PO 410

Mushroom-bodies regulate habit formation in *Drosophila*

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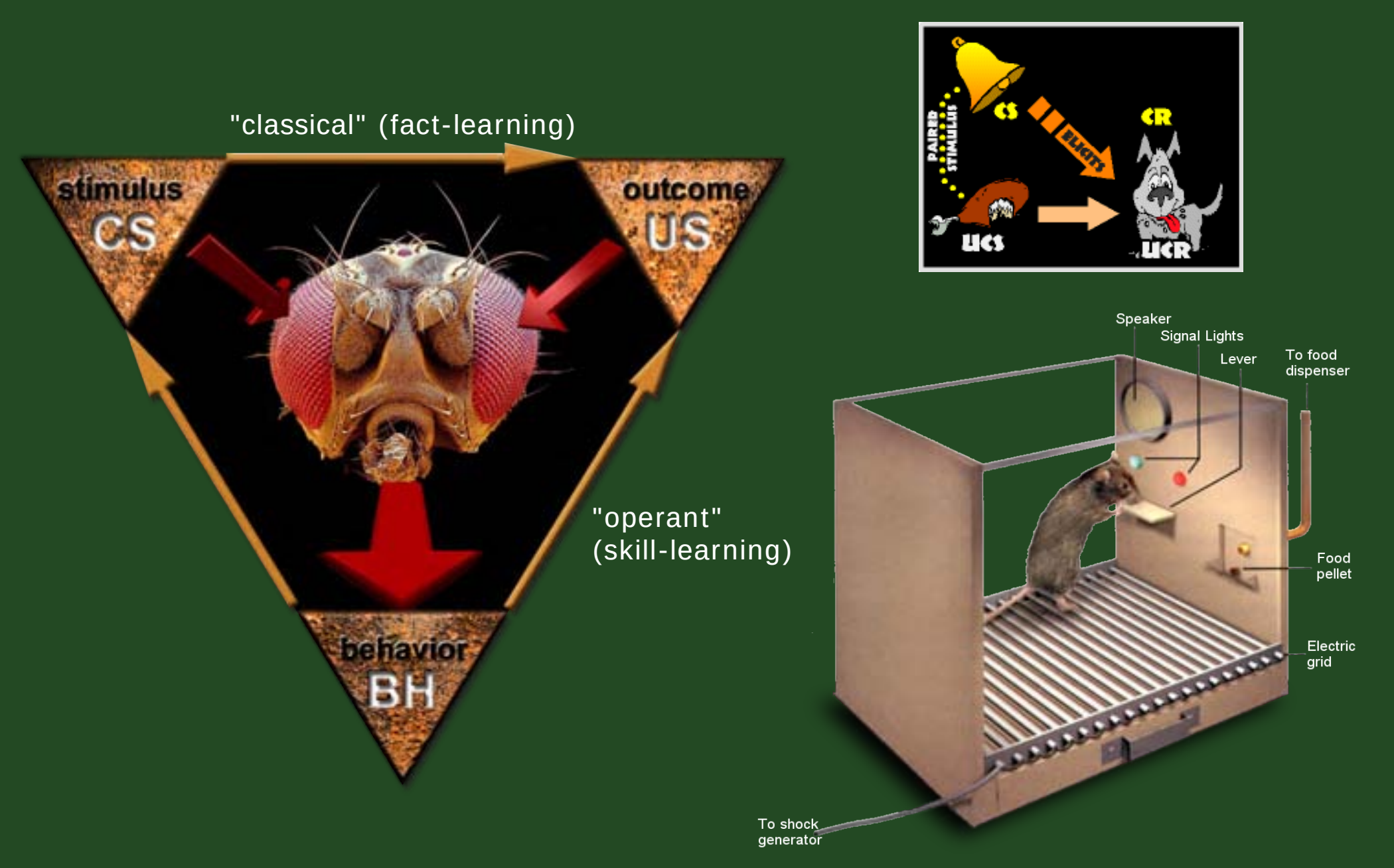
1. Introduction

Why do we have to practice so hard until we learn the fadeaway jump-shot or the right golf swing, when most other learning processes require much less time and effort? Neuroscientists have long known that the acquisition of skills and habits takes much longer than the acquisition of, say, facts and events. How can learning curves be so drastically different?

