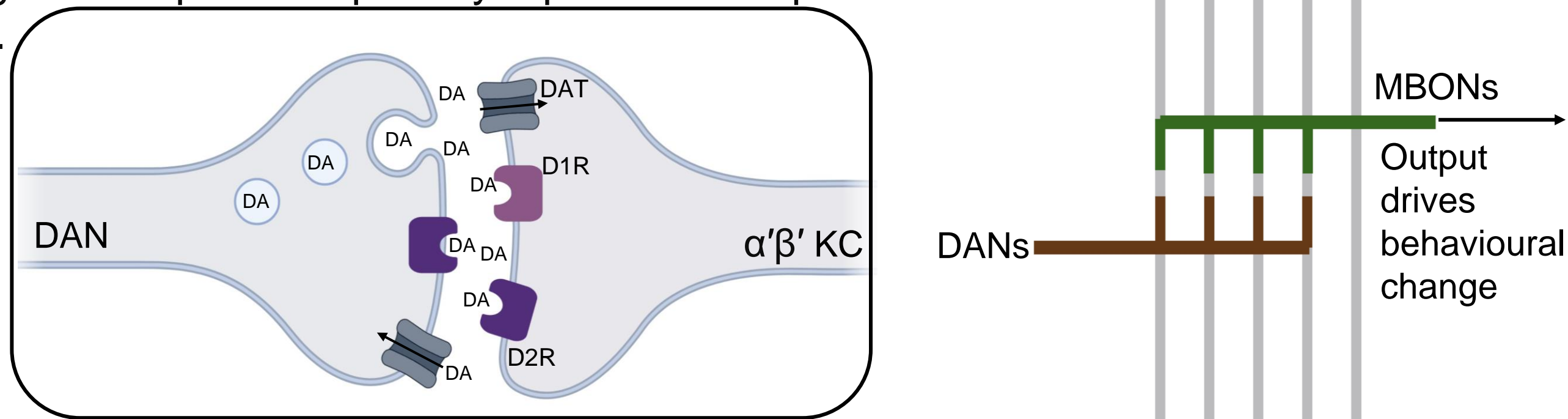


# Unravelling the role of dopamine transport in the modulation of *Drosophila* learning and memory

Tarika Sodhi, Karina M Piotrowska, Angus J Armstrong, Abigail C Stretch, Vincent Croset.  
Department of Biosciences, University of Durham, UK.

