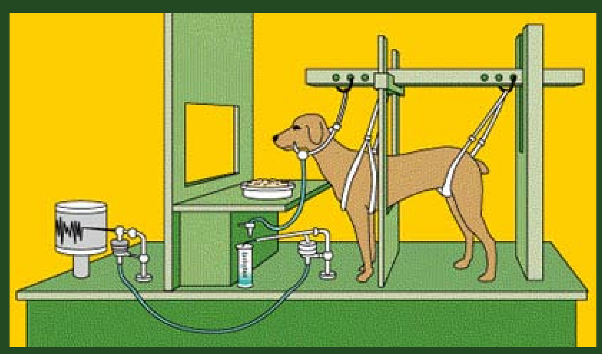
Double Dissociation of Protein Kinase C and Adenylyl Cyclase Manipulations on Operant and Classical Learning in *Drosophila*

Björn Brembs
FU Berlin, Institut für Biologie - Neurobiologie,
Königin-Luise-Strasse 28/30, 14195 Berlin, Germany
bjoern@brembs.net, http://brembs.net

1. Classical or Pavlovian learning



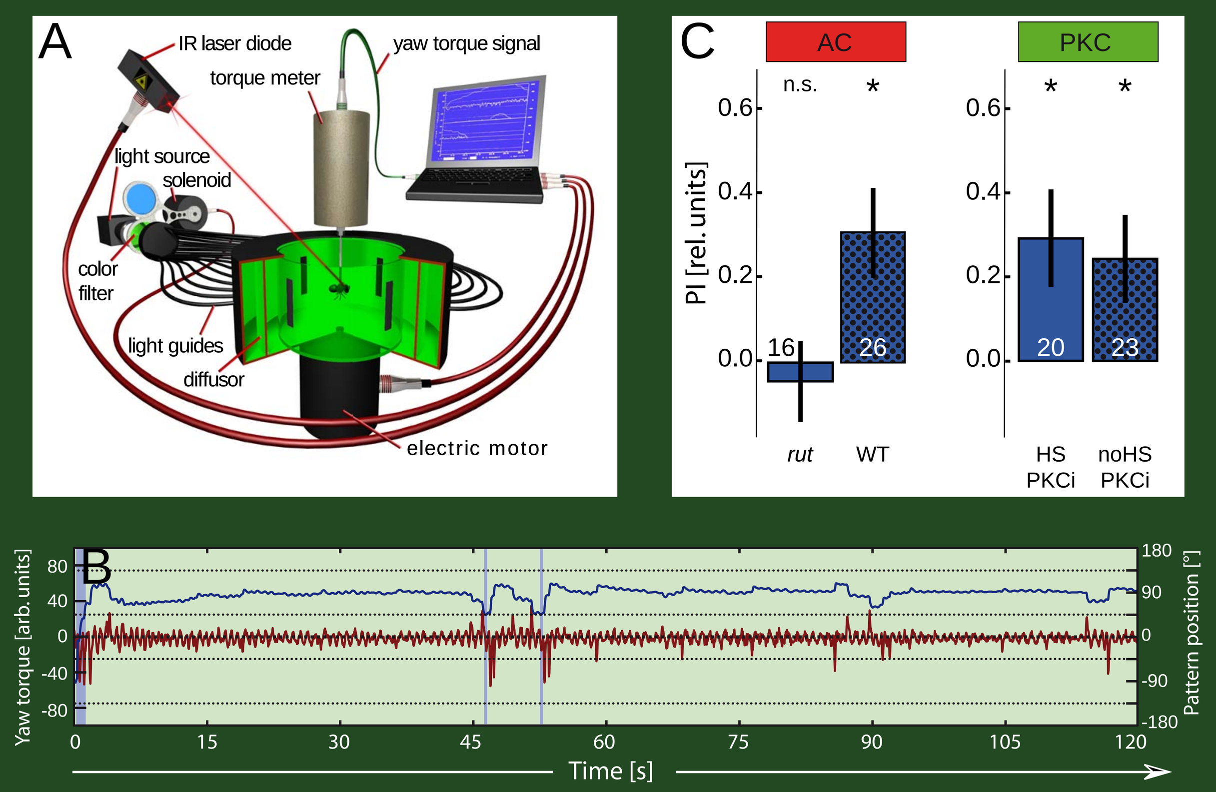
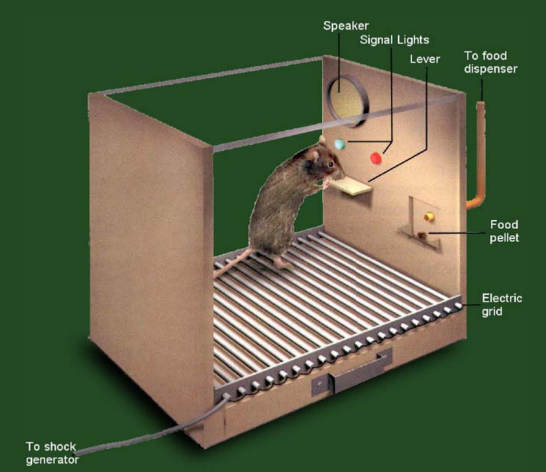


