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Pain is so close to pleasure: the same dopamine neurons can mediate approach and avoidance in Drosophila

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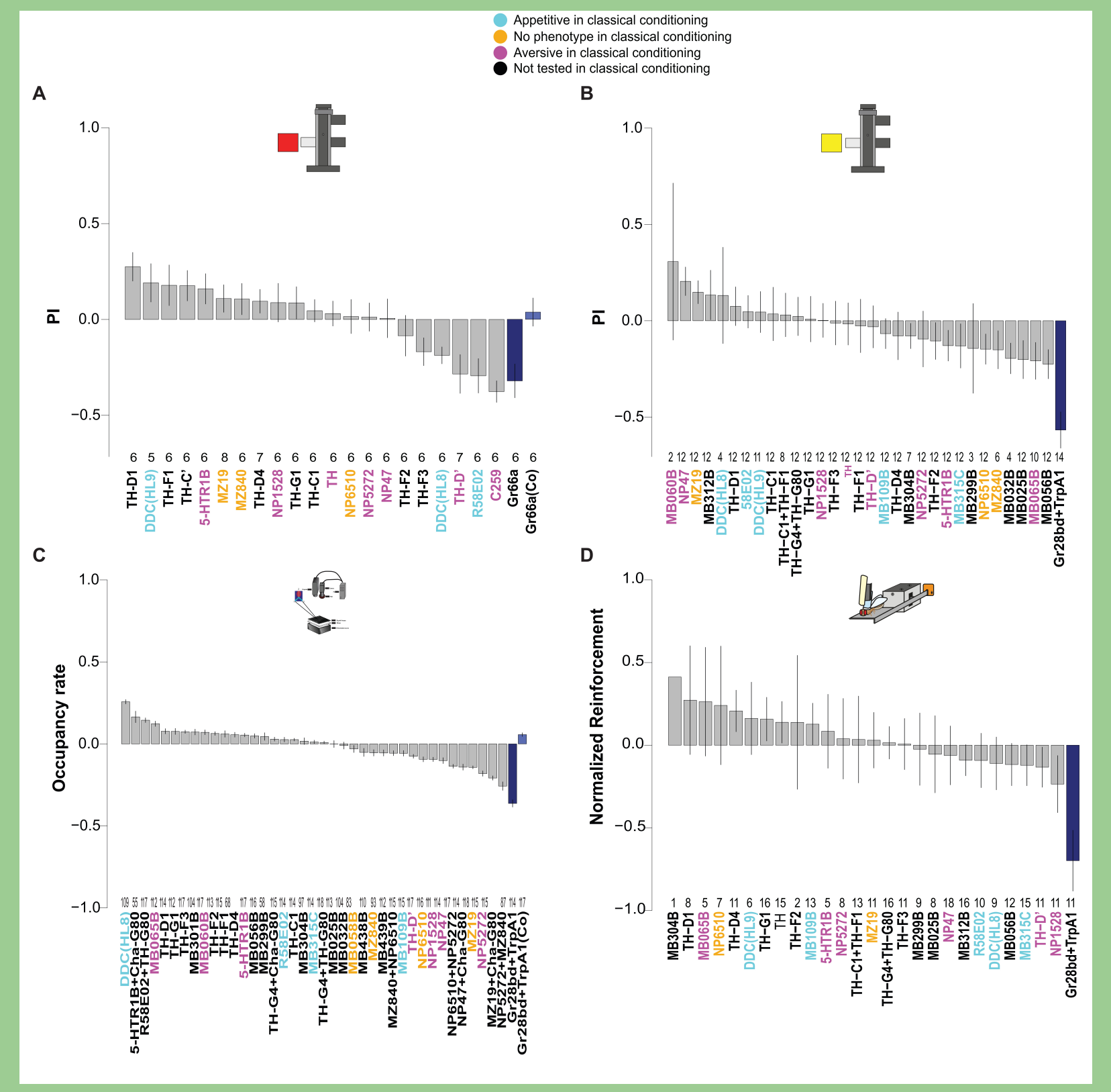
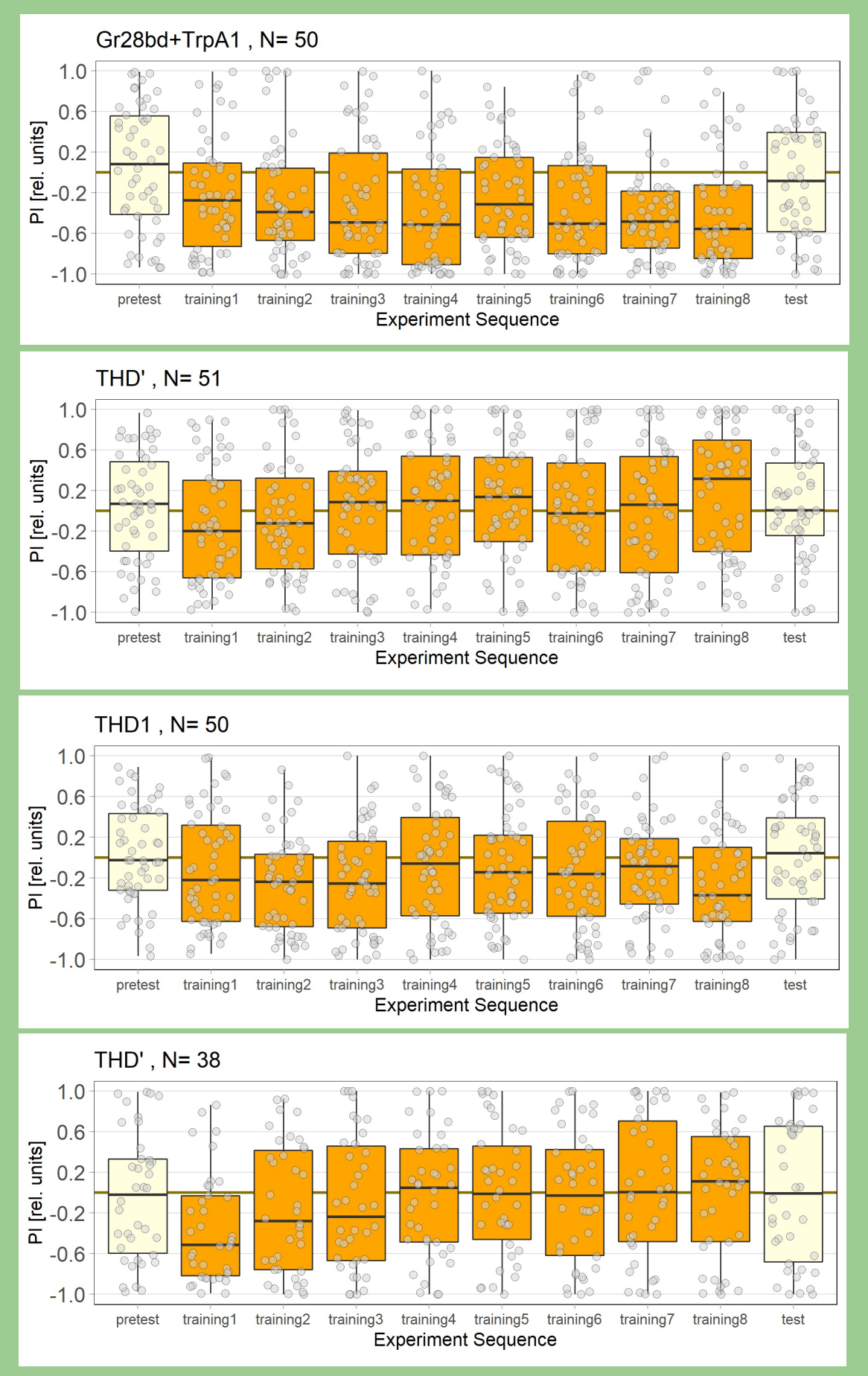
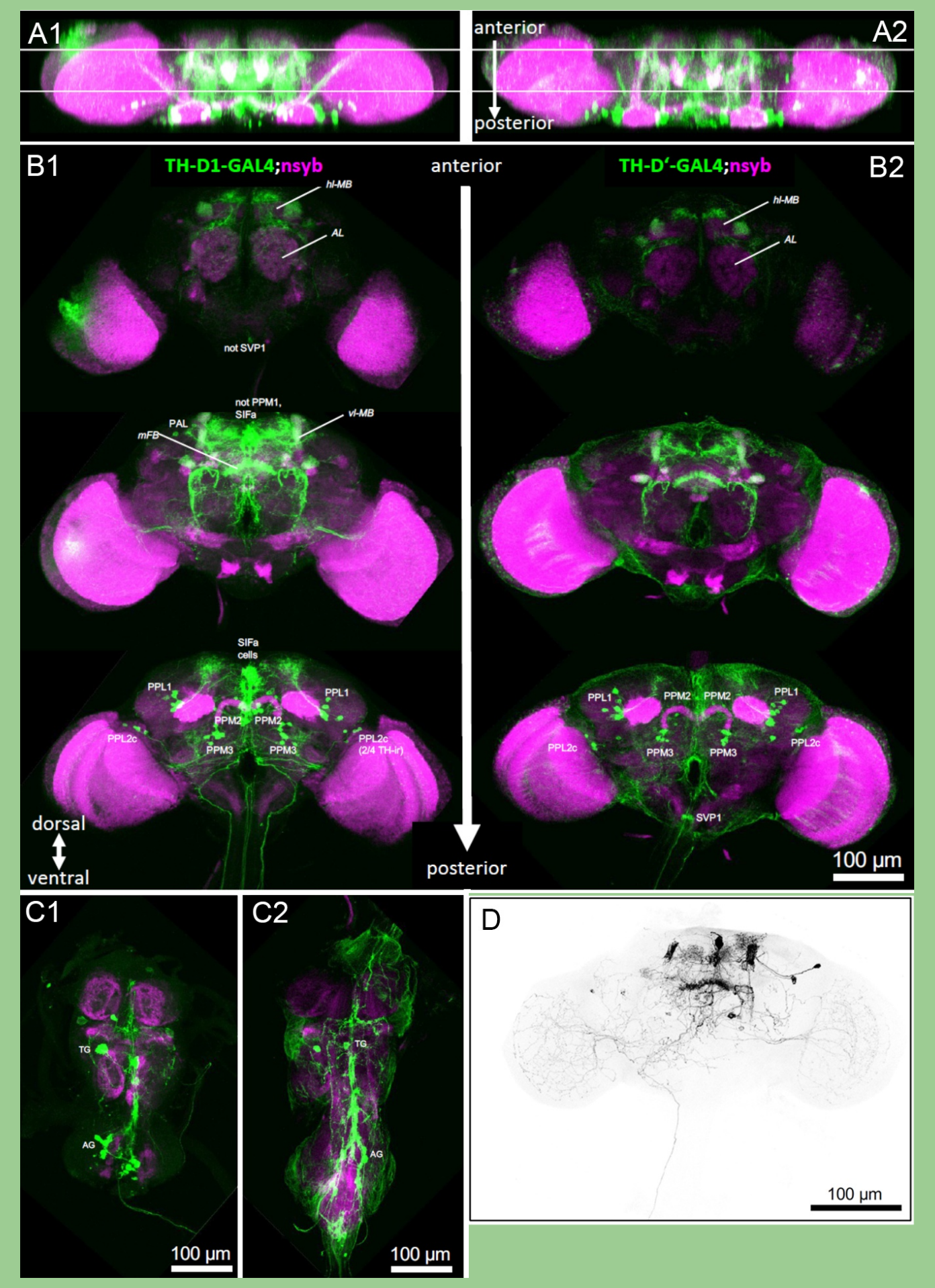
Background



