Operant and classical components interact hierarchically in *Drosophila* predictive learning

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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.

Introduction

Ever since operant and classical conditioning were distinguished in 1928, their relationship has been under intense debate. The discussion has varied between singular stimulus-response ("S-R") concepts, multi-process views and a variety of unified theories. The intensity and duration of the debate can in part be explained by the fact that most learning situations comprise operant and classical components to some extent (composite conditioning): one or more initially neutral stimuli (conditioned stimulus, CS), the animal's behavior (BH) and the reinforcer (unconditioned stimulus, US). A natural example is a frog attempting to prey on a wasp. Extending the tongue (BH) towards the striped wasp (CS) leads to the painful sting (US). The frog may later remember the pain associated both with the striped animal (classical conditioning, CS-US) and with the extension of the tongue (operant conditioning, BH-US) to predict the wasp's reaction at future encounters. "What is learned?" is the old but still unanswered question.

Tethered Drosophila suspended at a torque meter can be used to mimic this situation and to finally answer the question. 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.

"Learning by doing" is most effective

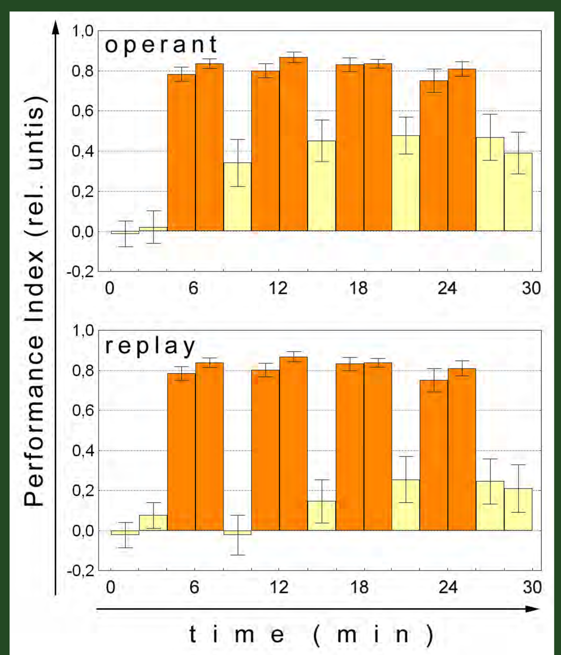
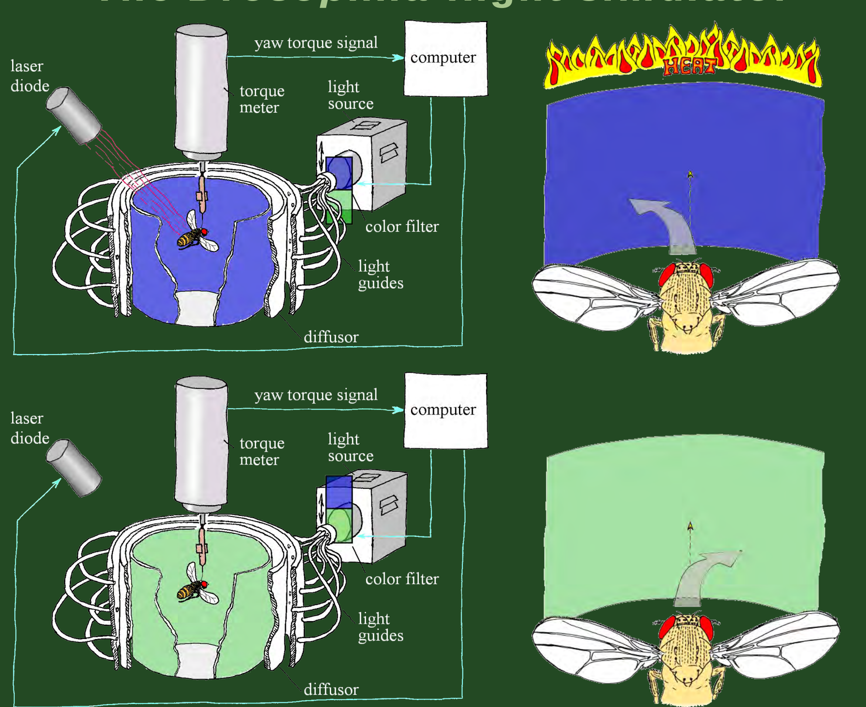


