FLEXIBLE VALENCE CODING BY DOPAMINERGIC NEURONS IN *DROSOPHILA*

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Background

Dopaminergic neurons (DANs) are widely implicated in encoding behavioral valence, yet it remains unclear whether specific DAN populations convey fixed appetitive or aversive signals across behavioral contexts. To address this question, we performed a large-scale screen of DAN driver lines in operant self-stimulation paradigms in *Drosophila melanogaster*. Contrary to the idea of a centralized and conserved valence system, we found no evidence that particular DAN populations consistently mediated the same valence across experimental conditions. Instead, one driver line (TH-D'-Gal4; ~40 DANs in the PPL1, PPM2 and PPM3 cluster) exhibited a striking time-dependent shift in behavioral preference: Flies initially avoided optogenetic DAN activation but later developed a mild attraction (Figure 1).

Here, we characterize the neuronal subpopulation underlying this effect and investigate its mechanistic basis. We test the contribution of dopaminergic signaling using the dopamine (DA) synthesis inhibitor 3-Iodo-L-tyrosine (3IY) and determine whether neuronal activation alone is sufficient to induce the valence shift.

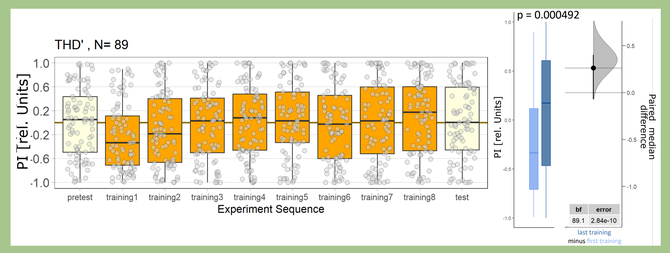


Figure 1: JoyStick experiment with TH-D'-Gal4>NorpA;20xUAS-Chrimson
Flies initially avoid optogenetic activation of targeted neurons but gradually increase their preference index (PI) across training periods. During the final training period, flies exhibit a weak preference for the optogenetically reinforced platform position. **Left:** Training and test periods. Yellow boxes indicate pre-test and test periods without optogenetic stimulation; orange boxes indicate training periods in which one platform position triggers optogenetic stimulation. **Right:** Boxplot and estimation statistics showing the behavioral shift from the first (light blue) to the last (dark blue) training period (last training – first training), with the corresponding Bayes factor (bf).
Boxplots depict medians (solid lines), quartiles (boxes) and non-outlier ranges (whiskers), with individual data points in gray.

Valence shift mediated by PPL1 and PPM2 dopaminergic neurons

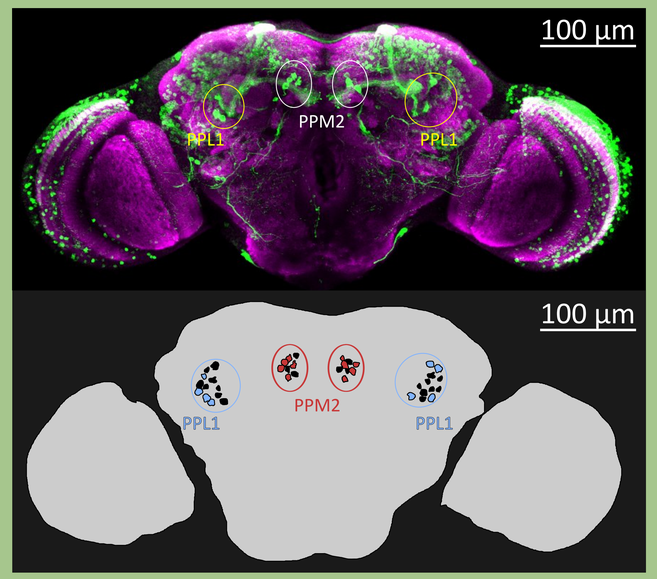


Figure 2: Expression pattern of TH-C-AD;TH-D-DBD>mCD8::GFP
Top: Maximum-intensity projection of confocal image substacks. Confocal images show expression in several DANs of the protocerebral posterior medial cluster 2 (PPM2) and protocerebral posterior lateral cluster 1 (PPL1). Additional expression is observed in putatively non-dopaminergic neurons of the optic lobes and subesophageal zone, as well as in Kenyon cells. Identification of individual neurons by connectomic analysis is ongoing. Magenta: neuropil marker; Green: GFP.
Bottom: Schematic summary of the expression pattern. Colored cells indicate the mean number of labeled neurons per dopaminergic cluster.

