

Description of FlyVac and Y-maze apparatus

FlyVac-2 is a high-throughput device designed for automatically assessing phototaxis in single flies, with 27 parallel behavioral modules (Krams et al., 2021). This system improves upon the original FlyVac developed by the de Bivort lab (Kain et al., 2012) (Fig. 1, 2, 3, 4). This system improves upon the original FlyVac by reducing the number of modules from 32 to 27 (Kain et al., 2012), providing more space for working with flies. The FlyVac experiment begins by loading individual flies from their storage vials into separate behavioral modules, each containing a phototactic T-maze. In each maze, both choice tubes terminate in a light-emitting diode (LED) (Fig. 5A; YouTube video: [<https://www.youtube.com/watch?v=ZFtIYY8oFZ0&t=7s>]). During each trial, one LED is randomly lit. Once a fly enters the maze and chooses to proceed left or right, FlyVac records the direction of the choice; randomizes the light and dark stimulus LEDs, and sends vacuum pulses to the module. After crossing an infrared beam in the choice tube, the associated vacuum line activates, pulling the fly back to the start tube's vacuum trap. This trap, designed to cushion the fly's landing, mitigates injury (Fig. 5B, C). The control field-programmable gate array (FPGA) unit records each fly's choice relative to the illuminated LED. Each experiment typically records 40 choices per fly. During testing, flies do not exhibit learning or adaptation to their light-choice probability, which is expected as they experience vacuum pulses regardless of their phototactic decisions. However, they do learn that they will be pulled back after choosing a T-maze tube.

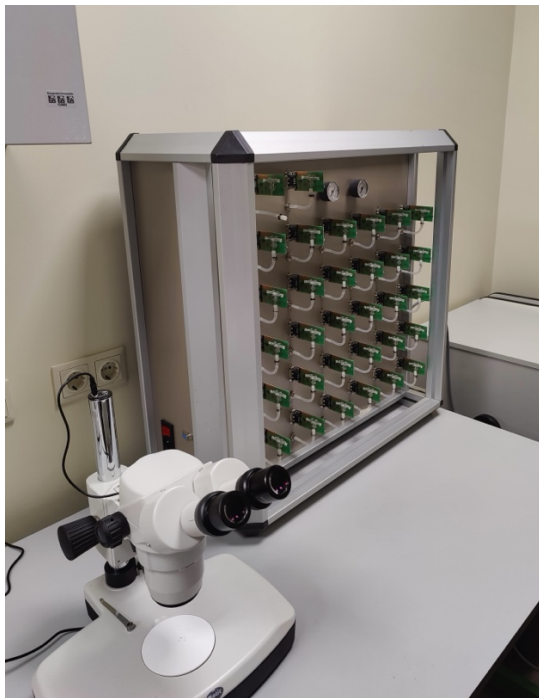


Fig. 1. The FlyVac-2 device in the Krams lab.



Fig. 2. The 27 individual modules to study phototaxis in 27 fruit flies simultaneously.



Fig. 3. A close-up of the FlyVac-2.

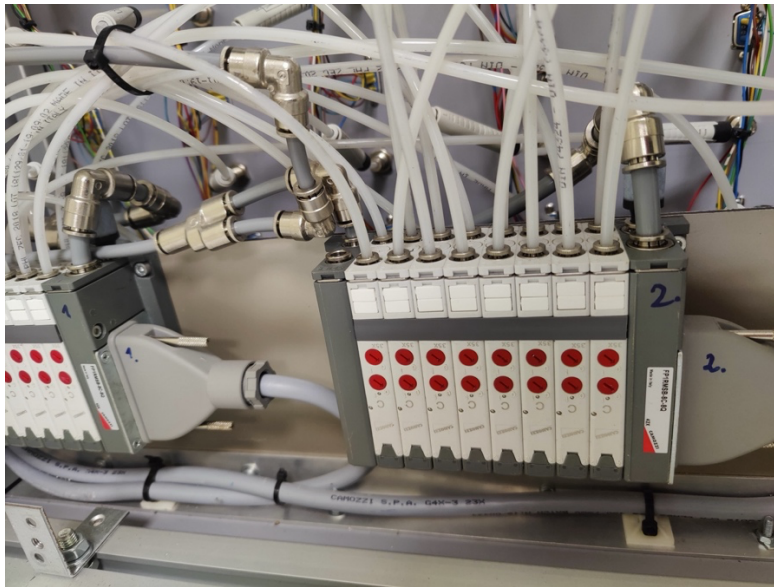


Fig. 4. Valves and vacuum tubes of FlyVac-2.

