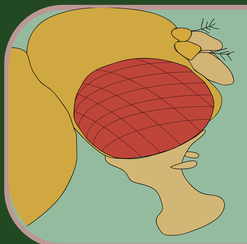

Screening for neuronal substrate of the inhibitory interaction between two genetically different learning systems in *Drosophila*

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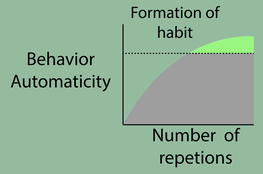
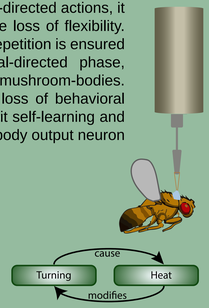
Introduction

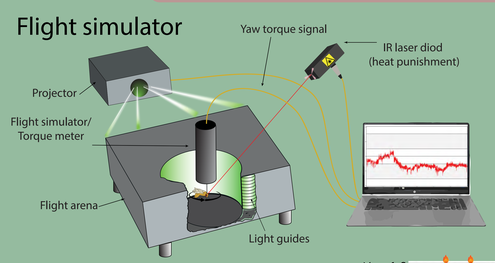
Habit formation is the mechanism by which animals including humans trade off flexibility and efficiency demands on their behavior. Goal-directed actions are flexible but slow, while habitual responses are fast but inflexible. Operant learning situations are commonly comprised of two stages: In the early goal-directed phase, the animal is learning about both the contingencies in its (new) environment (world-learning) as well as how to manipulate these contingencies for its own benefit (operant activity). In a later phase, the continued relationship between the animal's actions and their consequences (self -learning) transforms the behavior into efficient, habitual responses. One important factor regulating the transition from goal-directed behavior to habitual responses is repetition. Repetition not only ensures the behavior has been practiced enough to be more efficient than the more variable goal-directed actions, it also serves to ensure the environment is constant enough to warrant the inevitable loss of flexibility. Research in the fruit fly Drosophila has uncovered a mechanism by which sufficient repetition is ensured in order to prevent maladaptive, premature habit formation. During the goal-directed phase, world-learning inhibits self-learning via a prominent neuropil in the fly brain, the mushroom-bodies. Blocking this brain region leads to premature habit formation accompanied by a loss of behavioral flexibility. To decipher the neuronal mechanism by which the mushroom bodies inhibit self-learning and contribute to the crucial regulation of habit formation, we screened all 21 mushroom body output neuron (MBON) classes for their involvement in the inhibition.

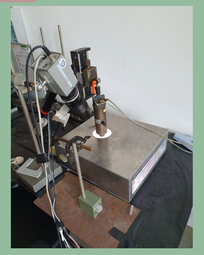


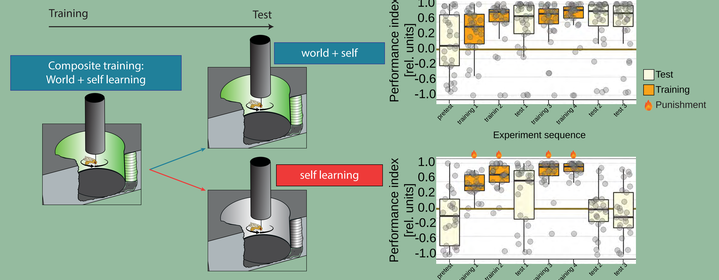


Methods

Flight simulator



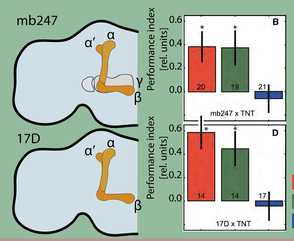




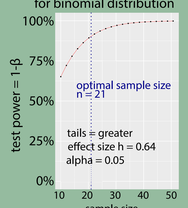
Flies with inhibited mushrom body form habits prematurely

The Gal4 line Mb247 labels neurons of all three mushroom body subsystems (α/β; α′/β′; and γ)

17D drives expression mainly in the MB α and β lobes (core and surface) but not in the γ lobes



Proportion power calculation for binomial distribution

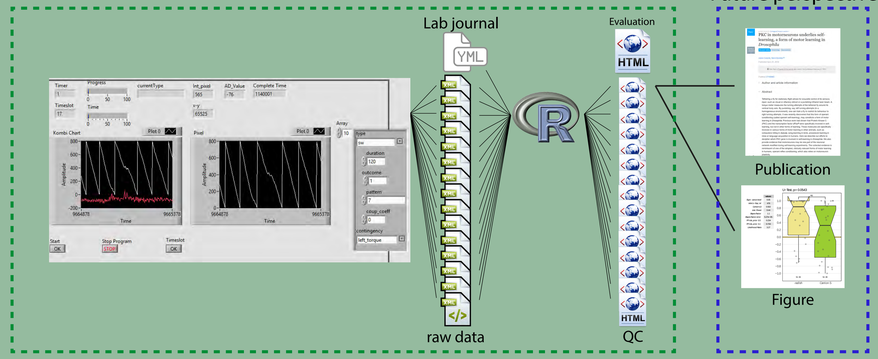


Composite control (world + self)
Isolated self learning component
Isolated world learning component