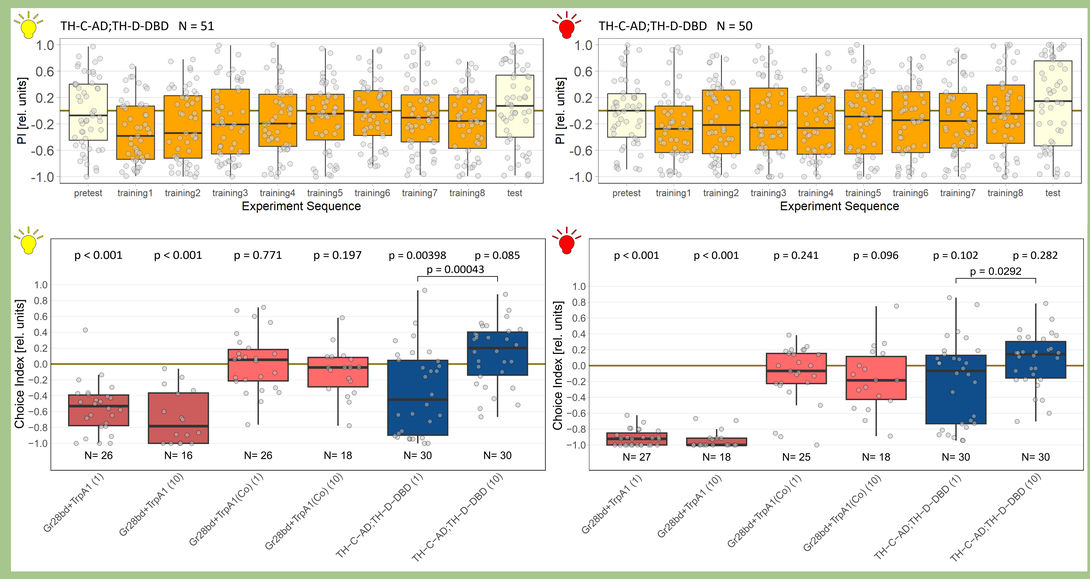
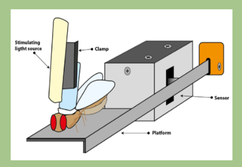


Figure 3: Valence associated with activation of TH-C-AD;TH-D-DBD positive neurons shifts over the course of ten minutes across behavioral paradigms
Top: Performance indices (PIs) in the JoyStick during test and training periods. Flies initially avoid optogenetic stimulation, but PIs increase over time, indicating a gradual shift towards approach behavior. Sample sizes are shown above the plots. Yellow bars indicate pre-test and test periods without optogenetic stimulation; orange bars indicate training periods in which one platform position triggers optogenetic stimulation.
Bottom: T-Maze choice incides (CI) after 1 min and 10 min. The control driver (Gr28bd-Gal4;TrpA1-Gal4) targets heat-sensing neurons and flies show robust avoidance with (dark red), but no preference without ATR (light red; '(Co)') at both timepoints, confirming optogenetic-stimulation–dependent behavior. Test group flies (blue) avoid the illuminated arm at minute 1, but exhibit weak approach of optogenetic stimulation at minute 10. Sample sizes are shown below plots. p-values above boxes indicate comparisons to zero; p-values above brackets indicate between-group comparisons. The icon in the upper left corner of each panel indicates the color of the stimulating light.

Experimental paradigms

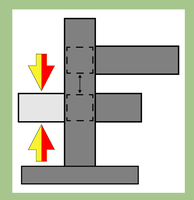
JoyStick

The joystick paradigm tests valence of optogenetic activation in flies making a non-locomotor motor decision. Individual flies are tethered on a platform which can be moved left or right, relative to the animals' longitudinal body axis. Displacement of the platform to one side will activate LEDs targeted to the fly's brain, while movement to the other side terminates stimulation.
The testing protocol consists of ten one-minute periods. Period 1 and 10 measure preference in the absence of stimulation, while periods 2-8 capture the animals' optogenetic preference (see Figure 1).