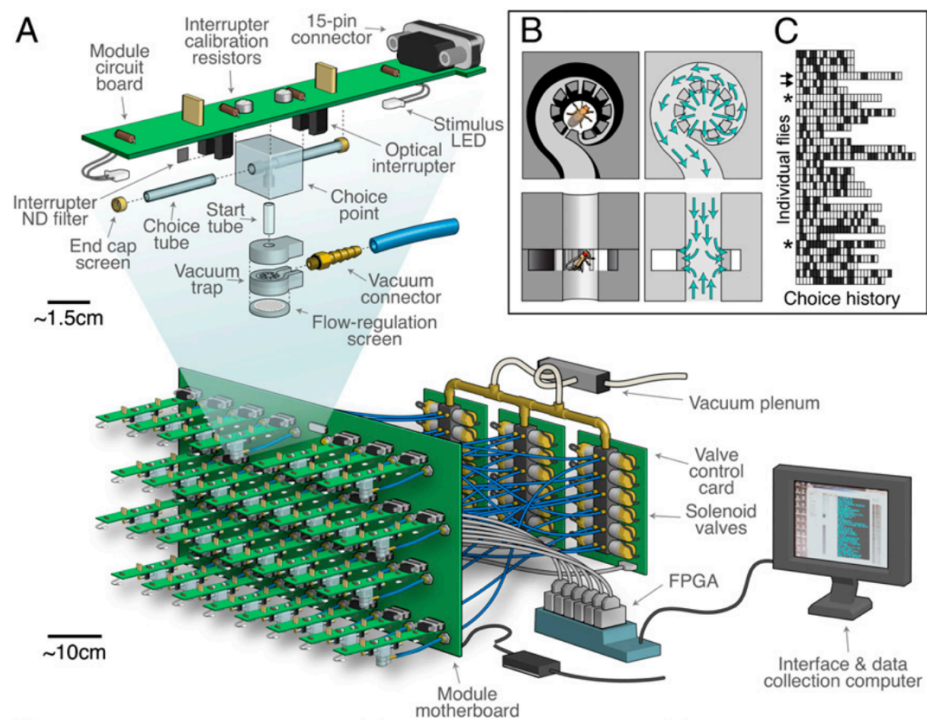


Fig. 5. A scheme of the FlyVac high-throughput device for automatically assessing phototaxis in 32 fruit flies in parallel (the de Bivort Lab).

The Y-maze equipment

The Y-maze system is an automated, high-throughput assay designed to analyze large sample sizes necessary for recording variance as a phenotypic trait. To determine whether fruit flies exhibit individual locomotor handedness and how erratic is the turning behavior, we developed a high-throughput assay to quantify turning behavior in the Y-maze, allowing us to assess several hundred flies in a single day (Ayroles et al., 2015; Buchanan et al., 2015; Krama et al., 2023) (Fig. 6, 7). During experiments, flies are individually placed in Y-shaped mazes, each covered by a translucent plastic cover (Fig. 8). We use two types of arrays, each containing either 60 or 95 individual Y-mazes, which consist of three symmetrical arms (each 12 mm long) constructed from laser-cut acrylic (Fig. 6, 7) (Krama et al., 2023). Once all flies are inserted into their respective mazes, the arrays are illuminated from below by a grid of 100 white LEDs (5500K) diffused through acrylic. High-resolution digital cameras capture the flies' movements (Fig. 9, 10), and their X-Y centroid positions are automatically tracked and recorded using custom-written software in LabView (National Instruments, USA) (Buchanan et al., 2015) or Ethovision (Noldus, Inc) (Krama et al., 2023). We record turning behavior for two hours (YouTube video for students: [<https://www.youtube.com/watch?v=ZFtIYY8oFZ0&t=7s>]). This Y-maze system enables the detection of individual handedness and the predictability of left-right choices. Each fly is tested only once. To quantify turning predictability—measuring variability in turning bias across individuals—we compute the median absolute deviation (MAD) from each observation's median (Buchanan et al., 2015; Krama et al., 2023), a robust metric resistant to outliers. MAD is estimated for each experimental group.



Fig. 6. Y-maze arrays of a high-throughput device for studying the body lateralization of 95 fruit flies simultaneously.

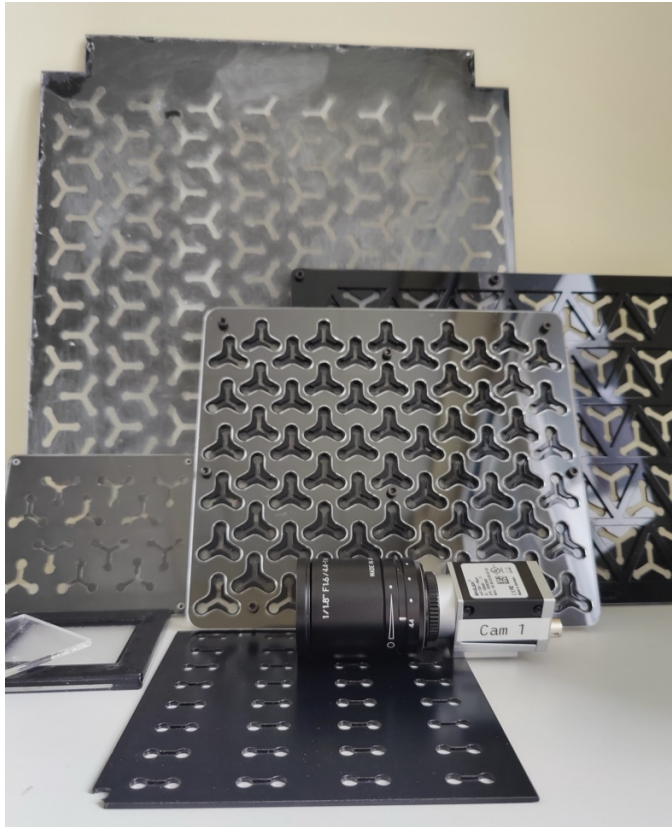


Fig. 7. Y-maze arrays of a high-throughput device for studying the body lateralization of 60 and 95 fruit flies simultaneously.

