A dedicated, non-olfactory mushroom body sub-circuit mediates the interaction between goal-directed actions and habit formation in *Drosophila*

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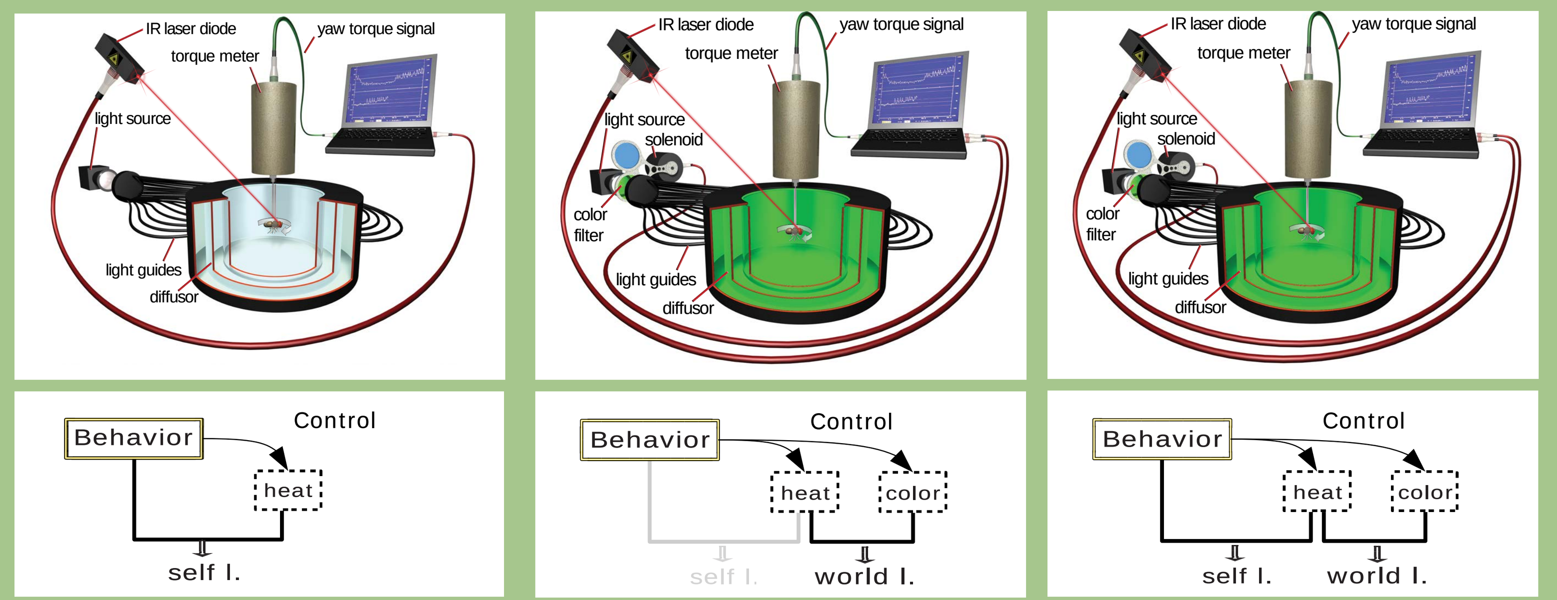
Introduction



Yaw torque learning: isolating the behavior from predictive stimuli leads to *self-learning*, a form of motor learning, after 8 but not 4 minutes of training.

Composite learning, goal-directed phase: adding predictive colors to the yaw torque experiment (left) leads to color learning, a form of *world-learning*.

