# Motor learning in fruit flies: what happens where and how to improve it

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## Operant self-learning: a form of motor learning

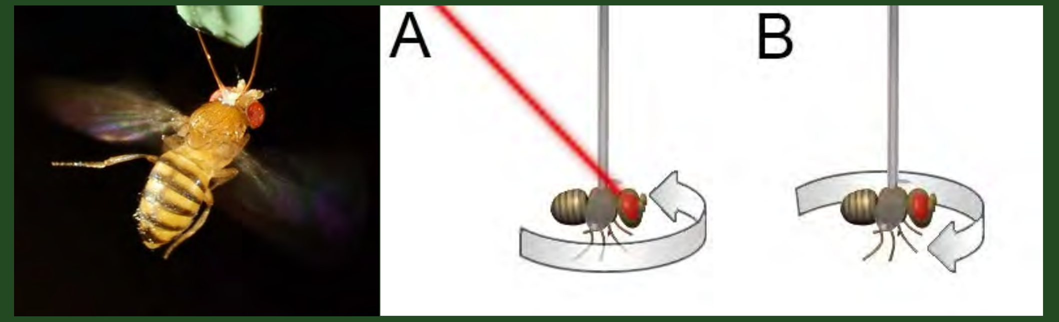


**Figure 1: Operant self-learning in *Drosophila* requires FoxP and PKC**
A fly is tethered by head and thorax flying stationarily in a featureless environment (photograph, left). The animal is tethered to a torque meter which measures the angular momentum around the fly's vertical body axis (yaw torque). The yaw torque range is divided into to torque domains of similar size, approximately corresponding to left and right turning attempts, respectively. In the example above, left turning attempts (A) are punished by an infra-red heat beam that is invisible to the fly, while right turning attempts (B) are not punished. Half of the flies are always punished for left, the other half for right turning attempts. Mutants and RNAi-mediated knock-downs of the gene FoxP are deficient in this task. Similarly, iCRISPR/Cas9-mediated knock-out of the gene for the atypical protein kinase C (aPKC) in all motor neurons, or FoxP expressing neurons also impairs this formn of motor learning.

