Project ID: ANIM024

Exploring the Role of *Frazzled* in *Drosophila* Neural Circuit Assembly and Foraging Behavior

Introduction

Connectome
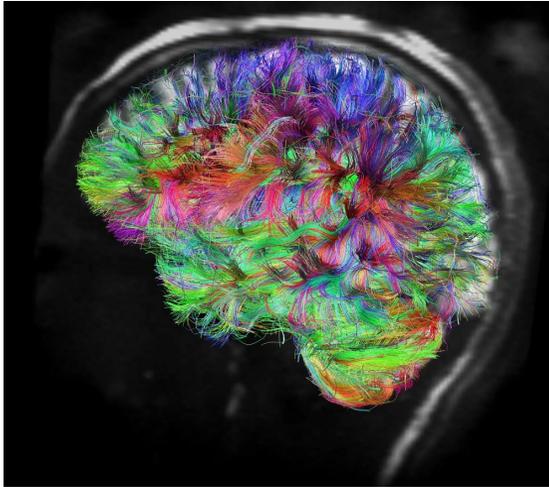
Human[1]
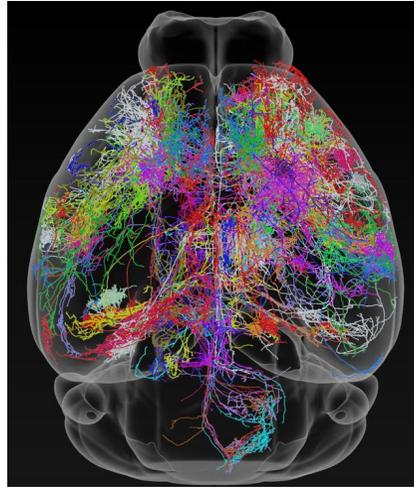
Mouse[2]
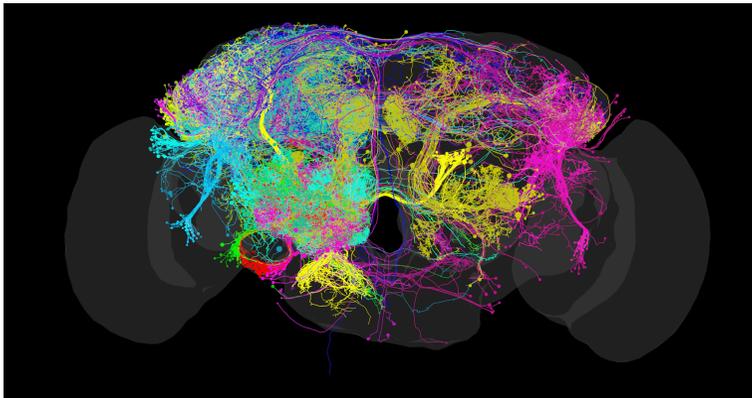
Drosophila[3]
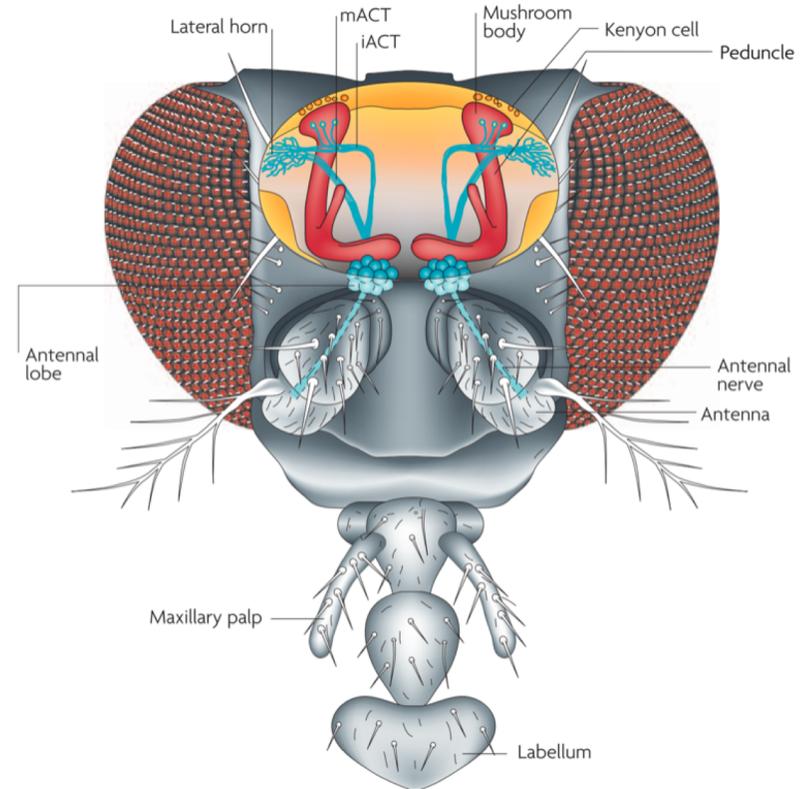
Drosophila brain[4]