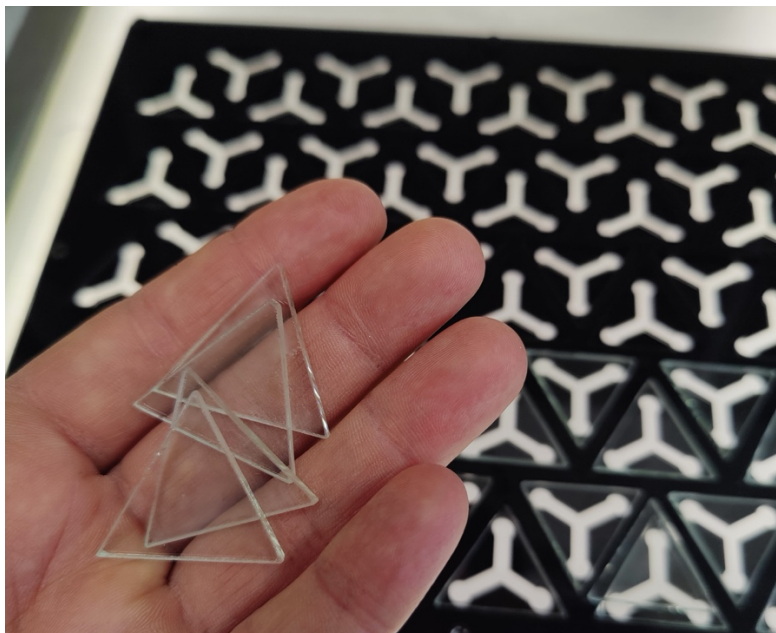


Fig. 8. Clear acrylic lids (one per maze) are lubricated with Sigmacote (Merck: Sigma Aldrich) to inhibit the flies from flipping upside down.



Fig. 9. An array of 60 individual Y-mazes and a video camera is set for a handedness experiment.

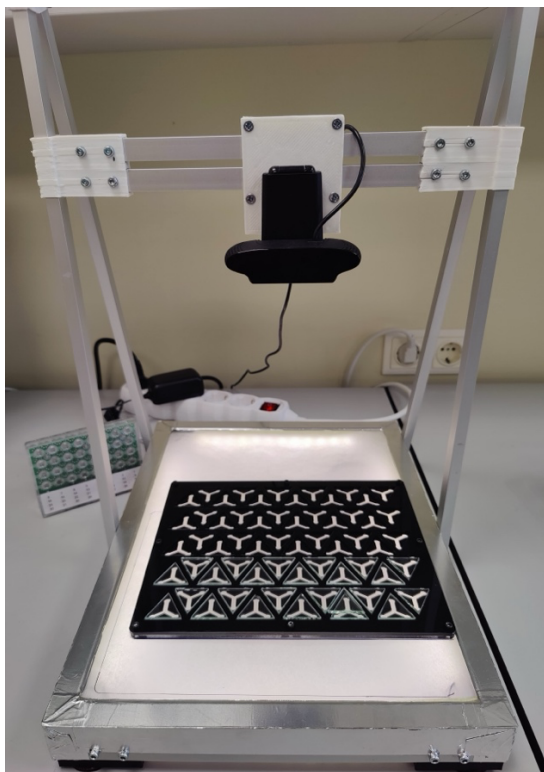


Fig. 10. Testing another kind of video camera.

References

Kain, J. S., Stokes, C. & de Bivort, B. L. Phototactic personality in fruit flies and its suppression by serotonin and white. *Proc. Natl. Acad. Sci.* **109**, 19834–19839 (2012).

Ayroles, J. F. *et al.* Behavioral idiosyncrasy reveals genetic control of phenotypic variability. *Proc. Natl. Acad. Sci.* **112**, 6706–6711 (2015).

Buchanan, S. M., Kain, J. S. & de Bivort, B. L. Neuronal control of locomotor handedness in *Drosophila*. *Proc. Natl. Acad. Sci.* **112**, 6700–6705 (2015).

Kain, J. S., Stokes, C. & de Bivort, B. L. Phototactic personality in fruit flies and its suppression by serotonin and white. *Proc. Natl. Acad. Sci.* **109**, 19834–19839 (2012).

Krama, T. *et al.* Development under predation risk increases serotonin-signaling, variability of turning behavior and survival in adult fruit flies *Drosophila melanogaster*. *Front. Behav. Neurosci.* **17**, (2023).

Krams, I. A. *et al.* Serotonergic modulation of phototactic variability underpins a bet-hedging strategy in *Drosophila melanogaster*. *Front. Behav. Neurosci.* **15**, (2021).