Fig. 1: ***Manipulation of AC, but not of PKC disrupts learning of a classical predictor.*** A – Experimental setup. The fly controls the angular position of a drum with four identical vertical bars in a flight simulator-like situation. The coloration of the arena is switched between bars, such that flying towards one pair of opposing bars leads to green coloration and towards the other pair to blue coloration. During training, heat is made contingent on one color, irrespective of the turning maneuver which changed flight direction. B – Sample data from a wildtype fly during the first test period after the final training with heat on blue coloration. The fly uses both left and right turning maneuvers (red trace) to change flight direction (blue trace) and hence coloration of the environment (background color of the graph). The fly shows a clear preference for green with only brief excursions into flight directions which lead to blue color, even though the heat is switched off. C – Pooled performance indices (PI) from the first test period after training. In this and all subsequent bar graphs: Displayed are means, error bars are s.e.m. Numbers at bars – number of animals; *rut* – *rut*-mutant flies affecting AC; WT – wildtype; HS PKCi – heat shock induced expression of the specific PKC inhibitor; noHS PKCi – PKCi expression not induced; n.s. – not significant, * – p<0.05.

2. Operant or instrumental learning



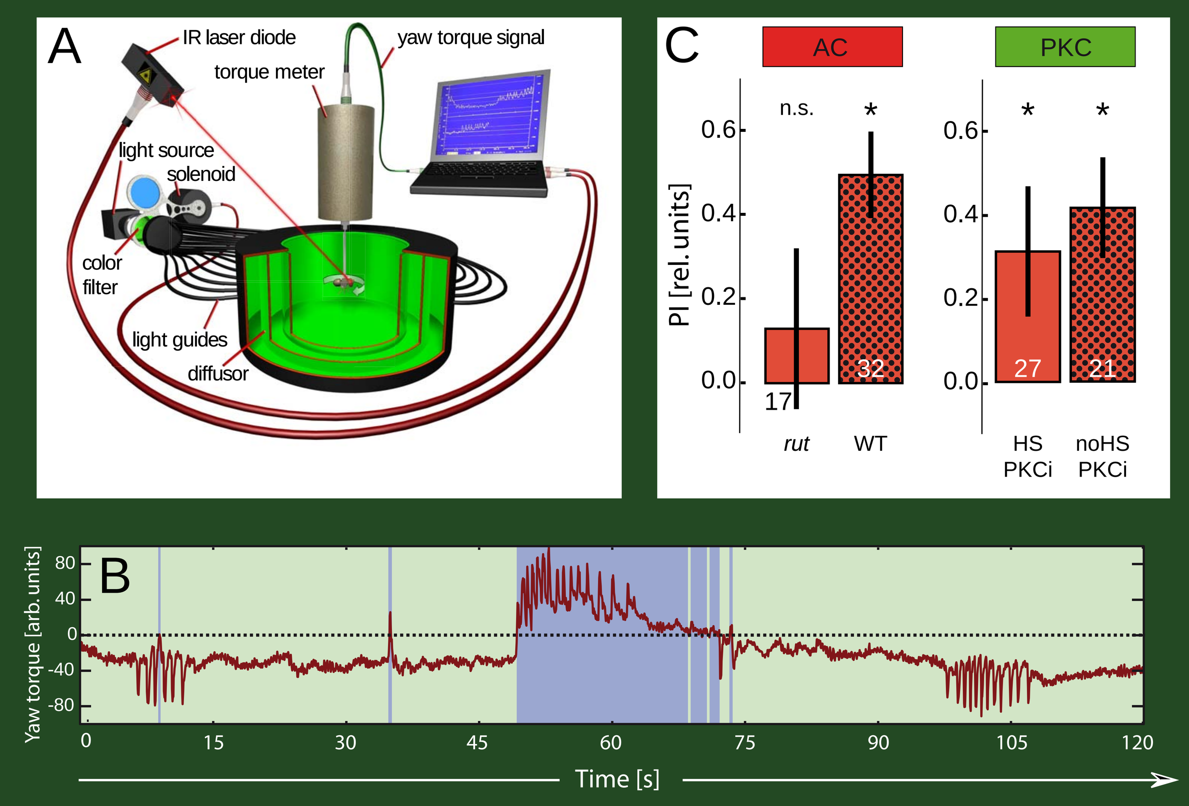
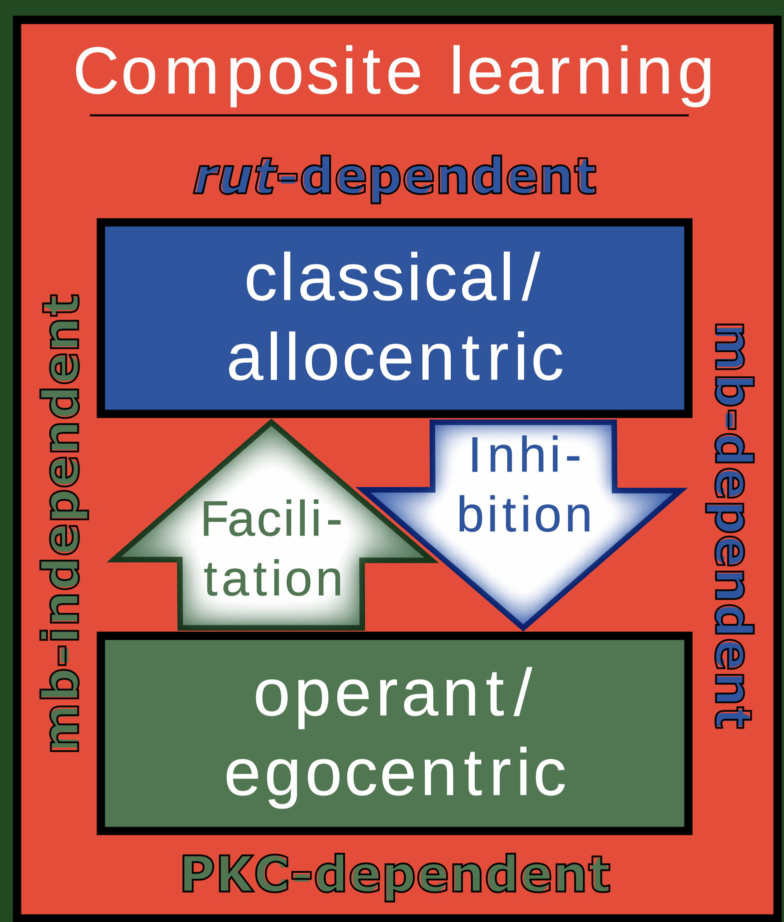


Fig. 2: ***Operant learning requires the same gene as learning a classical predictor.*** A – Experimental design. Throughout the experiment, one yaw torque domain is coupled to one color and the other to the other color (e.g., right turning causes green illumination and left turning blue illumination of the environment). During training, heat is made contingent on one of the two yaw torque/color combinations. B – Sample data from a wildtype fly during the first test period after the final training with heat on positive (right-turning) yaw torque (red trace) and blue illumination (background coloration). The fly shows the yaw torque domain/color preference and only briefly ventures into the previously punished situation, even though the heat is switched off. C – Pooled performance indices (PI) from the first test period after training.

6. Conclusion



Composite learning consists of two components with reciprocal, hierarchical interactions. The AC-dependent classical or allocentric learning system inhibits the PKC-dependent operant or egocentric learning system via the mushroom-bodies. Operant behavior controlling predictive stimuli facilitates learning about these stimuli by the classical system via unknown, non-mushroom-body pathways. These interactions lead to efficient learning, generalization and prevent premature habit-formation.