Drosophila mushroom body structure[5]
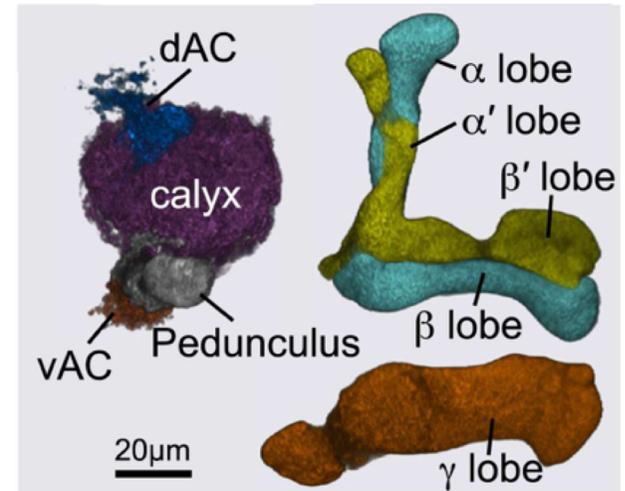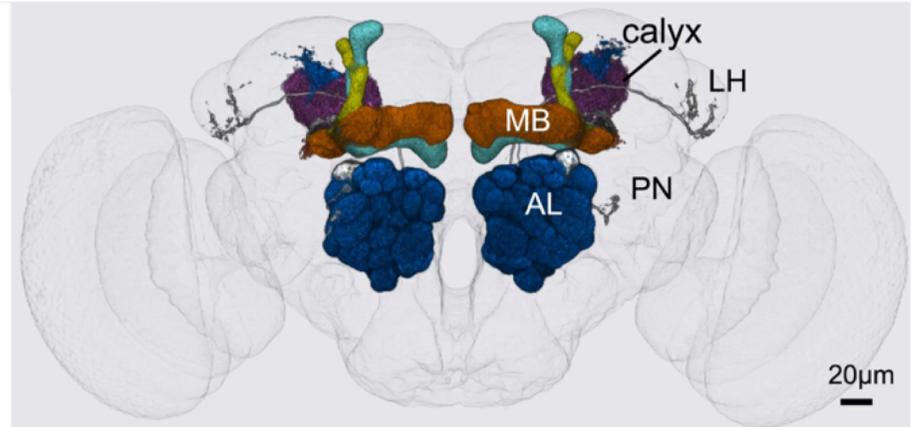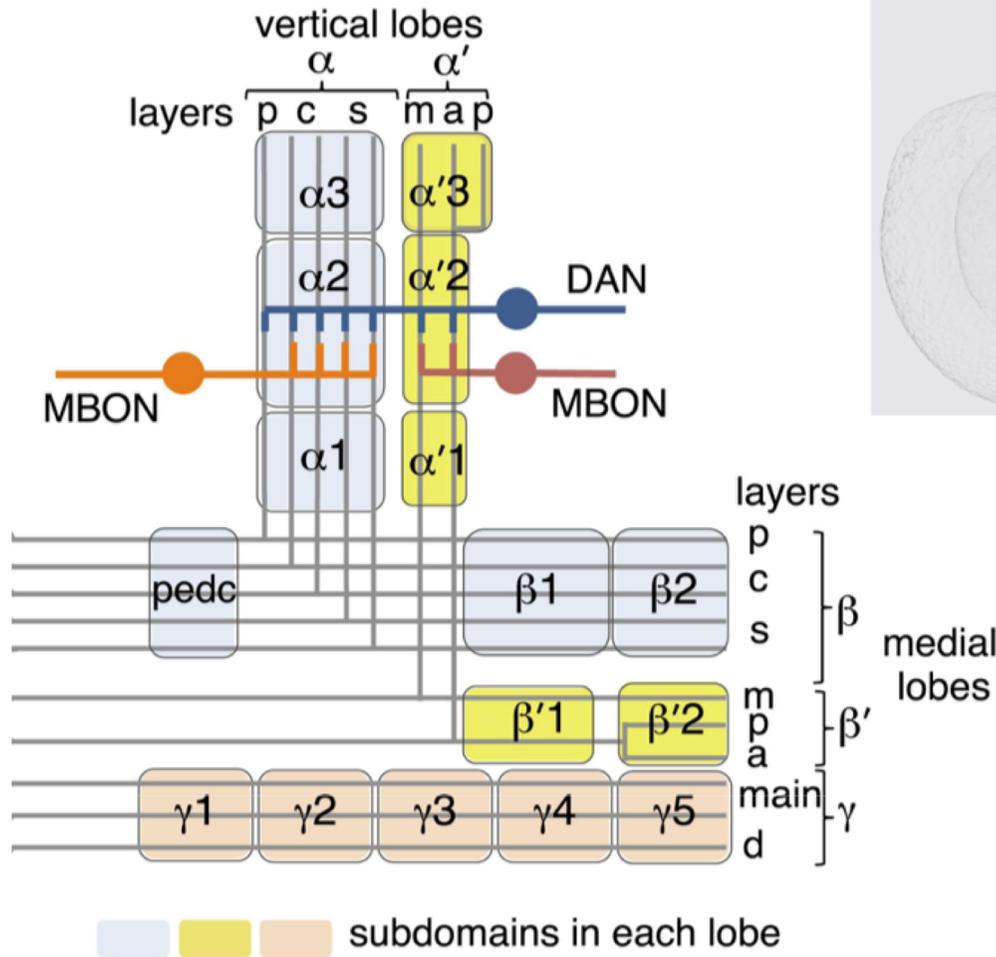
Research Questions:

