Neurogenetic dissection of learning-by-doing in *Drosophila*

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1. Introduction

Neurons constitute the most energy-hungry tissue, consuming energy at 10 times the rate of non-neural tissues. So voracious is our brain that we had to change our diet considerably just to be able to afford it. Assuming that brains mainly compute input/output transformations, one would expect the brain to drastically reduce its energy expenditure when there is no input to compute. However, even at rest our brain's energy consumption barely drops. Functional imaging experiments show spontaneous activity fluctuations to be responsible for most of the brain's drain. Moreover, less than 10% of all synapses carry sensory information and processing these sensory data only requires an additional 0.5-1% in energy expenditure. This accumulating evidence suggests that brains are active agents rather than passive computers. What is so important about being active that it would be worth 99% of the brain's energy?

In the 100 years since the term was coined, "learning-by-doing" has been recognized as a successful educational and economic strategy. At its core lies the psychological phenomenon which was described only a few years earlier: Active engagement of the brain provides learning capabilities which are difficult or impossible to achieve by passive observation alone. This phenomenon is today known as the generation effect and can also be observed in animals as diverse as monkeys, cats or fruit flies. Despite the impact learning-by-doing has on society and the evolutionary ubiquity of the generation effect, the mechanism by which activity enhances passive learning is as unknown as it is profound. Therefore, its potential contribution to "the brain's dark energy", can currently not be well assessed.

To study its neurobiological basis, we hypothesized that the generation effect may be brought about by an interaction of two components: an active, skill-learning component and a passive, fact-learning component. We tested this hypothesis by combining two simple experimental instances of fact- and skill-learning, respectively, in the fruit-fly Drosophila melanogaster.

2. Learning-by-doing is most effective (in flies, too)

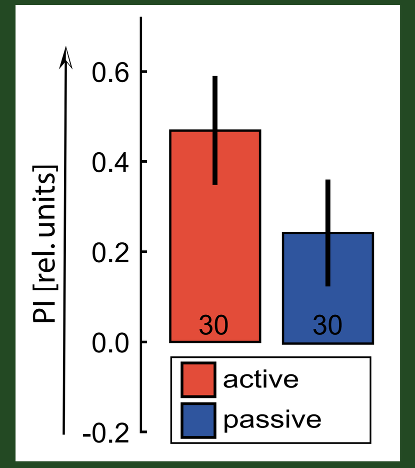
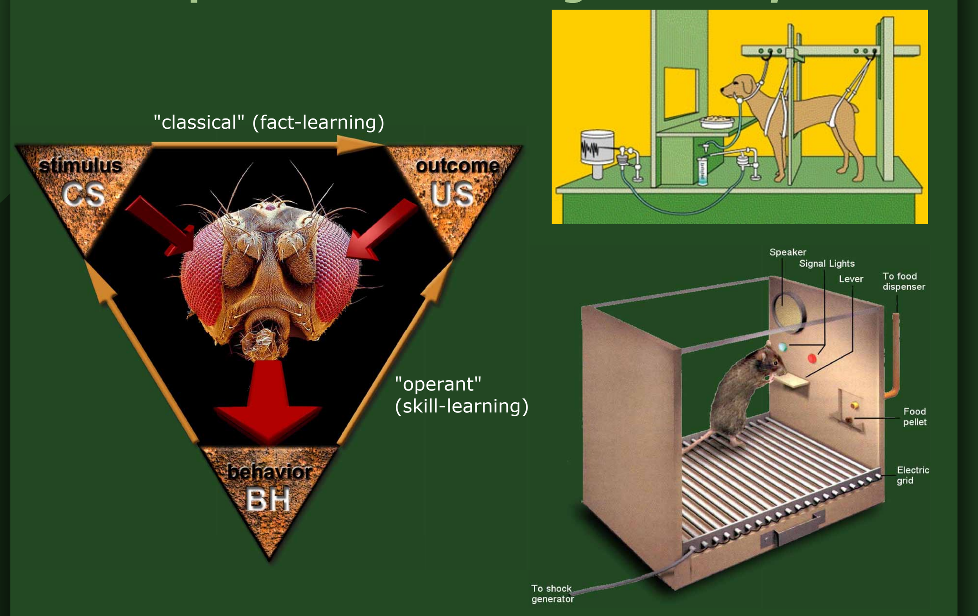


