How to improve motor learning in *Drosophila*

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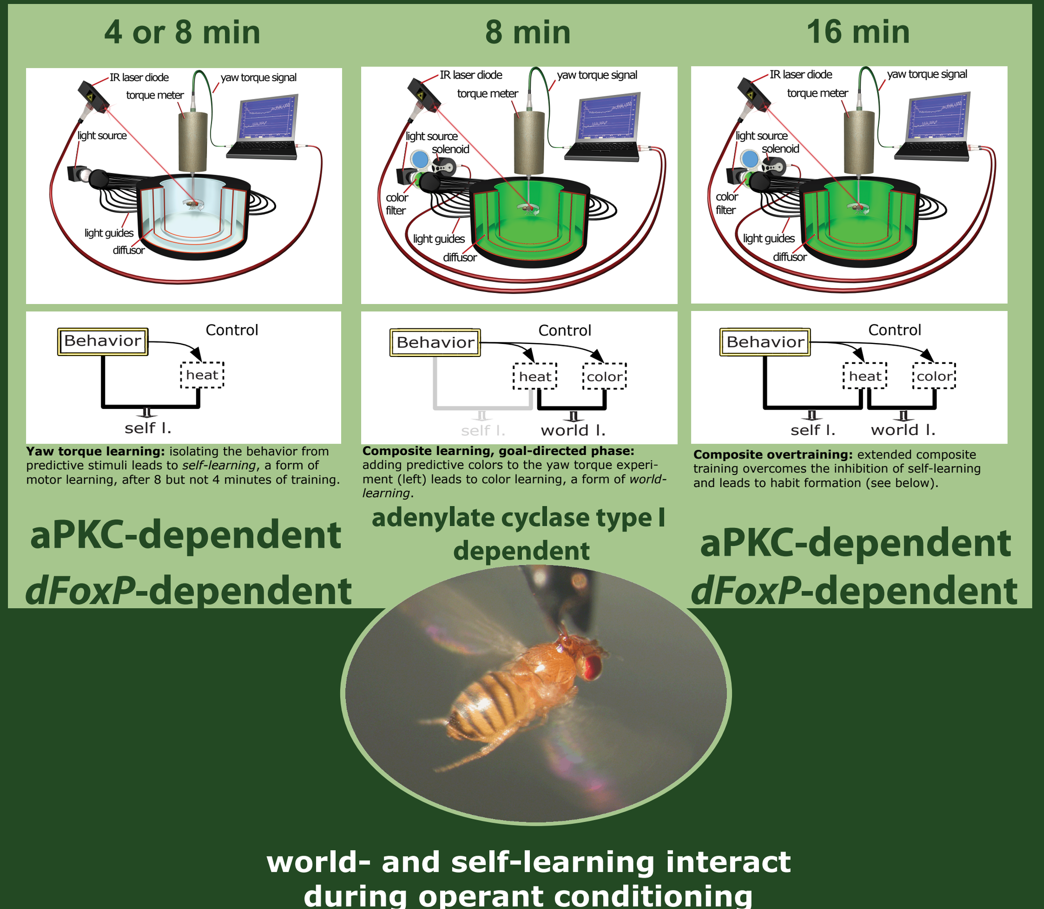
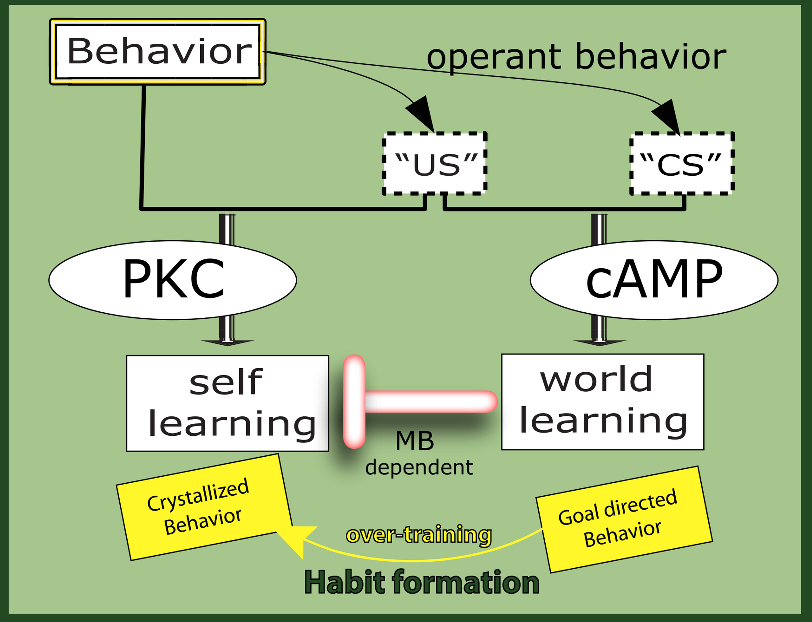
Introduction