1. Which genes control the neurite targeting of PPL1-α′2α2 neurons?
2. Does abnormal neurite targeting of PPL1-α′2α2 neurons influence food-seeking behavior?
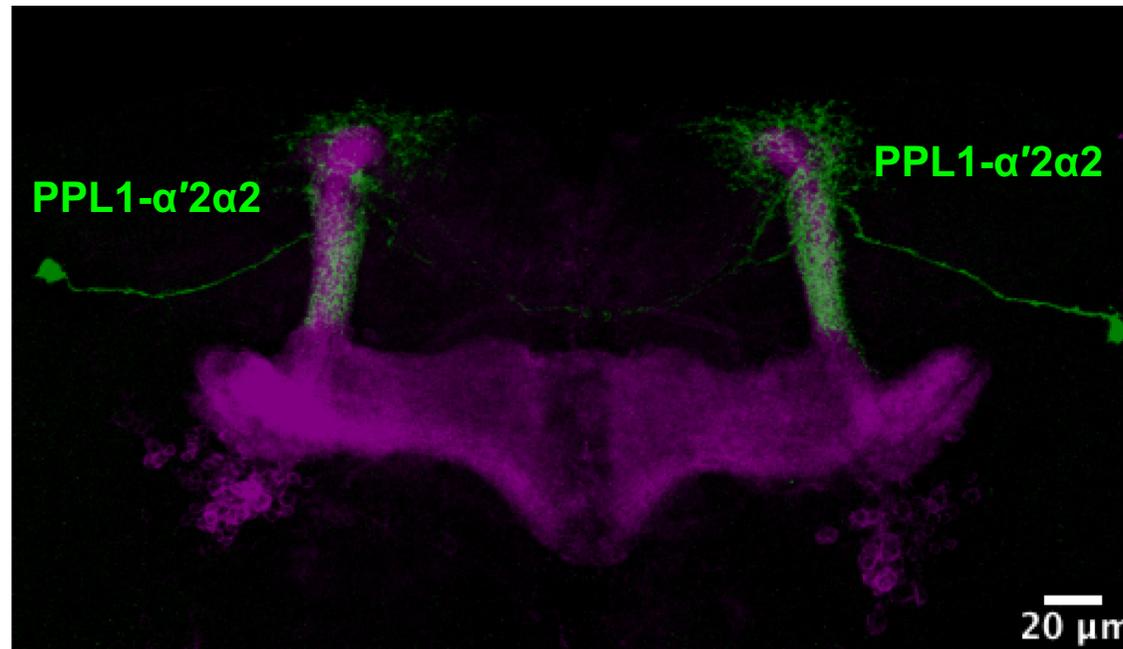
Fig. 1 The *Drosophila* brain mushroom body(magenta) and PPL1-α'2α2 neuron(green) under confocal microscopy.

