Behavioral Flexibility in *Drosophila* Phototaxis

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Abstract

Animals exhibit innate preferences for different stimulus modalities and intensities, which likely reflect evolutionary adaptations to specific ecological needs. For instantece, insects such as *Drosophila* move towards a light source when startled. Given the robustness of this response, positive phototaxis has been categorized as an example of a hard-wired input-output behaviors. However, classic experiments performed by McEwen in 1918 and Benzer in 1967 demonstrated that wing defects, caused by mutation or damage, profoundly affect phototaxis preferences in walking *Drosophila*[1,2]. The fact that manipulating an unrelated organ (wings) affects positive phototaxis contradicts the notion of it as hard-wired, and indicates the presence of a certain element of flexibility. Therefore, we evaluated flies in two different phototactic paradigms (Benzer Countercurrent Apparatus and T-Maze) after altering their flying ability using a range of mechanical and genetic manipulations.

Here we show that flies unable to fly exhibit a negative phototactic behavior. This reversal is not learned, as neither learning mutants nor transgenic flies deficient in various learning paradigms show any deficit, and the effect is immediate. The effect is neither due to injury, as injuries not affecting flight ability do not affect phototaxis. Genetic manipulations preventing the flies from flying but leaving wing-morphology intact also affect phototaxis. Finally, if flying ability is temporarily compromised and then restored, the phototactic behavior changes concomitantly, demonstrating the reversibility of the phototactic effect. These results reveal the flexibility of this taxis, and the existence of an evaluation step prior to behavioral performance.

We hypothesize that phototaxis is not just an automated response, but rather it has a central decision-making stage. Moreover, if even this iconic and simple hard-wired behaviour consist of an action selection step, more complex behaviours should be also built on decision-making blocks.

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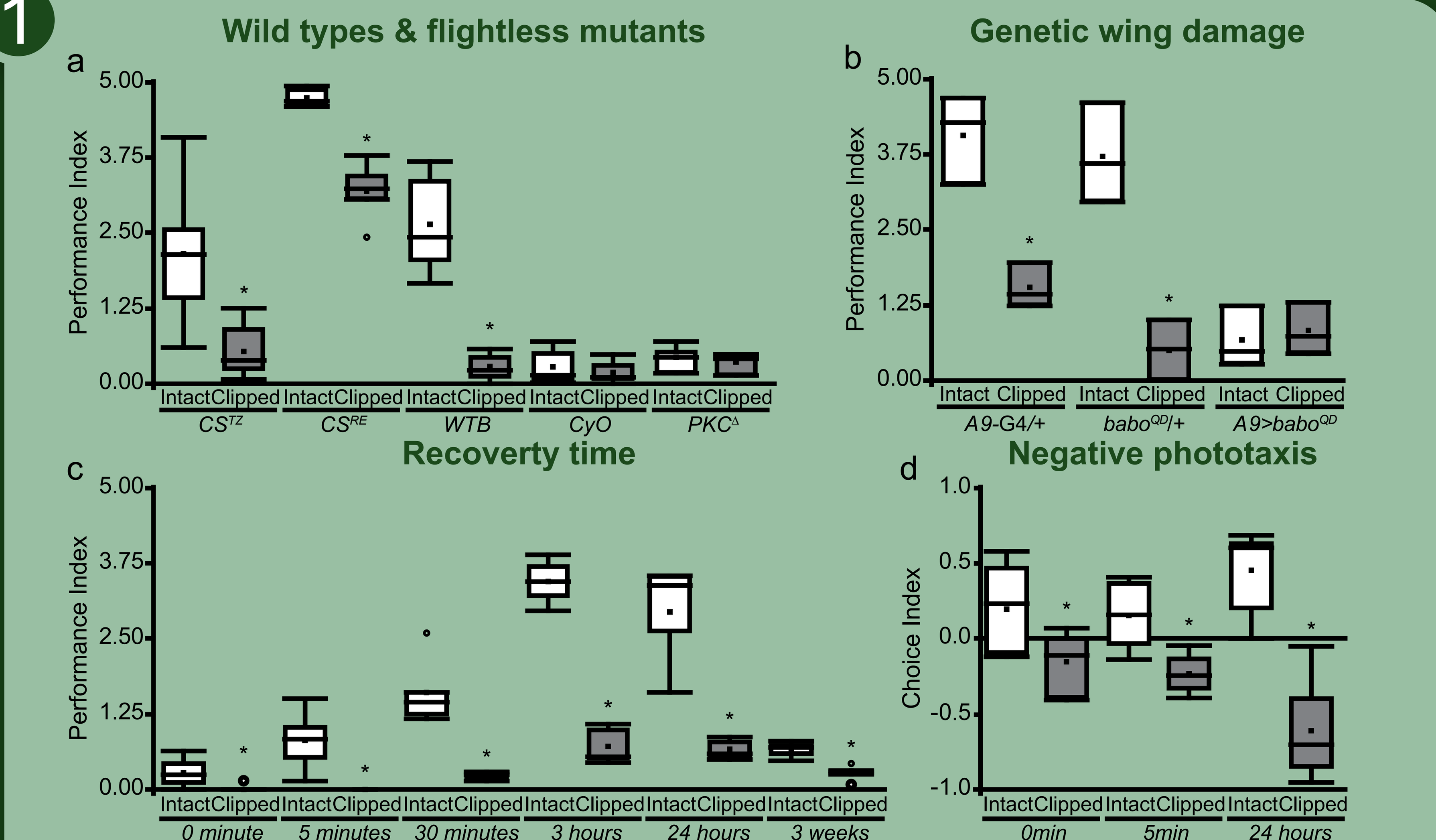