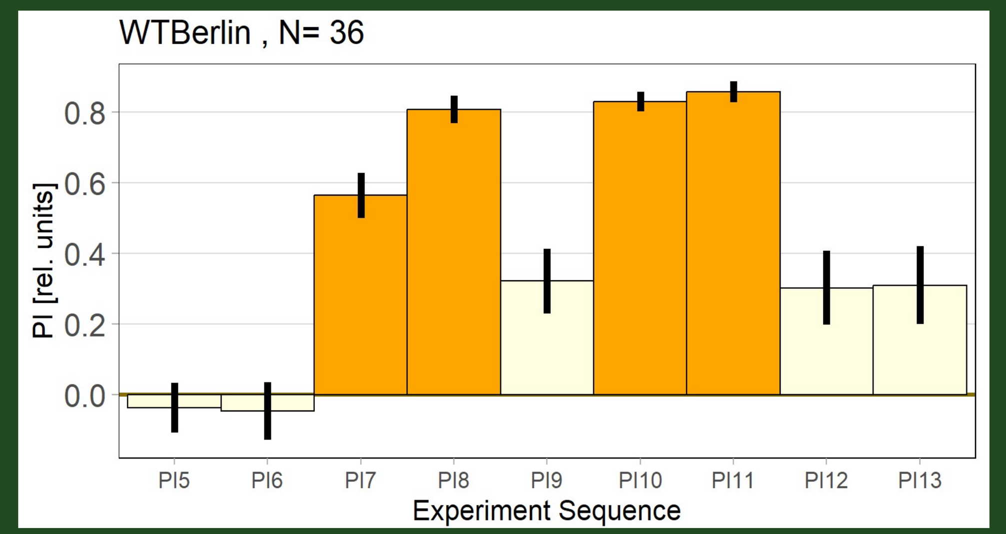


world- and self-learning interact during operant conditioning



Colomb J, Brembs B. The biology of psychology: "Simple" conditioning? Communicative & integrative biology. 2010;3(2):142-5

Experimental sequence of yaw torque/composite learning



Color coded sequence of the single periods throughout a learning experiment in the flight simulator.
Plotted are PIs indicating torque preference in each single period of the experimental sequence. The first two periods represent naive preference without any external feedback. Periods 3, 4, 6 and 7, indicated by the orange color, represent the training periods where the fly's behavior controls the punishing laser, e.g., right turning attempts leading to punishing heat. Period 5 is an intermediate test (heat off) between training sessions. During the final two periods the heat is again permanently switched off and torque preference tested without external feedback. For statistical evaluation in the following figures the first test after the last training period is used. Positive PIs indicate preference of the unpunished torque, negative the opposite and PIs close to zero indicate no preference.