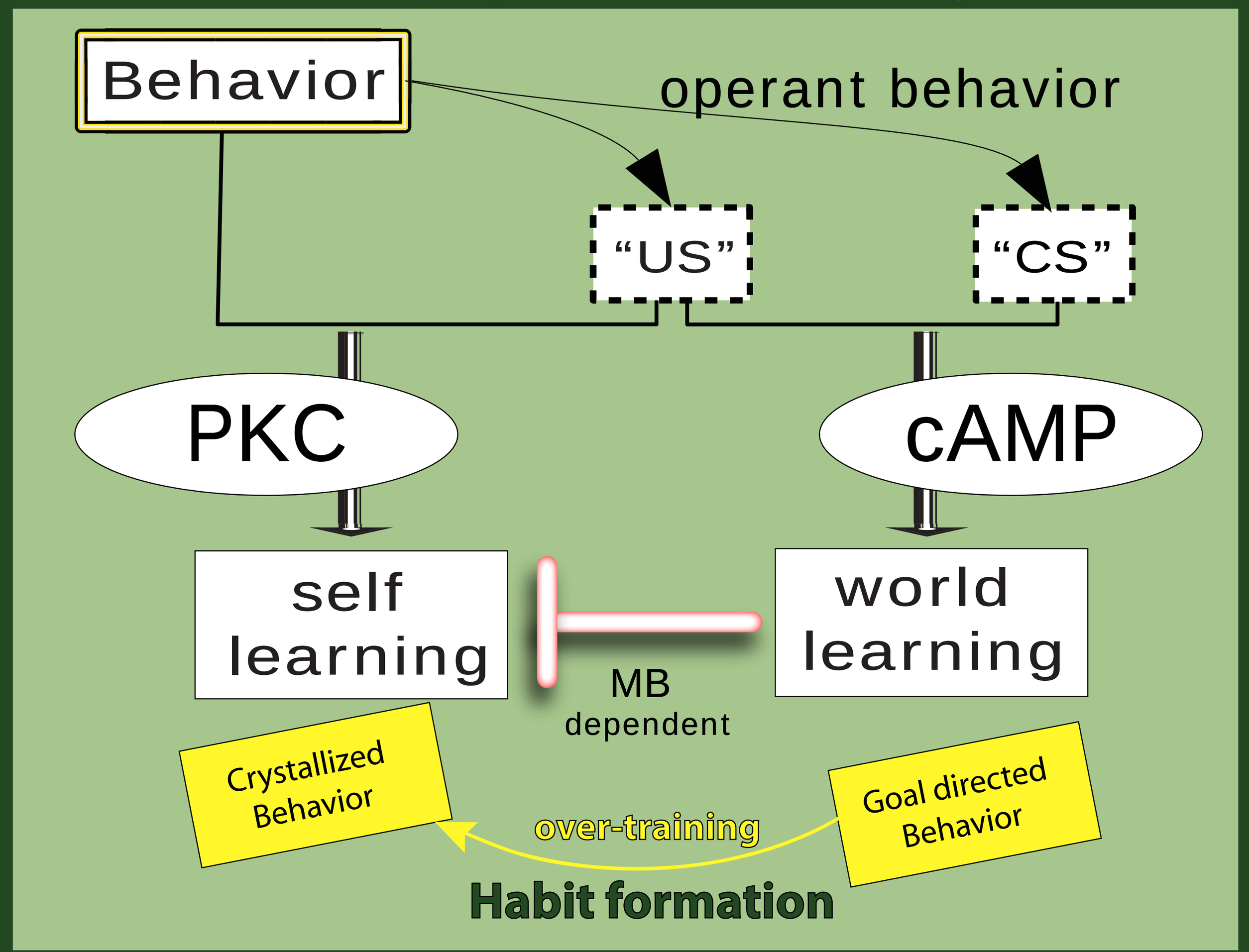
Composite overtraining: extended composite training overcomes the inhibition of self-learning and leads to habit formation (see below).

