Insights from Comparative Connectomics in *Drosophila*

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Overview

1. The new & shiny FlyWire connectome
2. Lessons learned from comparing two connectomes
3. What is a cell type and why does that matter?
ommatidium
cortex
neuropil
optic lobes
central brain
eye
ventral nerve cord
0.5 mm
bipolar
20mm cable
3,190 postsynapses

dendrites

soma

axon

unipolar
2,5mm cable
887 postsynapses

dendrites
- sexually dimorphic behaviour
- decision making
- sensory integration

- motor control & motor sequences (e.g. Card, Lee, Simpson, Tuthill, Dickinson)

- olfactory processing & innate behaviour (e.g. Jefferis, Wilson)

- visual processing (e.g. Card, Kim, Turaga, Reiser)

- learning & memory (e.g. Rubin, Waddell)

- navigation (e.g. Jayaraman)
3,063 genetic driver lines targeting specific neuronal cell types

A split-GAL4 driver line resource for Drosophila CNS cell types


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This article is a preprint and has not been certified by peer review [what does this mean?].
The Janelia “hemibrain” connectome

22,704 neurons
5,235 cell types

Scheffer et al., eLife (2020)
FAFB, “Full Adult Fly Brain” image volume

ssTEM volume, 4x4x40nm resolution


Z. Zheng
D. Bock
FlyWire

Collaboration between Princeton, Cambridge and others

Dorkenwald et al., Nature Methods (2021)
Popovych et al., Nature Comm. (2024)
FlyWire
Collaboration between Princeton, Cambridge and others

>200 users with 100+ edits
contributions from 50 different lobe

proofread neurons

30 person-years

2019-09-28
4,700 edits
FlyWire
Collaboration between Princeton, Cambridge and others

- 3.1M total edits
- Estimated 30 person-years
  (hemibrain: 50 person-years)
- 0.02mm³
  (1/25,000 of a mouse brain)
- 139,000 neurons
- 150m of cable
- 130,000,000 synapses

Schlegel et al. (2023)
Dorkenwald et al. (2023)
FlyWire.ai
FlyWire

Collaboration between Princeton, Cambridge and others

860,000 annotations in total
(hemibrain: ~64,000)
estimated 3 person years
FlyWire
Collaboration between Princeton, Cambridge and others
Where can I get the data?
Codex

FlyWire Dataset (v783)
Codex provides access to proofread static snapshots of the FlyWire full-brain connectome - wiring diagram of adult Drosophila brain. Latest connections snapshot is v783 and it includes:

- 139,356 probed cells
- 2,701,401 connections
- 134,076 (96%) typed or labeled cells
- 34,170,824 synapses

Other snapshots are available from search settings. See FlyWire Info & Credits and FAQs pages for more details.

APPLIANCES

- Search
  - Find patterns using free-form or structured queries
- Stats
  - See statistics and charts for various attributes of all or subset of neurons in the dataset
- Annotations
  - Browse core tools, labels, and groupings of the neurons in the dataset

Arie Matsliah
R

```r
> library(fafbseg)
> dl4df = flytable_meta('DL4.*')
> dl4_p = flywire_partner_summary(dl4df)
```

Python

```python
>>> from fafbseg import flywire
>>> NC = flywire.NeuronCriteria

>>> dl4_p = flywire.get_connectivity(
...     NC(type='DL4_adPN'),
... )
```

https://natverse.org/fafbseg/
https://github.com/natverse/fafbseg/
https://fafbseg-py.readthedocs.io/
https://github.com/navis-org/fafbseg-py
How stereotyped are brains?

Are we collecting snowflakes?
mapping

FlyWire
within brain comparisons
left
right

across brains comparisons

hemibrain
NBLAST similarity matrix
FlyWire transform into FlyWire space
within brain comparisons
left right

22,704 neurons
5,235 cell types
within brain comparisons

across brains comparisons

manual review

NBLAST
FlyWire similarity matrix
hemibrain morphology match
type match
analyses

high conf. low conf.
NBLAST for rapid comparison of neurons

Costa et al., Neuron (2016)
99% of neurons have a match in another brain.
“cell type” is the smallest unit of conservation across brains/hemispheres
FlyWire neuron
hemibrain type
NBLAST scores

hemibrain cell types identified:
• 55% matched 1:1
• 12% found but modified
• 33% likely need revision
3.6k cell types
15k neurons / hemisphere
coverage ~50% of central brain graph
individual neurons collapsed into cell types

mean = 2.3
median = 1
3,6k cell types
15k neurons / hemisphere

node = cell type
edge = synaptic connections (weight = # of synapses)
Does this edge exist across all hemispheres?
If so, how consistent is its weight?
fraction of edges present across hemispheres

FlyWire
right

FlyWire
left

hemibrain

FlyWire
right

left

58%
60%

54%
61%

61%
52%
stronger edges are more likely to be present across hemispheres

FlyWire
right ← left
hemibrain

97% chance
30

29% chance
1
edges with >10 synapses
(or >=0.9% of target's input)
can be reproducibly (>90% chance) found across datasets
16% of edges are >10 but contain ~80% of all synapses
7% of edges are stronger than 1% but contain ~50% of all synapses
The graph shows the edge weight distribution in FlyWire right hemibrain compared to the hemibrain edge weight distribution.

- **Q5** to **Q75** to **Q95**

- **Brain 1** (30)
- **Brain 1-100** (30) (on average)
- **Brain 23** (3) (~5% chance)
across brains

within brain
Why does this matter?

control connectome

experiment connectome

30

3
Why does this matter?

female brain

male brain
Reasons connections could be different between brains?

- neurons proofread to different degrees
- synapse detection
- developmental abnormality
- biological variability
presynapses
postsynapses

FAFB/FlyWire synapse

Scheffer et al., eLife (2020)

accuracy of synapse detection across neuropils in hemibrain
FAFB/FlyWire synapse

completion rates per brain area

presynapses

postsynapses
$P(x_i, y_i)$

brain A

- sample prep.
- imaging
- segmentation
- proofreading

connectome

brain B

- sample prep.
- imaging
- segmentation
- proofreading

+ biological variability

+ technical noise
modelling impact of technical noise

observed connectome

extrapolate

fictive ground truth

remove false negative synapses

add false positive synapses

randomly drop synapses according to completion rates

fictive connectome

remove false negative synapses

add false positive synapses

randomly drop synapses according to completion rates

fictive connectome

compare
modelled technical noise

edge weight differences of +/- 30% could be entirely due to technical noise
Cell types

Why are cell types useful?

1. Easier to think about types than individual cells
2. Compress the data
3. Link neurons across datasets/modality (“unit of conservation”)

How to define cell types?
Cell types

How to define cell types?

dataset 1
dataset 2
dataset 3
Cell types

How to define cell types?

dataset 1
dataset 2
dataset 3

cell types = clusters with representatives from all datasets
Cell types

How to define cell types?

“A cell type is a group of neurons that is more similar to a group of neurons in another brain than to any other neuron in the same brain”
Cell types
Take homes

1. New adult fly brain connectome now publicly available (as well as complete nerve cord)
2. (Insect) brains aren’t snowflakes but the observed variability includes both biological and technical components
3. Robust cell types have to be defined in a way that takes inter-individual variability into account
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