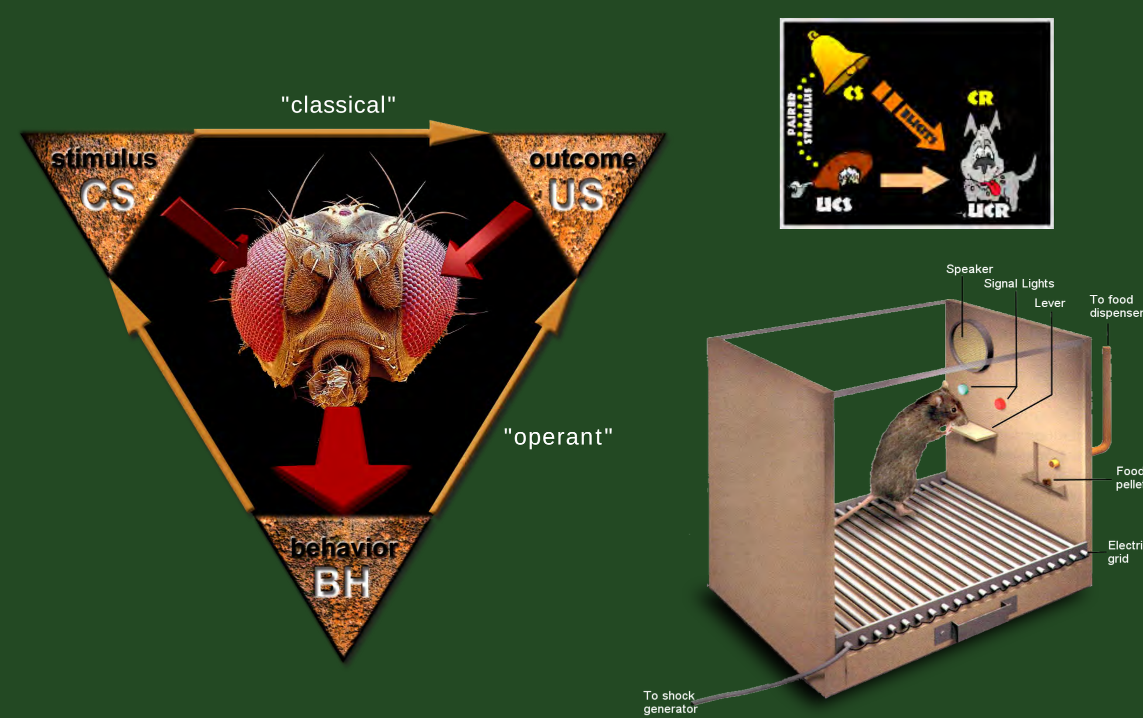
Fig. 1: Comparison of mean operant and classical pattern learning performance indices. above - Operant 'master' flies. N=30. below - Classical 'replay' flies. N=30.
Note that the same sequence of CS-US perceptions sufficient for inducing an early, asymptotic learning effect if produced operantly (above), only induces a small learning score if it is presented classically (below). Thus, operant control over a CS-US relationship is more effective than experiencing the exact same relationship independently of the fly's behavior.
Orange bars - training, yellow bars - test. Error bars (as in all figures) are S.E.M.s.

The *Drosophila* flight simulator



Composite Conditioning: Yaw Torque + Color

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.