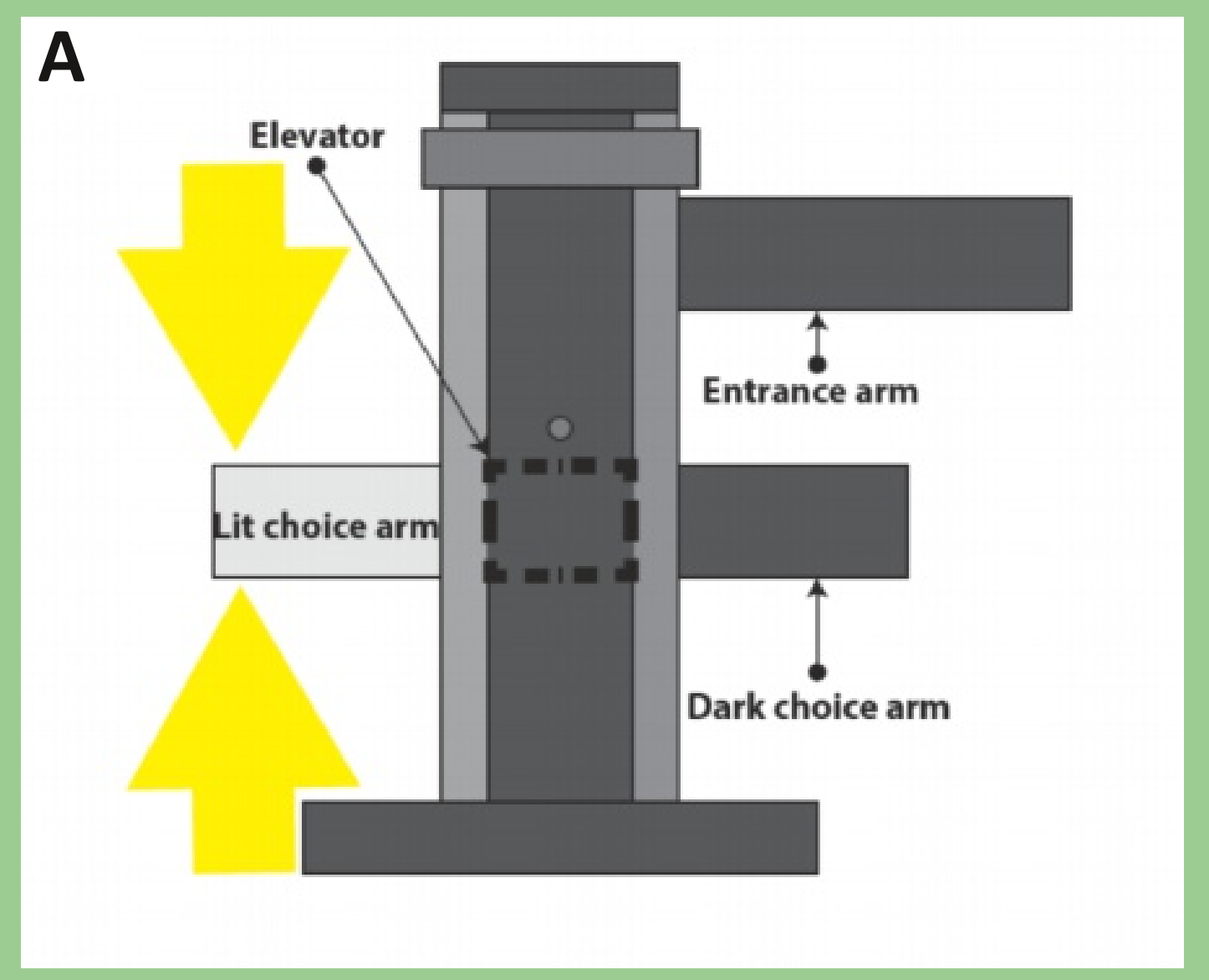


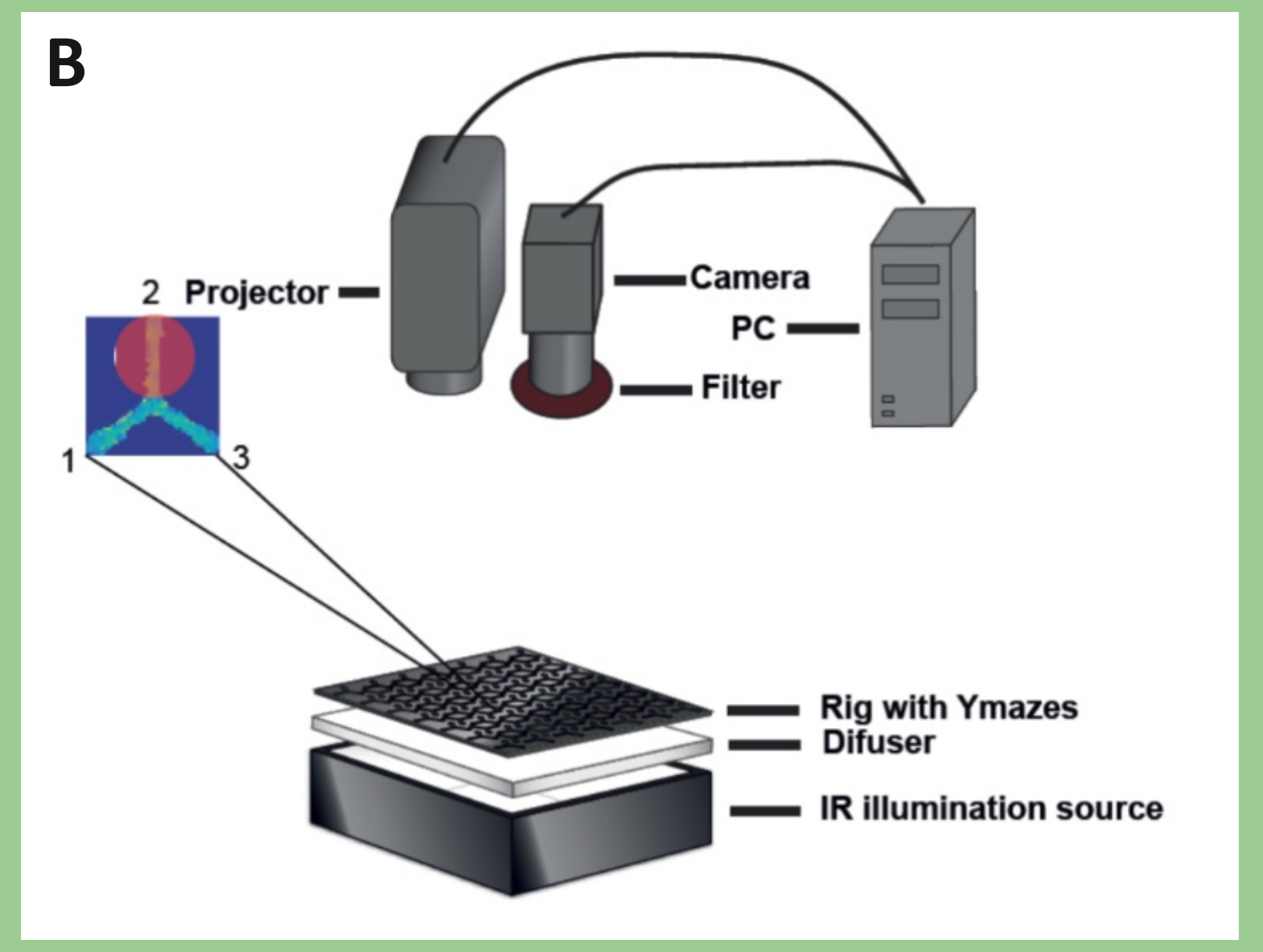
Left: Results from four different operant self-stimulation screens driving Chrimson expression in dopaminergic sub-populations. A: T-maze screen with red light. B: T maze screen with yellow light. C: Y-maze screen. D: Joystick screen. Barplots depict each driver line means with standard error of the mean (SEM). Positive controls with and without ATR are colored in dark- and light blue, respectively. Driver line fonts are color-coded according to classical conditioning phenotypes reported in the literature. All lines contained a norpAP24 mutation, rendering them blind. Right: Experimental time course of the Joystick experiment. Shown are the Performance Indices (PI) of the lines that showed consistent behavior in the four screens on the left. Each boxplot represents one minute of behavioral recording. Positive PIs indicate the optogenetic light was chosen to be switched on, negative PIs indicate the fly preferred the optogenetic light to be off. Orange denotes periods when the optogenetic light was controlled by the fly (training) and yellow denotes periods where the optogenetic light was permanently switched off (test). Small grey circles denote individual flies. Boxes denote quartiles, solid black lines means and whiskers non-outlier range.

