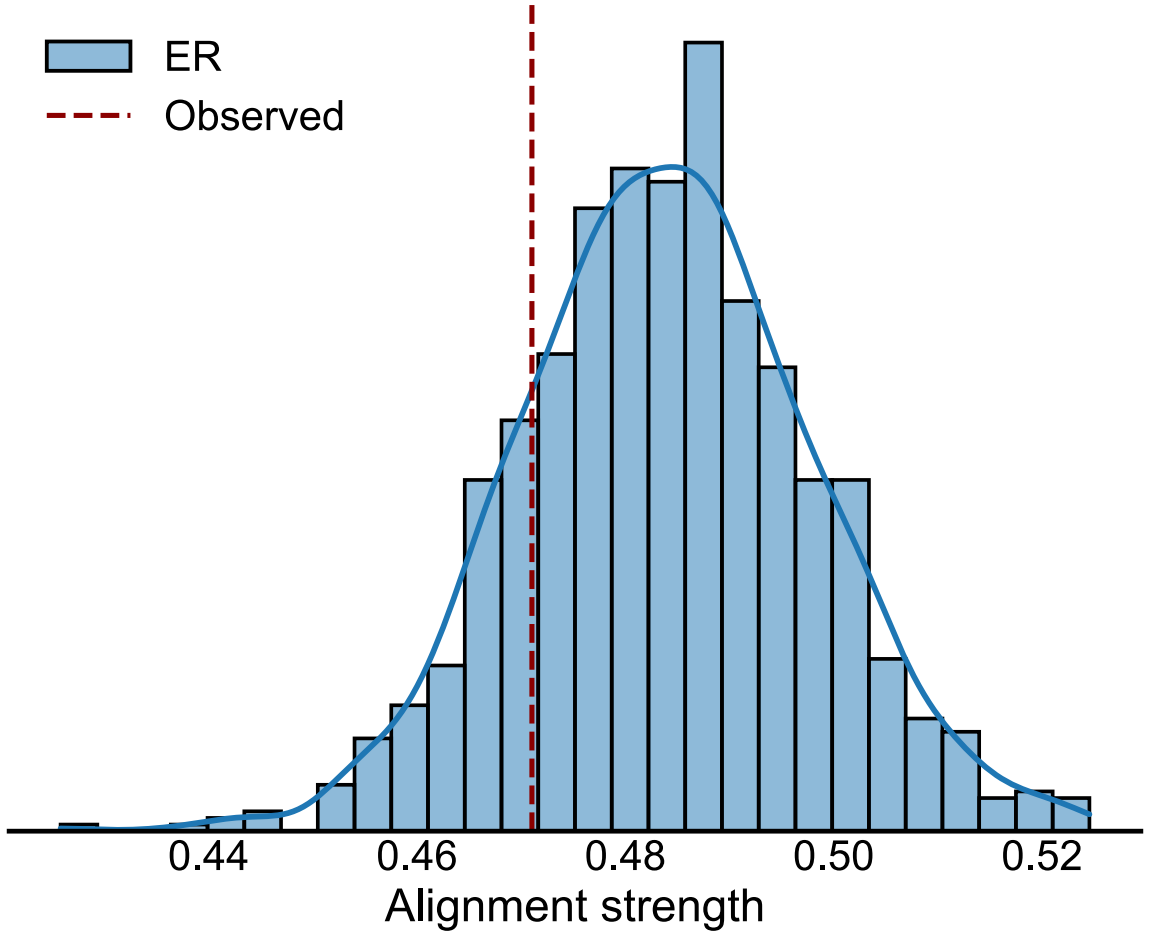
- Generate an Erdos-Renyi network (A)
- Perturb a copy of it (B) (add edges)
- Test for differences between A and B