Methods

1. GAL4-UAS system was used to drive
- **RNAi knockdown** (e.g. *frazzled, octβ2r*)
- **Gene overexpression:** *frazzled*
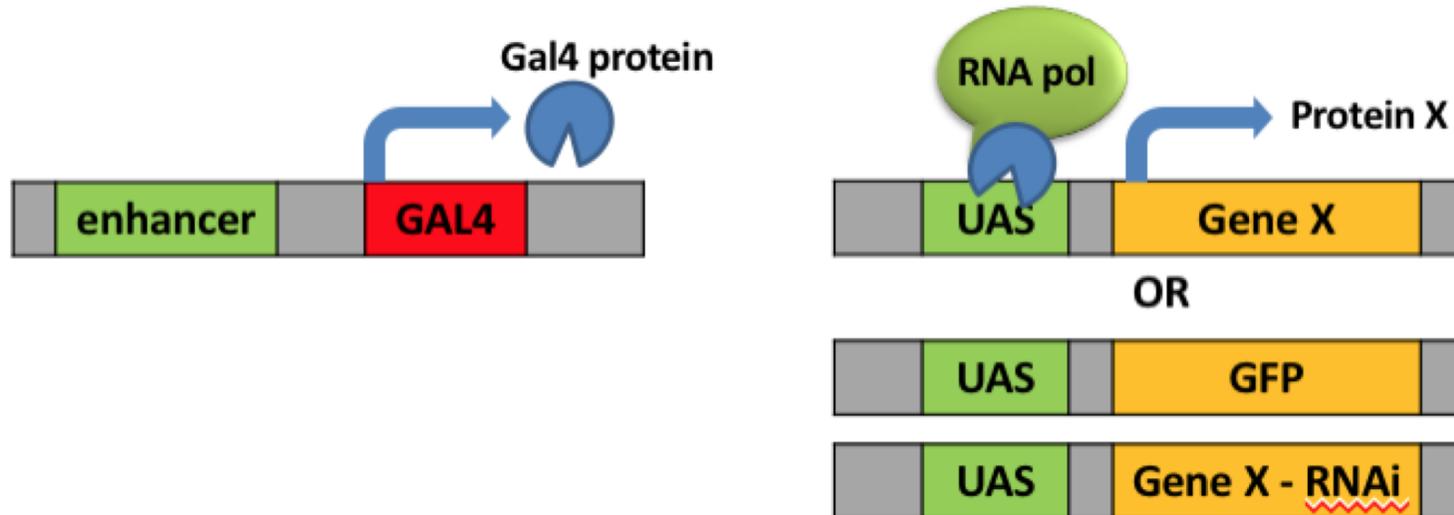
2. Foraging assay
Starve fly for 48hrs and analyze food-seeking behavior

Result-1 Knockdown of *frazzled* causes lost of innervation in the α2 and α'2 zones

PPL1-α'2α2 MB **Single focal pane**

Stacked image of MB

Fig. 2a
PPL1-α'2α2 neuron model[5]