## Motor learning modifies motor neurons expressing both aPKC and FoxP

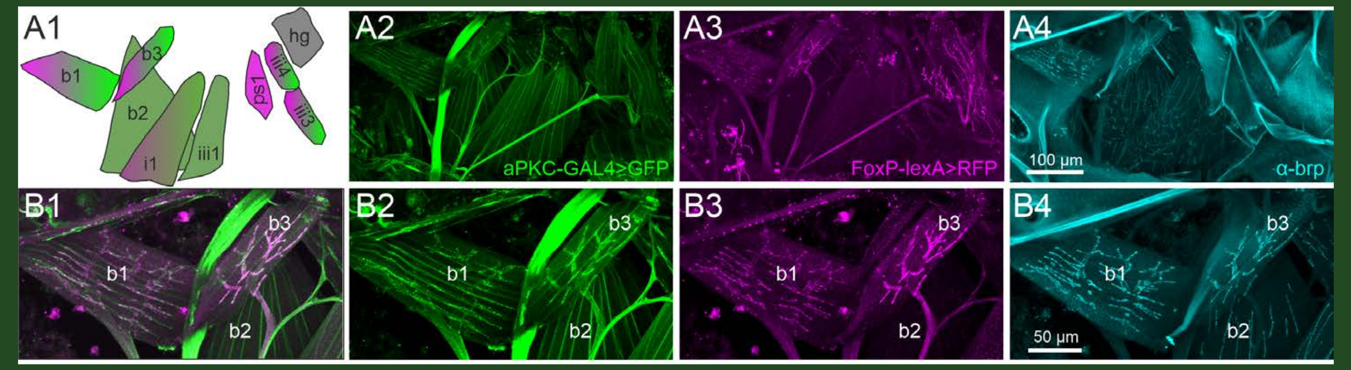


**Figure 2: A subset of direct wing muscles is innervated by FoxP / aPKC co-expressing MNs.**
Representative projection view of MN terminals on the direct flight steering muscles in animals with GFP label in aPKC expressing cells (aPKC-Gal4>CD8::GFP, green), RFP expression in FoxP expressing cells (FoxP-LexA>CD8::RFP, magenta), and immunolabeling for the presynaptic active zone marker bruchpilot (brp, cyan) reveal which direct flight steering MNs express either aPKC, FoxP, or both, or none of them but only brp in presynaptic active zones. (A1) depicts the orientation, shape, and abbreviated names of direct flight steering muscles and summarizes which ones are innervated by aPKC expressing MNs (green), by FoxP-expressing MNs (magenta), or by MNs without FoxP and aPKC expression (grey). (A2) Projection view of direct flight muscles and their innervation with GFP expression under the control of aPKC-GAL4 (green) in 20x magnification. (A3) Same preparation, image stack, and field of view but with RFP expression under the control of FoxP-lexA (magenta). (A4) Same preparation, image stack, and field of view with brp immunolabel (cyan) in presynaptic active zones of flight steering MNs. (B1-B4). Same preparation but with selective enlargement of the three basalare muscles (b1-b3), with all three labels in (B1), GFP label in aPKC-expressing cells (green, B2), RFP label in FoxP expressing cells (magenta, B3), and Brp label in presynaptic active zones (cyan, B4). Muscles b1 and b3 are innervated by steering MNs with aPKC and FoxP expression, but b2 is devoid of FoxP-expressing innervation.

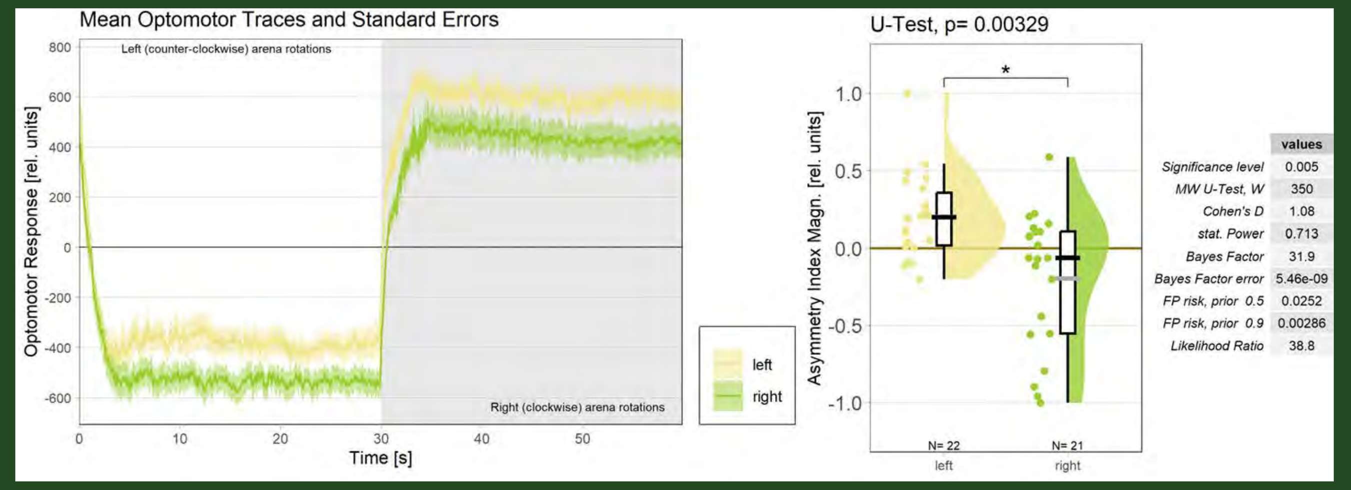


**Figure 3: Associating one torque domain with heat changes optomotor behavior.**
**Left:** Averaged optomotor traces of flies punished either on the 'left' (yellow) or on the 'right' (green) torque domain. A reduction in the OMR magnitude can be observed on the punished, but not on the unpunished side. Errors are standard errors.
**Right:** Optomotor asymmetry indices for flies punished either on the 'left' (yellow) or on the 'right' (green) torque domain. Positive values indicate a shift towards positive (right-turning) torque. The values for each group have now shifted towards the unpunished side compared to the values before training. Both frequentist and Bayesian analyses are displayed to the right of the plots and indicate a significant difference between groups.