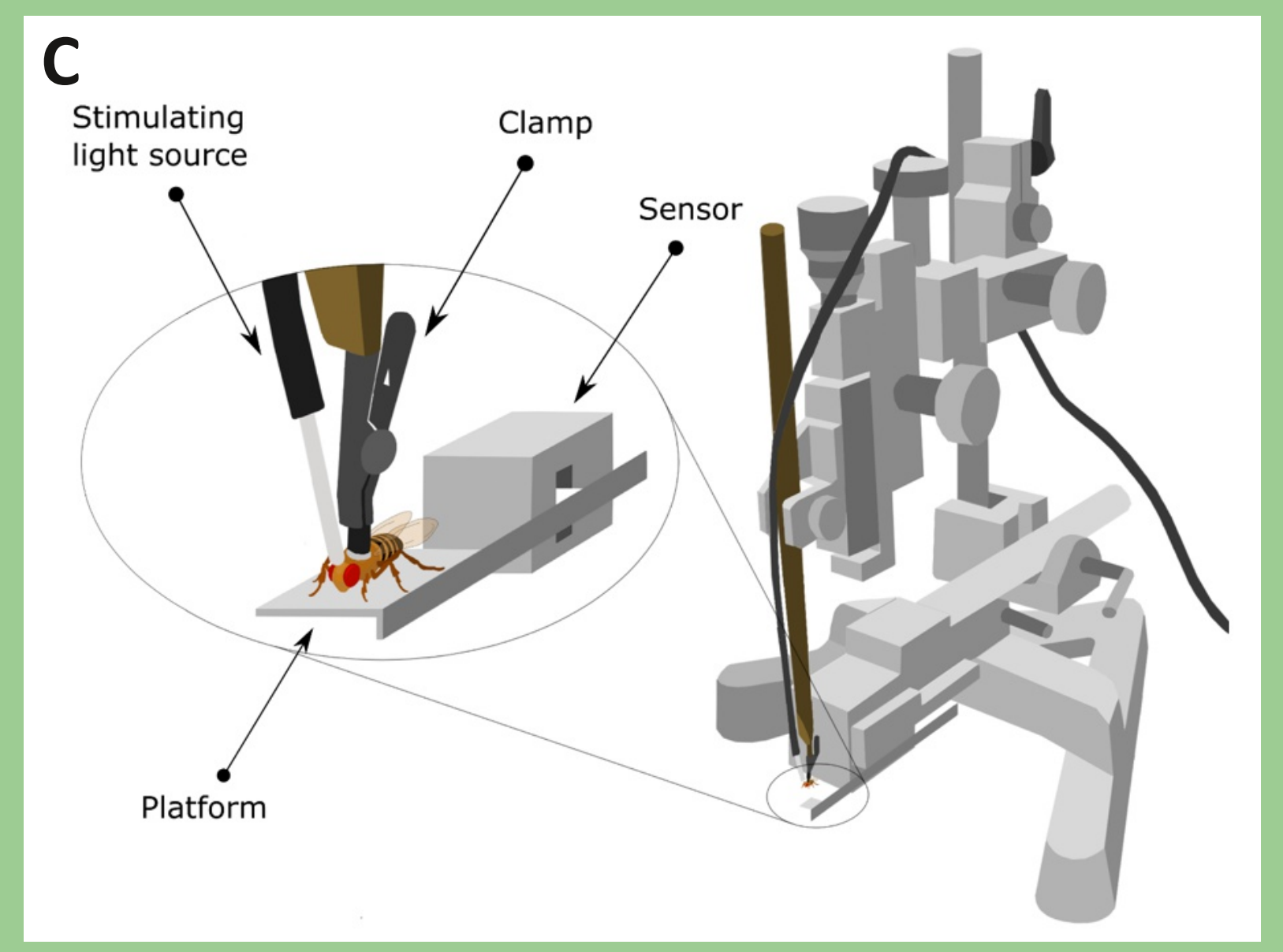


Two dopaminergic lines emerged from the screens that showed consistent behavior in all four experiments: TH-D1 (A1, B1, C1) and TH-D‘ (A2, B2, C2). Both lines show expression in three dopaminergic clusters: PPL1, PPM2 and PPM3. A-B: Brain, C: Ventral nerve cord. Cell clusters and neuropils (bold italics) in white abbreviations: PPL: paired posterior lateral; PPM: paired posterior medial; dFB: dorsal fanshaped body; vFB: ventral fan-shaped body; LAL: lateral accessory lobe; vMB: vertical lobes of the mushroom bodies. The THD-1 line also appears to stain SiFa peptidergic neurons (D). Scale bar = 50µm