Experimentally, environmental (fact-)learning has been conceptualized as classical conditioning and behavioral (skill-)learning as operant conditioning. Skills or habits are commonly acquired through extended operant training. However, virtually all traditional operant preparations contain classical (fact-learning) components complicating he biological study of skill-learning. Tethered *Drosophila* suspended at a torque meter is currently the only intact paradigm where fact- and skill-learning can be separated. The fly is fixed in space with head and thorax, but is free to beat its wings, move its legs, etc., while its yaw torque is being recorded. The visual panorama around the fly is featureless, but can be illuminated in any color. During so-called switch-mode (sw-)learning, one half of the fly's yaw torque range is coupled with, say, green panorama illumination, while the other half is coupled with blue illumination. These yaw torque domains approximately correspond to left and right turns in free flight. A punishing heat-beam is associated with one of the colors/yaw torque domains. In this situation, the fly can learn that one of the colors is associated with heat (e.g., “blue-hot”: fact-learning) and that turning only in one direction will keep the heat off (e.g., “right-turning-safe”: skill-learning). Both components predict the heat equivalently. What is learned?

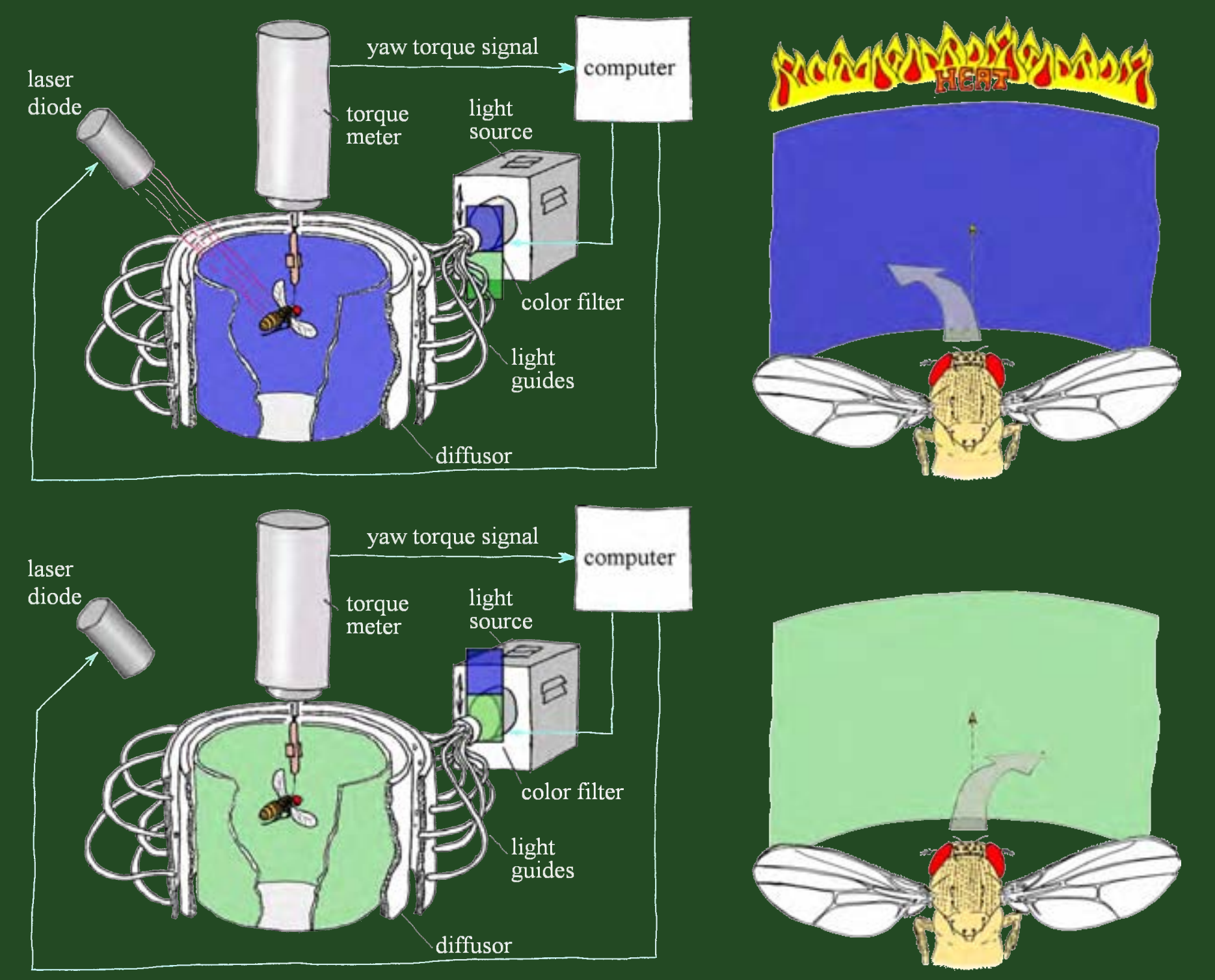
Similarly, most real-world learning situations consist of inseparable fact- and skill-learning components. The research presented here shows that fact-learning in flies supresses skill-learning such that skill-learning appears to be slower than fact-learning. If fact-learning is absent or compromised, fast skill-learning processes are engaged which are otherwise suppressed.