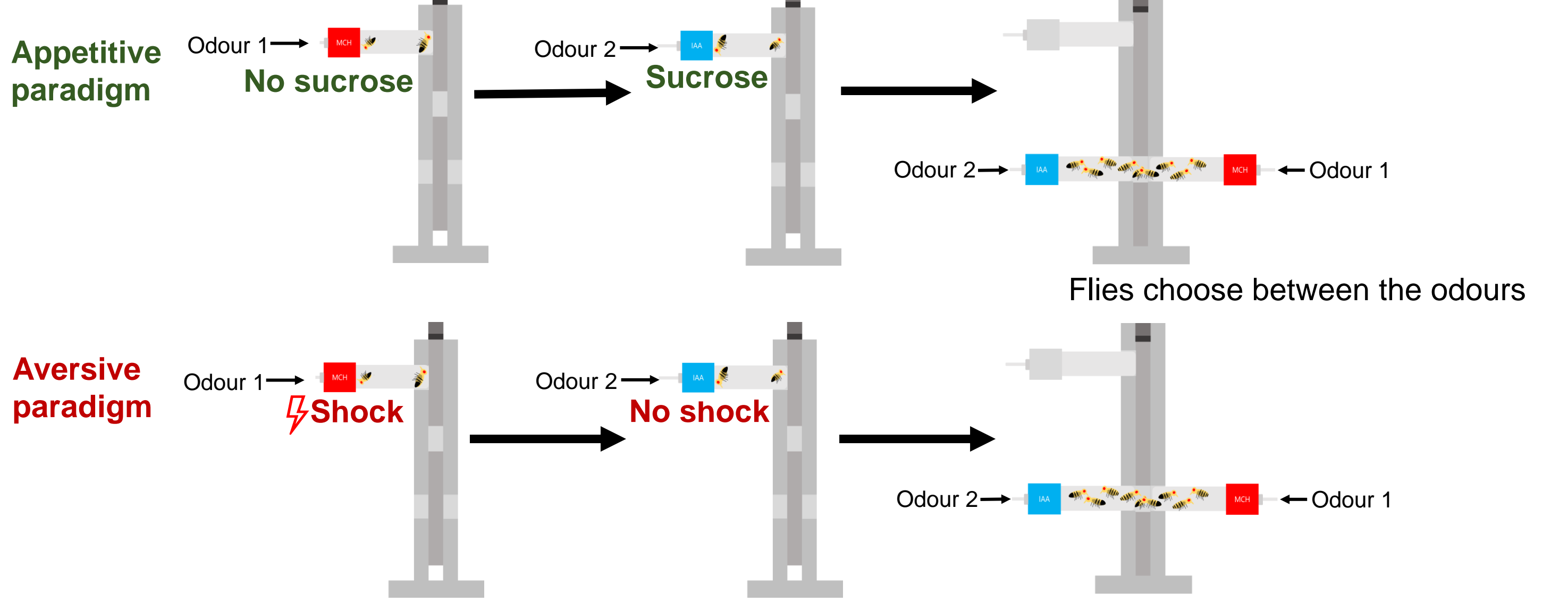
## Introduction

The dopamine transporter (DAT) is a target of both recreational and therapeutic psychostimulants and is involved in conditions including depression and ADHD. DAT mediates dopamine reuptake from synapses, limiting its lifetime and diffusion<sup>1</sup>. Though DAT is known to be expressed in dopaminergic neurons (DANs), recent sc-RNAseq data has uncovered its expression in  $\alpha\beta'$  Kenyon cells (KCs), which are not dopaminergic. Synapses between KCs and mushroom body output neurons (MBONs) are modulated by distinct subsets of reward or punishment DANs<sup>3</sup>. Here, we investigate how pre- and post-synaptic DA transport controls memory.