Pavlovian learning mutants show improved motor learning

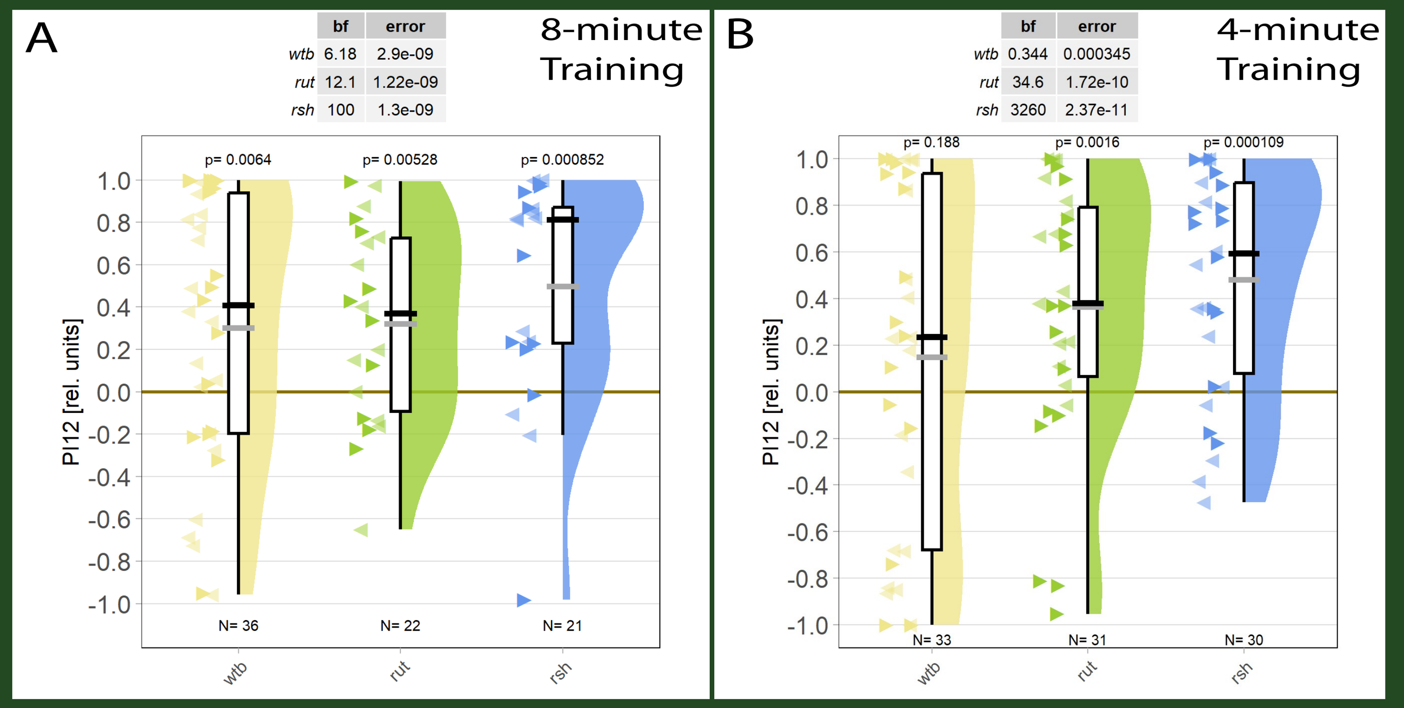


Figure 1: Both *rutagaba* and *radish* show motor learning under conditions not sufficient for learning in wild type flies.
A. After 8 minutes of yaw torque training, mutant and wild type control flies show normal motor learning. In the first training after the last test period, wild type Berlin flies (yellow, left) show performance indices that differ from zero both using frequntist and bayesian statistics. Flies mutant for the *rutabaga* gene (green, middle) show very similar performance indices as wild type flies, indicating they also learned the task well. Also *radish* mutants show high learning scores, a low p-value and a high Bayes-Factor, indicating they also learned the task.
B. After 4 minutes of yaw torque training, only the learning mutants show high performance indices. As reported previously, 4 minutes of training are insufficient for wild type Berlin flies to show high learning scores in the first test period after training. However, for both Pavlovian learning mutants, this reduced training is sufficient for high performance indices, low p-vlaues and high Bayes-Factors.
Triangles indicate individual PIs and punished torque direction. Black bars denote medians and grey bars means. Boxes indicate quartiles and whiskers non-oulier range.

