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Identifying the role of neurotransmitter receptors in neuronal remodeling of *Drosophila* mushroom body γ neurons

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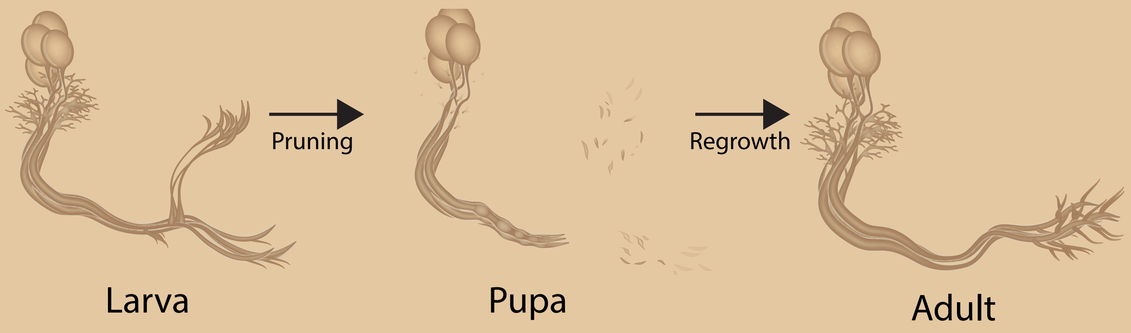
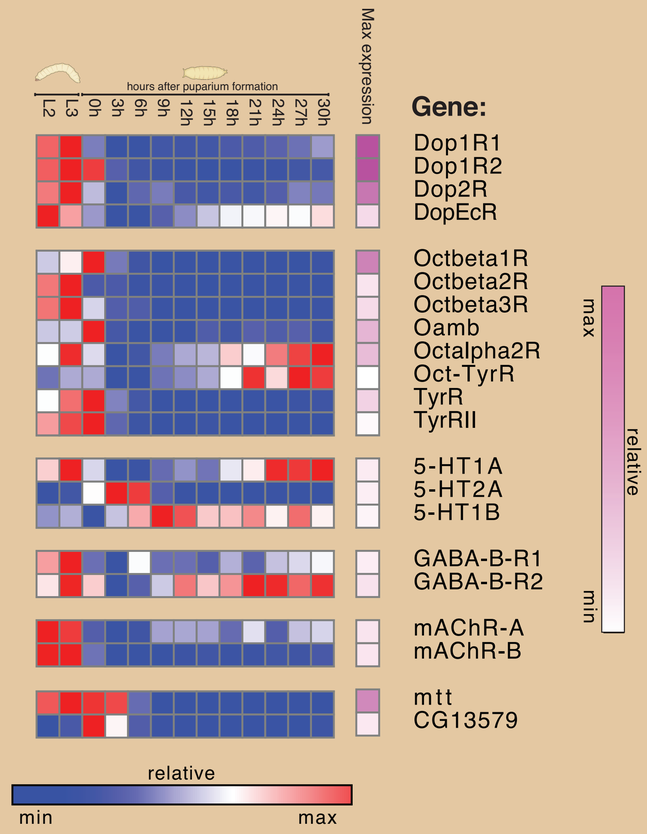
Introduction

- Neuronal remodeling is a conserved developmental process used to refine the nervous system after its initial establishment.
- The stereotypic remodeling of *Drosophila* Mushroom Body (MB) γ neurons during metamorphosis includes pruning of larval axons followed by regrowth to form the mature adult specific γ lobe.
- A developmental expression atlas of MB γ neurons generated in our lab (Alyagor et al, 2018) highlights dynamic expression of neurotransmitter G-coupled Protein Receptors (NT-GPCRs) during development. This suggests their involvment in regulating these developmental processes.



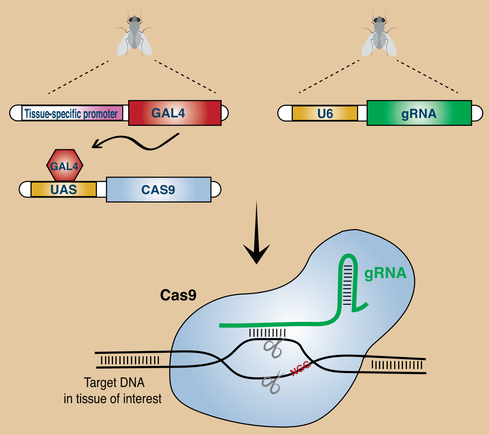
G-protein coupled Neurotransmitter receptors present dynamic expression throughout development