PKC-dependent
***dFoxP*-dependent**

adenylate cyclase type I dependent

***dFoxP*-dependent**

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

FoxP knock-out affects self-learning after 14 days

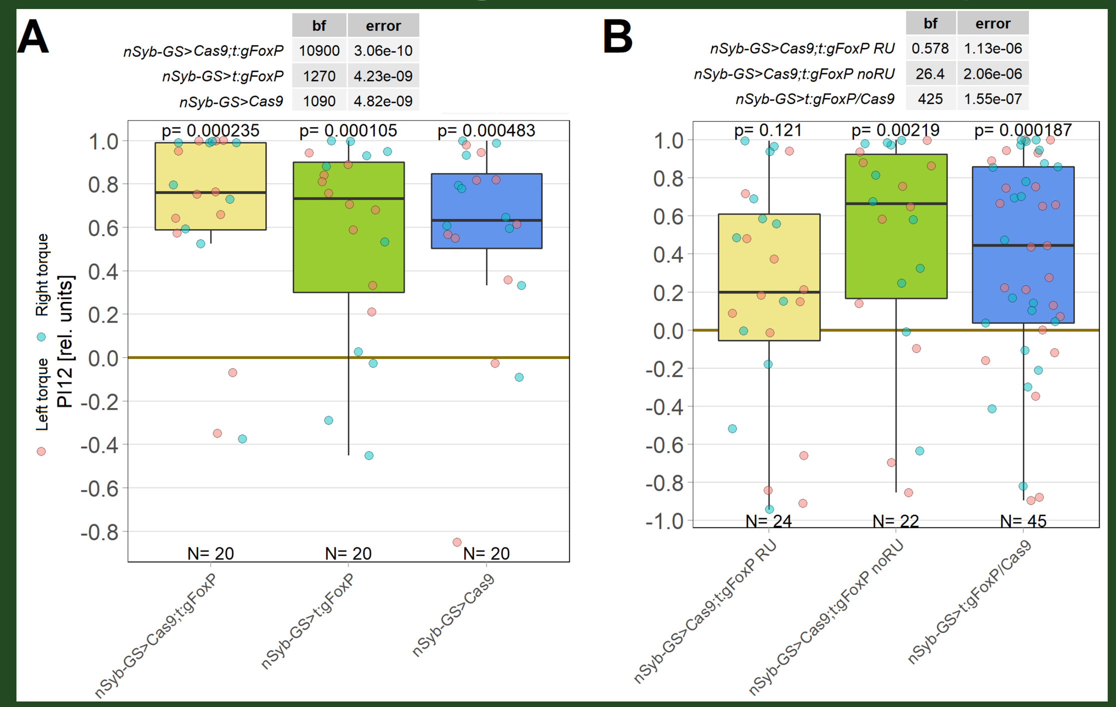


Figure 1: Knockout of *FoxP* in adult *Drosophila* shows yaw torque learning impairments only after 14 days.
Plotted are performance indices for the first period after training (PI12). Each colored dot represents a single fly experiment. Red dots denote experiments where the fly was punished on its left turning torque domain, blue dots denote flies that were punished on their right turning domain. Box plots denote medians, quartiles and non-outlier range. Statistical analyses test for differences of PIs against zero.
A. Self-learning two days after FoxP knock-out induction with RU486. Experimental animals (left, yellow) as well as gRNA (middle, green) and Cas9 (left, blue) control animals show both large Bayes Factors above 1000 and p-values below 0.005, indicating that all groups showed unimpaired self-learning.
B. Self-learning 14 days after FoxP knock-out induction with RU486 and 12 days after cessation of RU486 administration. Experimental animals (left, yellow) show a Bayes Factor of less than one and a large p-value, whereas both the genetic control animals without RU486 treatment (middle, green) and the pooled RU486-treated gRNA and Cas9 controls (right, blue) show large Bayes Factors and p-values smaller than 0.005, indicating an impairment in self-learning in the experimental group.