Testing for "stereotypy" in edge structure

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

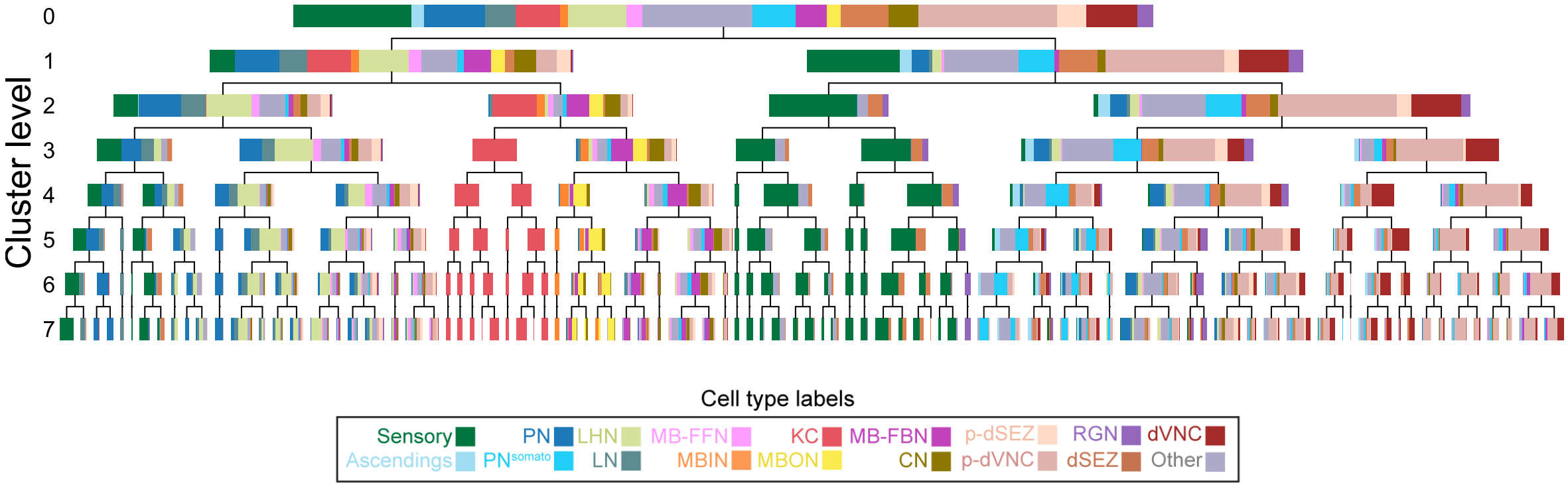




Eichler et al. Nature (2017), Fishkind et al. Applied Network Science (2021)

Neurons clustered by connectivity using recursive spectral clustering

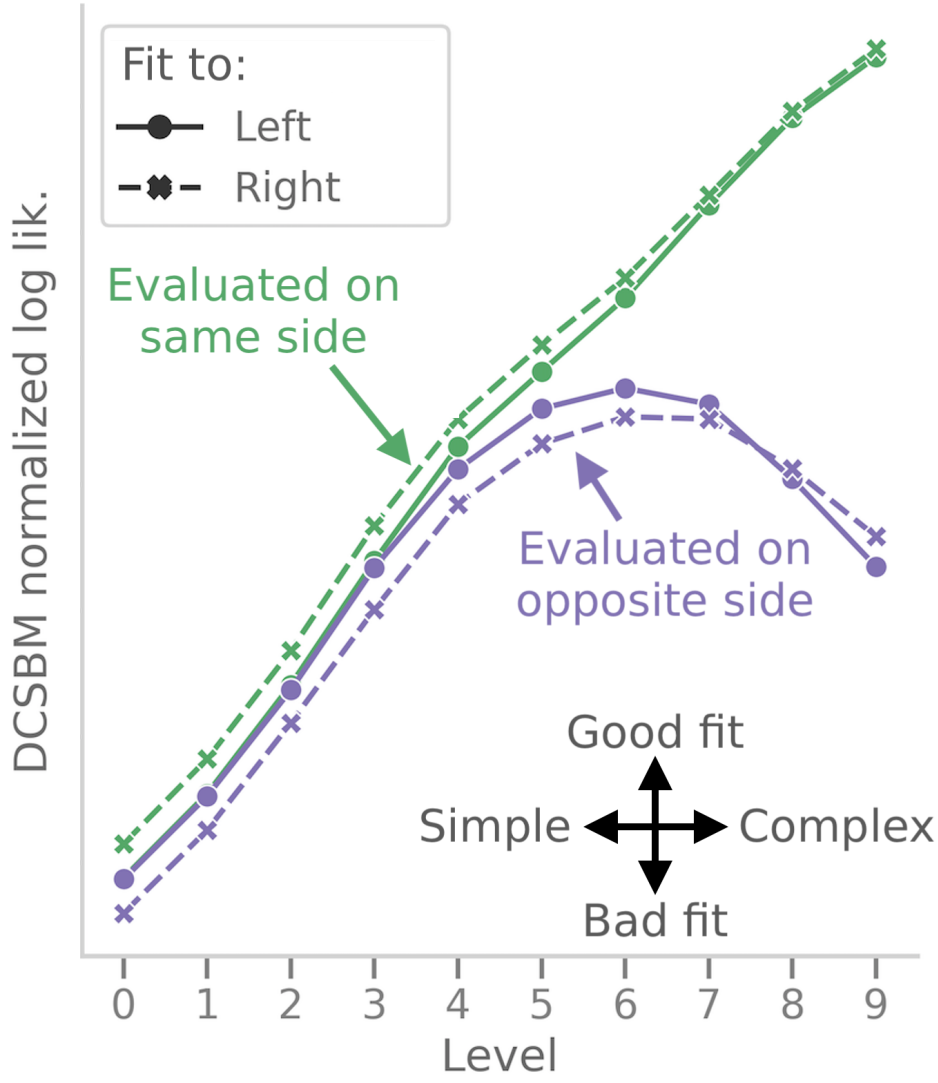
Where to stop splitting?