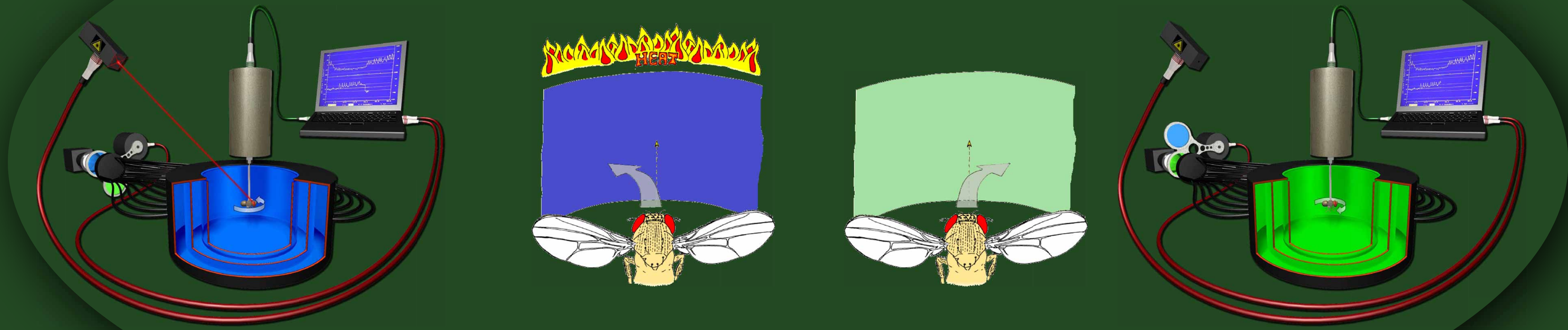
Fig. 1: *Comparison of active and passive pattern learning in flies.* The same sequence of sensory input sufficient for inducing a substantial learning effect if controlled operantly ("ative", left), only induces a small learning score if it is perceived passively (right). Thus, active learning ("by doing") is more effective than passive learning.
Left/red - Operant 'active' flies. N=30. Right/blue - Classical 'passive' flies. N=30.
Error bars (as in all figures) are S.E.M.s.

3. Composite Conditioning in *Drosophila*



At the Drosophila flight simulator, operant and classical components can be combined and dissociated at will. The fly's behavior can be made contigous with an arbitrary number of different stimuli, enabling the experimenter exquisite control over classical (CS-US) and operant (BH-US) contingencies.

