Characterising genetic regulators of auditory and proprioceptive neuron differentiation in the *Drosophila* ear

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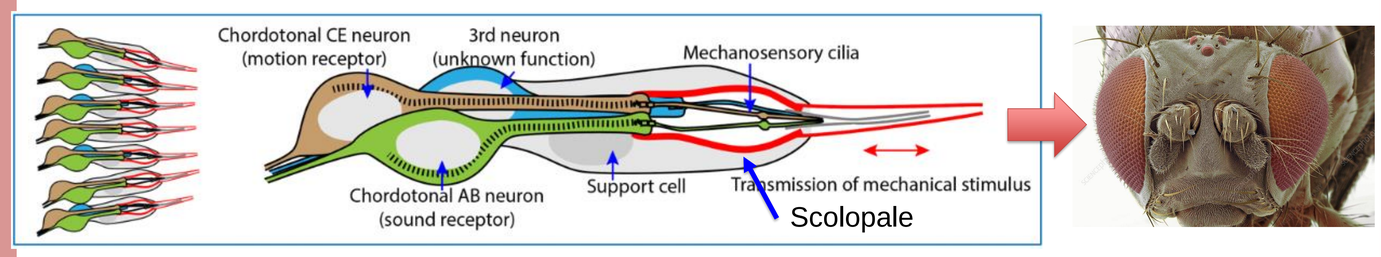
1) Introduction



Johnston's organ is a mechanosensory organ found in the antennae of *Drosophila*. It contains differentiated neurons which act as receptors for motion or sound (CE and AB respectively). *Drosophila* distinguishes between motion and sound based on the stimuli that activate these different types of neurons. Specifically, the AB neurons are particularly important in the fly's ability to court and mate.

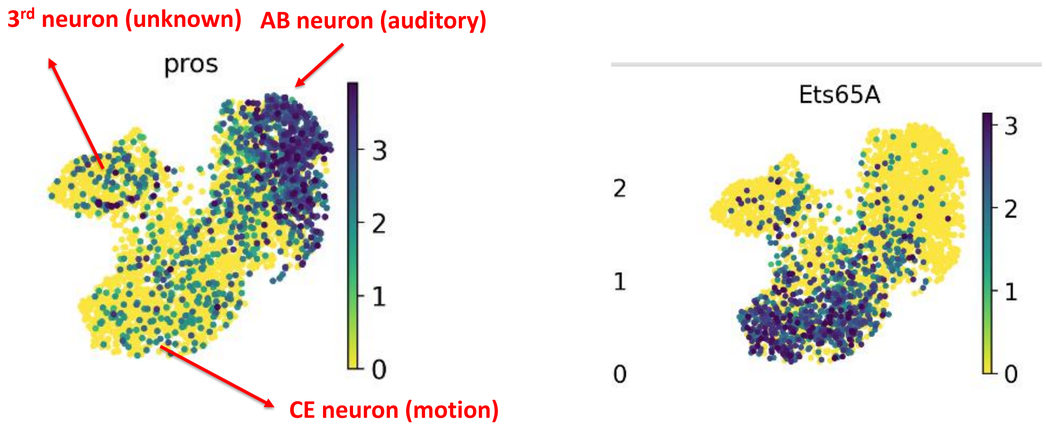
Key question: What controls the differentiation between these two types of neurons?

2) Potential genetic regulators of AB and CE neuron differentiation

Ets65A
Prospero } transcription factors expressed in the antennae

Key question: Are these transcription factors expressed in specific neurons?

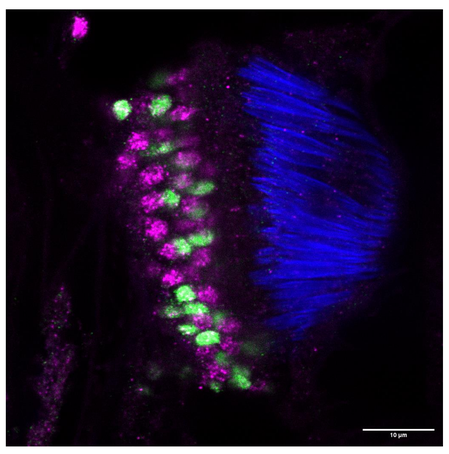
Single cell RNA sequencing of all genes expressed across the neurons in the *Drosophila* antennae, followed by cluster analysis



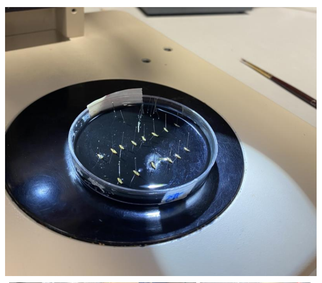
Conclude: Ets65A and Prospero are expressed predominantly in CE and AB neurons respectively

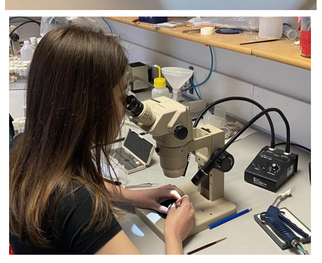
This is mRNA in isolated cells, now check for protein in the antenna