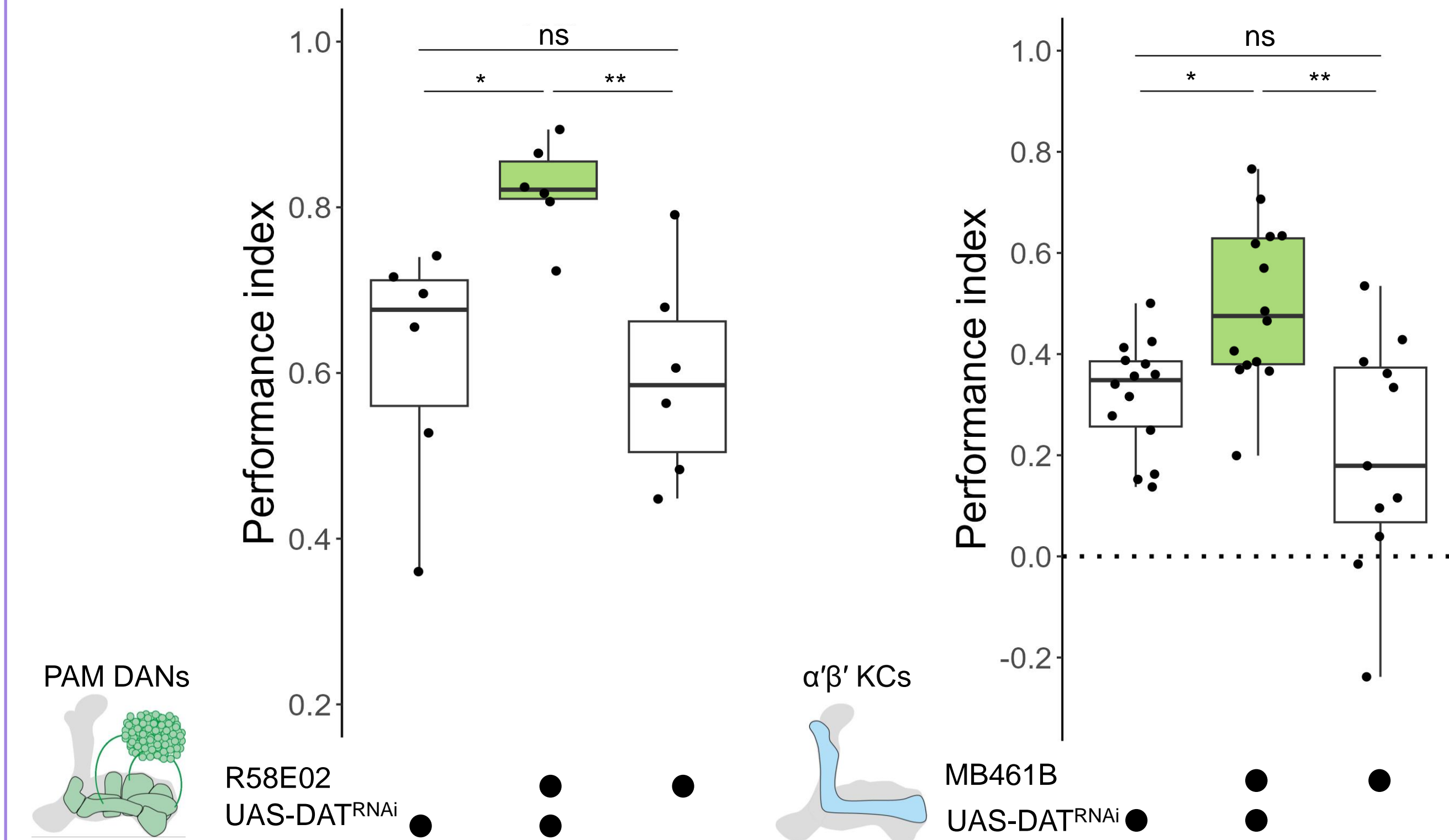
## Methods

Flies were trained in the T-maze to associate odours with **appetitive** (sucrose) or **aversive** (electric shock) cues.



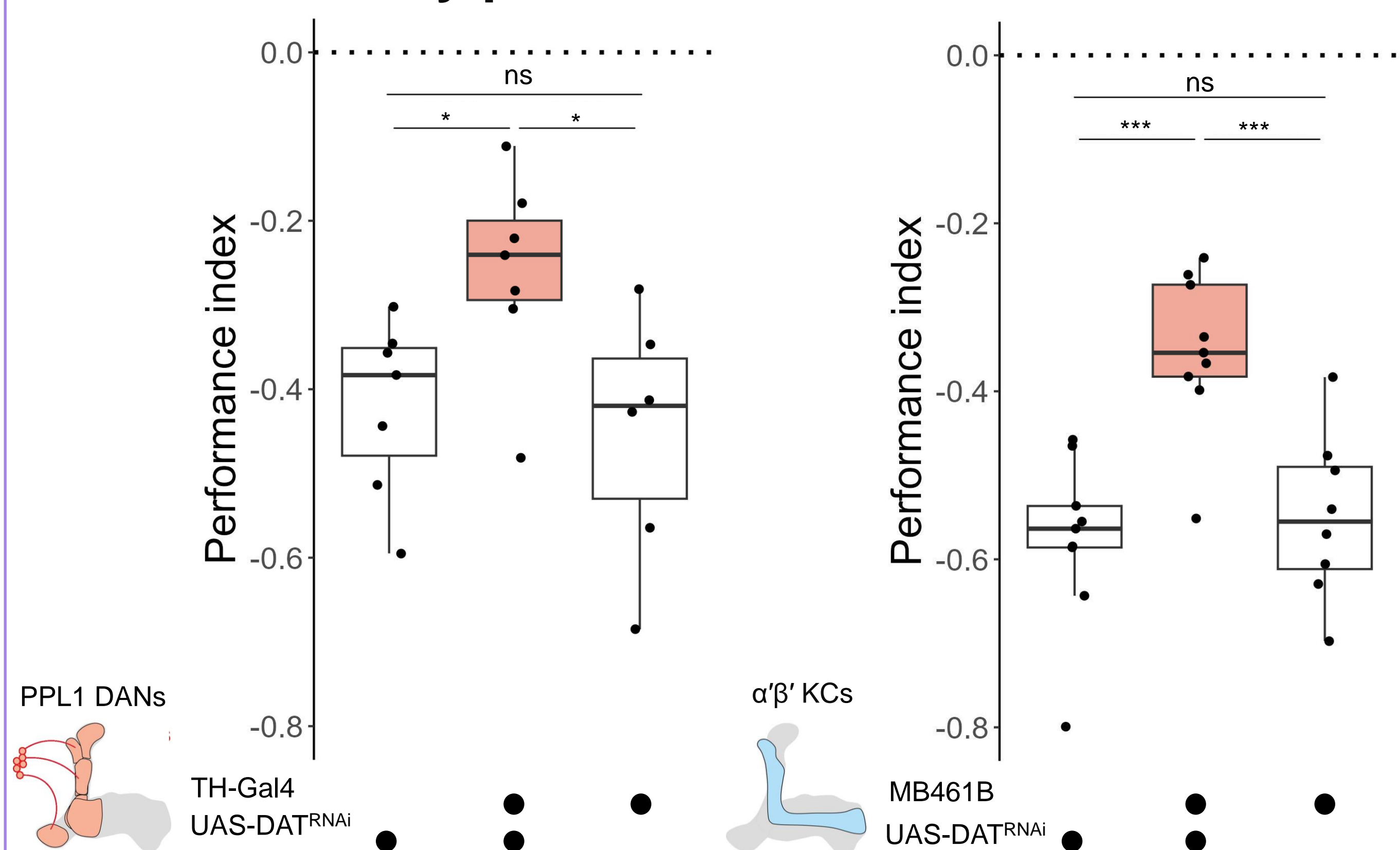
## Pre- and post-synaptic DAT knockdown increases immediate appetitive memory