Transgenic flies

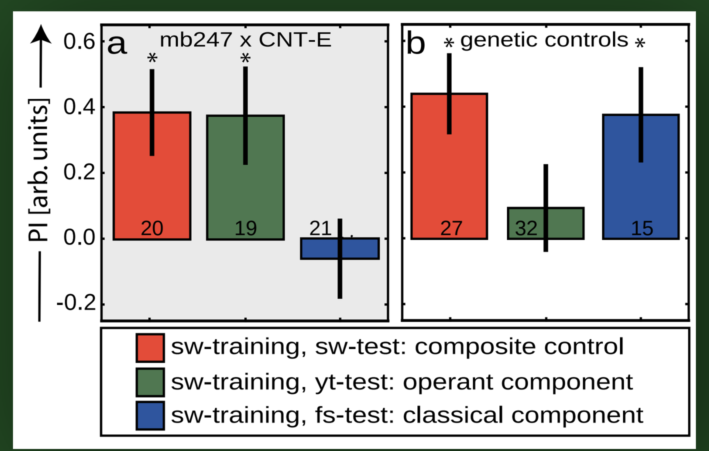
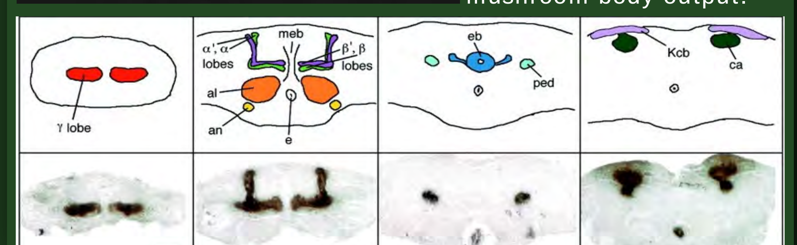


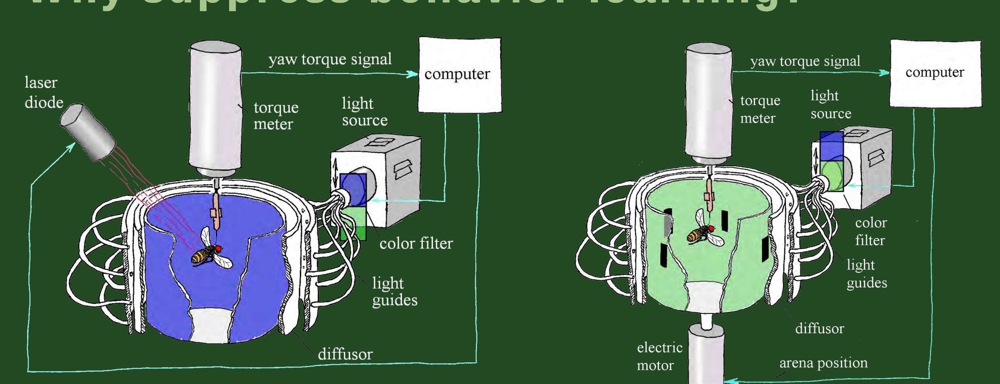
Fig. 4: *Without suppressing the operant component, there is no generalization of the classical component.* **a,** Flies expressing the bacterial tetanus toxin light chain in most mushroom-body intrinsic Kenyon cells perform well in sw-learning (red), but do not suppress the operant component in sw-learning (green). Without the suppression of the operant component, these transgenic flies are unable transfer the classical component to a different behavior, even with 60s of familiarization training (blue). b, Genetic control flies do suppress the operant component and thus can transfer the classical component. Numbers at bars - number of animals. * - p<0.05.

Blocking mushroom bodies

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



Why suppress behavior learning?