◄ (cyan)	Ectopic innervation
◄ (yellow)	Loss of innervation

WT

octβ2r-RNAi

fra-RNAi

20 µm

Fig. 2b Knockdown of genes in PPL1-α'2α2

p<0.0001
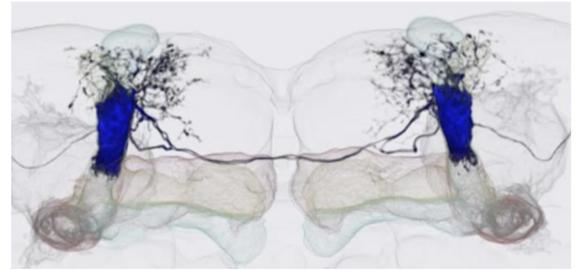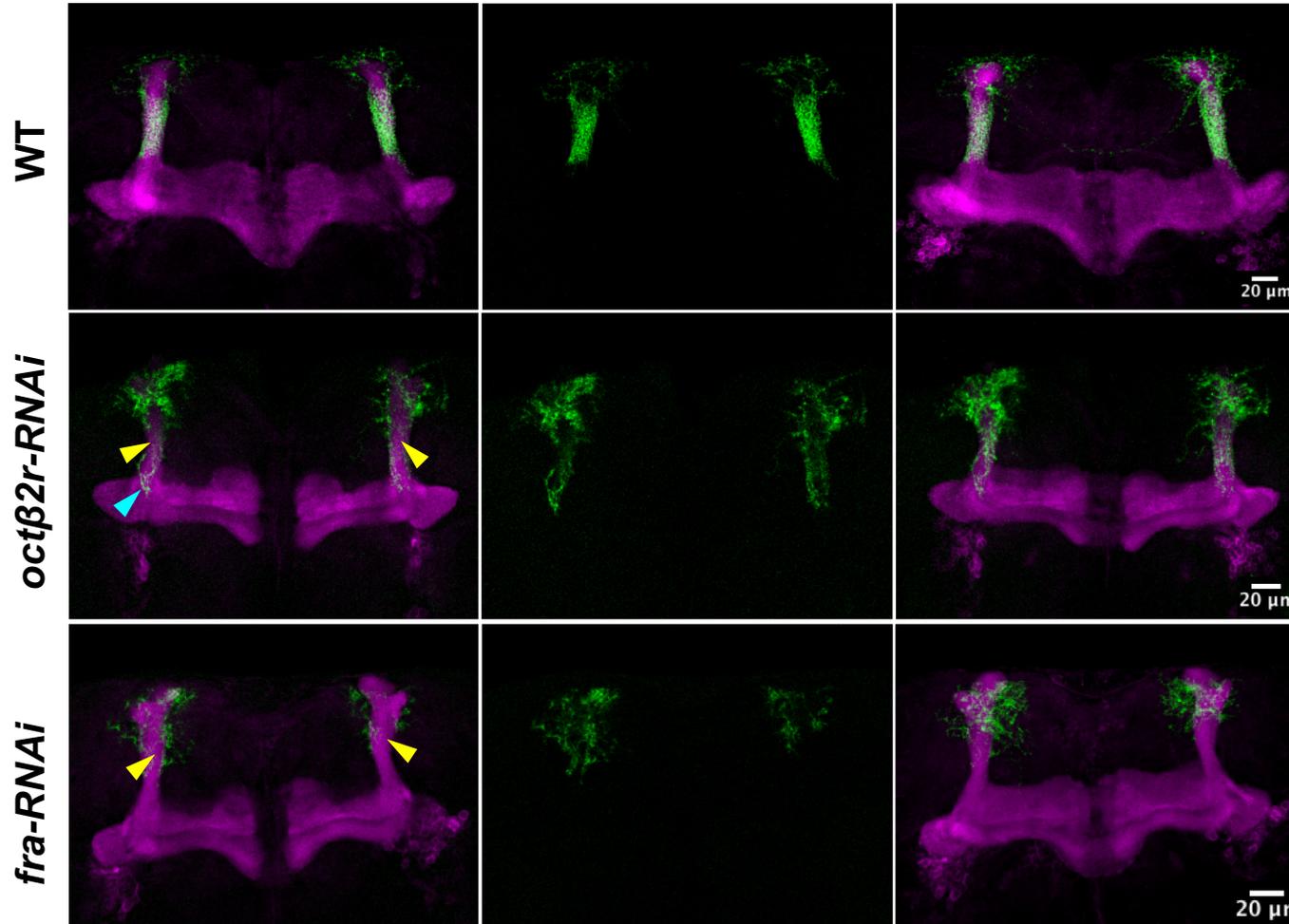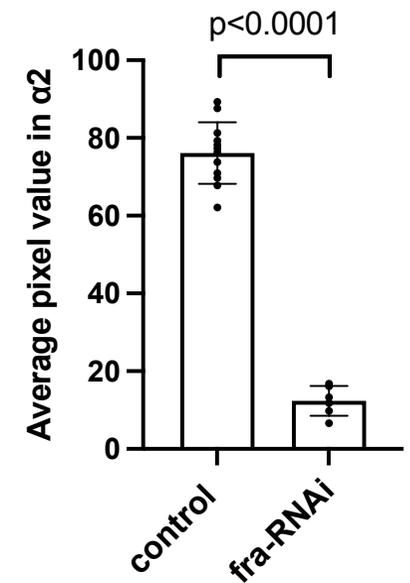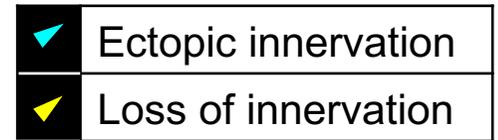
Fig. 2c Quantification of PPL1-α'2α2 innervation density in α2 zone

6

Functions of *frazzled* gene

- Netrin receptor on neuron surface.
- Embryo commissural axons in ventral nerve cord.
- Differentiation, development, and maturation of visual system.