## aPKC is required for optomotor modulation after motor learning

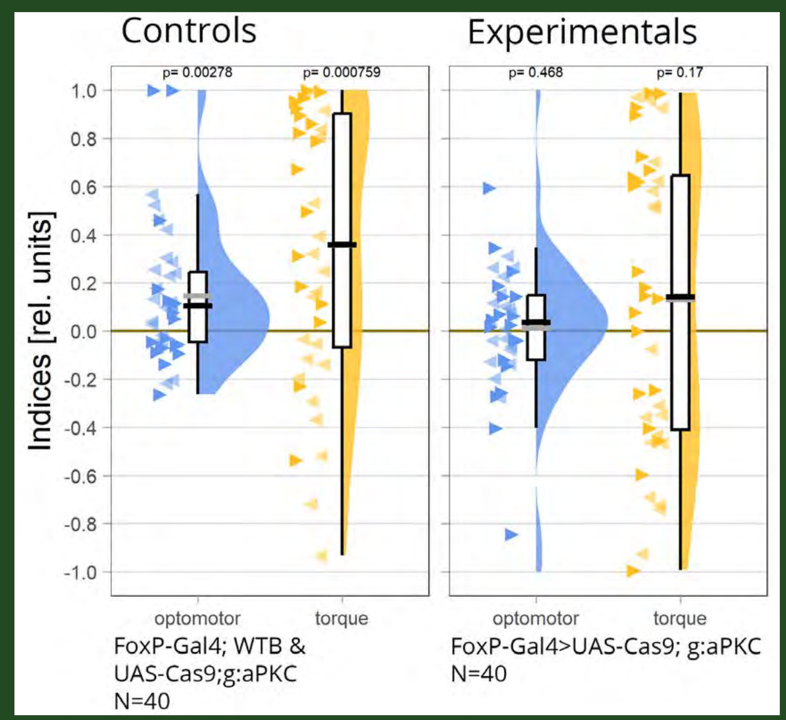


**Figure 4: Knocking out aPKC abolishes optomotor asymmetry after motor learning.**
**Left:** After motor learning, genetic control animals shows both optomotor asymmetry (blue) and a preference for the unpunished turning directions / torque domain (yellow).
**Right**: After motor learning, animals in which aPKC was knocked out in FoxP neurons showed neither optomotor asymmetry (blue), nor a preference for the unpunished torque domain (yellow).
Shown are data from a 2-min period without heat immediately after eight minutes of training.
Triangles indicate individual PIs and punished torque direction. Black bars denote medians and grey bars means. Boxes indicate quartiles and whiskers non-oulier range.

## ML is unaffected by aPKC ko in either b1 or b3 alone

| | bf | error |
|---|---|---|
| b1>aPKC | 155 | 6.79e-10 |
| b3>aPKC | 650 | 1.17e-10 |
| aPKC_WTB | 9.13 | 7.36e-10 |

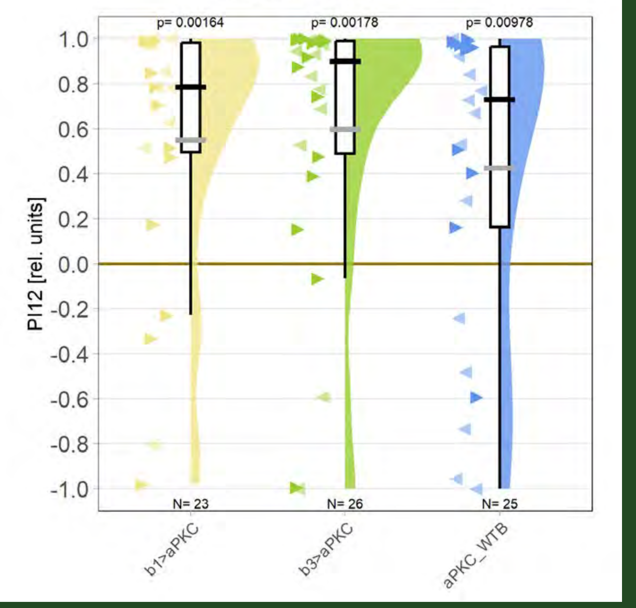


**Figure 5: CRISPR / Cas9-mediated aPKC knock-out in single motor neurons leaves motor learning intact.**
Plotted are performance indices for the first test period after training (PI12). Knocking out aPKC in eiter b1 (left, yellow) or b3 (green middle) motor neurons alone does not impair motor learning. If anything, genetic control flies (blue, right) show slightly lower PIs. In all three groups, however, WIcoxon tests against zero show low p-values,. while Bayes Factors (bf) are high throughout.
Triangles indicate individual PIs and punished torque direction. Black bars denote medians and grey bars means. Boxes indicate quartiles and whiskers non-oulier range.

