HOW DO THE MUSHROOM BODIES REGULATE HABIT FORMATION IN *DROSOPHILA*?

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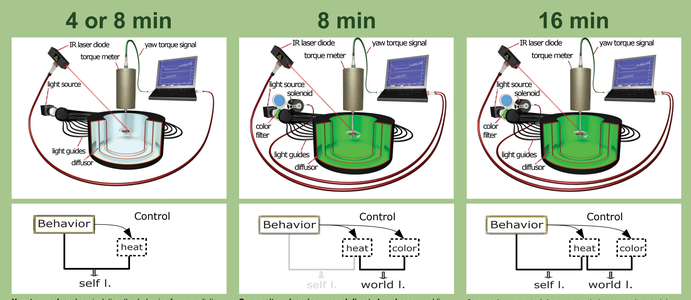
Introduction

In classical learning experiments, an association between a neutral external stimulus and a consequence of innate value is formed. Conversely, in operant learning experiments, the animal's own behavior is associated with such consequences; eventually, the animal forms habits which ensure fast and efficient behaviors. In an experiment that aims to create both types of associations simultaneously, two biologically distinct learning mechanisms interact and the classical association is prioritized. In ***Drosophila***, doubling the training period shifts learning to the operant component, enabling habit formation in flies.

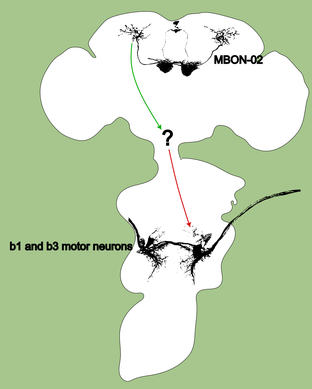


Yaw torque learning: isolating the behavior from predictive stimuli leads toself-learning, a form of motor learning, after 8 but not 4 minutes of training.

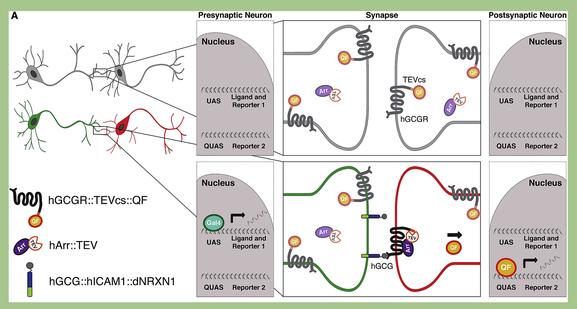
Composite learning, goal-directed phase: adding predictive colors to the yaw torque experi-ment (left) leads to color learning, a form of world-learning.

Composite overtraining: extended composite training overcomes the inhibition of self-learning and leads to habit formation.

In our preliminary studies, we have found that silencing the α/βc and α/βs lobes of Mushroom Bodies (MBs) eliminates the requirement for extended training and allows flies to form premature habits. This suggests MBs provide an inhibitory output signal that delays habit formation. Screening the output from MBs, which is being re-screened in this study, showed that this signal is mediated by MBON-02 neurons.



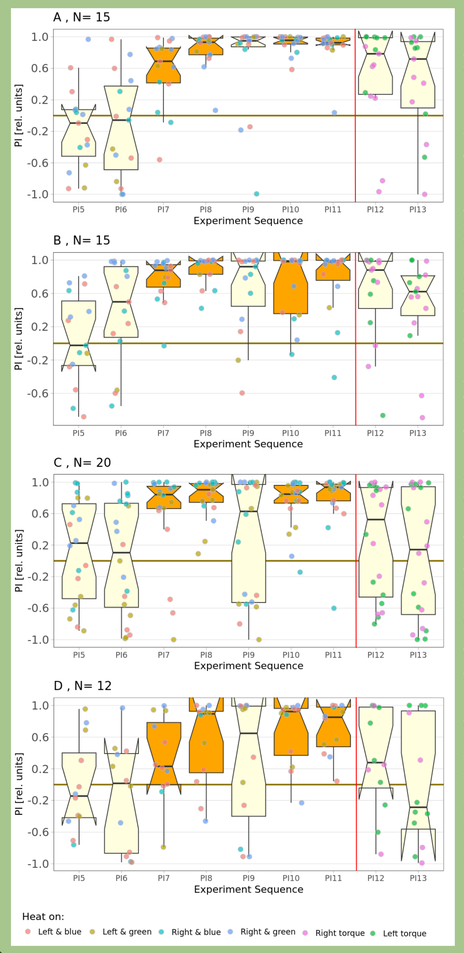
A previous study by Ehweiner et al. (2024) established that FoxP and aPKC genes are necessary for operant self-learning but dispensable for other types of learning. It has also been found that these two genes are co-expressed in b1 and b3 motor neurons, which act as wing-steering neurons in the ventral nerve cord. Our hypothesis is that memory is formed in the neurons that co-express FoxP and aPKC, and the inhibitory signal to switch over different learning paradigms comes from MBON-02 to these neurons. Preliminary connectomic analysis showed weak direct synaptic connections from MBON-02 to b1 and b3 motor neurons; here we investigate whether the signal from MBON-02 is transmitted to motor neurons via neuromodulatory signal-producing neurons. Then we will analyze the connections between MBON-02 and these neurons via connectomic analysis.



Trans-tango staining allows the visualization of post-synaptic cells of our neurons of interest. We aim to use trans-tango to visualize the post-synaptic cells of the MBON-02 and create a candidate list of neurons that might be playing a role in signal relay.