Hypothesis:

The α2 and α'2 zones attract frazzled-expressing neurites.

Result-2 Overexpression of *frazzled* misguided **MBON-α3** and **MBON-β2β'2a** neurites towards the α2 and α'2 zones
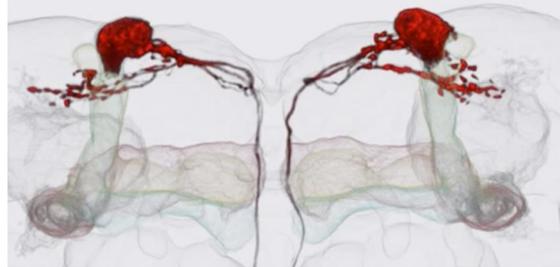
Fig. 3a
MBON-α3
neuron model[5]

Fig. 4a
MBON-β2β'2a
neuron model[5]

MBON-α3 MB

WT

fra OE

20 μm

20 μm

$p<0.0001$
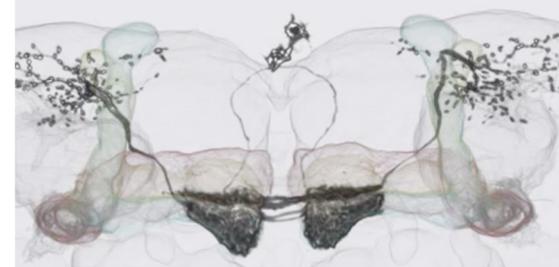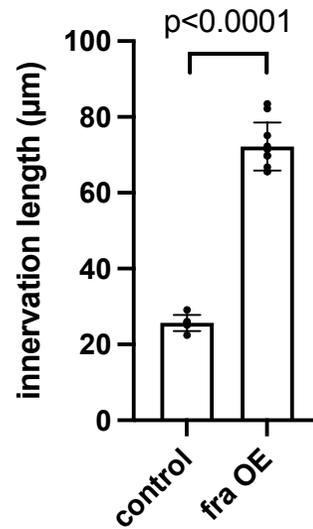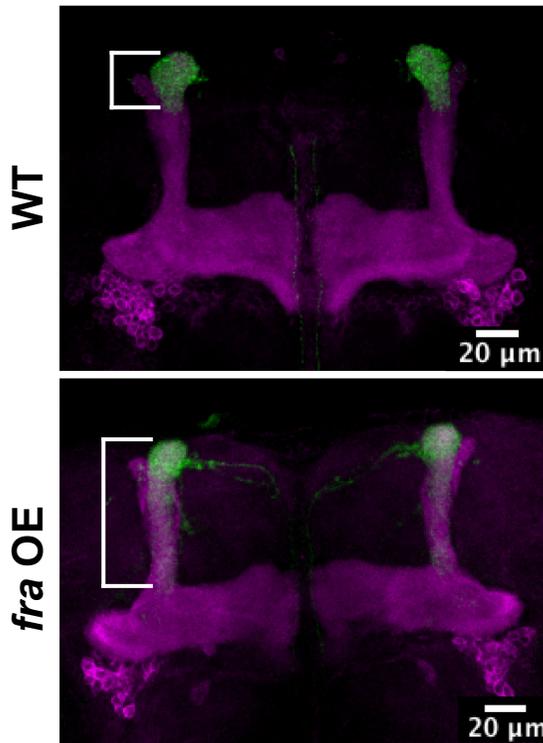
Fig. 3c Quantification of MBON-α3 innervation length