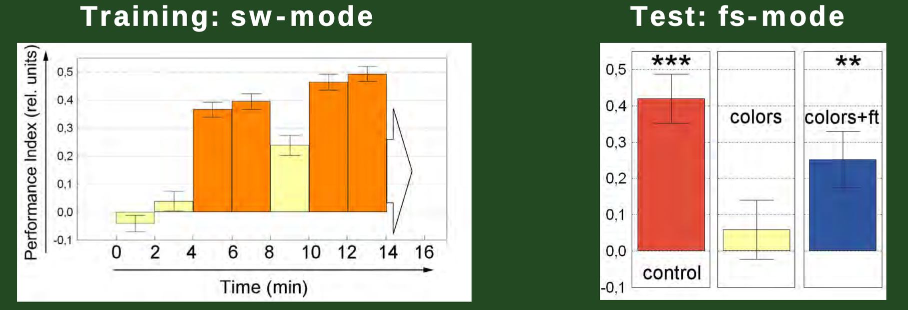


Fig. 4: *Classical components can be generalized for access with a different behavior.* **Left:** Training in sw-mode. **Right:** Test in flight-simulator mode. Only after a 60s familiarization (reminder) training do the flies show the conditioned color preference.

Classical stimuli dominant

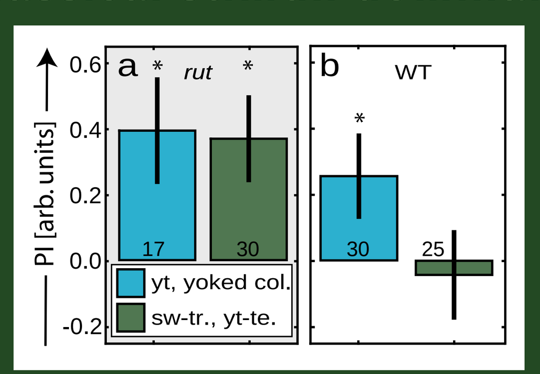


Fig. 3: *Classical components suppress acquisition of operant memory.* **a.** Performance indices (PI8) of rut mutant flies. Neither ex-afferent (i.e., yoked to the sw-learning flies in Fig. 3d; left), nor re-afferent (i.e., sw-training; right) color changes during training can disrupt operant learning 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.

Hierarchical interactions

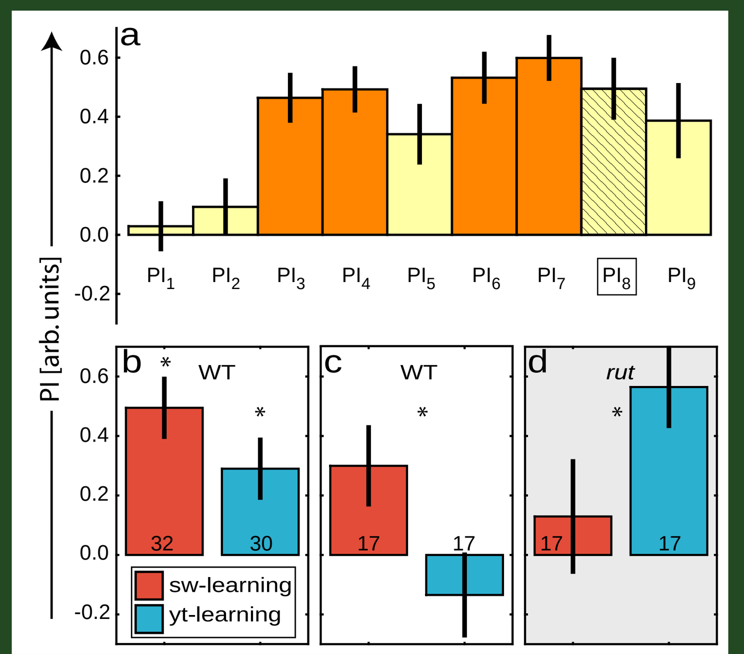


Fig. 2: *Operant and classical components show a hierarchical interaction during sw-mode learning.* **a,** Course of experiment. Bars show performance indices (PI) of successive 2-min intervals of pretest (yellow bars; PI_1, PI_2), training (orange bars; PI_3, PI_4, PI_6, PI_7) and memory test (yellow 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.

Presented at the annual meeting of the Society for Neuroscience in Atlanta, Ga. October, 2006