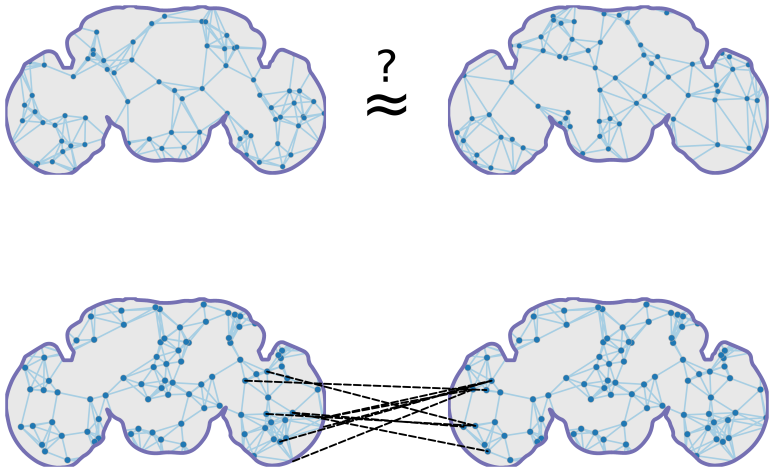
Winding, Pedigo et al. Submitted (2022)

Using *pairs* and *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)?



Summary



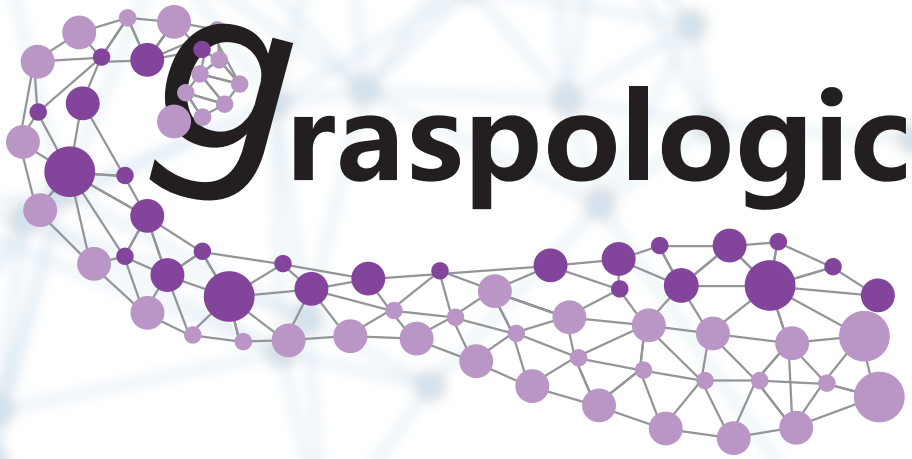
- Model-based network comparison enables testing (and refining) hypotheses about connectomes
  - We proposed a few tests, but just the beginning!
- Graph matching can pair neurons across datasets
  - Helpful to adapt off-the-shelf algos. to use biological info (e.g contralaterals, edge types)

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

How to use these (and other) tools?

graspologic

github.com/microsoft/graspologic



Model-based testing

github.com/neurodata/bilateral-connectome

Improved matching

github.com/neurodata/bgm

(Or for WIP final implementation see github.com/microsoft/graspologic/pull/960)

Chung, Pedigo et al. JMLR (2019)