Drosophila at the torque compensator



Composite Conditioning: Yaw Torque + Color

4. Fact- and skill-learning interact hierarchically

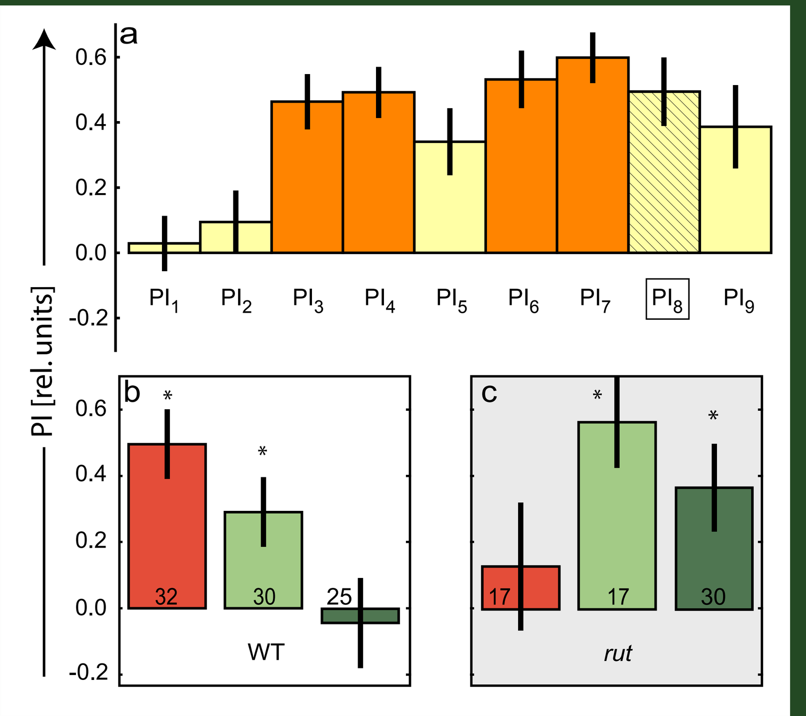


Fig. 2: *Experiments with wildtype, mutant and transgenic flies reveal hierarchical interactions between fact- and skill-learning.* **a,** Course of experiment. Bars show performance indices (PI) of successive 2-min intervals of pretest (yellow bars; PI_1, PI_2), training (orange bars; PI_3, PI_4, PI_6, PI_7) and memory test (yellow bars; PI_5, PI_8, PI_9) (see experimental procedures for details and definition of PI). The following bar graphs all show PI_8 (hatched bar). **b,** Significant composite and skill-learning in wildtype (WT) flies (red, composite: $t_{31}=5.1$, $p<0.001$; light-green, skill-learning: $t_{29}=3.0$, $p<0.006$). After composite training, the skill-learning score is not significant (dark green: $t_{24}=-0.3$, $p<0.8$) indicating inhibition of skill-learning during acquisition. **c**, Abolished composite and unaffected skill-learning *rut* mutant flies (composite: $t_{16}=0.7$, $p<0.5$; skill-learning: $t_{16}=4.3$, $p<0.001$). After composite training, the skill-learning component is significant ($t_{29}=2.9$, $p<0.007$) indicating skill-learning inhibition at the level of retrieval. Numbers at bars - number of animals. * - $p<0.05$.