5. Mushroom-bodies prevent premature habit formation

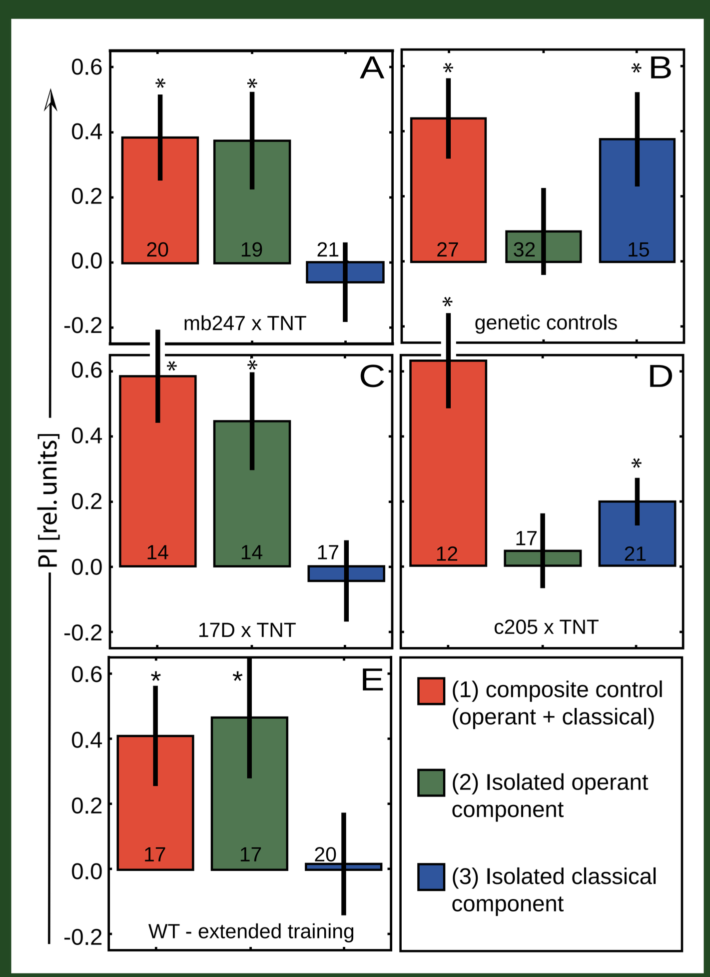


Fig. 5: ***The mushroom-body α and β lobes but not the γ lobes are necessary for inhibition of the operant component and generalization of classical memory.*** **A**. Flies with blocked MB output perform well in composite learning (red), but do not inhibit the operant component during composite training (green). Without inhibition of operant system, these transgenic flies are unable to generalize the isolated classical component to a novel behavior (blue). **B**. The genetic control flies (the two heterozygote strains did not differ and were pooled) reproduce the wild-type results: significant composite learning, inhibition of the operant system and successful generalization of the isolated classical component. **C**. Flies with blocked output only from the α and β lobes of the MB mimic the flies expressing tetanus toxin in all MB lobes. They perform well in composite learning, do not inhibit the operant system and do not generalize. **D**. Specificity of our mushroom-body effects is provided by expressing TNT in the fan-shaped body. These flies behave as wildtype and control heterozygote flies with significant composite learning and inhibition of the operant system, which in turn allows for a successful generalization of the classical component to a novel behavior. **E**. Extended training in wildtype flies constitutes a phenocopy of the transgenic animals (A). The longer training duration does not lead to an overtraining decrement. Testing for the operant component shows a release from the inhibition of operant learning. Without inhibition of the operant system, the flies are unable to generalize.

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

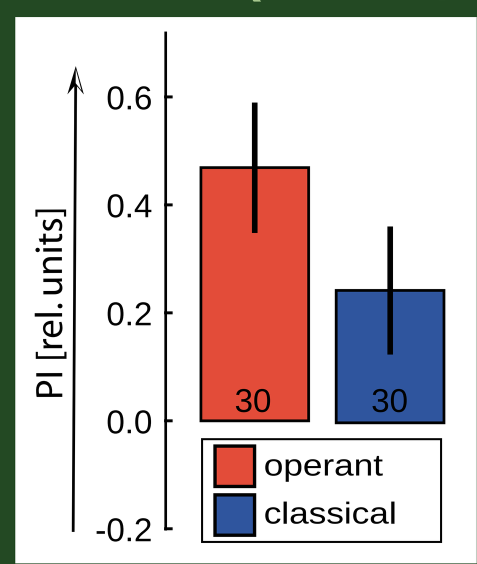


Fig. 3: ***Comparison of operant and classical pattern learning in flies.*** The same sequence of sensory input sufficient for inducing a substantial learning effect if controlled operantly (left), only induces a small learning score if it is perceived passively ("classical", right). Thus, active learning ("by doing") is more effective than passive learning. Left/red - Operant flies. N=30. Right/blue - Classical flies. N=30.

