SUPPLEMENTARY MATERIAL

MEMORY, ANTICIPATION, ACTION - WORKING WITH TROY D. ZARS

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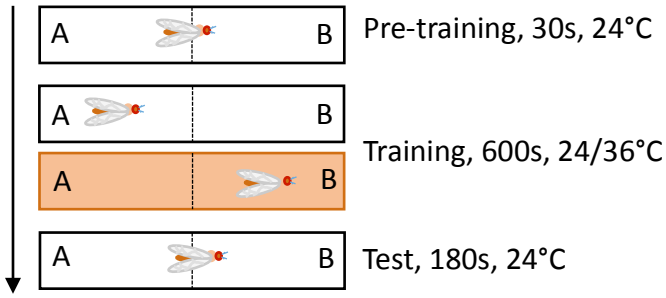
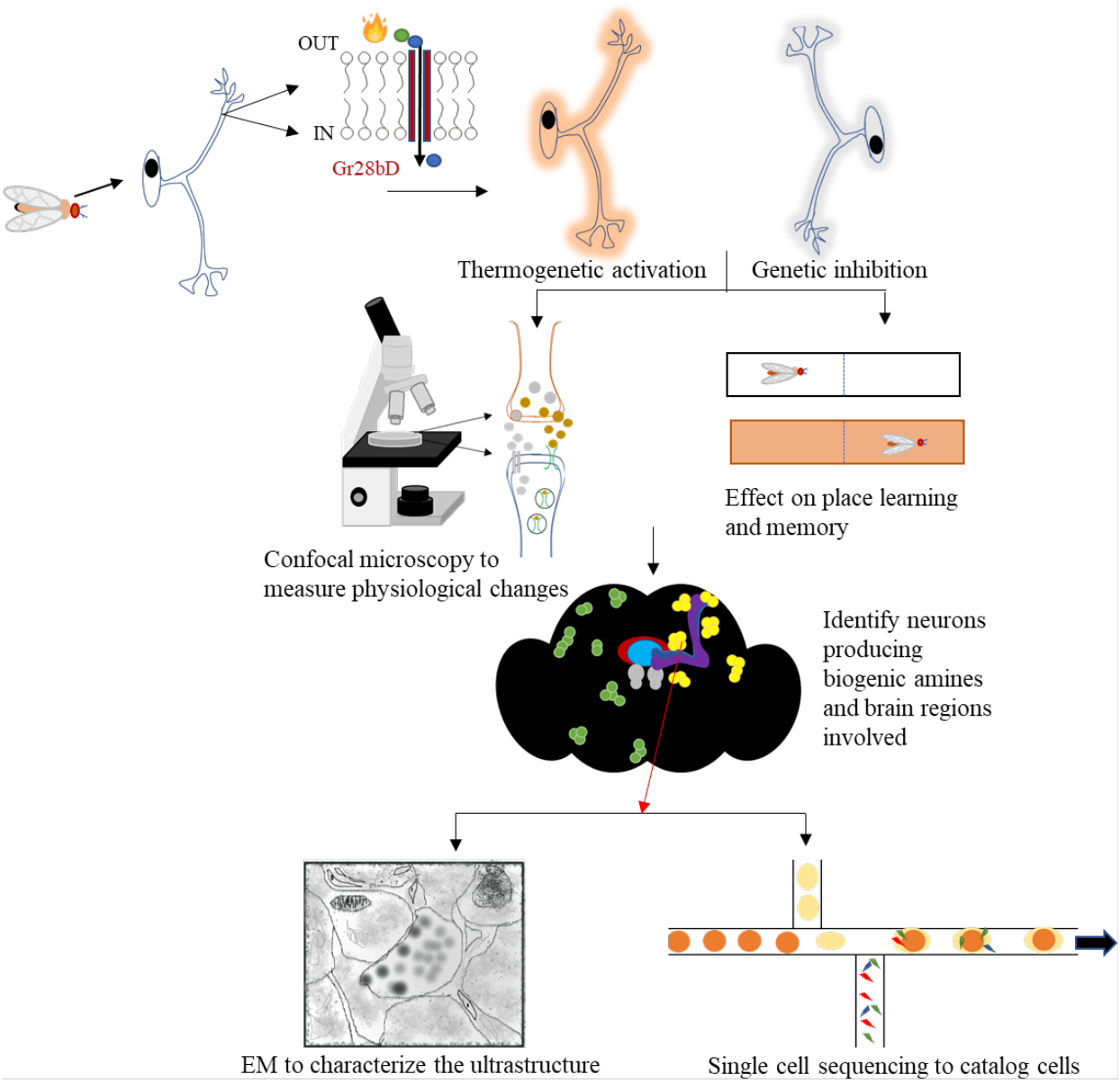


Supplementary figure 1: Schematic of the place learning and memory paradigm in the heat-box. Pre-training: Flies acclimate for 30s in a small chamber maintained at 24°C. Training: Following acclimation, the temperature of the chamber increases to a punishing temperature (for example, 36°C) when the fly crosses an imaginary midline to side B. For 10 minutes, flies thus drive their own learning in the heat-box. Test: In the final 3 minutes, memory of the flies is assessed by measuring the fly's side preference when the whole chamber is maintained at 24°C. The PI (time spent on the 24°C-associated side minus time spent in the 36°C-associated side, divided by the total time) during training provides insight into a fly's place learning, and the PI during the test indicates place memory.



Supplementary figure 2: A rationale to test the role of the dopamine system in place learning and memory that represents Troy's "Do it all" approach.

To understand the role of the dopamine system in place learning and memory, our lab used novel thermogenetic tools to activate small clusters of dopaminergic neurons or inhibit them with a inward rectifying potassium channel Kir2.1. The resultant effect on behavior was studied using the heat-box paradigm. Simultaneously, physiological experiments were underway that utilized confocal imaging and two-photon microscopy to examine changes in activity of these neurons at the cellular level. In addition, changes in ultrastructure of the neurons in mutants and after

conditioning were being investigated with electron microscopy, while the targeted clusters of neurons were set to be isolated for single-cell RNA sequencing to identify the molecular markers that define the cell type. This approach also would allow changes in gene expression associated with learning and memory, as well as mutations that affect these processes, to be quantified and analysed. This approach from behavior to molecule (and back again) was a hallmark of the integrated approach to understanding learning and memory in our lab.