5. Suppression of skill-learning allows generalization

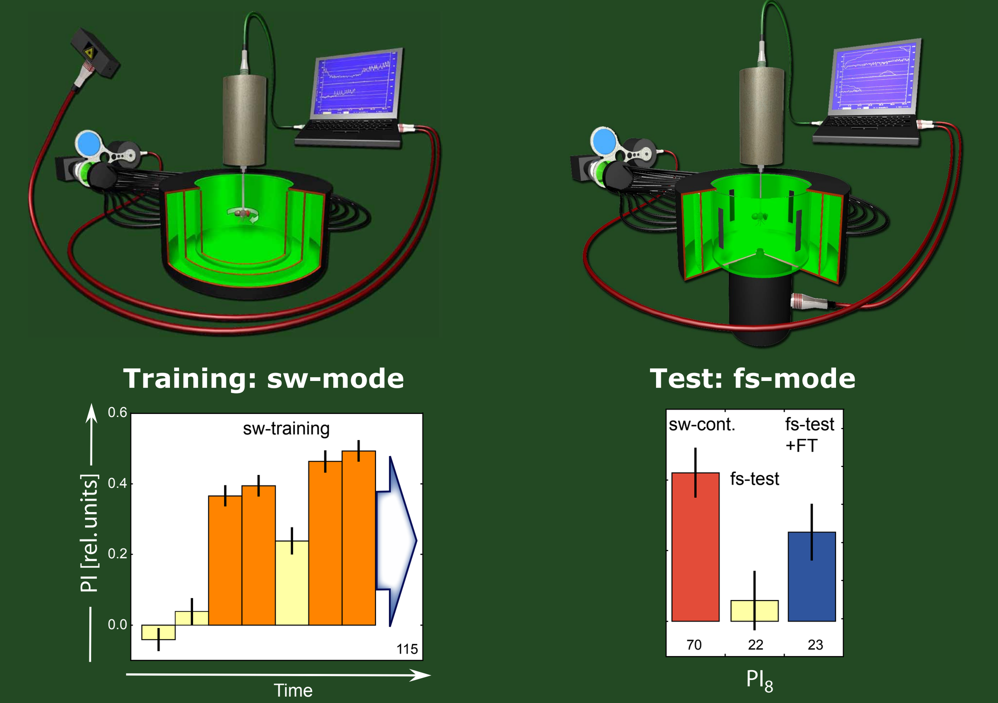
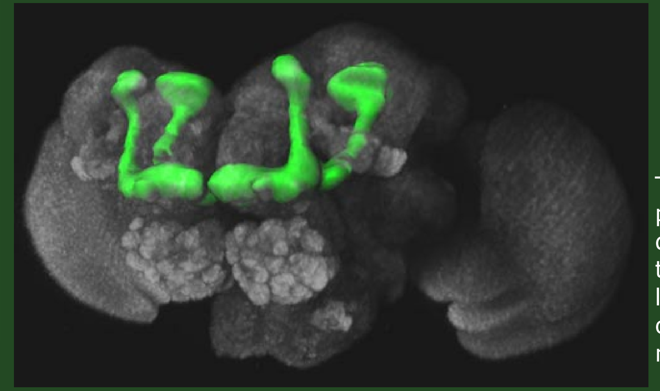
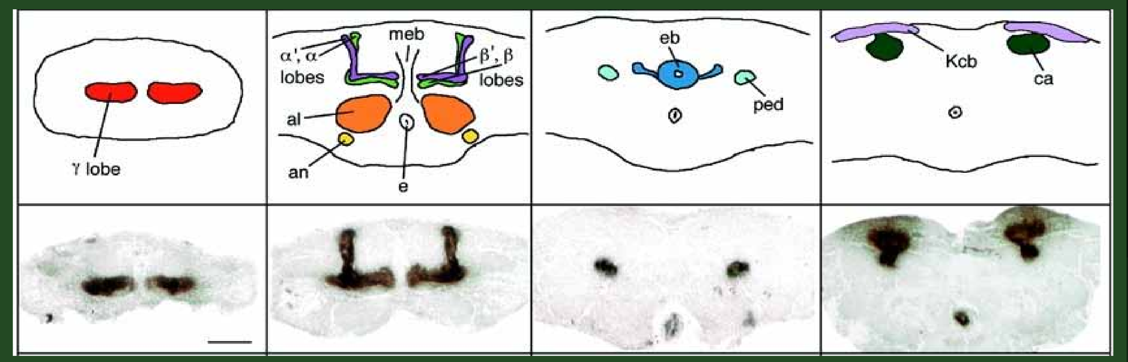


Fig. 3: *Classical components can be generalized for access with a different behavior.* **Left:** Training in sw-mode. **Right:** Test in flight-simulator mode. Only after a 60s familiarization (reminder) training do the flies show the conditioned color preference.

6. Blocking mushroom bodies



The mushroom bodies (left) are a prominent insect neuropil. The GAL4 driver line mb247 was crossed (below) to a line expressing the tetanus toxin light chain under the UAS promotor to obtain offspring with blocked mushroom body output.