3) Expression patterns using confocal microscopy

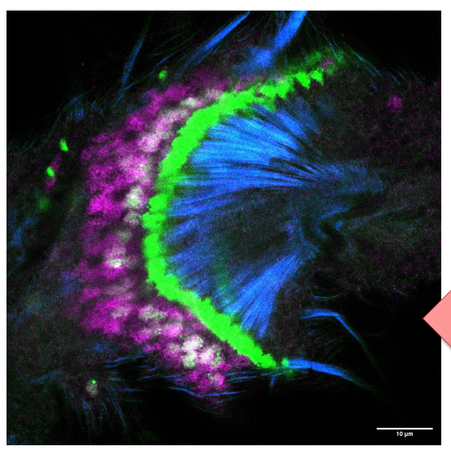


Antenna Ets65A staining





Dissecting antennae



Antenna Prospero staining

- Ets65A GFP reporter
- Elav (stains all neuronal cell nuclei)
- Phalloidin (stains actin in scolopale surrounding the mechanosensory dendrite)

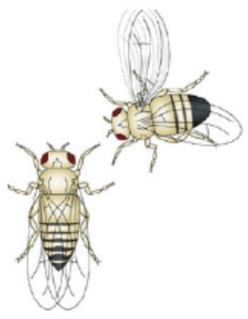
- Prospero
- Elav
- Phalloidin

4) Mating assay for testing hearing ability

Key question: Are the transcription factors required for function of these neuron subsets?

We tested this for **Prospero** by knockdown of the gene in **AB neurons** and conducting a mating assay. Males 'sing' to females during courtship song produced by the males. Females must hear this stimulus via AB neurons in order to be receptive to mating.

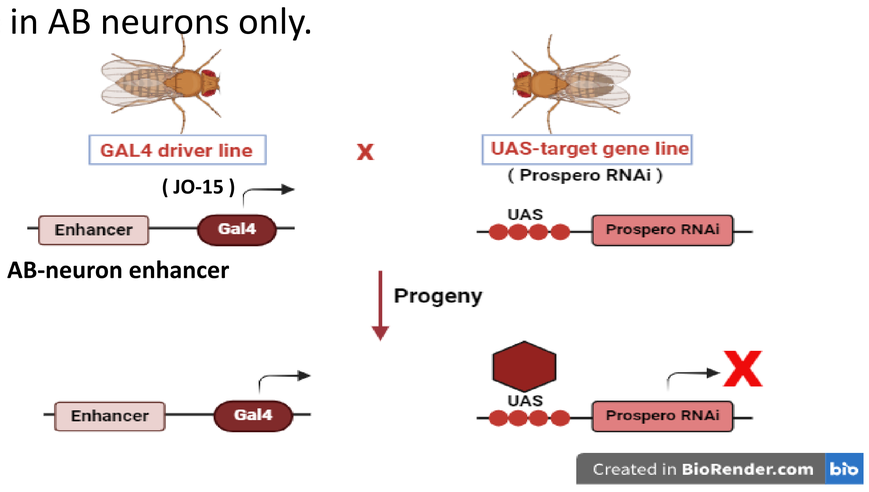
Predicted outcome: AB (sound) neurons will be defective upon Prospero knockdown, and there will be a reduction in mating pairs.



« Singing »

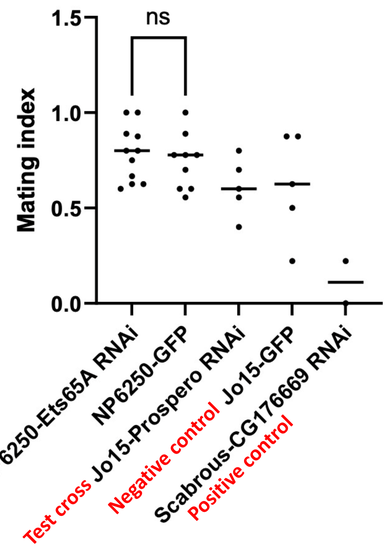
5) Knockdown using UAS/GAL4 system

The UAS/GAL4 system was utilized to knock down the Prospero gene in AB neurons only.

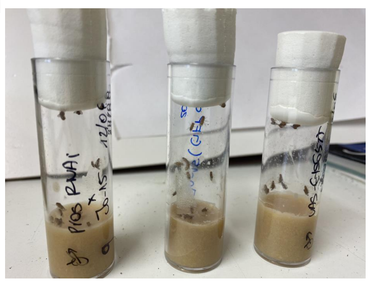


These flies were tested for mating behaviour

6) Results



- Mating index = proportion of pairs mating after 15 mins.
- Each dot represents one experiment with 12 pairs
- Knockdown of Prospero in AB neurons (Jo15-Prospero RNAi) did NOT result in mating reduction



7) Conclusions

1. We successfully show that these Ets65A and Prospero transcription factors are expressed in CE and AB neurons respectively, suggesting they might regulate the differentiation of these neuron subsets.

2. The results provide no evidence for the function of Prospero in AB neurons. However, as the results for the positive control (hearing gene, *CG17669*) are strong, we suggest that the JO-15 driver line does not efficiently knock-down Prospero. Further work is required.

8) Future implications

As the fruit fly shares approximately 60% of its protein-coding genes with humans, it can be implied that there could be a potential homologue of the gene Prospero in human hearing. With further positive investigations regarding Prospero knock-down, it could be a promising candidate for investigating the underlying causes of human deafness and related disorders, which can be applied to potential gene therapies.