## Motor learning requires the *foraging* PKG

| | bf | error |
|---|---|---|
| elav>forRNAi | 0.155 | 0.000687 |
| wtb_forRNAi | 123 | 2.33e-11 |
| elav_WTB | 627 | 4.7e-07 |

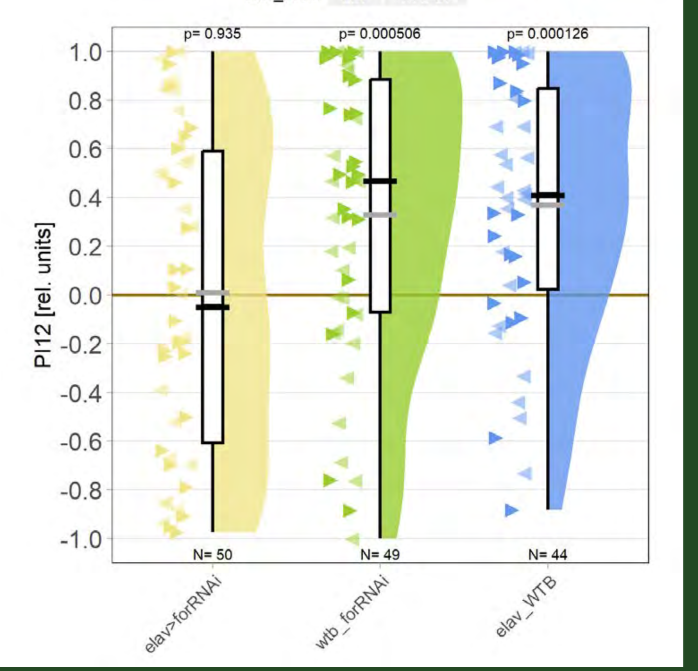


**Figure 6: RNAi-mediated knock-down of the *foraging* PKG impairs motor learning.**
Plotted are performance indices of a preference test without reinforcement for the first 2-min period after eight minutes of training. Flies with for PKG knocked down (yellow, left) do not show any preference for any turning direction, with a high-palue and low Bayes Factor (bf) whenh PIs are tested against zero. Both control groups (green and blue) show a significant preference for the unpunished torque domain, each group with a low p-avlue and high Bayes Factors when tested against zero.
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

| | bf | error |
|---|---|---|
| forS | 5510 | 1.02e-11 |
| forR | 3.35 | 5.07e-09 |
| WTB | 0.341 | 0.000193 |

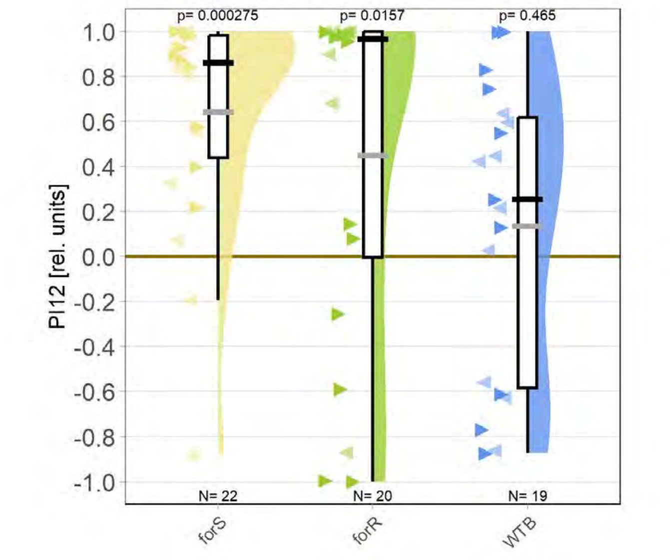


**Figure 7: Both variants of the *foraging* gene show motor learning after 4-minutes training.**
Plotted are performance indices for the first period after training (PI12). When both alleles of the for gene (rover: forR, sitter: forS) are isolated in separate populations, flies show a torque preference 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.