7. Mushroom-bodies prevent premature habit formation

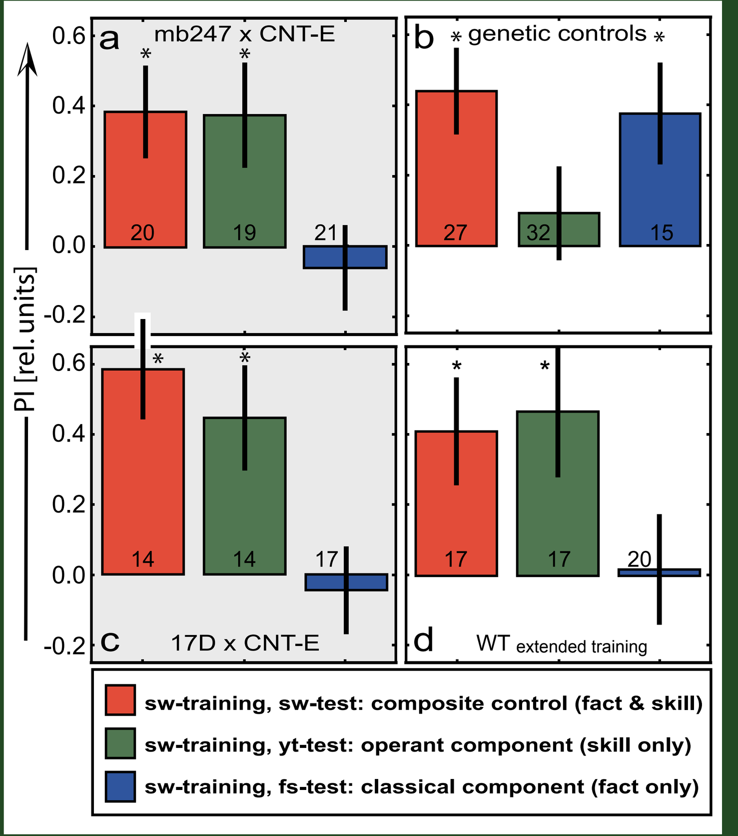
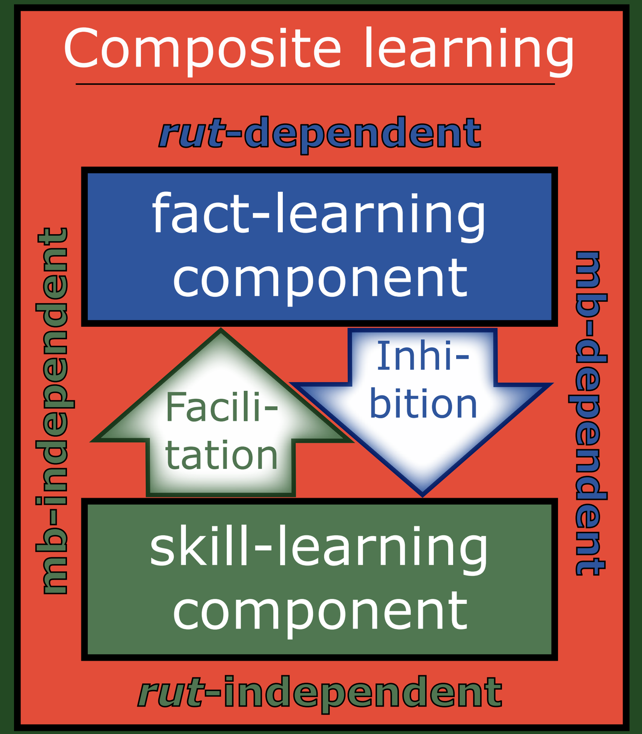


Fig. 4: *Mushroom-body mediated suppression of skill-learning is necessary for the generalization of fact-learning.* **a**. Flies with blocked mushroom-body output perform well in sw-learning (red), but do not suppress the operant component in sw-learning (green). Without the suppression of the operant component, these transgenic flies are unable to transfer the classical component to a different behavior (blue). **b**. The genetic control flies reproduce the wild-type results. **c**. Flies with blocked output only from the α and β lobes of the mushroom-bodies mimic the flies expressing tetanus toxin in all mushroom-body lobes. **d**. Extended training overcomes the suppression of the operant component in wildtype (WT) flies. The results constitute a phenocopy of the transgenic animals (a, c). Numbers at bars – number of animals. * – significant difference from zero.

8. Conclusion

Our results allow for the first time to establish a mechanistic model of how active and passive learning systems interact in composite learning situations and which biological substrates mediate the processes resulting in the generation effect. Acquisition of the *rut*-dependent fact-learning component suppresses acquisition of the *rut*-independent skill-learning component via the MB. The skill-learning component facilitates fact-learning via still unknown, non-MB pathways. This interaction leads to efficient learning, enables generalization and prevents premature habit-formation. Habit formation after extended training reveals the gate-keeping role of the MB, allowing only well-rehearsed behaviors to consolidate into habits. This two-stage process of skill-learning – an initial, variable phase and a later, stereotyped phase – mirrors the way cortico–basal ganglia circuits are thought to promote learning of action sequences through trial-and-error learning in vertebrates.



Presented at the Gordon Research Conference "Genes & Behavior"in Lucca, Febrary 24-29, 2008.