PKG variants show improved motor learning

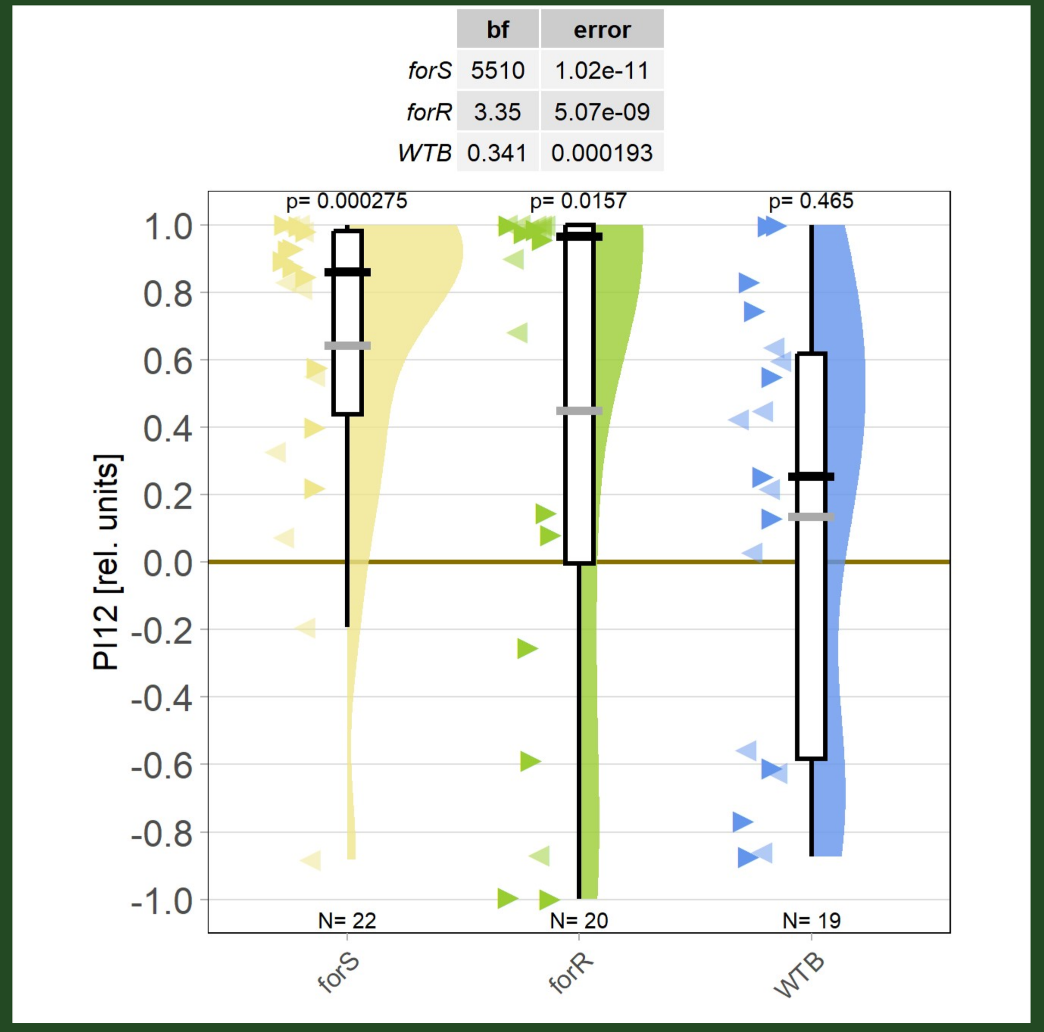


Figure 2: Both variants of the *foraging* gene show motor learning after 4-minutes training.
Plotted are performance indices for the first period after training (PI12). When both alleles of the for gene (rover: forR, sitter: forS) are isolated in separate populations, flies show a torque preference already after short training, insufficient for wild type flies. Significant motor learning is indicated by high PIs, low p-values and high Bayes Factors. Triangles indicate individual PIs and punished torque direction. Black bars denote medians and grey bars means. Boxes indicate quartiles and whiskers non-oulier range.

