Conditional knockout strategy to quantify dopamine transporter function in memory

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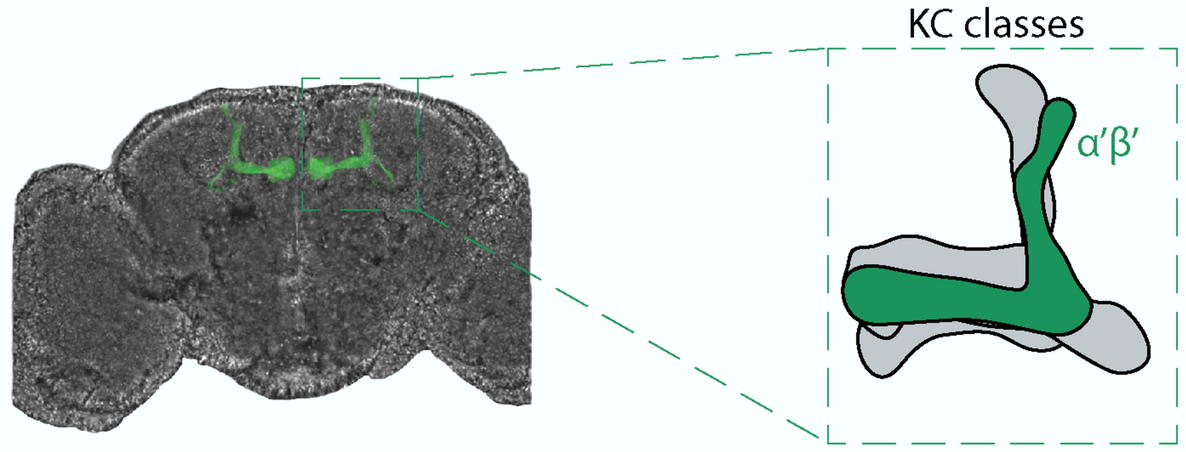
Introduction

The **dopamine transporter (DAT)** is a protein that brings secreted **dopamine** back into neurones and is the main target for psychostimulants such as cocaine and methamphetamine. The prevailing dogma is that only neurones that make and release dopamine have the ability to transport dopamine. However, single-cell transcriptomics has revealed **unexpected dopamine transporter expression** in a group of neurones in the fruit fly (Drosophila melanogaster) brain that do not make or release dopamine. These neurones, called **α'β' Kenyon cells**, are crucial in **memory** and receive multiple dopamine signals during **learning**.

In Drosophila, the mushroom body functions as the associative centre of the brain. Kenyon Cells (KCs) are the neurones that make up the MB. KCs receive input that encodes olfactory information from antennal lobe projection neurones (PNs). Different odours activate different distinct populations of KCs, so that the unique pattern of KCs activation is what encodes the identity of the odour. Nearby dopaminergic neurones (DANs), which linger close to KC-to-MBON synapses, release DA, **which assigns a positive or negative valence to the odour during associative learning**.

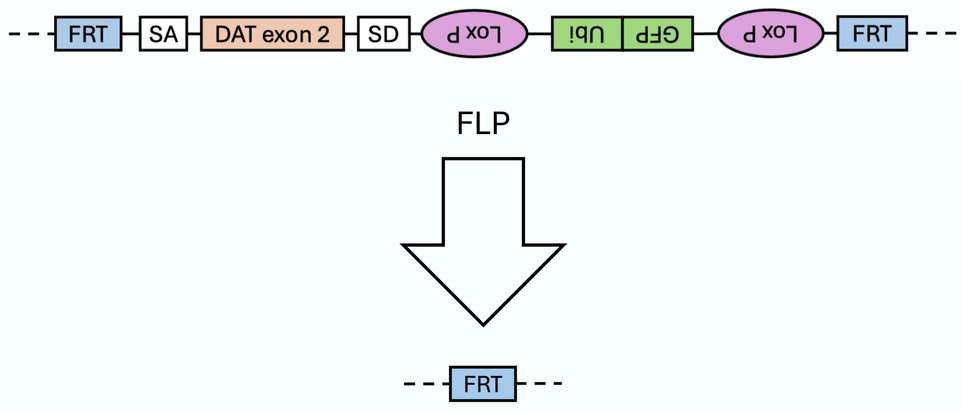


Conditional Knockout Strategy

Exon 2, which contains the start codon of the *dat* gene, is **flanked by two Flippase Recognition Target (FRT) sites.**

Flippase recombinase (FLP) binds to FRT sites and triggers the **excision of the DNA between the FRT sites via recombination**. We conditionally expressed FLP in the α'β' KCs using the VT030604-GAL4 driver and in the PAM-DANs using the R58E02-GAL4 driver, thus upon excision DAT expression would theoretically be abolished in those regions.