## Overexpressing aPKC improves motor learning

A

| | bf | error |
|---|---|---|
| nsybGal4>aPKCdelta(+RU) half | 351 | 3.27e-07 |
| nsybGal4>aPKCdelta(-RU) half | 3.29 | 6.95e-09 |

B

| | bf | error |
|---|---|---|
| Cas9gBaz x nsybGs RU half time | 3820000 | 9.94e-13 |
| Cas9gBaz x nsybGs noRU half time | 5.06 | 3.55e-09 |

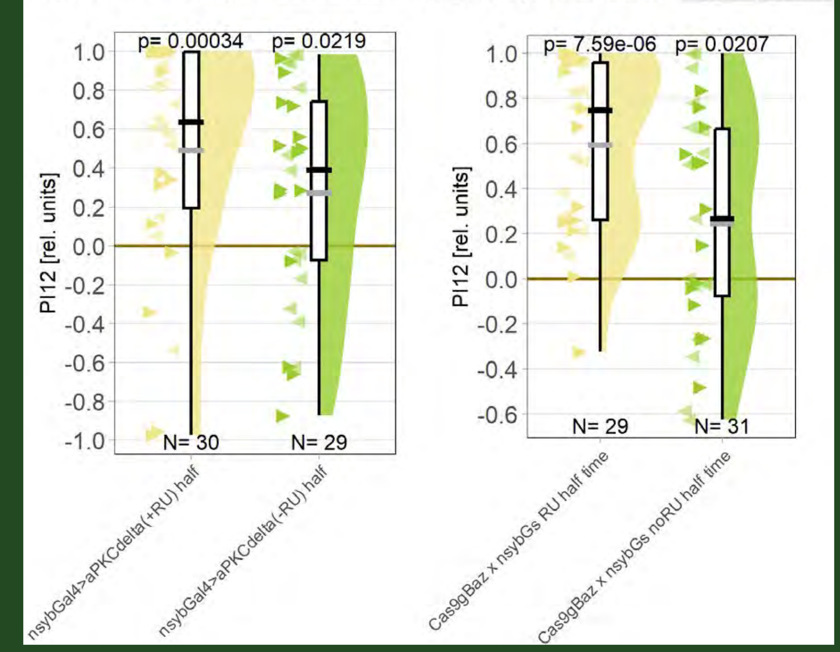


**Figure 9: Overexpressing the constitutively active aPKCdelta or *bazooka* knock-out both improve motor learning.**
**A. Short yaw torque training.** Expressing the constitutively active form of aPKC in all adult neurons (left, yellow) leads to high PIs, a low p-value and a high Bayes Factor, even with 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.

## Pavlovian learning mutants show improved motor learning

A — 8-minute Training

| | bf | error |
|---|---|---|
| wtb | 6.18 | 2.9e-09 |
| rut | 12.1 | 1.22e-09 |
| rsh | 100 | 1.3e-09 |

B — 4-minute Training

| | bf | error |
|---|---|---|
| wtb | 0.344 | 0.000345 |
| rut | 34.6 | 1.72e-10 |
| rsh | 3260 | 2.37e-11 |