PKG is required for motor learning

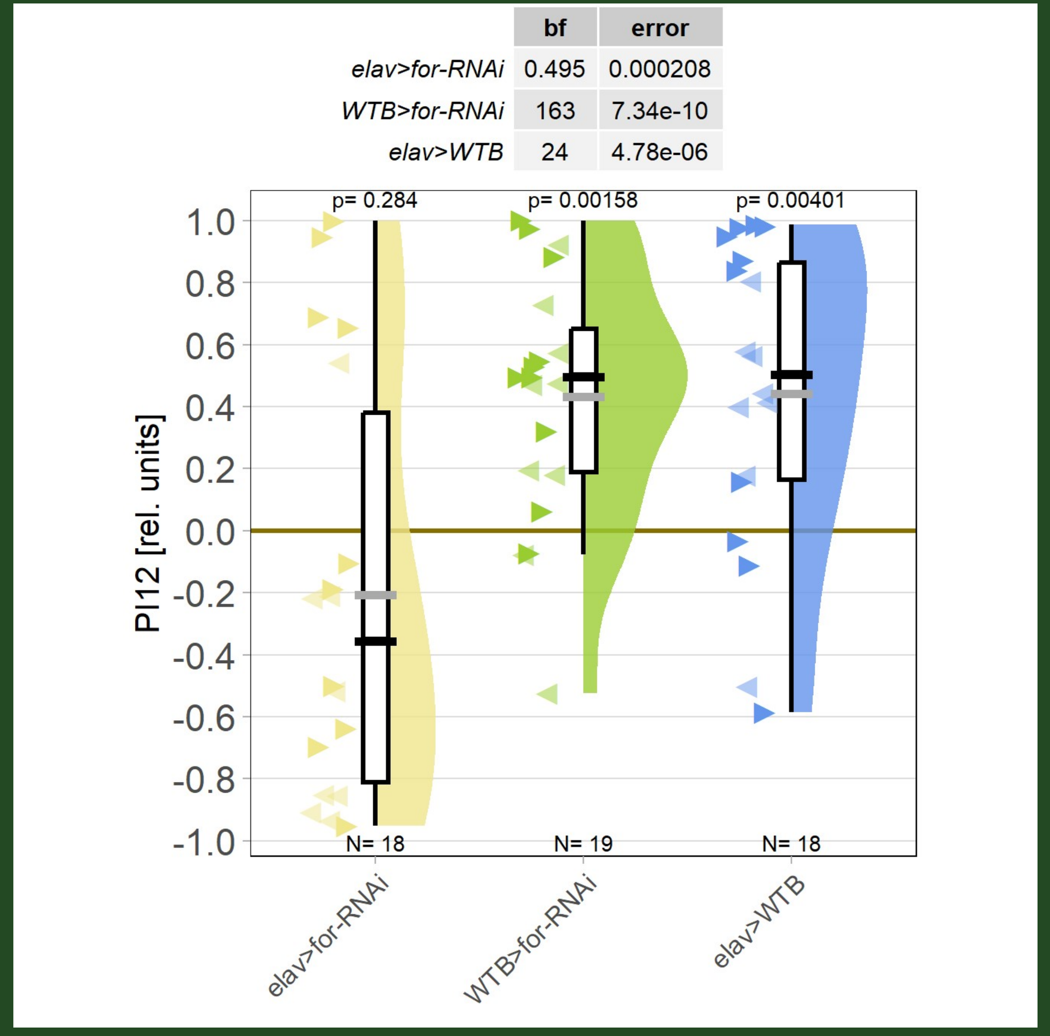


Figure 3: Downregulation of the *foraging* gene impairs motorlearning.
Plotted are performance indices for the first period after training (PI12). When PKG levels are downregulated after panneuronal expression of *foraging-RNAi* flies do not show motor learning after 8-minutes training while genetic control flies show torque preference towards the non punished domain. Significant motor learning is indicated by high PIs, low p-values and high Bayes Factors.
Triangles indicate individual PIs and punished torque direction. Black bars denote medians and grey bars means. Boxes indicate quartiles and whiskers non-oulier range.