FoxP likely not required in brain

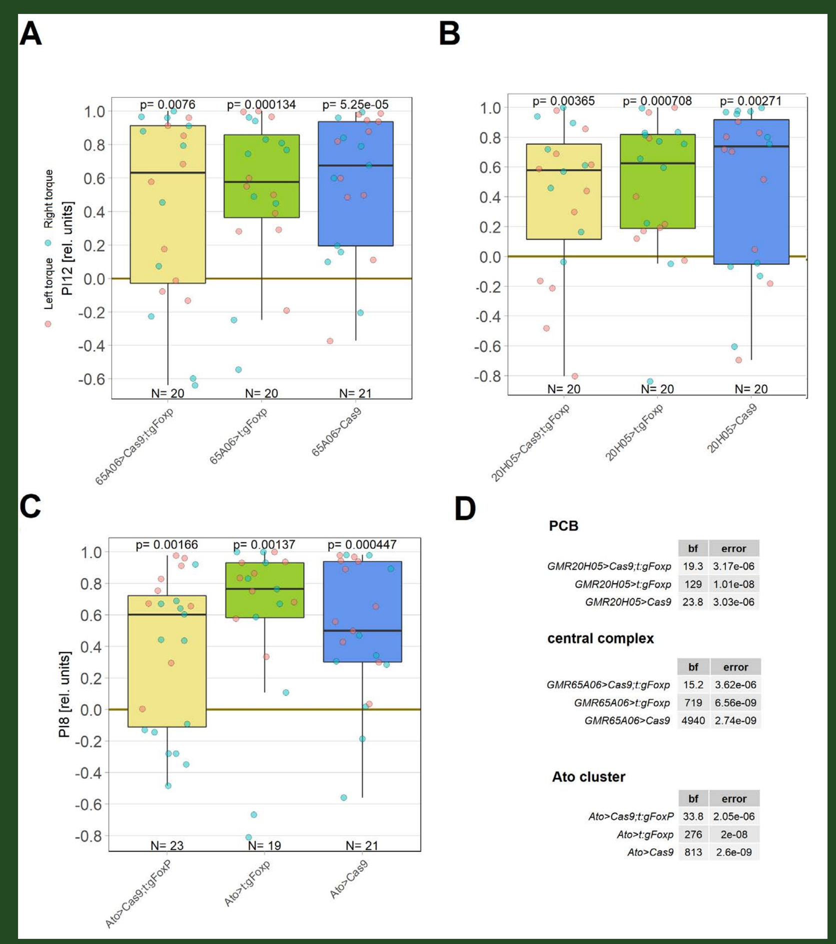


Figure 2: For operant self-learning, *FoxP* is likely not required in the brain.
Plotted are performance indices for the first period after training (PI12). Each colored dot represents a single fly experiment. Red dots denote experiments where the fly was punished on its left turning torque domain, blue dots denote flies that were punished on their right turning domain. Box plots denote medians, quartiles and non-outlier range. Statistical analyses test for differences of PIs against zero (above plots and D).
A. Flies with FoxP knocked out in the protocerebral bridge (left. yellow) as well as the gRNA (middle, green) and Cas9 (right, blue) control flies show large Bayes factors (D) and small p-values, indicating their self-learning is intact
B. Flies with FoxP knocked out in the protocerebral bridge and additional components of the central complex (left. yellow) as well as the gRNA (middle, green) and Cas9 (right, blue) control flies show large Bayes factors (D) and small p-values, indicating their self-learning is intact.
C. Flies with FoxP knocked out in the dorsal cluster neurons (left. yellow) as well as the gRNA (middle, green) and Cas9 (right, blue) control flies show large Bayes factors (D) and small p-values, indicating their self-learning is intact.