2. 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.

The *Drosophila* flight simulator



Composite Conditioning:
Yaw Torque + Color

3. Fact- and skill-learning interact hierarchically

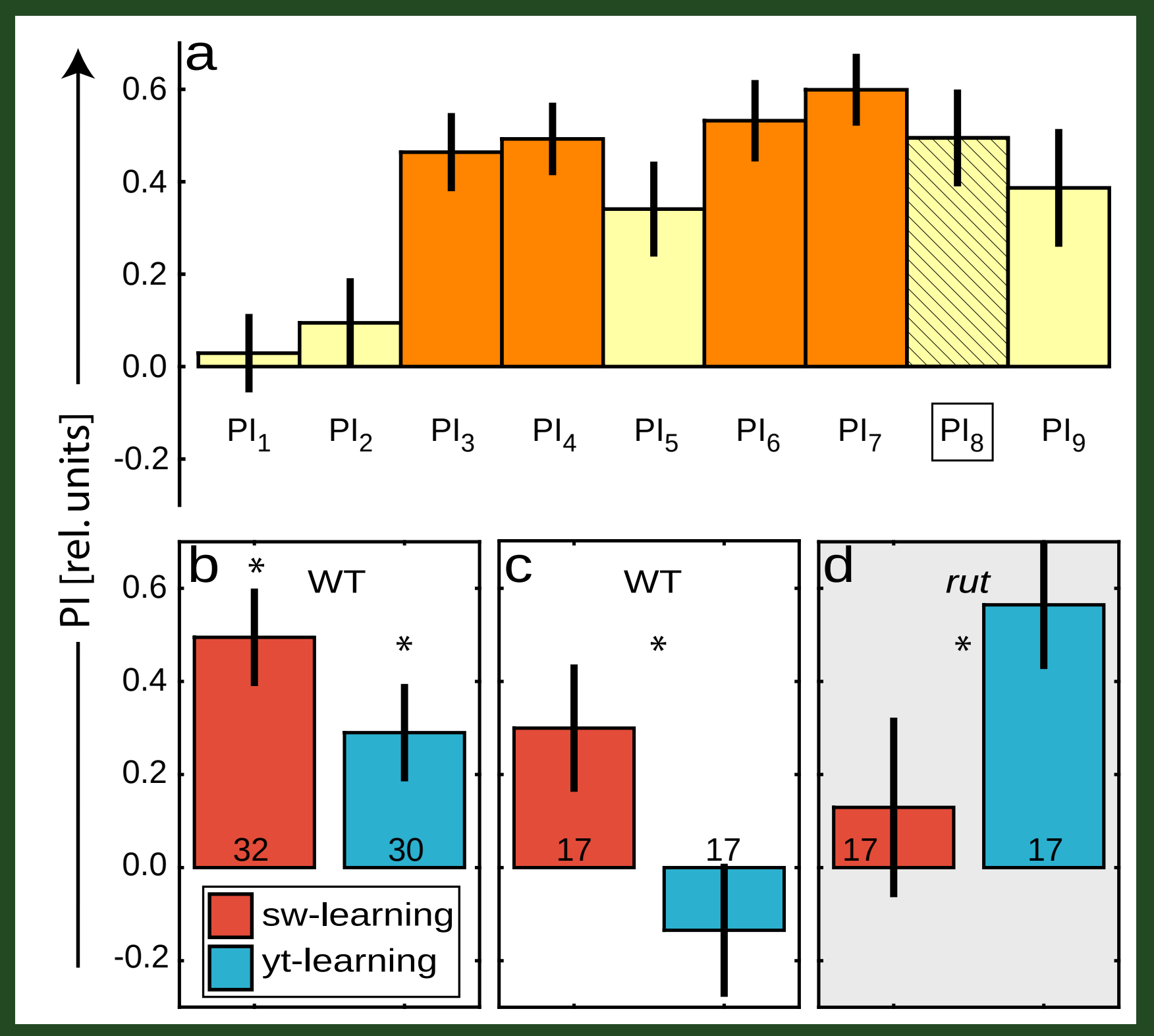


Fig. 1: *Operant and classical components show a hierarchical interaction during sw-mode learning.* **a,** Course of experiment. Bars show performance indices (PI) of successive 2-min intervals of pretest (yellow bars; PI_1, PI_2), training (orange bars; PI_3, PI_4, PI_6, PI_7) and memory test (yellow 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 sw- and yt-learning in WT flies. **c,** Reducing period duration (compared to the otherwise identical experiments in b) by 50% unmasks the difference between sw- and yt-learning. **d,** Reversed relationship of yt- compared to sw-learning in rut mutant flies (period duration as in b and c). Numbers at bars - number of animals. * - $p<0.05$.