MBON-β2β'2a MB

WT

fra OE

20 μm

20 μm

$p<0.0001$
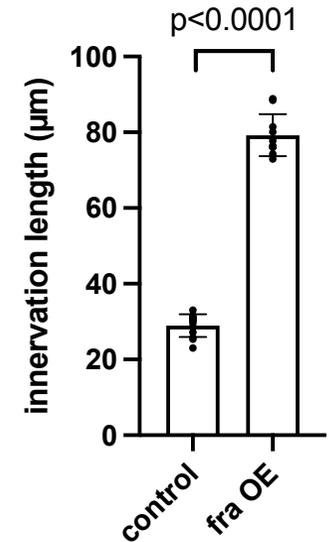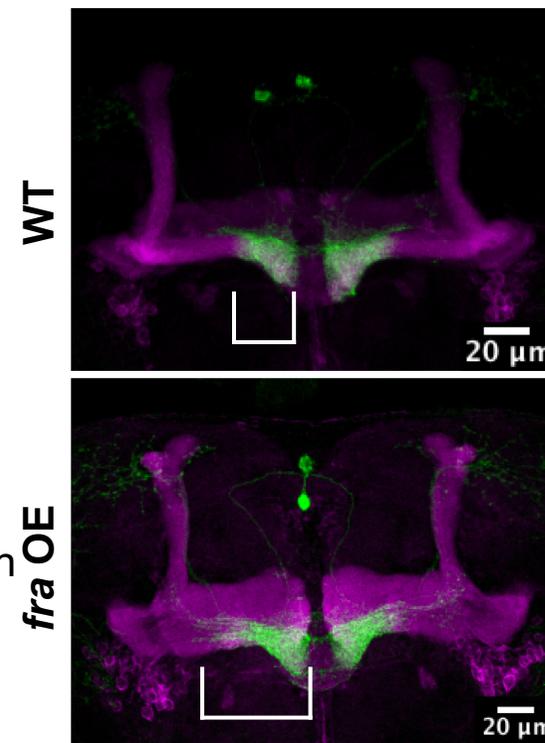
Fig. 4c Quantification of MBON-β2β'2a innervation length

Fig. 3b *Frazzled* overexpressed in MBON-α3

Fig. 4b *Frazzled* overexpressed in MBON-β2β'2a

Result-3 Overexpression of *frazzled* has no effect on PPL1-γ2α′1 and MBON-α′2 neurite targeting
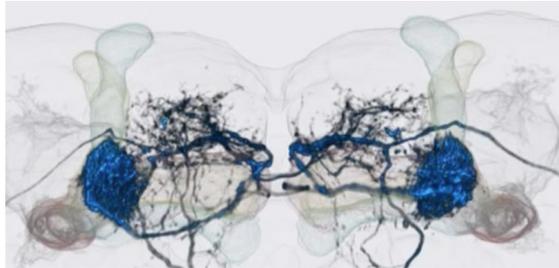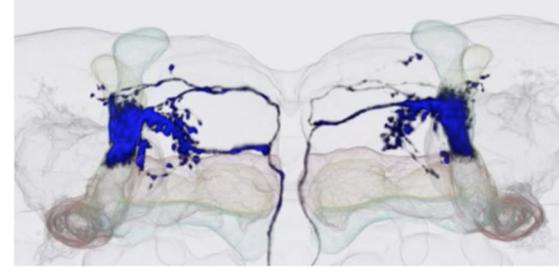
Fig. 5a
PPL1-γ2α′1
neuron model[5]

PPL1-γ2α′1 MB

WT

fra OE

20 µm

20 µm
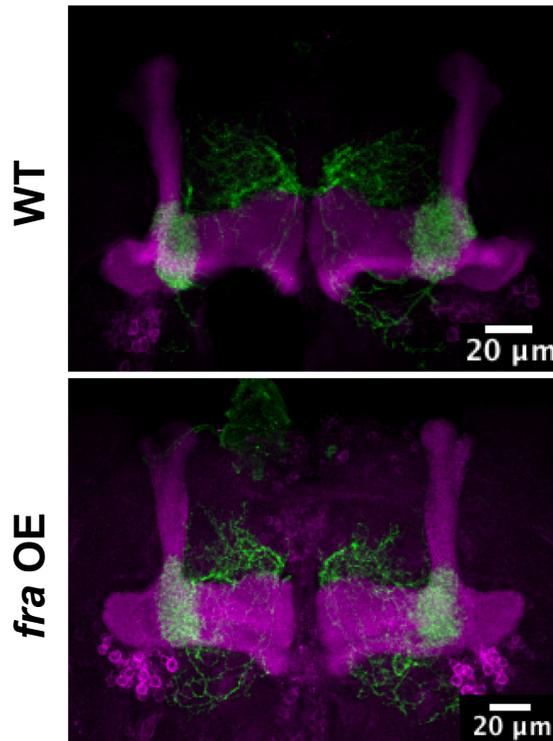
Fig. 5b *Frazzled* overexpressed in PPL1-γ2α′1

Fig. 6a
MBON-α′2
neuron model [5]