4. Purely operant learning is different

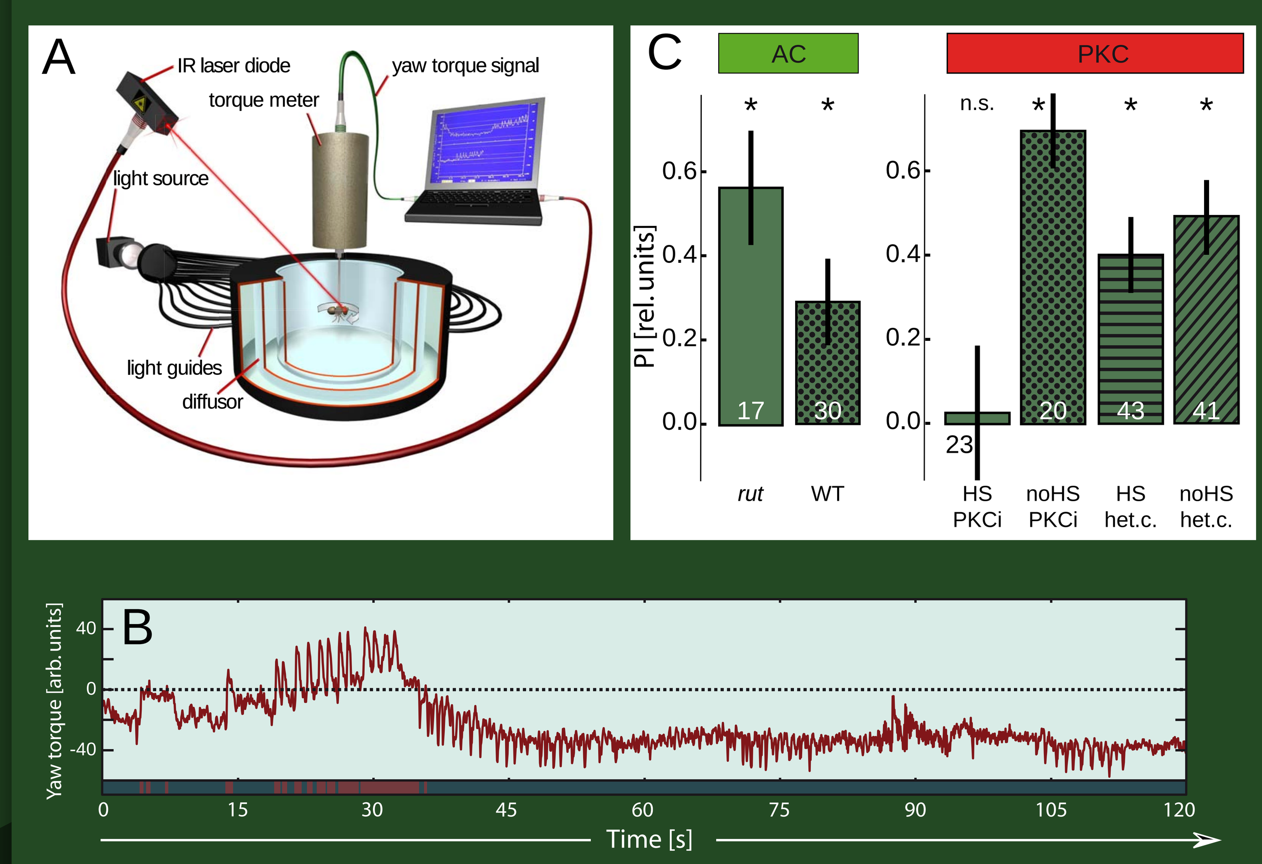
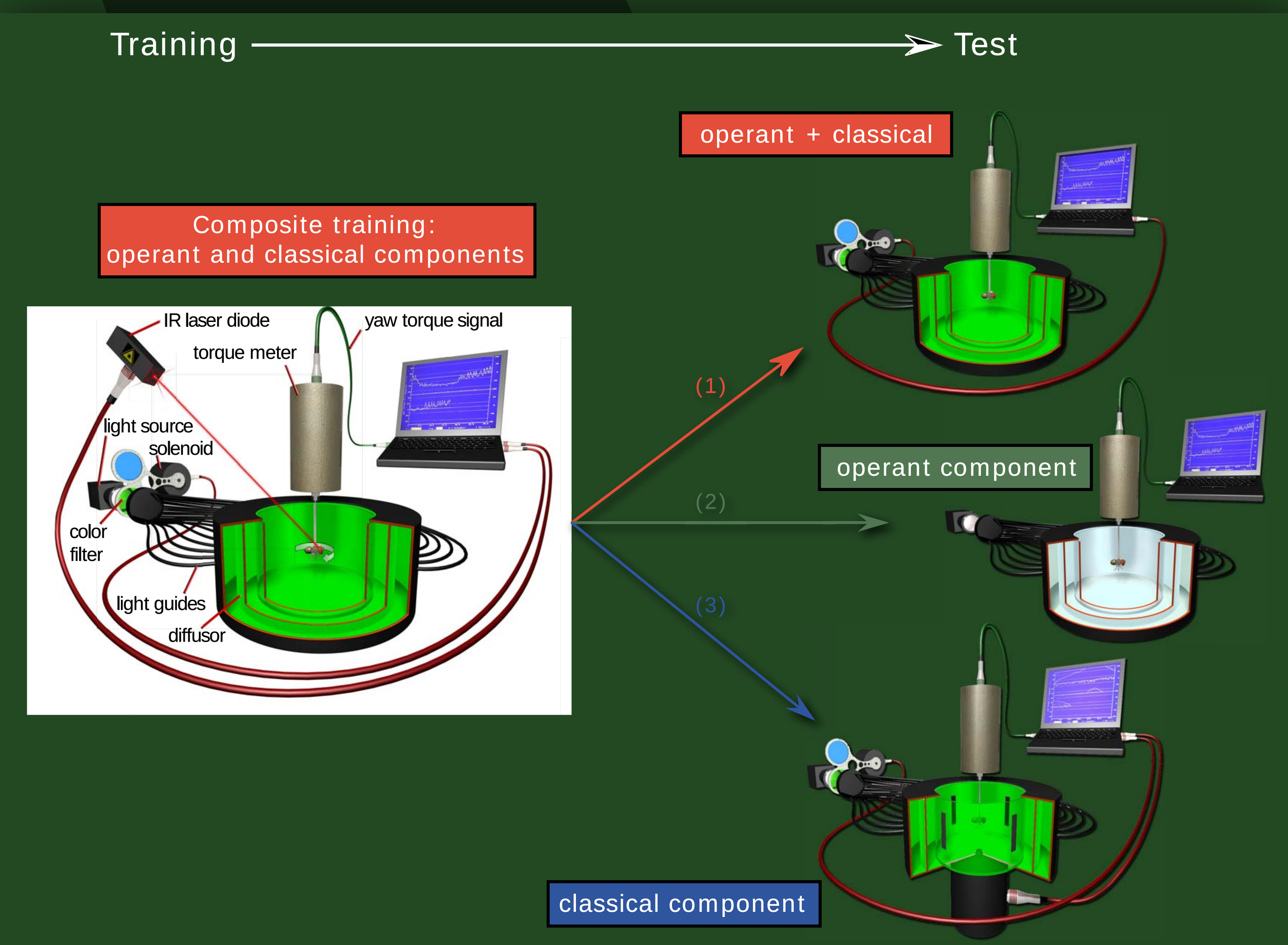


Fig. 4: ***Manipulation of PKC but not of AC disrupts learning of a purely operant predictor.*** A – Experimental setup. There are no visual cues for the fly. During training, heat is made contingent on either left- or right-turning yaw torque. B – Sample data from a wildtype fly during the first test period after the final training with heat on positive (right-turning) yaw torque. The fly only briefly generates right-turning yaw torque during the test phase (unsaturated red/blue bar underneath dark red yaw torque trace), even though the heat is switched off. C – Pooled performance indices (PI) from the first test period after training. HS het.c. – Heat shock-treated heterozygous parental controls strain; noHS het.c. – Heterozygous parental control strain without heat shock.



Presented at the Neurofly 2008 conference in Würzburg, Germany, September 9, 2008.