Overexpressing aPKC improves motor learning

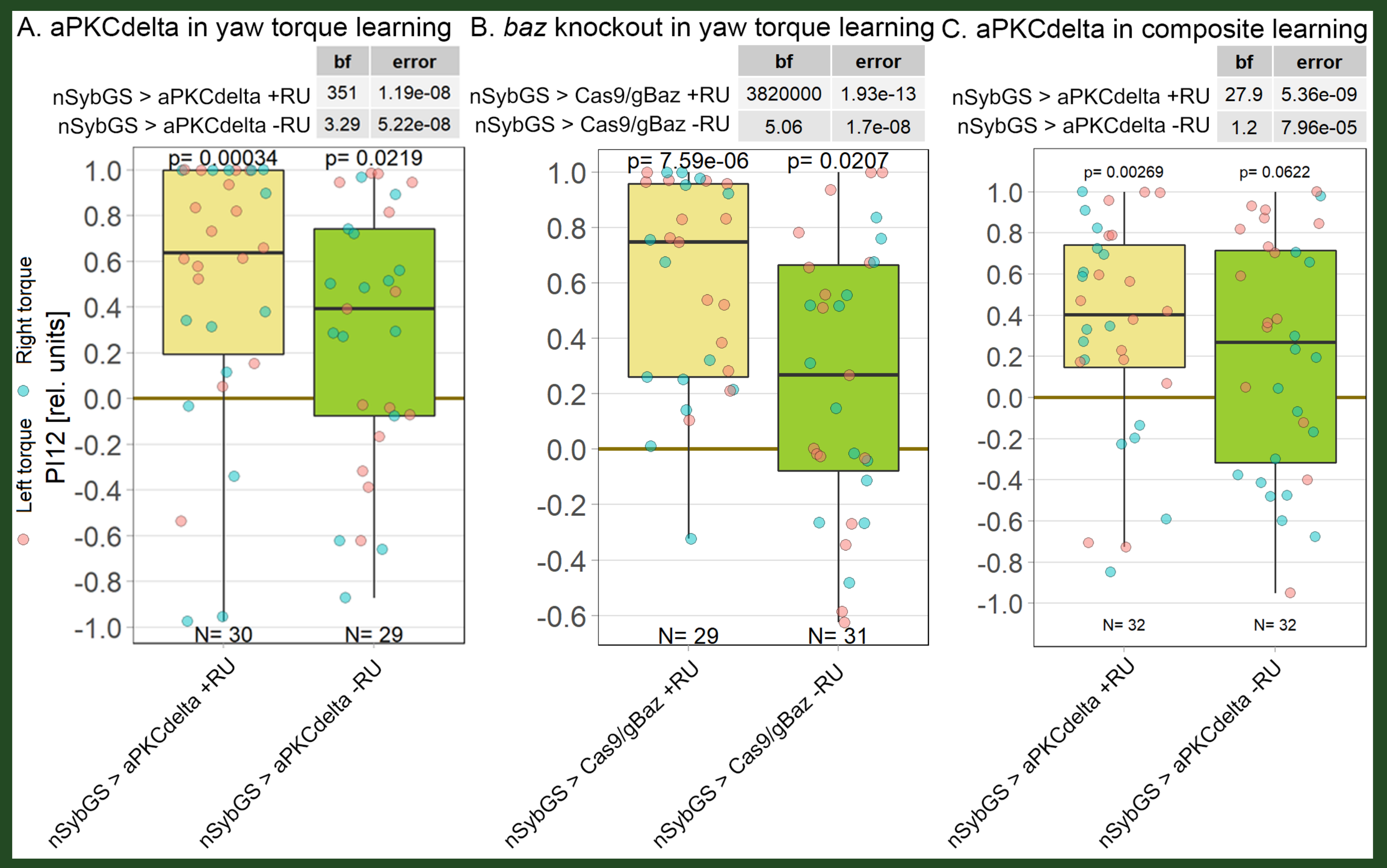


Figure 4. Overexpressing the constitutively active aPKCdelta or *bazooka* knock-out both improve motor learning.
A. Short yaw torque training. Expressing the constitutively active form of aPKC in all adult neurons (left, yellow) leads to high PIs, a low p-value and a high Bayes Factor, even with half the regular yaw torque training duration, indicating that these flies perform better at self-learning than wild type flies which do not learn with such short training. Genetic control flies without RU486 treatment (right, green) show weak PIs with a higher p-value and a lower Bayes Factor, indicating they show only very weak learning after the reduced training time
B. Short yaw torque training. Knocking out aPKC interaction partner *bazooka* in all adult neurons (left, yellow) yields flies with high PIs, a low p-value and a high Bayes Factor, even with half the regular training duration, indicating that these flies perform better at self-learning than wild type flies which do not learn with such short training. Genetic control flies without RU486 treatment (right, green) show weak PIs with a higher p-value and a lower Bayes Factor, indicating they show only very weak learning after the reduced training time.
C. Composite training, goal-directed phase (test without colors). Expressing the constitutively active form of aPKC in all adult neurons (left, yellow) leads to high PIs, a low p-value and a high Bayes Factor, even if the colors are removed after composite training with torque and colors as predictors of punishment. Wild type flies do not show learning in such experiments. Genetic control flies without RU486 treatment (right, green) show weak PIs with a higher p-value and a lower Bayes Factor, indicating they show only a very weak preference after composite training.