Acknowledgements

Team

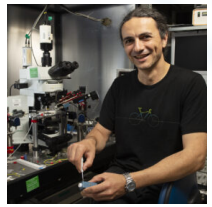

Michael Winding


Mike Powell


Eric Bridgeford


Ali Saad-Eldin


Marta Zlatic


Albert Cardona


Carey Priebe

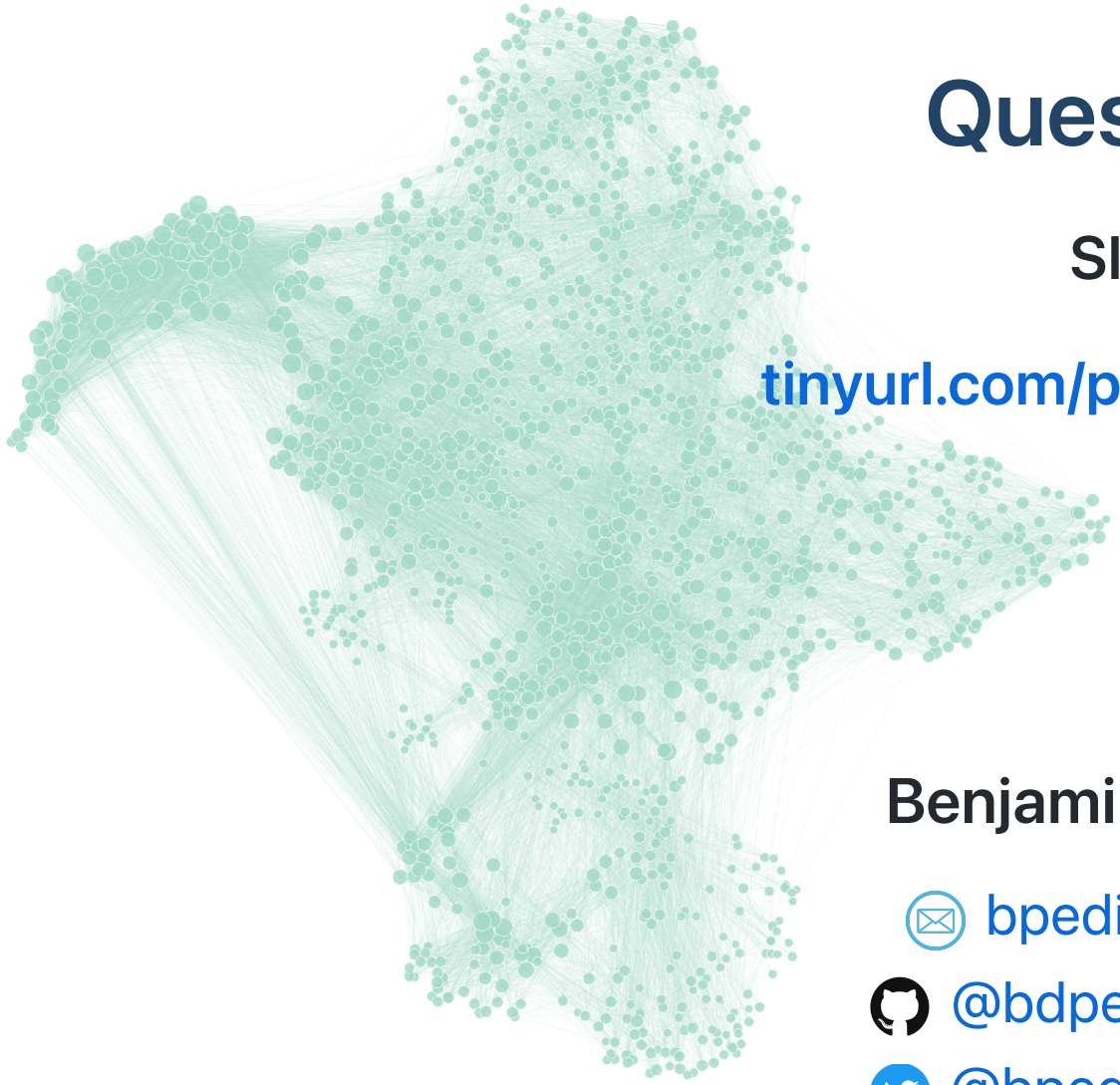

Joshua Vogelstein

Tracers who contributed to larva connectome, Heather Patsolic, Youngser Park, NeuroData lab, Microsoft Research

Figures from Scidraw + Noun Project (Alexander Bates, Xuan Ma, Gil Costa, Vivek Kumar, Leslie Coonrod)

Funding

NSF Graduate Research Fellowship (B.D.P.), NSF CAREER Award (J.T.V.), NSF NeuroNex Award (J.T.V and C.E.P.), NIH BRAIN Initiative (J.T.V.)