MB & MBONs

Silencing the Kenyon cells (KC) of the mushroom bodies (MBs) replicated the effect of overtraining and caused operant learning to be prioritized. In a preliminary screen (not shown) of all mushroom body output neurons (MBONs), MBON-02, 15, and 17 seemed to show a phenocopy of silencing the MBs. Our research focuses on tracking this phenomenon upstream and downstream of the MBs.



MBON Screen: Results from an ongoing premature habit formation screen of 3 MBON lines and WT control. (2nd rescreen)

The time periods are tracked on the x-axis, each 2 minutes long. (PI5-PI13)

A higher performance index (PI) score (y-axis) indicates that the fly avoided the punished domain. The color of the individual dots indicates the punished domain and light color.

The first two periods are unpunished, and the fly is allowed to fly undisturbed to express preference. Orange periods indicate that one domain was punished with a hot laser. The red line indicates when the arena color turns off and premature habit formation is measured.

An elevated PI-score at the last two periods (PI12 & PI13) compared to the pretest (PI5 & PI6) would indicate that the fly showed premature habit formation.

WT flies show no preference at PI12 & 13.

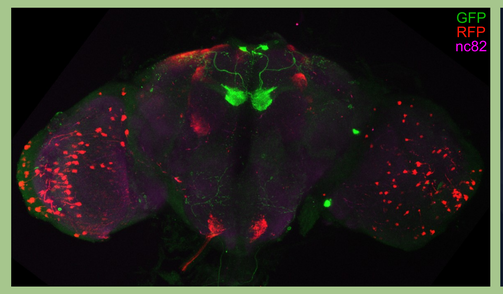
MBONs are silenced with a tetanus toxin effector and MBON split-Gal4 driver lines, selected after an overall screen of all MBON lines.

Driver lines:
MB499B: MBON-02
MB050B: MBON-15
MB027B: MBON-17

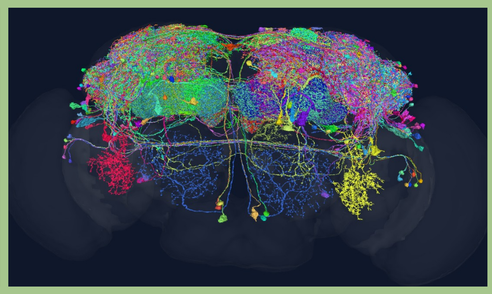
Descending Circuit

For comparison, we verified the trans-tango staining with the MB399B driver line (left). Active MBON-02 post-synaptic cells from the BANC dataset (right). From comparison with this and other available connectomic datasets, we will create a list of candidate neurons with varying priorities assigned by trans-tango staining. The role of selecting neurons in habit formation will be tested by silencing the neurons and testing the flies' behaviorally.

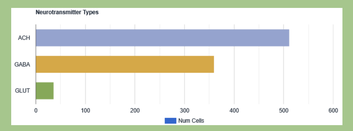
MBON-02 trans-tango imaging showing the postsynaptic neurons



MBON-02 postsynaptic cells on the BANC dataset

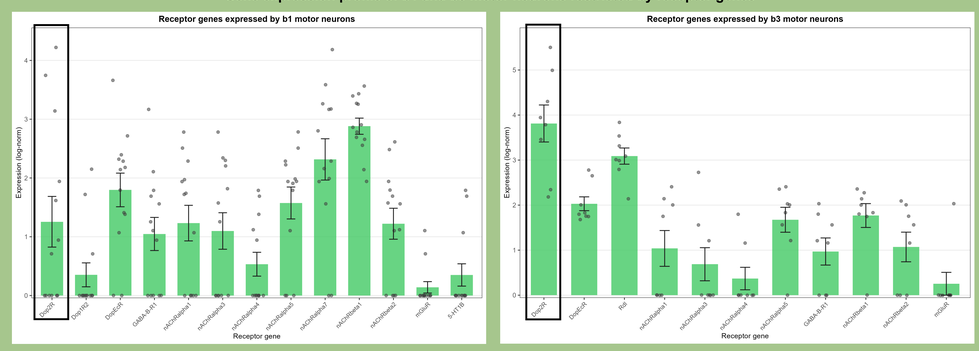


Neurotransmitter profile of presynaptic cells of b1 and b3 motor neurons



Connectome analysis suggests a high probability of the presence of neuromodulation on b1 and b3 motor neurons. To address that probability, we used a publicly available single-cell RNAseq dataset (Allen et al., 2020). To search for receptor candidates among b1 and b3 motor neurons, we selected the expression profile of the cells that express FoxP, aPKC, and VGlut as well as specific transcription factors used to create split-Gal4 drivers to target b1 (CG31345 and Awh) and b3 (ct and Ubx) motor neurons. We have found that dopamine receptors, especially Dop2R, were expressed in these cells, as well as acetylcholine, GABA, and glutamate receptors. When compared with the neurotransmitter identity of pre-synaptic neurons in the BANC dataset, we found acetylcholine, GABA, and glutamate but not dopamine.

Gene expression profile of b1 and b3 motor neurons restricted by receptor genes



Outlook

➔ Considering the presence of Dop2R receptors but no presynaptic dopaminergic cells, we will knock down the Dop2R receptors using driver lines targeting b1 motor neurons (IS69306), b3 motor neurons (SS98650), or both of them (IS38497) and test the flies behaviorally.

➔ Using BANC and MaleCNS datasets, we will create a list of potential neurons that might be releasing the dopamine in the ventral nerve cord and test the flies behaviorally while silencing the neurons.

➔ Silence specific KC subpopulations and test the flies behaviorally for premature habit formation.

➔ Once the relevant KCs are identified, we will knock down their acetylcholine receptors and innexin genes and test separately to investigate the impact of chemical and electrical synapses.

➔ Prevent maturation of short neuropeptide F in KC and test the flies behaviorally.

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