Knock down of DAT in reward (PAM) DANs (left) or  $\alpha\beta'$  KCs (right) increases immediate appetitive memory. This suggests an involvement of both pre- and post-synaptic DAT in mediating memory acquisition by modulating dopamine dynamics.



**Figure 1:** The effects of a DAT knockdown on immediate appetitive sucrose memory. Left: The effect of a DAT knockdown in PAM DANs, Right: The effect of a DAT knockdown in  $\alpha\beta'$  KCs. Statistical significance was determined using a Kruskal-Wallis test, ns = non-significant, \* $p < 0.05$ , \*\* $p < 0.01$ .

## DAT in punishment DANs and in $\alpha\beta'$ KCs is required for aversive memory performance



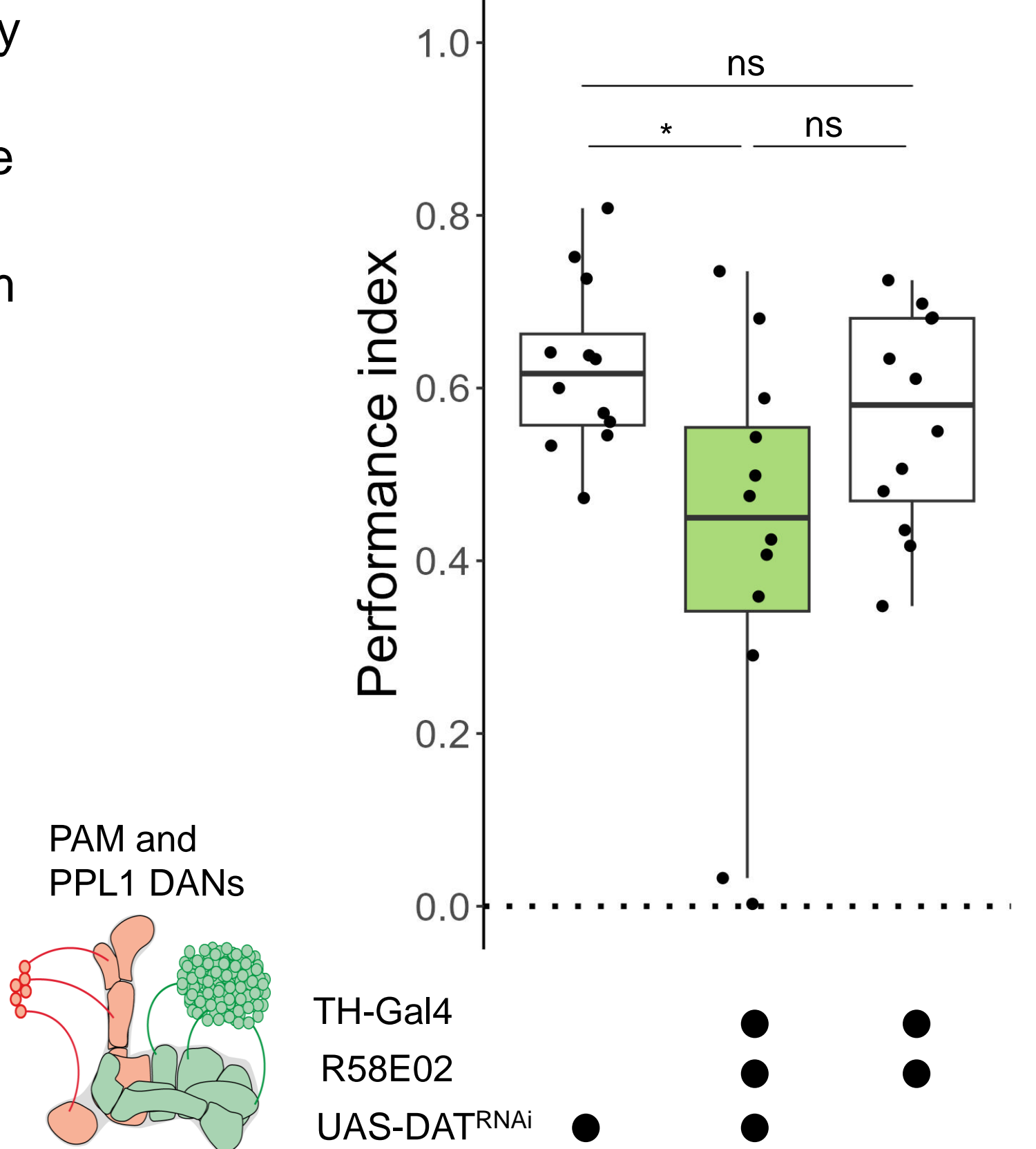
The role of DAT in aversive memory was then investigated. Methylphenidate (MPH) is a psychostimulant that inhibits DAT. Flies fed with 5 mM MPH for 24 hours prior to the experiment displayed impaired aversive memory performance (right). Further, knockdown of DAT in punishment (PPL1) neurons (top, left) or  $\alpha\beta'$  KCs (top, right) reduced aversive memory performance. Here too, we show a joint role of pre- and post-synaptic DAT to control aversive memory.