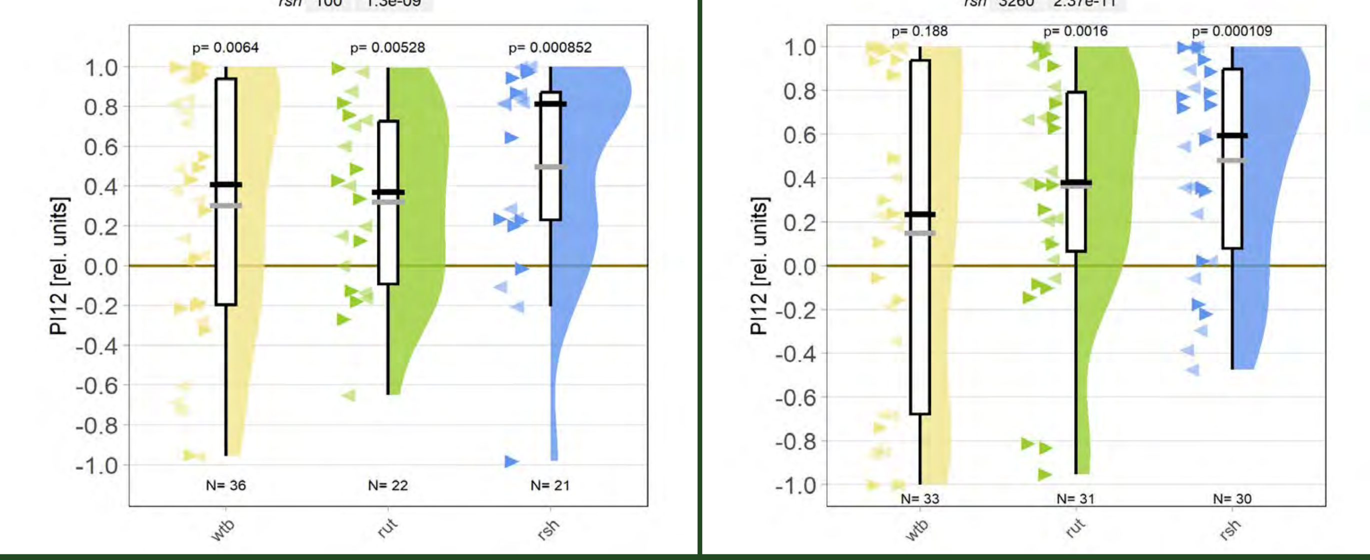


**Figure 8: Both *rutagaba* and *radish* show motor learning under conditions not sufficient for learning in wild type flies.**
**A. After 8 minutes of yaw torque training, mutant and wild type control flies show normal motor learning.** In the first training after the last test period, wild type Berlin 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.

## What is the role of dopamine in the ellipsoid body?

| | ERN>DopR2 ERN>TNT-E | ERN>DopR2 ERNxWTB | ERN>TNT-E ERNxWTB |
|---|---|---|---|
| Significance level | 0.005 | 0.005 | 0.005 |
| MW U-Test, W | 582 | 570 | 438 |
| U-Test p-value | 0.00496 | 0.00372 | 0.621 |
| Cohen's D | 0.524 | 0.669 | 0.225 |
| stat. Power | 0.184 | 0.347 | 0.0236 |
| Bayes Factor | 1.34 | 3.41 | 0.362 |

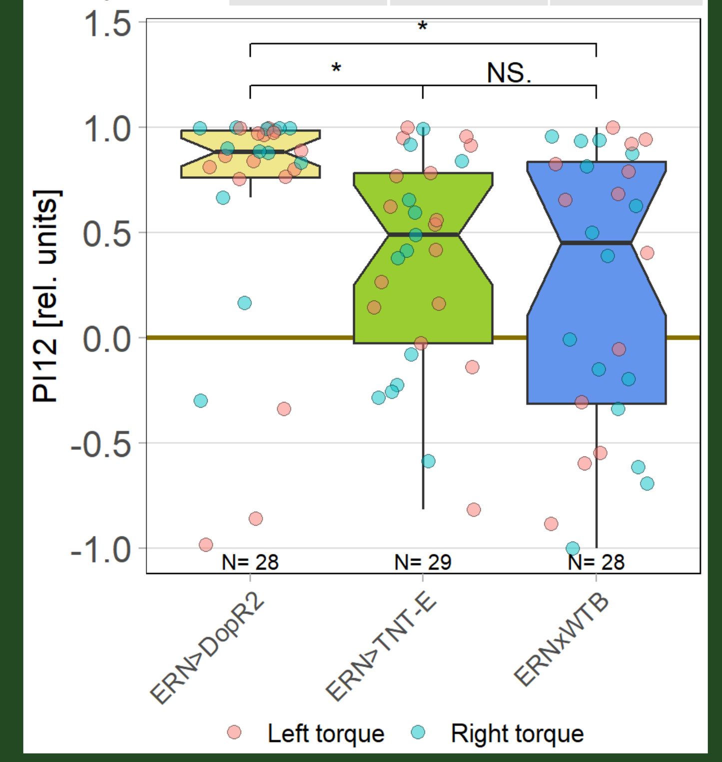


**Figure 10: Probing ellipsoid-body function.**
Plotted are the torque preferences as measured by performance indices (PIs) of the 2-min period immediately following eight minutes of training. Flies with RNAi-mediated knock-down of the dopamine receptor DopR2 in ellipsoid body ring neurons (yellow, left, driver RRID:BDSC_48870) show significantly higher preference for the unpunished torque direction (i.e. more learning) than flies either with tetanus-toxin light chain expression in ring neurons (middle, green) or driver control flies (right, blue). Plots denoite medians, quartiles and non-oulier range. Table above the graphs displays frequentist and Bayesian statistics indicating higher learning in DopR2 knock-down flies.