T-Maze

The T-maze tests the valence of optogenetic DAN activation in groups of freely moving flies making a binary spatial choice. Pushing down the elevator transfers flies to the choice point, where they can move freely between two arms for either one or ten minutes. One arm is illuminated by LEDs, inducing optogenetic stimulation of target neurons, while the other arm is devoid of light, allowing the animals to operantly control stimulation.

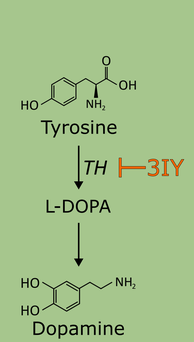


Index calculation

To determine the valence associated with optogenetic stimulation of neurons we calculate preference (PI) or choice indices (CI) based on platform position and animal count per tube, respectively.

$$PI\ /\ CI = \frac{(Light - Dark)}{(Light + Dark)}$$

Valence shift is DA dependent; Heat-avoidance is not



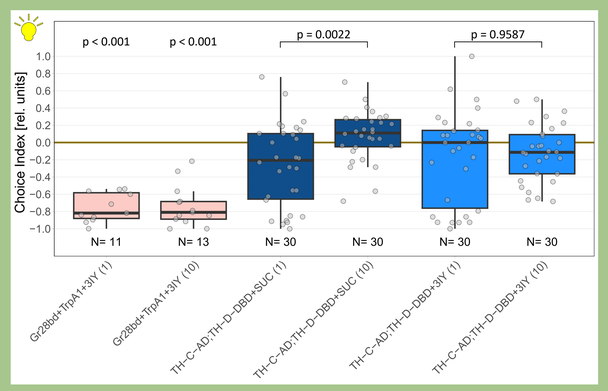
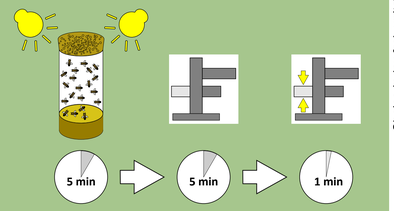


Figure 4: Dopamine depletion experiments with 3-Iodo-L-Tyrosine (3IY) show dopamine dependency of the behavioral switch
Left: Inhibition of dopamine synthesis by 3IY.
Right: Choice indices of T-maze experiments following dopamine depletion. Strong avoidance in the Gr28bd-Gal4;TrpA1-Gal4 flies (pink) confirms the ability to express optogenetic preference in DA depleted flies. In addition, this observation indicates that the evaluation of heat-sensation and the resulting avoidance behavior are DA independent. In contrast, inhibiting DA synthesis in TH-C-AD;TH-D-DBD flies (light blue) abolished both initial avoidance and the behavioral shift from one to ten minutes. Undepleted flies (dark blue) retain the behavioral shift.

No valence shift without operant control of stimulation



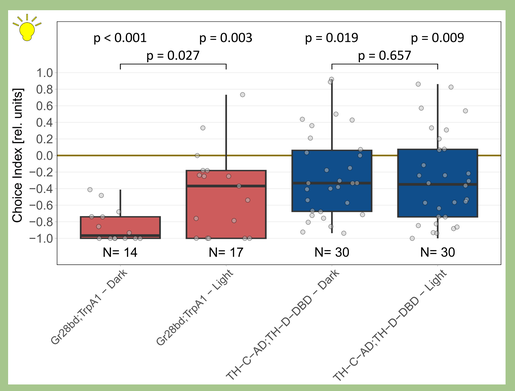


Figure 5: One-minute T-maze choice following prior light-exposure shows no change without operant control
Left: Stimulation protocol: Flies were exposed to 5 minutes of uncontrollable optogenetic stimulation, followed by a 5 minute resting period. Subsequently, we record the behavior in the 1-minute choice test.
Right: Choice indices of naive and exposed flies: Control flies (red) avoid the illuminated arm under standard conditions ('Dark') and show reduced but still significant avoidance following prior exposure to the stimulating light ('Light'). Naive and exposed TH-C-AD;TH-D-DBD flies (blue) show similar avoidance, indicating that operant control over the stimulation is necessary for a shift in DAN activation associated valence. Sample sizes are shown below the plots. p-values above individual boxes indicate comparison to zero; p-values above brackets indicate between-group comparisons.

Reference

Rohrsen, Christian; Kumpf, Aida; Semiz, Kader; Aydin, Ferruh; deBivort, Benjamin; 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. DOI: 10.1101/2021.10.04.463010.

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