Influence of Dopamine Transporter Knockout from Kenyon Cells on Associative Memory

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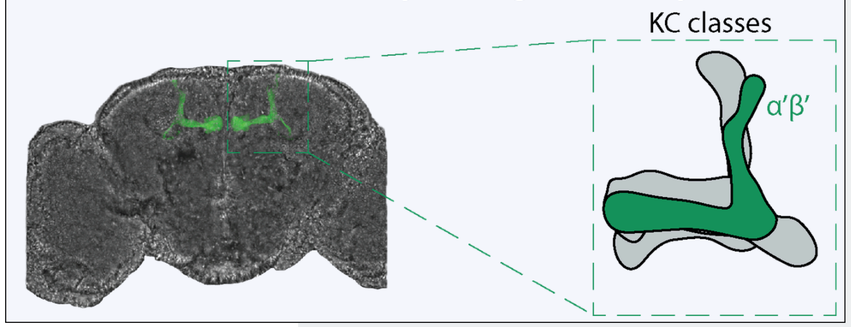
Summary of Research Topic

Dopamine (DA) is a catecholamine neurotransmitter that mediates essential cognitive processes and behaviours such as learning and memory [1]. The **DA transporter (DAT)** is a transmembrane protein that regulates the sphere of influence of dopaminergic volume transmission by uptaking secreted DA back into **dopaminergic neurons (DANs)**. These DANs allow the radius of dopaminergic transmission to be tightly regulated, which is important for the control of neural pathways.
DAT expression has recently been identified in a class of **non-dopaminergic Kenyon cells (KCs)** - the principal neurons of the **mushroom body (MB)** - called **α'β' KCs** [2]. This suggests that these neurons may contribute to the modulation of extracellular DA. The MB is the associative centre of the fly brain and the centre for olfactory learning and memory, which implies that the DAT expression in these α'β' KCs may have an influence on the flies' **olfactory learning and memory**.



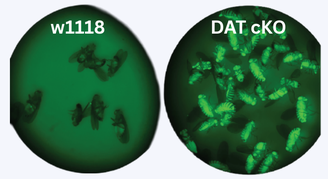
Goals

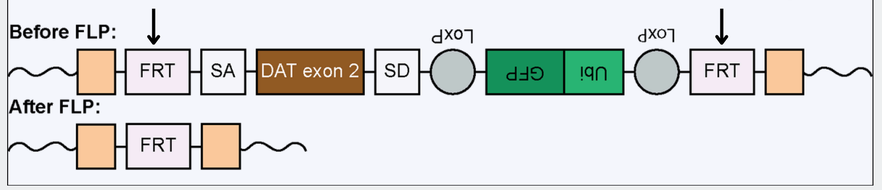
- Obtain immunostaining images to show functionality of DAT conditional knockout (cKO) method.
- Analyse learning and memory of α'β' KCs DAT cKO flies using behaviour training.
- Understand the role of DAT expression in α'β' KCs on learning and memory.

Full Research Paper

cKO Expression

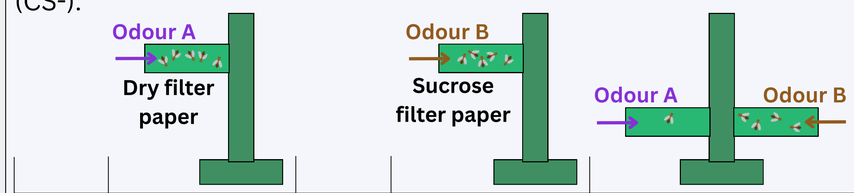
GFP tag in DAT cKO strain shows that the knock-in cassette used to make the line is in the strain. When FLP is expressed, the sequence between the two FRT sites is deleted, so DAT is non-functional.





Olfactory Learning and Memory

A T-maze was used to test appetitive associative conditioning - a type of learning where a signal is paired with a positive event. Flies were exposed to two odours: one paired with a positive stimulus (sucrose) (CS+) and one paired with a neutral stimulus (no sucrose) (CS-).



$$\text{Performance Index} = \frac{\text{number of CS+ flies - number of CS- flies}}{\text{number of CS+ flies + number of CS- flies}}$$

Acknowledgements

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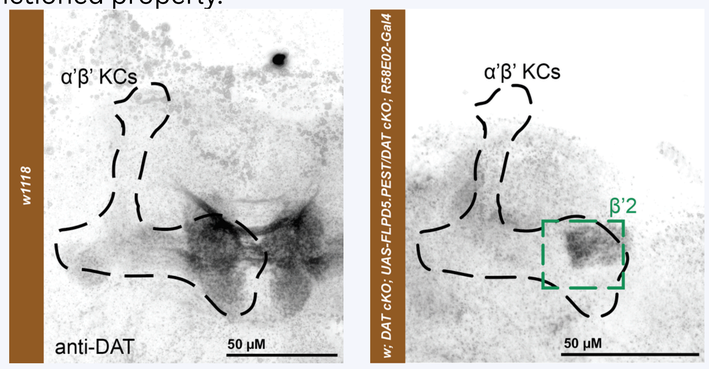
References

[1] Burke CJ, Huetteroth W, Owald D, et al. Layered reward signalling through octopamine and dopamine in Drosophila. Nature. 2012;492(7429):433–437. **[2]** Croset V, Treiber C D, Waddell S. Cellular diversity in the Drosophila midbrain revealed by single-cell transcriptomics. eLife. 2018;7. **[3]** Kume K. Dopamine is a regulator of arousal in the fruit fly. Journal of Neuroscience [Internet]. 2005 [cited 2026];25(32):7377–7384.

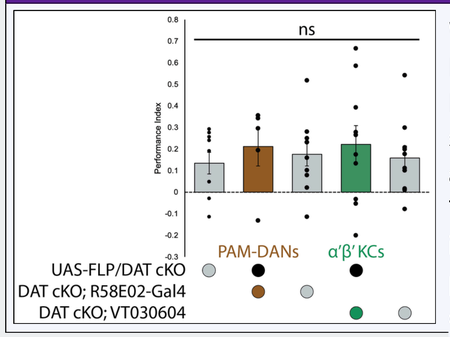
cKO suppresses DAT expression

We used anti-DAT immunostaining to prove that the cKO functioned properly.



- DAT cKO in PAM-DANs (reward DANs) had clearly decreased the amount of DAT in the MB.
- DAT strongly localises in the β'2 compartment of α'β' KCs, suggesting that effect of DAT in the α'β' KCs is likely represented by this section.

Evidence for DAT redundancy



We tested the effect when DAT was removed from PAM-DANs or α'β' KCs. Our results show that **DAT cKO does not affect learning and memory**. This contrasts with the impaired memory performance following complete KO of DAT [3].

Conclusions and open questions

→ cKO allows near-complete cell-specific DAT suppression - could this strategy be used in other neurons?
→ DAT localises in the β'2 compartment - why?
→ DAT KO in PAM-DANs/α'β' KCs does not affect short-term appetitive memory - would this change with long-term and/or aversive memory, or PAM-DAN and α'β' KC KO simultaneously?