4. Fact-learning suppresses skill-learning

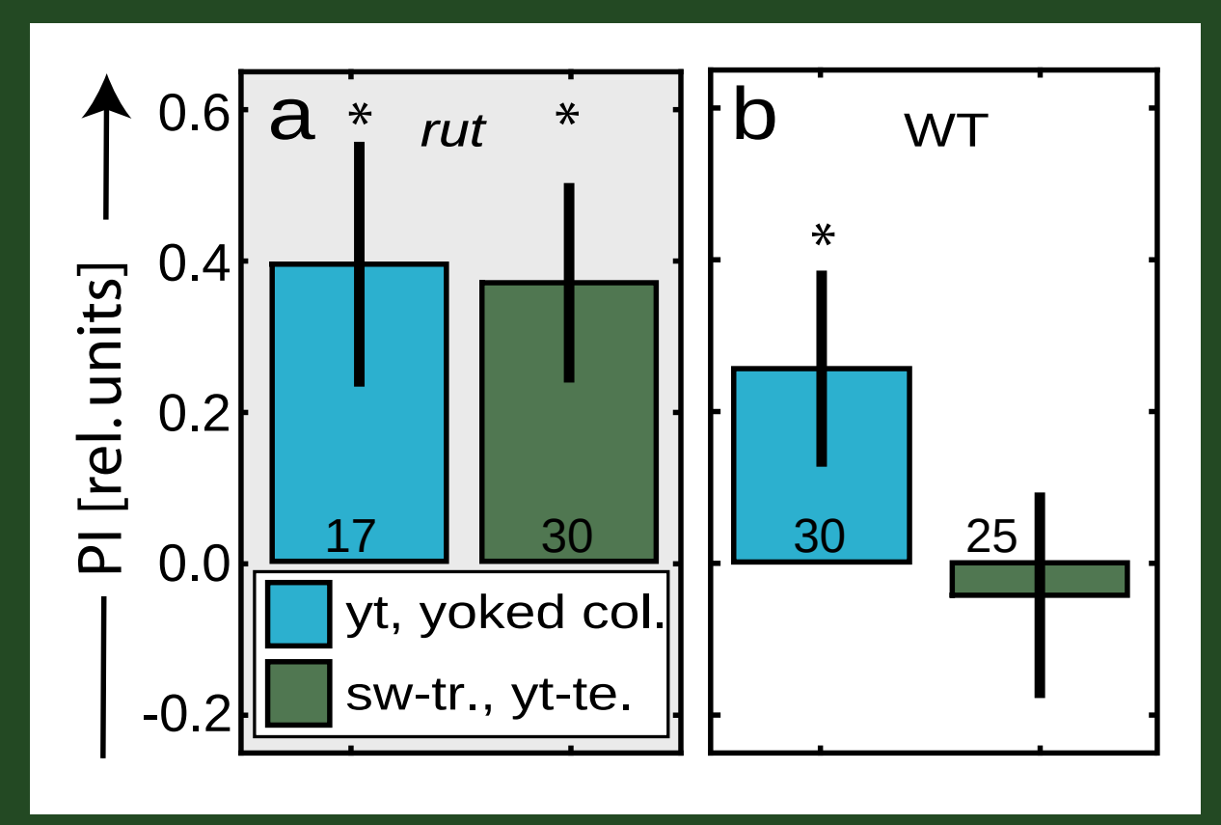


Fig. 2: *Classical components suppress acquisition of operant memory.* **a.** Performance indices (PI8) of rut mutant flies. Neither ex-afferent (i.e., yoked to the sw-learning flies in Fig. 3d; left), nor re-afferent (i.e., sw-training; right) color changes during training can disrupt operant learning in *rut* flies. The colors can not be learned by the mutant flies and thus do not suppress the operant component. Nevertheless, the colors have to be either ex-afferent (left) or absent (right) in the final test phase to reveal retrieval of the operant component. **b.** Performance indices (PI_8) of WT flies. If the color changes are not predictive of the heat, they do not disrupt operant learning (left). No operant learning takes place during sw-training, when the colors can be learned as predictors of the heat (right). Numbers at bars - number of animals. * - $p<0.05$.