**Figure 3:** The effects of a DAT knockdown on immediate aversive memory. Top left: The effect of a DAT knockdown in PPL1 DANs, Top right: The effect of a DAT knockdown in  $\alpha\beta'$  KCs. Bottom left: Aversive short-term memory in flies fed methylphenidate (MPH) and controls. Statistical significance was determined using a Welch's t-test, ns = non-significant, \* $p < 0.05$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ .

## DAT regulates the balance between learning and forgetting

DAT knock down enhances appetitive memory but decreases aversive memory. Since some PPL1 neurons are essential for forgetting<sup>5</sup>, we hypothesise that decreased memory performance when DAT is knocked down from these cells might be due to immediate activation of forgetting pathways.

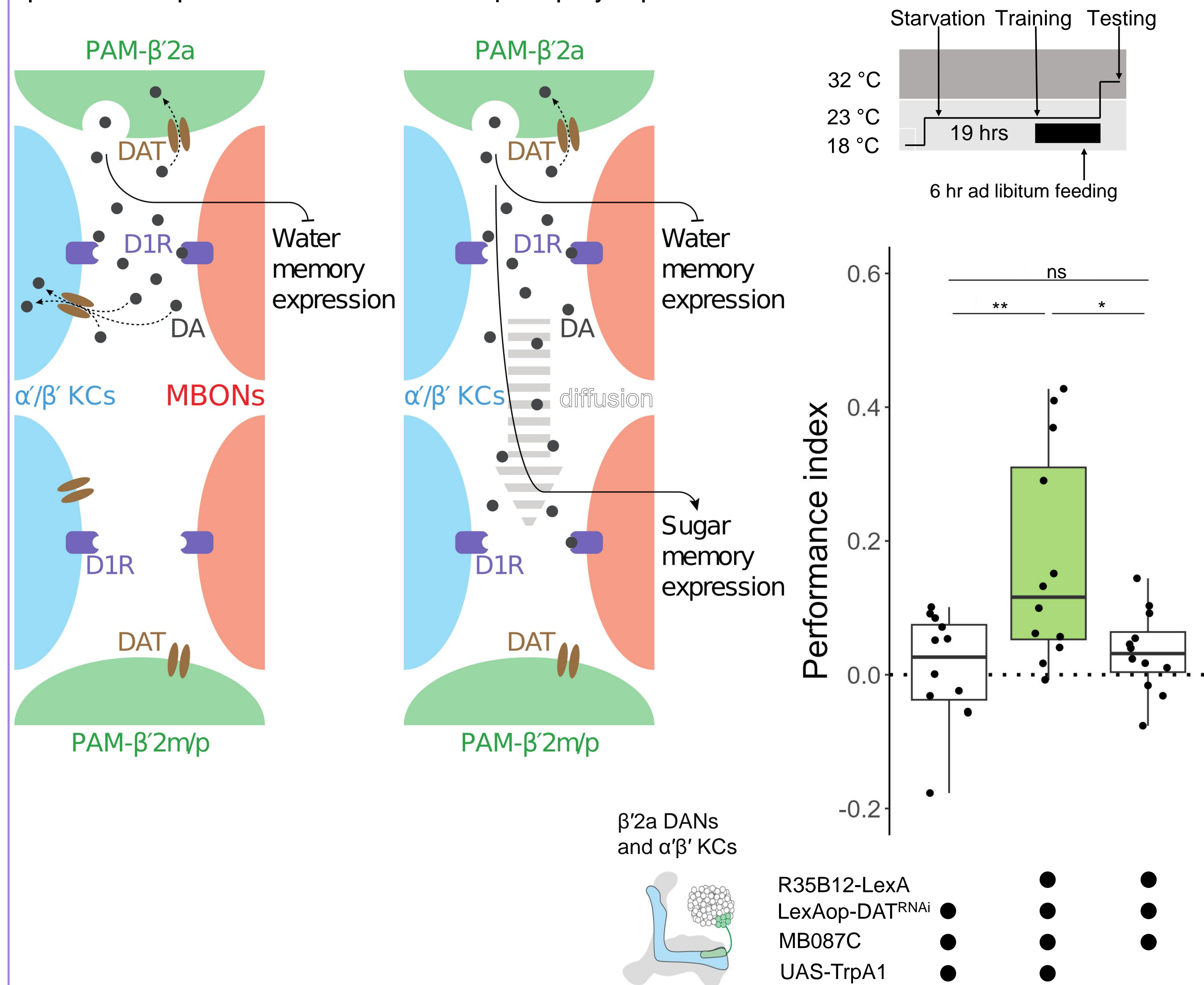
To test this, we knocked down DAT from both reward and punishment DANs, and trained flies for appetitive memory. This rescues the memory enhancement that we observed in experiments where DAT was knocked down from reward DANs alone. We conclude that DAT knock down in punishment DANs leads to increased forgetting, and that DAT acts in regulating the balance between learning and forgetting.



**Figure 4:** DAT knocked down in PAM DANs and PPL1 DANs with immediate aversive memory. Statistical significance was determined using a Welch's t-test, ns = non-significant, \* $p < 0.05$ .

## Post-synaptic DAT controls spillover in the $\beta'2$ compartment

The  $\beta'2$  compartment of the mushroom body receives input from functionally distinct DANs<sup>6</sup>. We hypothesise that DAT in  $\alpha\beta'$  KCs may contribute to preventing spillover between their synapses. Artificial activation of PAM- $\beta'2a$  only triggers sugar memory expression in fed flies when DAT is knocked down from  $\alpha\beta'$  KCs, therefore suggesting that post-synaptic DAT prevents dopamine diffusion to PAM- $\beta'2mp$  synapses.



**Figure 5:** The effects of DAT on DA spillover. Left: A schematic explaining the mechanism of DA spillover between the synaptic clefts of PAM-  $\beta'2a$  and PAM- $\beta'2mp$  DANs. Right: The effect of a DAT knockdown in  $\alpha\beta'$  KCs and simultaneous activation of  $\beta'2a$  DANs on appetitive memory. Statistical significance was determined using a Welch's t-test, \*\*\*\* $p < 0.0001$ .

## Conclusions

- Pre- and post-synaptic dopamine transport regulates both appetitive and aversive memories.
- DAT controls the balance between learning and forgetting.
- DAT prevents spillover in the  $\beta'2$  compartment of the Mushroom body.

### References

- Cragg and Rice, 2004, Trends Neurosci
- Croset et al., 2018, eLife
- Cognigni et al., 2017, Curr Opin Neurobiol
- Zhang et al., 2008, Biochem Biophys Res Commun
- Berry et al., 2012, Neuron
- Senapati et al., 2019, Nat Neurosci

### Acknowledgements

James Evans  
Sophie Waldron  
Helen Siddle  
Scott Waddell  
Ruth Brain