MBON-α′2 MB

WT

fra OE

20 µm

20 µm
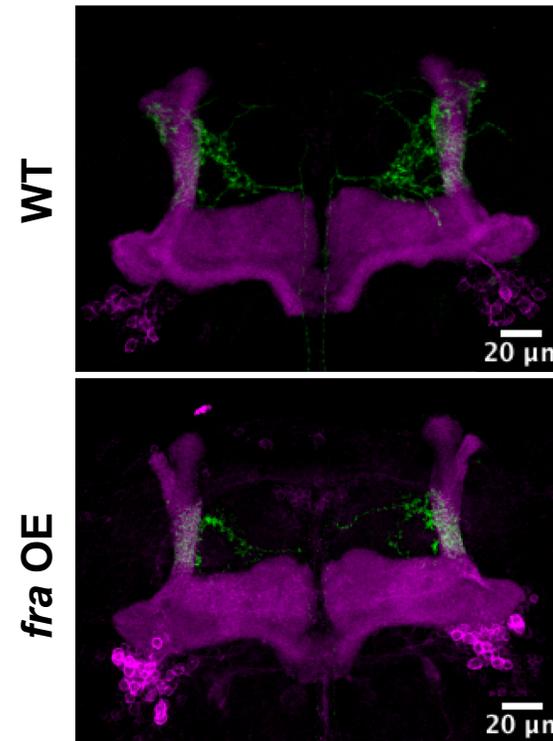
Fig. 6b *Frazzled* overexpressed in MBON-α′2

Result-4

Loss innervation of PPL1-α'2α2 into α'2 and α2 zones under *frazzled* knockdown does not affect food-seeking behavior

Previous studies show that silencing neurotransmission in PPL1-α'2α2 neurons disrupts foraging behavior[6]

Representative single-fly food-seeking trajectory

Control 1

Control 2

fra-RNAi
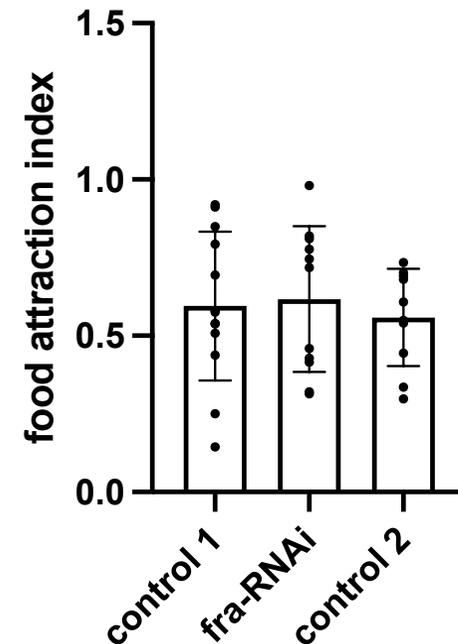
Fig. 7 The food attraction index is not significantly different between groups

Conclusions

1. *Frazzled* expression is required cell-autonomously for the neurite targeting of **PPL1-α'2α2** neuron.

2. Overexpression of *frazzled* in some MB neurons is sufficient to **redirect their neurites** towards α2 and α'2 zones.

3. The genetic effect of *frazzled* on neurite targeting might be **context-dependent** in *Drosophila* brain.

4. Loss innervation of PPL1-α'2α2 into α'2 and α2 zones under *frazzled* knockdown does not affect food-seeking behavior, suggesting that **developmental plasticity** may compensate for the miswiring of PPL1-α'2α2 neurons.

Future work:

1. Investigate the role of Frazzled-Netrin pathway in PPL1-α'2α2 neurite targeting.

2. Explore the compensatory mechanism for neural circuit miswiring.

References

[1] V.J. Wedeen and L.L. Wald, Martinos, Center for Biomedical Imaging
(https://www.discovermagazine.com/mind/36-new-project-maps-the-wiring-of-the-mind)
[2] Janelia Research Campus, MouseLight project team
(https://www.eurekalert.org/multimedia/pub/154461.php?from=375126)
[3] LK Scheffer, et al. (2020). A connectome and analysis of the adult *Drosophila* brain. *eLife*, 9:e57443.
[4] Hakim, Y., Yaniv, S. P., & Schuldiner, O. (2014). Astrocytes play a key role in Drosophila mushroom body axon pruning. *PLoS One*, *9*(1), e86178.
[5] Aso, Y., Hattori, D., Yu, Y., Johnston, R. M., Iyer, N. A., Ngo, T. T. & Rubin, G. M. (2014). The neuronal architecture of the mushroom body provides a logic for associative learning. *Elife*, *3*, e04577.
[6] Tsao, C. H., Chen, C. C., Lin, C. H., Yang, H. Y., & Lin, S. (2018). Drosophila mushroom bodies integrate hunger and satiety signals to control innate food-seeking behavior. *Elife*, *7*, e35264.