5. Suppression of skill-learning allows generalization

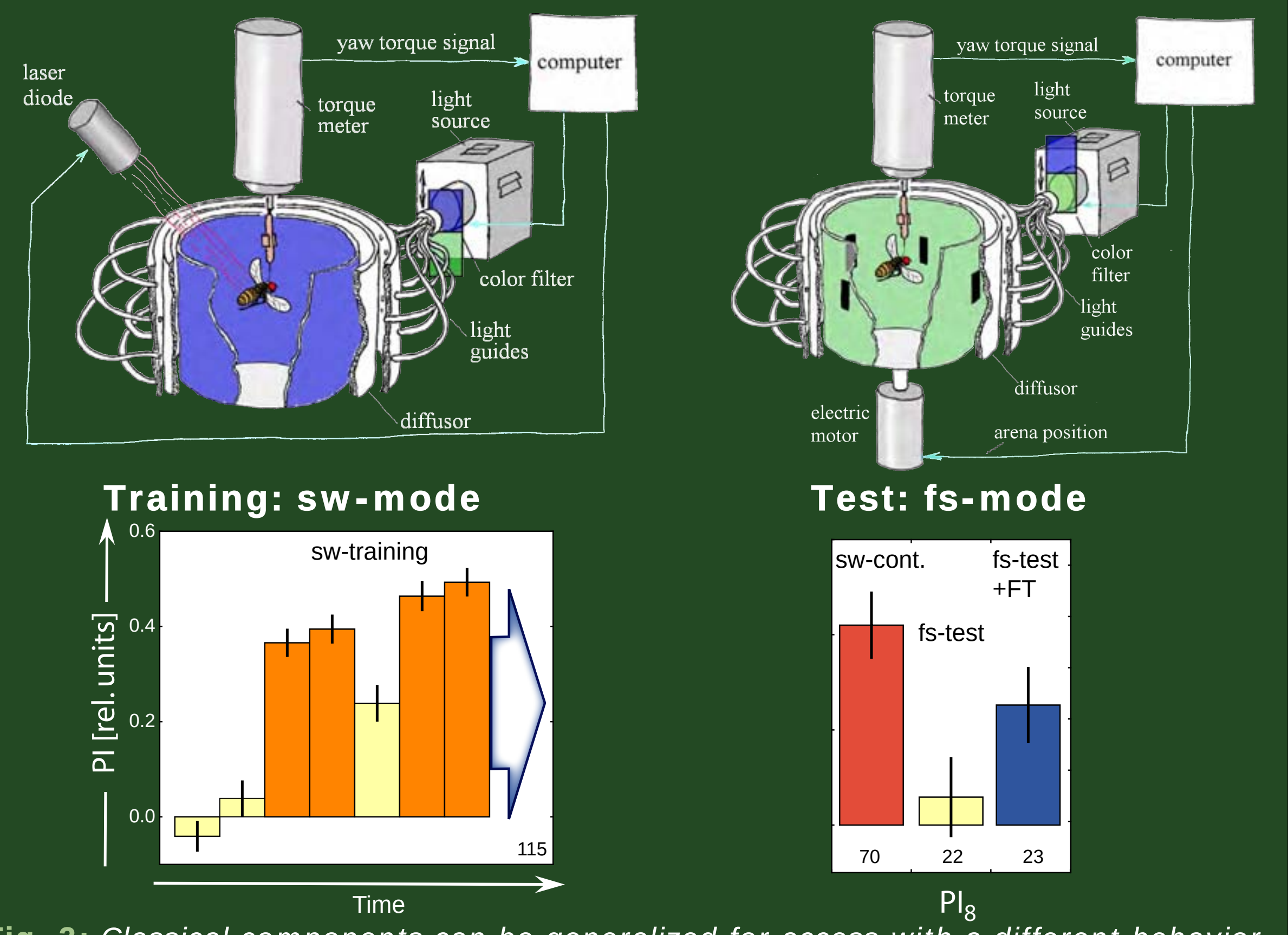