Self-learning requires *aPKC* in motor and *FoxP* neurons

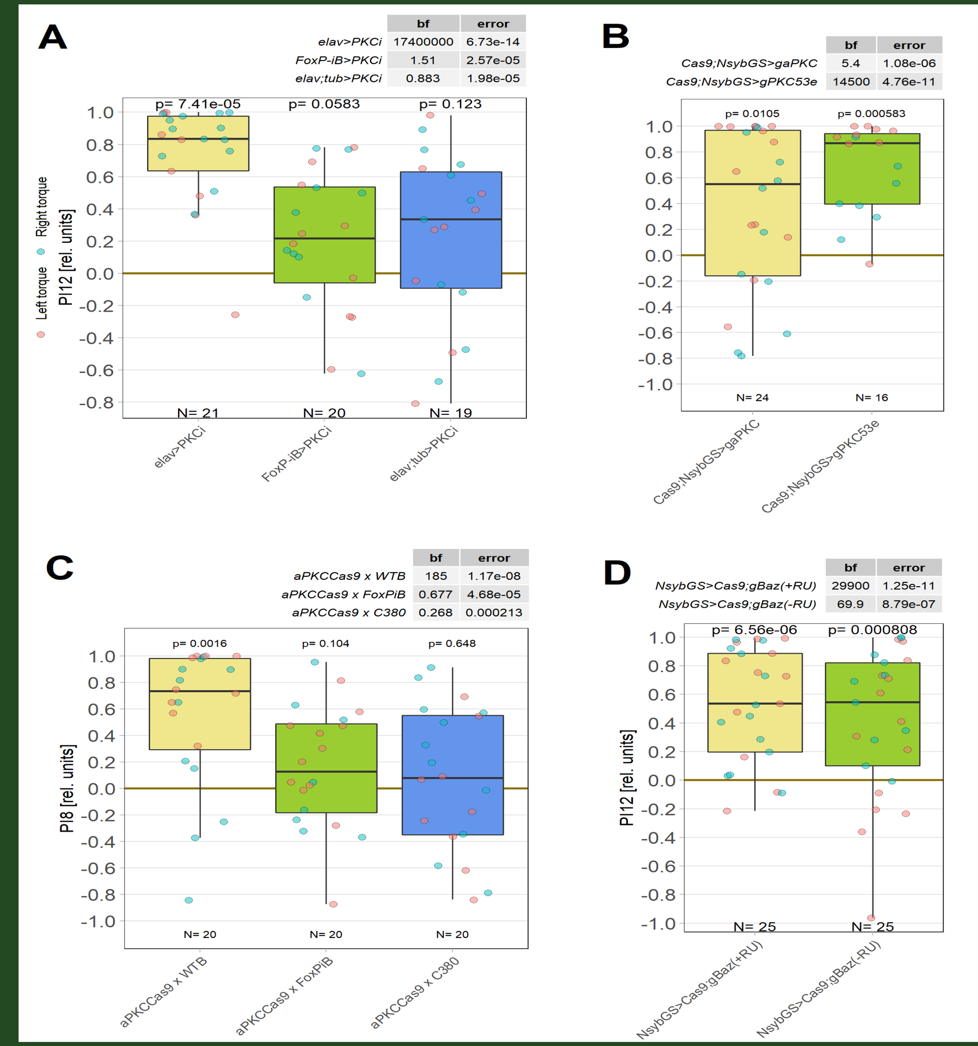


Figure 3: For operant self-learning, aPKC is required in motor and *FoxP* neurons.
A. Inhibiting all protein kinase C isoforms with the inhibitory peptide PKCi reproduces published results. Constitutive, pan-neuronal expression PKCi (left, yellow), leads to high PIs and a large Bayes Factor, indicating this manipulation left self-learning intact. Expressing PKCi either in FoxP-isoform B positive neurons (middle, green), or in all neurons but restricted to adulthood (right, blue) yields low PIs, high p-values and low Bayes factors, indicating self-learning is impaired.
B. Pan-neuronal knock-out of two different PKC genes with CRISPR/Cas9 in adulthood suggests aPKC is necessary for operant self-learning. Knocking out atypical PKC (yellow, left) yields moderate PIs, p-values and Bayes factors, indicating some effect on operant self-learning, while the high PIs, low p-values and high Bayes factor of the group where PKC53E was knocked out (right, green) indicate their self-learning is intact.
C. Knocking out aPKC in motor neurons or FoxP-neurons impairs operant self-learning. Expressing the CRISPR/Cas9 components either in FoxP isoform B-positive neurons (green, middle) or in motor neurons (blue, right) leads to low PIs, high p-values and low Bayes Factors, indicating their self-learning is strongly impaired. Control flies with only the CRISPR/Cas9 genetic elements but no driver, show high PIs, a low p-value and a high Bayes Factor, indicating their self-learning is intact. This result suggests aPKC and *FoxP* may interact.
D. Knocking out aPKC interaction partner *bazooka* does not impair self-learning. Flies containing the genetic elements for an RU486-triggered *bazooka* knockout show intact learning both with (left, yellow) and without (right, green) RU486 in the medium.