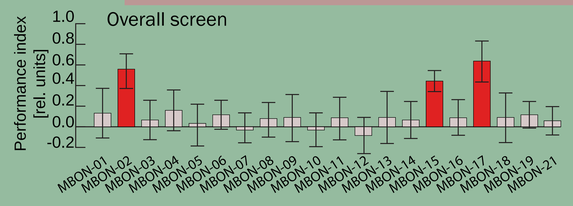
Results

Software development and data analysis

The in-lab custom written data collection and evaluation software automatically records and evaluates the data using a lab journal. The software provides the user with the results combined with appropriate statistics, it also supports a quality control of the data.

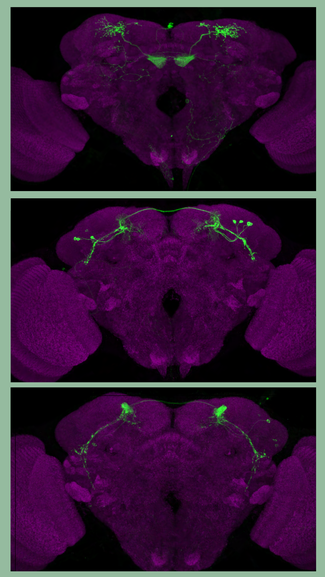


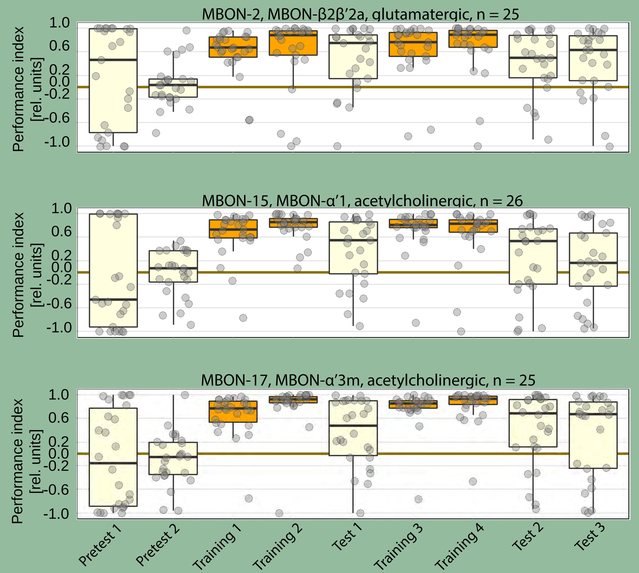
Preliminary mushroom body output neuron type screen



Each individual mushroom body output neuron type was blocked using tetanus toxin. A flight simulator was used to measure the premature habit formation in the flies.

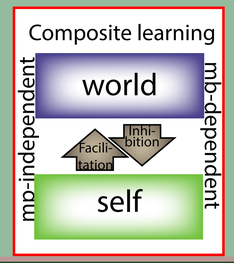
Expression pattern from FlyLight (3)





Summary and Conclusion

In the early phases of operant learning situations in Drosophila, world-learning inhibits self-learning via the mushroom bodies. This mechanism prevents the immediate stereotypization of the behavior which would otherwise take place and thereby keeps the behavior flexible. We set out to scan which subtypes of the mushroom body output neurons (MBONs) contribute to regulating habit formation. We blocked synaptic output from all 21 MBON classes expressing Tetanus toxin under the control of class-specific driver lines. Each of these lines was tested for premature habit formation using operant self-learning in a flight simulator. We used custom-written software for data collection and evaluation. Three different MBON classes showed premature habit formation when their synaptic output was blocked: MBON-2, 15 and 17. MBON-2 is glutamergic, whereas both MBON 15 and 17 are acetylcholinergic. The driver line for MBON 2 encompasses only a single neuron whereas MBON 15 and 17 each label two neurons.



Future Perspective

- Finish the data-collection and analysis software
- Validate the three positive MBON subtypes
- Rather than deactivate these MBONs, perform experiments while activated
- Perform rescue experiments with the three subtypes
- Explore the connectivity, both pre and post-synpatic of the three MBON types
- Investigate how the transition from having a goal-directed behavior to habitual responses takes place
- Explore the pathway that facilitates self-learning in a composite learning task

References

1. Björn Brembs, Mushroom Bodies Regulate Habit Formation in Drosophila Current Biology, Volume 19, Issue 16, 25 August 2009, Pages 1351-1355

2. Aso et al, The neuronal architecture of the mushroom body provides a logic for associative learning eLife 2014;3:e04577 DOI: 10.7554/eLife.04577

3. https://www.janelia.org/project-team/flylight

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