Silencing Kenyon cells improves motor learning

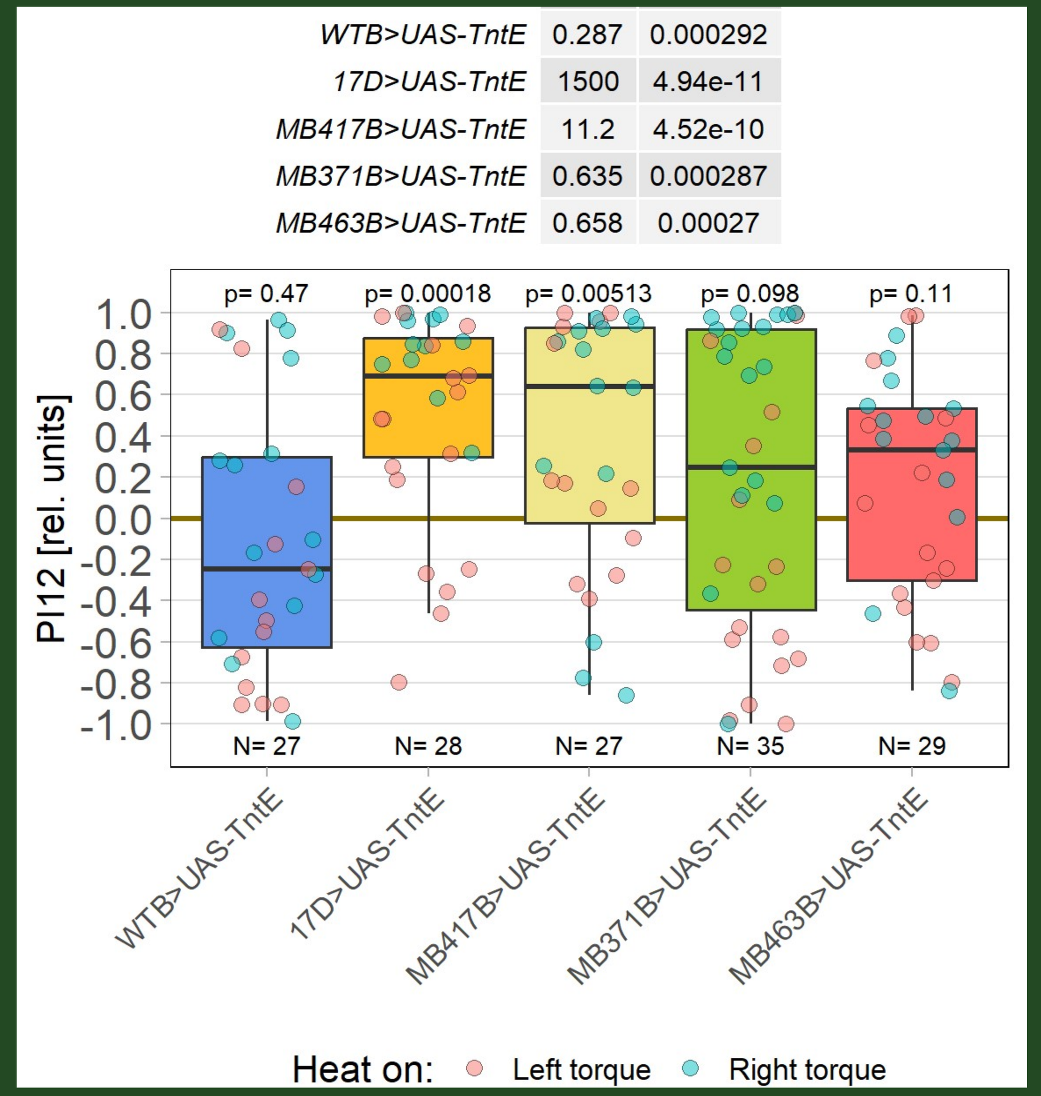


Figure 5: Silencing a small group of mushroom-body Kenyon cells improves motor learning such that it can be detected after 8-minute composite training.
Plotted are PIs indicating torque preference without colors in the first test after training. Expression of tetanus toxin in α/βc and α/βs KCs (17D) as well as KCs within the dACA and lACA simultaneously (and α/βs; MB417B) leads to high PIs, a low p-value and a high Bayes Factor. Silencing of either dACA KCs (MB371B) or lACA KCs (MB463B) separately does not result in premature habit formation.