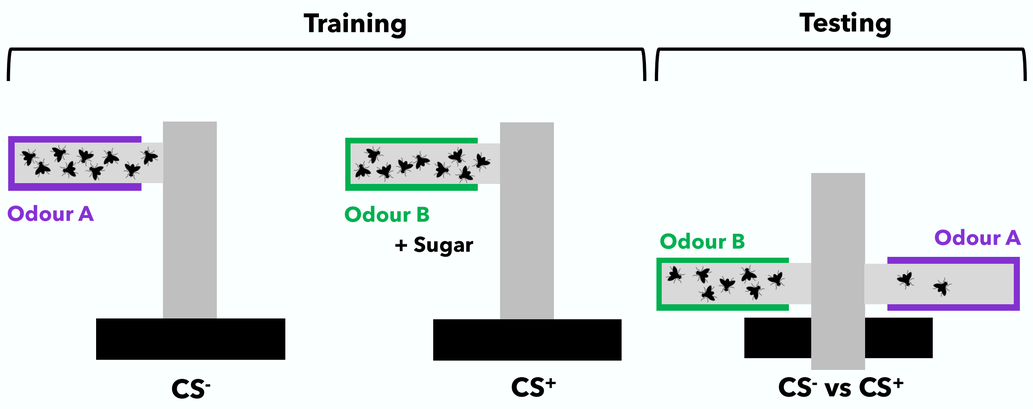
Moreover, we inserted a ubiquitin-GFP cassette alongside exon 2 that allowed us to screen for the mutants using fluorescence microscopy.



Behavioural Experiment Paradigm

During conditioning training, one odourant was presented with **dry filter paper** (unconditioned odour, CS-) for 2 min, before a 30 sec break, and presentation of a second odourant with filter paper coated with **sucrose** (conditioned odour, CS+).

That forms one half-replicate for this genotype, and a full replicate is derived by swapping the odours and repeating the above with a new cohort.



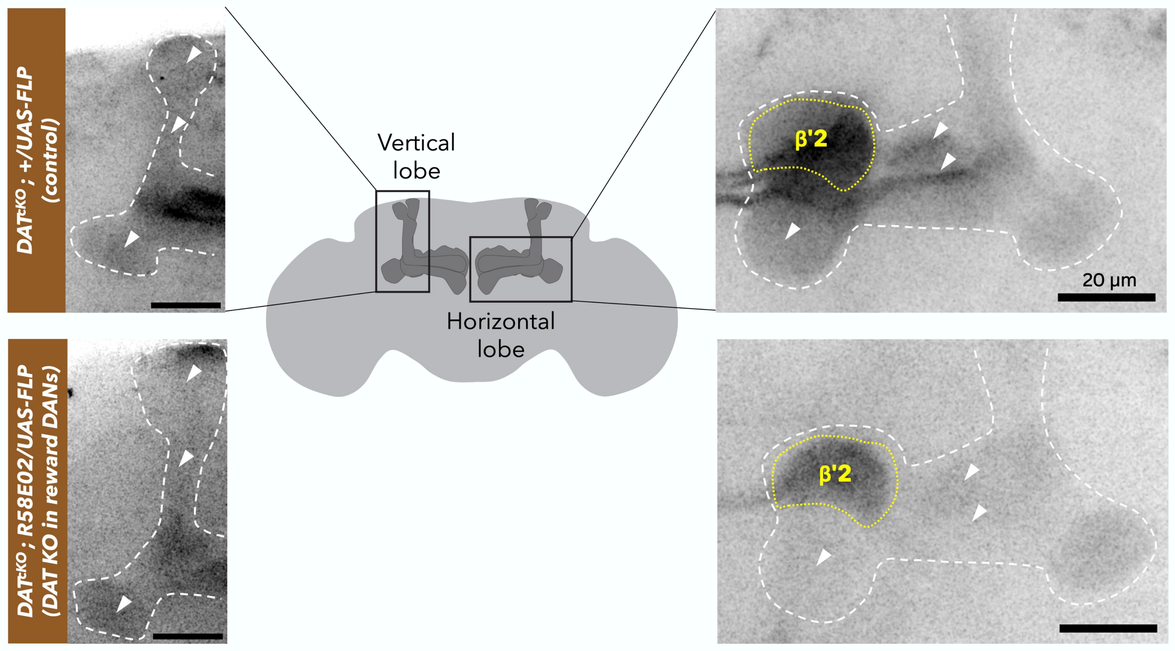
$$\text{Performance Index (PI)} = \frac{(CS^+) - (CS^-)}{(CS^+) + (CS^-)}$$