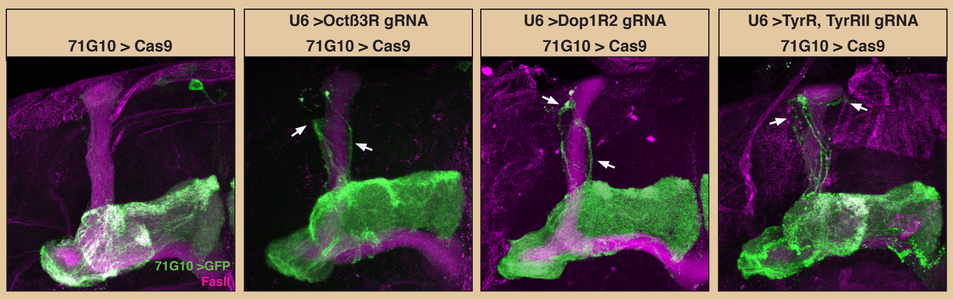
- Out of the 29 annotated NT-GPCRs in the *Drosophila* genome, 21 are differentially and dynamically expressed by γ neurons during development.
- Many of the these receptors, including all Dopamine receptors and most of the Octopamine/ Tyramine receptors, display a peak of expression prior to the onset of γ axon pruning.



Tissue specific CRISPR for Octopamine/Tyramine and Dopamine receptors results in axon pruning defects

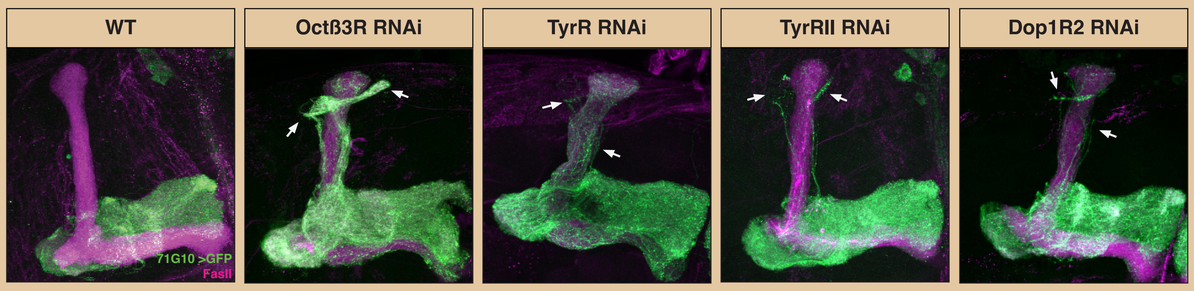
- Tissue specific CRISPR (ts-CRISPR) was optimized for our purposes. As shown in the scheme, this method allows simple and efficient screening by crossing a fly expressing the desired gRNA with a fly expressing CAS9 driven by the γ specific 71G10 Gal4 driver.





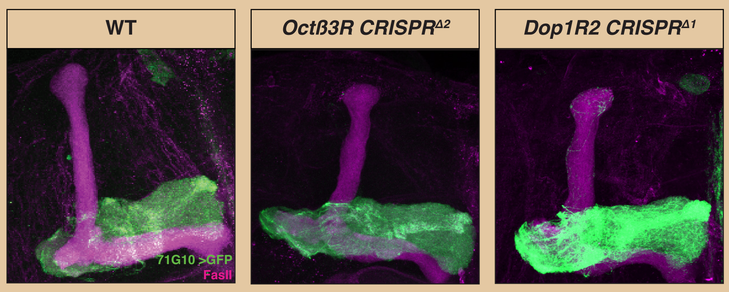
- Expressing gRNAs targeting **Octß3R** and **Dop1R2** results in pruning defects. In the case of the Tyramine receptors, an effect on pruning was seen only in flies expressing gRNAs targeting both **TyrR** and **TyrRII** (white arrows).

RNAi targeting Octopamine/Tyramine and Dopamine receptors further support a role in MB remodeling



- RNAi mediated knock down of Octopmaine receptor **Octß3R**, tyramine receptors **TyrR** and **TyrRII**, and Dopamine receptor **Dop1R2** in a tissue specific manner results in MB γ pruning defects (white arrows).

Whole animal mutants of Octß3R and Dop1R2 prune normally



- Homozygous germline mutants of **Octß3R** and **Dop1R2** generated in our lab prune normally and exhibit a WT- like adult phenotype.
- This could suggest that in this context a compensatory mechanism allows the γ neurons to overcome the loss of these receptors.

Conclusions

- RNAi and ts-CRISPR establish a potential role for Octopamine, Tyramine and Dopamine NT-GPCRs in MB γ pruning, suggesting that signaling from other cells may be required for proper MB development.
- The normal pruning of MB γ neurons in the germline mutants suggests a compensatory mechanism may arrise to ensure correct remodeling of the MB.

Future work

- Examine the function of other NTRs, such as GABA and 5-HT receptors
- Explore the possibility of compensation:
  - By testing the RNA levels of other receptors in Octß3R and Dop1R2 mutants
  - By generating flies mutated for several NTRs.
  - By testing flies with mutations in the Octopamine, Tyramine and Dopmaine biosynthesis pathways.