Silencing specific MBONs improves motor learning

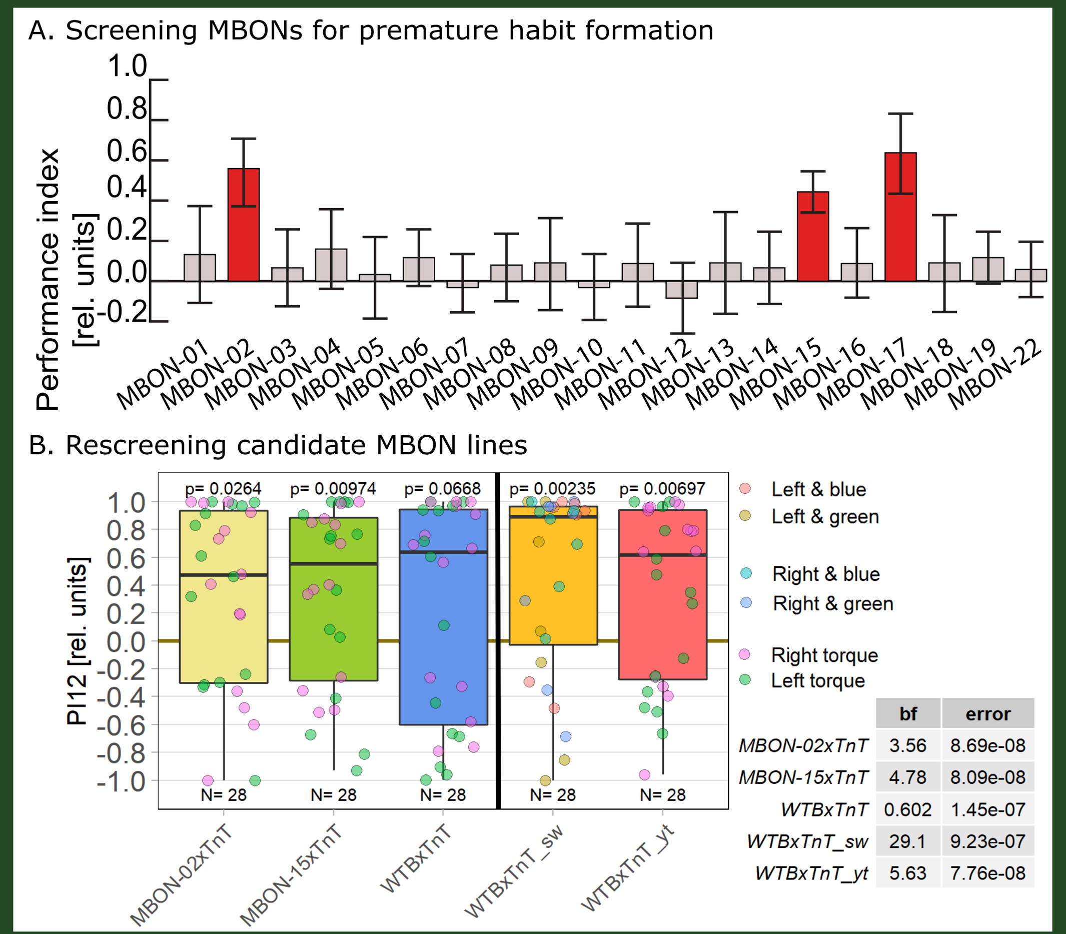


Figure 6. Some MBONs are involved in regulating habit formation.
A. Composite training, goal-directed phase. Screening MBON driver lines exressing tetanus toxin. Plotted are PIs indicating torque preference without colors after eight minutes of composite training with colors (goal-directed phase). Wild type flies do not show any preference with such training, revealing that no habits are formed after this kind of training. Three MBON lines show premture habit formation (red).
B. Rescreen, composite training, goal-directed phase. Two candidate lines (left, yellow, green) were rescreened with the corresponding genetic control flies (middle, blue) under identical experimental conditions: eight minutes of composite training, goal-directed phase. One additional group tested composite memory by keeping the colors also in the test after training (orange). A final group was trained
and tested without colors (yaw torque learning, right, red).
Both MBON-02 and MBON-15 seem to show premature habit formation, but due to pre-test differences, further experiments are required.