Immunostaining Results

We used immunofluorescent staining to compare the extent of DAT expression between flies that had DAT conditionally knocked out of reward DANs and wild-type flies.

We see that the faint expression of DAT in the vertical portion of the α'β' lobe is **hardly affected** by the knockout. **Immunoreactivity in the horizontal lobe, however, is significantly lower than in our wild-type control.** Despite the reduced presence of DAT in the horizontal lobe, we see **residual expression of DAT in the β'2 compartment**.

This residual expression could represent **DAT expression in α'β' KCs**. These images provide the first evidence of DAT's presence in non-dopaminergic neurones and reveal the subcellular location of DAT in α'β' KCs.

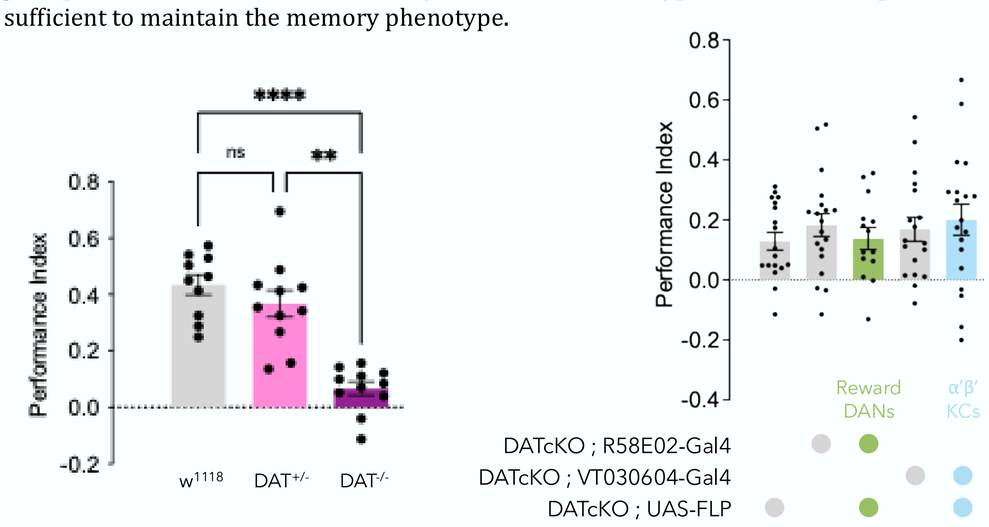


Behavioural Results

Finally, we conducted behaviour experiments to show how the knockout of DAT affected the mutants' ability to form **immediate appetitive memories**.

Our behavioural data suggests that **the presence of DAT in PAM-DANs or KCs doesn't significantly impact the strength of appetitive sugar memories**. In contrast to whole-brain knockouts such as the *fmn* mutant, which display decreased appetitive sugar memory scores.

This suggests **possible functional redundancy** between these cell types, where DAT expression in either group is sufficient to maintain the memory phenotype.



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Contact Details

References and Full Paper

Conclusions and Future Directions

✓ **The conditional knockout strategy allows us to achieve near-complete DAT suppression in a cell-specific manner.**
- Could the same strategy be leveraged to knockout DAT expression in other neurones?

✓ **We also see that the DAT in α'β' KCs is localised to the β'2 compartment.**
- Why here?

✓ **Knockout in either the DANs or α'β' KCs did not produce an immediate memory phenotype**
- Would memory be affected by simultaneous DAT knockout in both PAM-DANs and KCs?
- Would this phenotype be maintained for long-term, aversive, or water memory?