Questions?

Slides:

tinyurl.com/princeton-bilarva

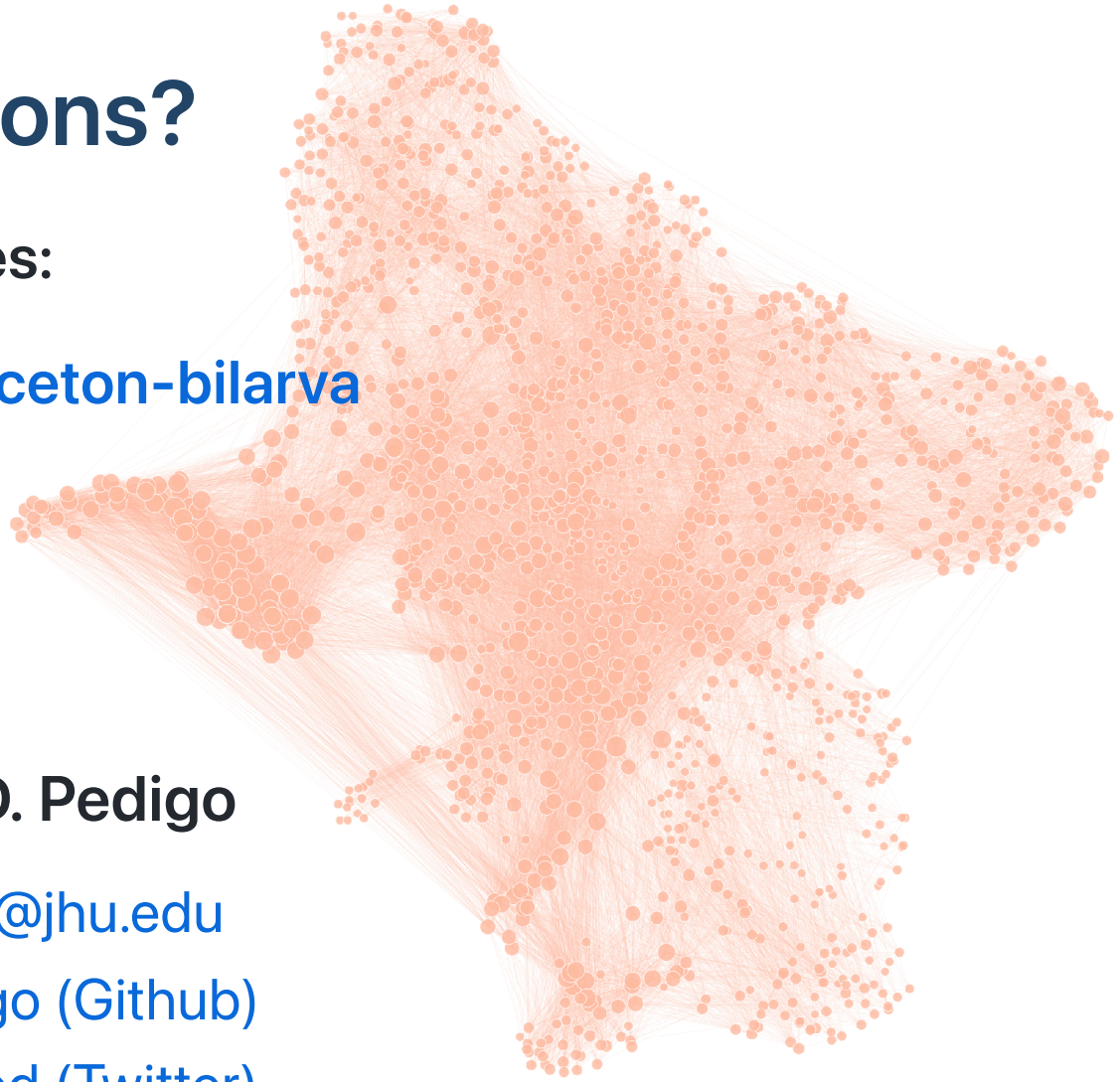
Benjamin D. Pedigo

bpedigo@jhu.edu

@bdpedigo (Github)

@bpedigod (Twitter)

bdpedigo.github.io



Left

Right