Increasing aPKC improves self-learning and leads to premature habit formation

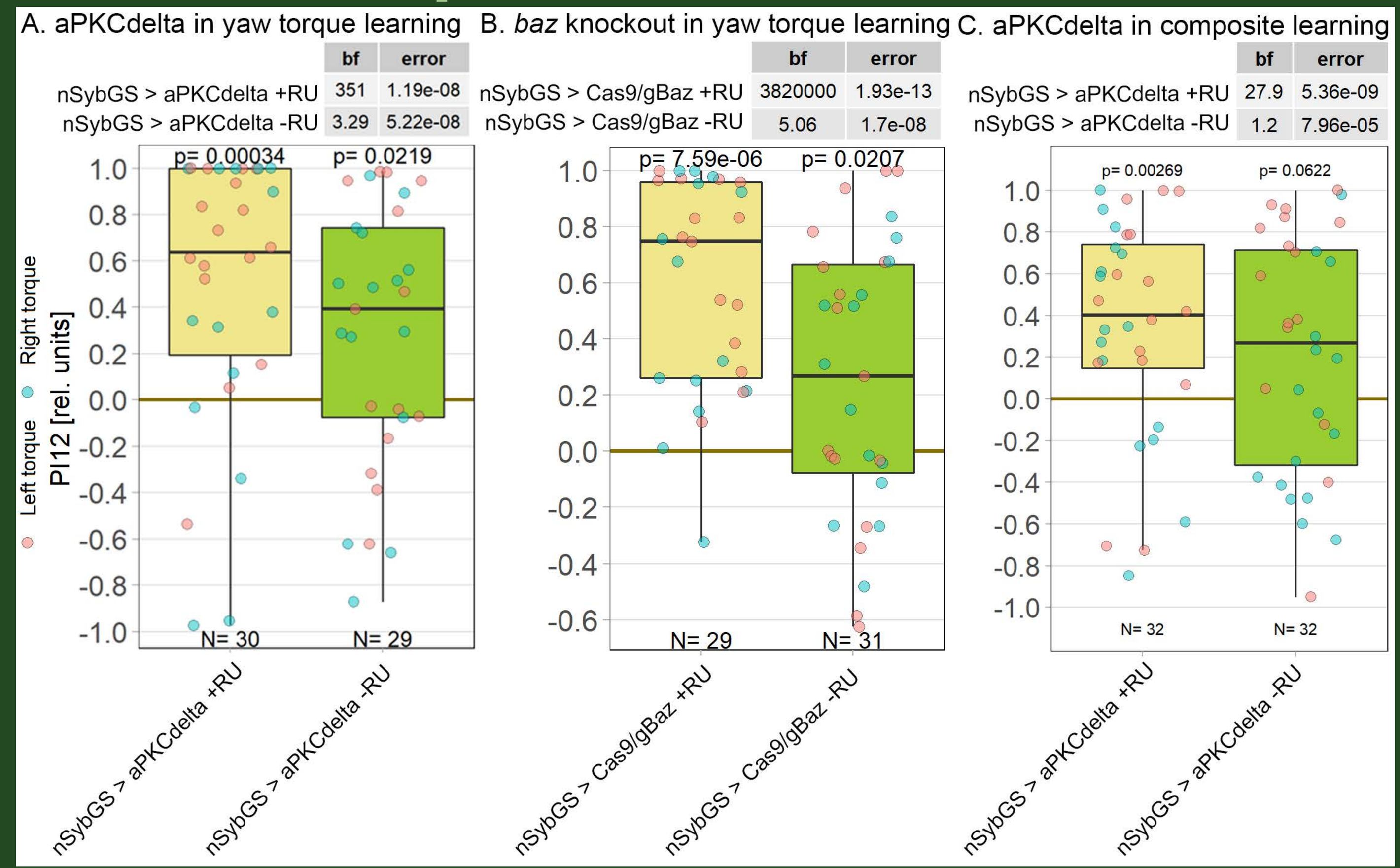


Figure 4. Expressing aPKCdelta and knocking out *bazooka* improves self-learning.
A. Short yaw torque training. Expressing the constitutively active form of aPKC in all adult neurons (left, yellow) leads to high PIs, a low pvalue and a high Bayes Factor, even with half the regular yaw torque training 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 very weak learning after composite training.