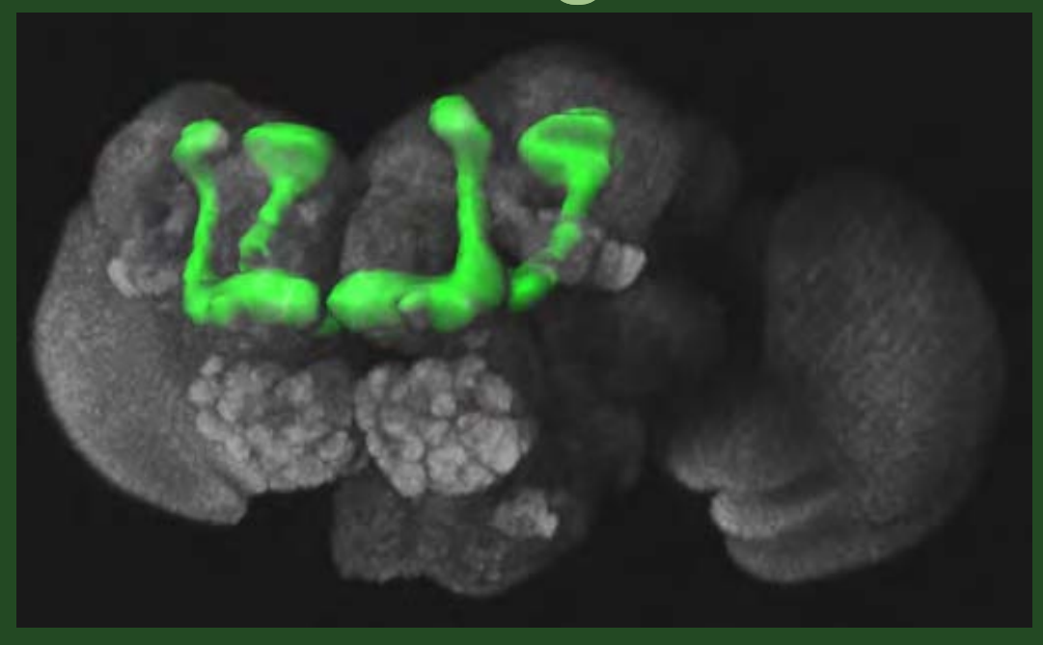
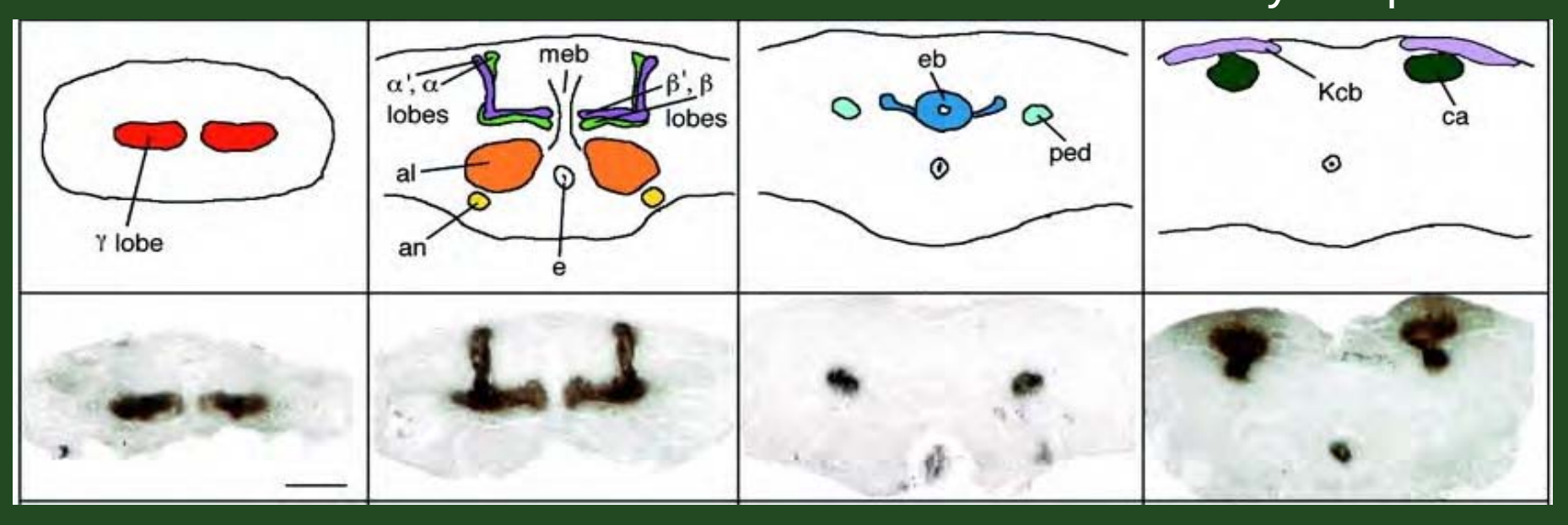