Experimental setups

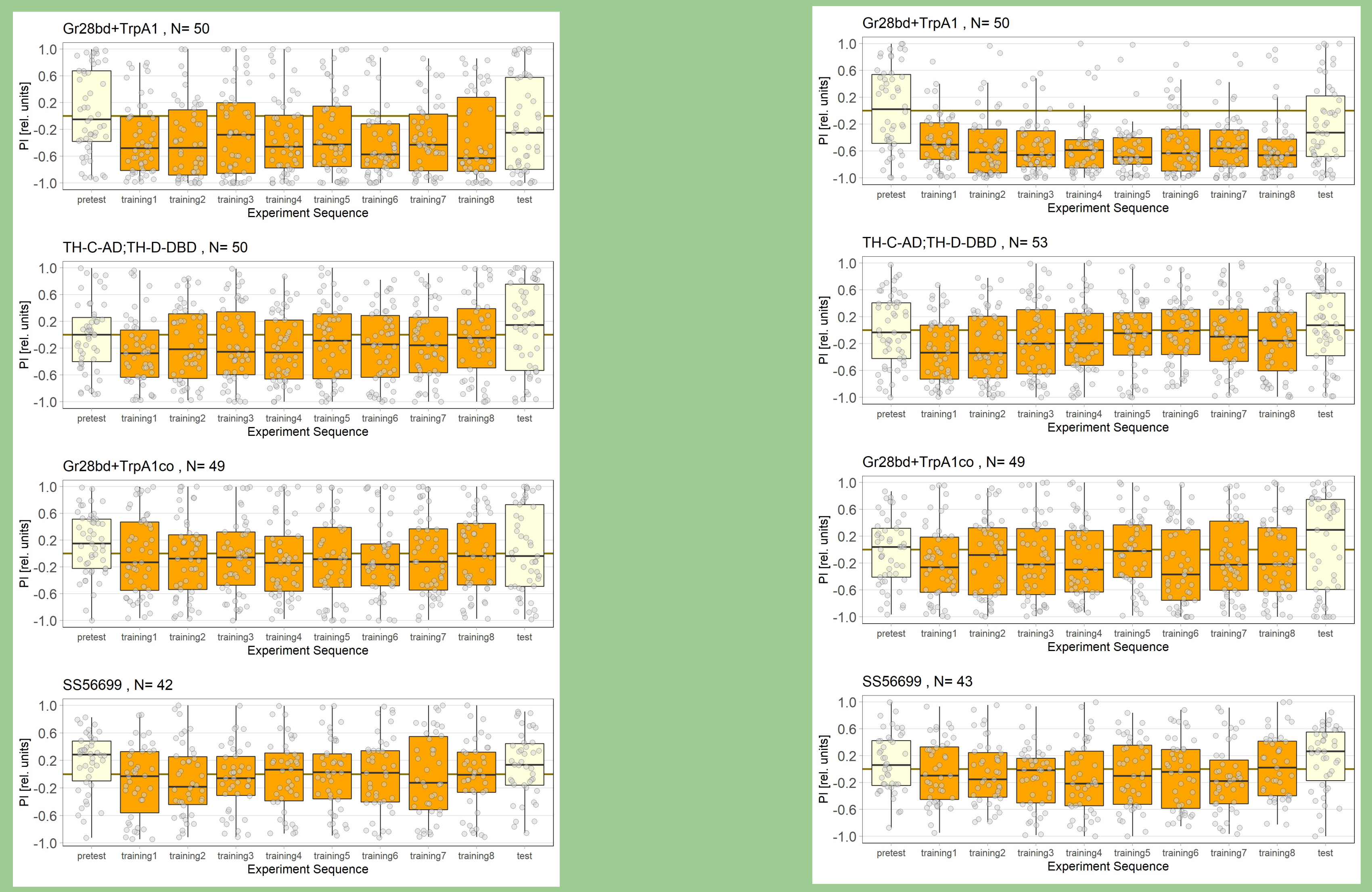




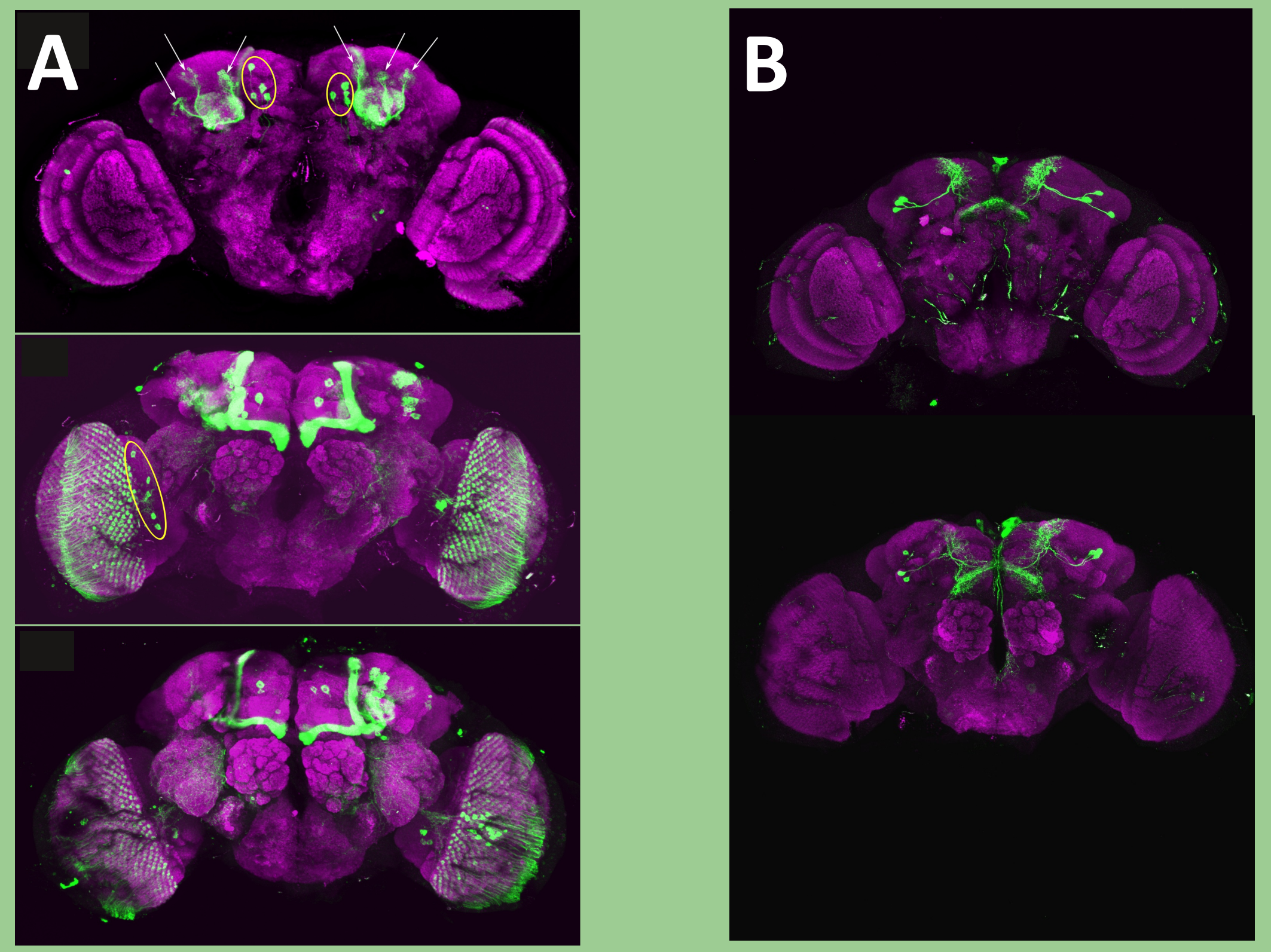


A: The T-mazes are composed of a core and three removable arms. An elevator transfers the flies from the entrance arm to the choice arms. One arm is illuminated and the other one dark. After 60 seconds choice time the flies get anaesthetized and counted. B: The Y-maze-setup consists of a Perspex block containing 120 Y-mazes. They are backlit with an infrared LED panel and a diffuser to scatter light homogeneously. Single walking flies can freely explore three arms, one of which is illuminated with the optogenetically stimulating light. Each arm gets reinforced for 20 minutes. C: The Joystick consists of a small platform that can be moved laterally and a clamp with an attached light source and light guide. A single fly tethered to the clamp gets lowered onto the platform and is allowed to freely move it with its legs. A sensor records the platform’s position and one side is reinforced by