Silencing specific MBONs leads to premature habit formation

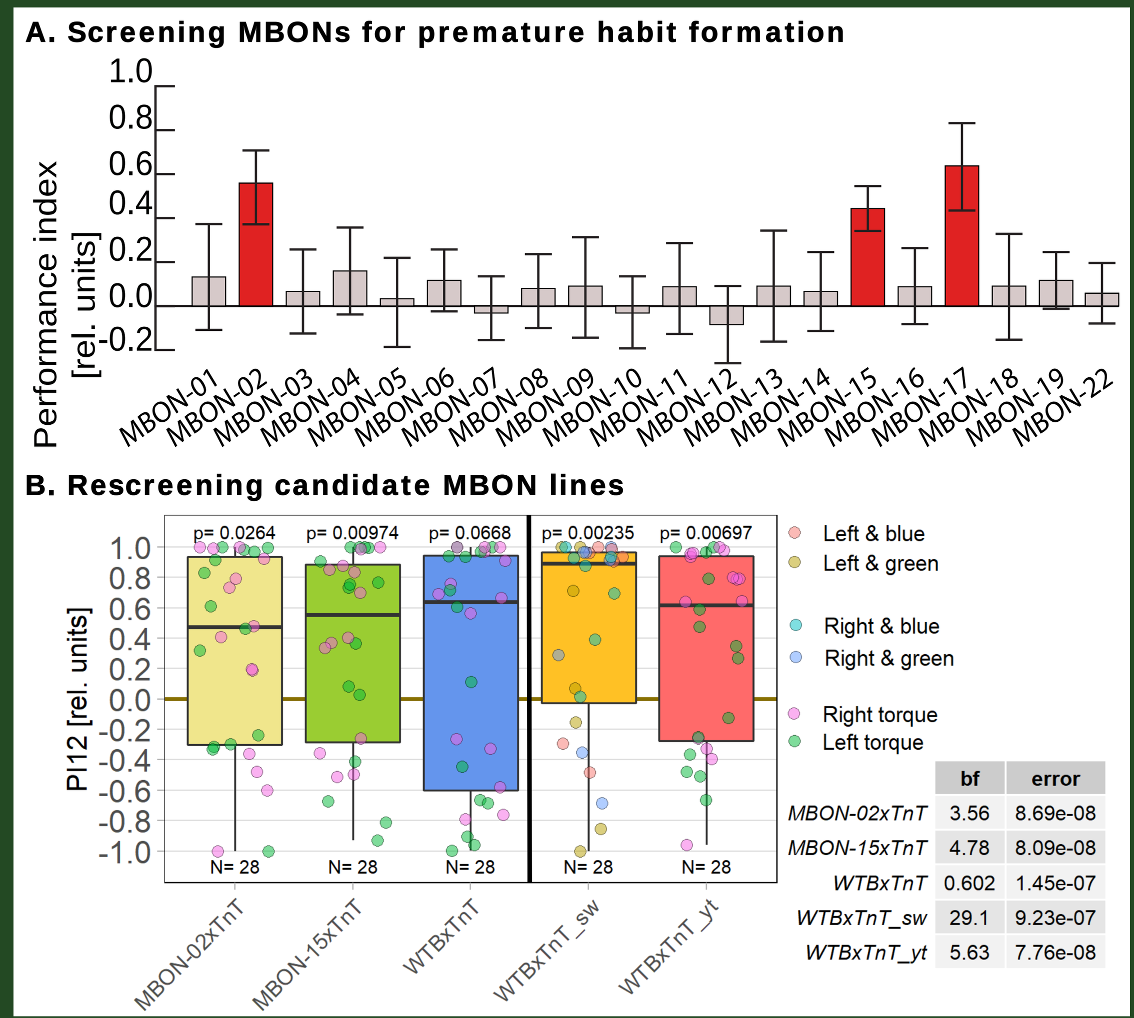


Figure 5. Some MBONs are involved in regulating habit formation.
A. Composite training, goal-directed phase. Screening MBON driver lines exressing tetanus toxin. Plotted are PIs indicating torque preference without colors after eight minutes of composite training with colors (goal-directed phase). Wild type flies do not show any preferrence 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.

SfN meeting 2022, San Diego