Fig. 3: *Classical components can be generalized for access with a different behavior.* **Left:** Training in sw-mode. **Right:** Test in flight-simulator mode. Only after a 60s familiarization (reminder) training do the flies show the conditioned color preference.

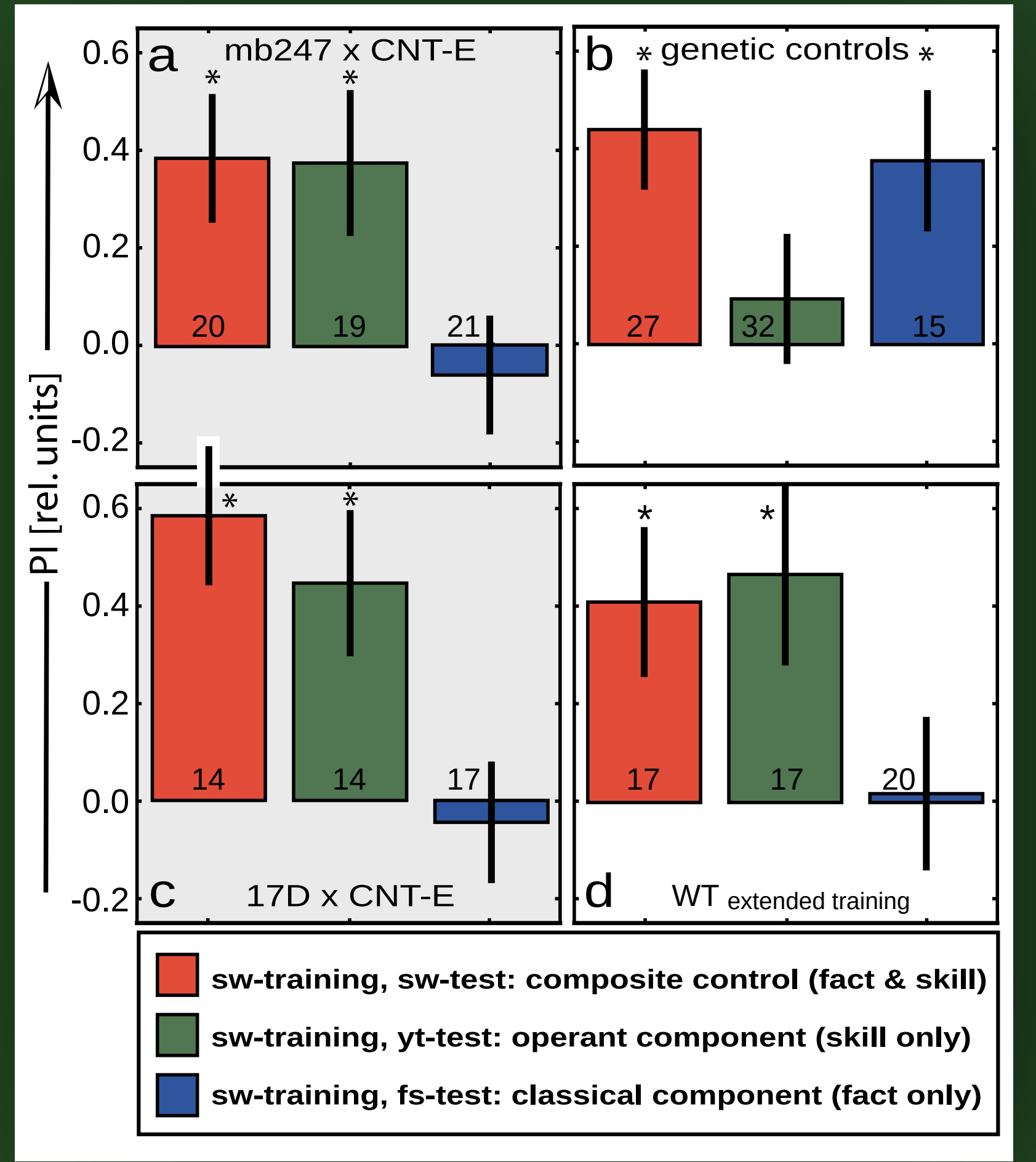
6. Blocking mushroom bodies



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



- 🟥 sw-training, sw-test: composite control (fact & skill)
- 🟩 sw-training, yt-test: operant component (skill only)
- 🟦 sw-training, fs-test: classical component (fact only)

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

Fixed flying *Drosophila melanogaster* at the torque meter provide one of the very few systems where the relationship of operant and classical predictors in associative learning can be studied with sufficient rigor. Experiments with wildtype and rutabaga (*rut*) mutant flies show that there is a hierarchical interaction between predictive stimuli (classical component) and behavior (operant component) which makes composite conditioning more effective than the operant and classical components alone. Wildtype flies suppress learning about the behavior when the stimuli are present, while *rut* mutants are impaired in learning about the stimuli, leaving behavior learning intact. Despite learning about the behavior, *rut* mutant flies retrieve the operant component only if the stimuli are either non-predictive or absent. These results indicate that despite the facilitating effect of operant behavior controlling the predictive stimulus, classical stimulus-learning dominates and suppresses learning about the operant behavior with which it was acquired. Experiments with transgenic flies suggest that his suppression is mediated by the mushroom-bodies and serves to ensure that the classical memories can be generalized for access by a variety of behaviors. Thus, in *Drosophila* composite conditioning, acquisition of a *rut*-dependent classical component is facilitated by a *rut*-independent operant component. Learning about this operant component is suppressed by the mushroom-bodies to render the classical component more flexible.

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