Results

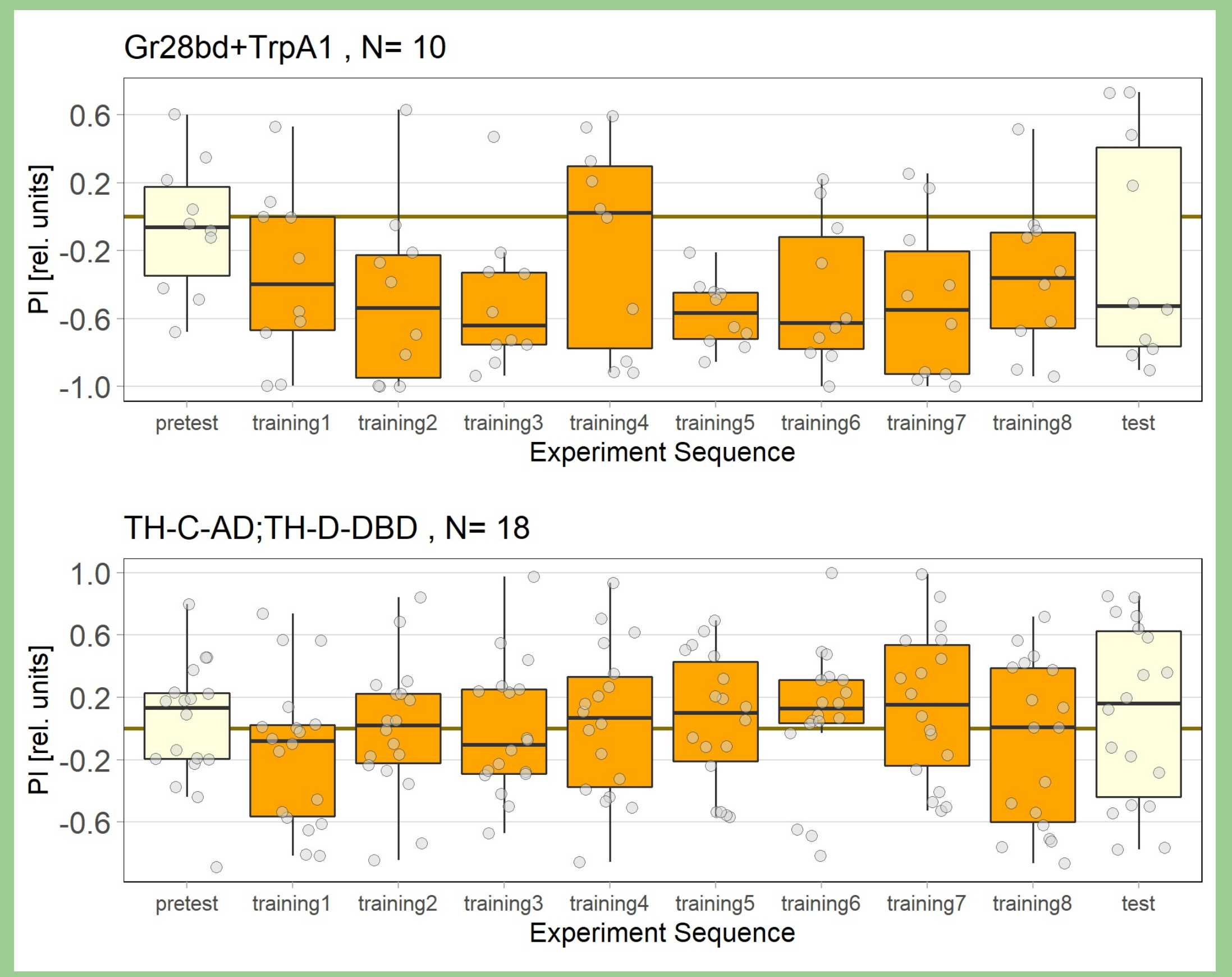


Testing the PPL1 and the PPM2 cluster individually, with red **(left)** and yellow **(right)** light in the Joystick apparatus. The experiment is divided into ten periods of 1 minute duration. In the first and last minutes (test, light yellow) only the spontaneous preference of the fly where to hold the platform is recorded in darkness. In the other periods (training, orange) the fly uses platform position as a light-switch for the optogenetic light. For half of the flies, light is on with a platform position to the right, for the other half the contingency is reversed. **Gr28bd+TrpA1**: Control flies expressing CsChrimson in heat-sensing neurons avoid optogenetic activation throughout the training periods, with an after-effect on platform position with the light permanently switched off (light yellow). **Gr28bd+TrpA1co**: Negative control flies expressing CsChrimson in heat-sensing neurons with no optogenetic activation. **TH-C-AD;TH-D-DBD** Flies expressing CsCHrimson in the PPM2 cluster. **SS56699**: Flies expressing CsChrimson in the PPL1 cluster. For detailed expression patterns see below.



Expression pattern of dopaminergic driver lines used above: **A: TH-C-AD;THD-DBD** targeting the PPM2 cluster. **B: SS56699** targeting the PPL1 cluster.

Preliminary



Is dopamine responsible for the optogenetic behavior? Comparing **Gr28bd+TrpA1** positive control flies (above) with **TH-C-AD;TH-D-DBD** (PPM3, below) flies fed with the dopamine synthesis inhibitor 3-iodo-tyrosine.

Outlook

- Test whether **3-iodo-tyrosine** inhibits DA synthesis using the chosen method of feeding flies to confirm wether the observed behavior is DA-dependent
- **PPL1** neurons will be tested with the line: **TH-Flp-p10;R64H06** to complete the testing of different DAN-clusters targeted by **THD'.**

References

- Rohrsen, Christian; Kumpf, Aida; Semiz, Kader; Aydin, Ferruh; deBivort, Benjamin; Hütteroth, Wolf; Brembs, Björn (2021): Pain is so close to pleasure: the same dopamine neurons can mediate approach and avoidance in Drosophila. In: bioRxiv 2021.10.04.463010.

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