Flies become negatively phototactic after losing their wings. **a,** BCP Performance Index from three wild type strains and two flightless mutants with intact and clipped wings. Paired T-test; *CS*^{TZ}: N=6, p=0.0036; *CS*^{RE}: N=5, p=0.0003; *WTB*: N=12, p<0.0001; *CyO*: N=14, p=0.0663; *PKC*$^{\Delta}$: N=4, p=0.4130. **b,** BCP Performance Index from flies with a genetic manipulation of wing development (*A9>babo*^{QD}) and their parental control (*A9*-G4/+, *babo*^{QD}/+). Randomize Blocked ANOVA; N=3; Block p<0.0001, Interaction Genotype vs Wings Integrity: p<0.0001, simple effect Genotype: *A9*-G4/+: p<0.0001, *babo*^{QD}/+: p<0.0001, *A9>babo*^{QD}: p=0.4014. **c,** BCP Performance Index of *WTB* flies after different recovery time lengths. Paired T-Test, 0 minutes: N=6, p=0.0227; 5 minutes: N=6, p=0.0084; 30 minutes: N=5 , p=0.007 ; 3 hours: N=5, p<0.0001 ; 24 hours: N=5, p=0.0052 ; 3 weeks: N=5 , p=0.0042 . **d,** T-Maze Choice Index after different recovery time lengths. Paired T-Test, 0 minutes: N=7, p=0.0033; 5 minutes: N=6, p=0.0255; 24 hours: N=6, p=0.0004.* indicates significant differences. Box plot show quantiles 0.05, 0.25, 0.75 and 0.95, median, mean (black square), and outliers (circle).

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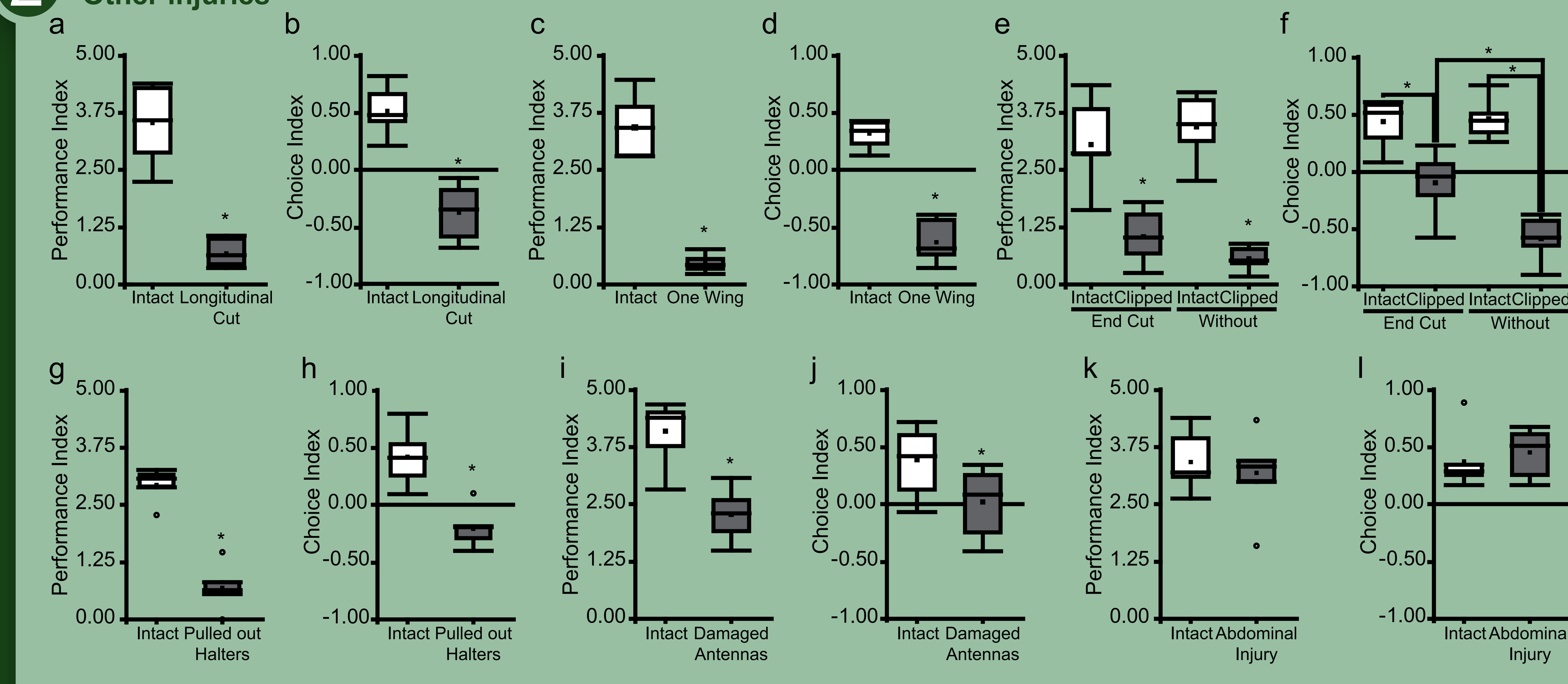
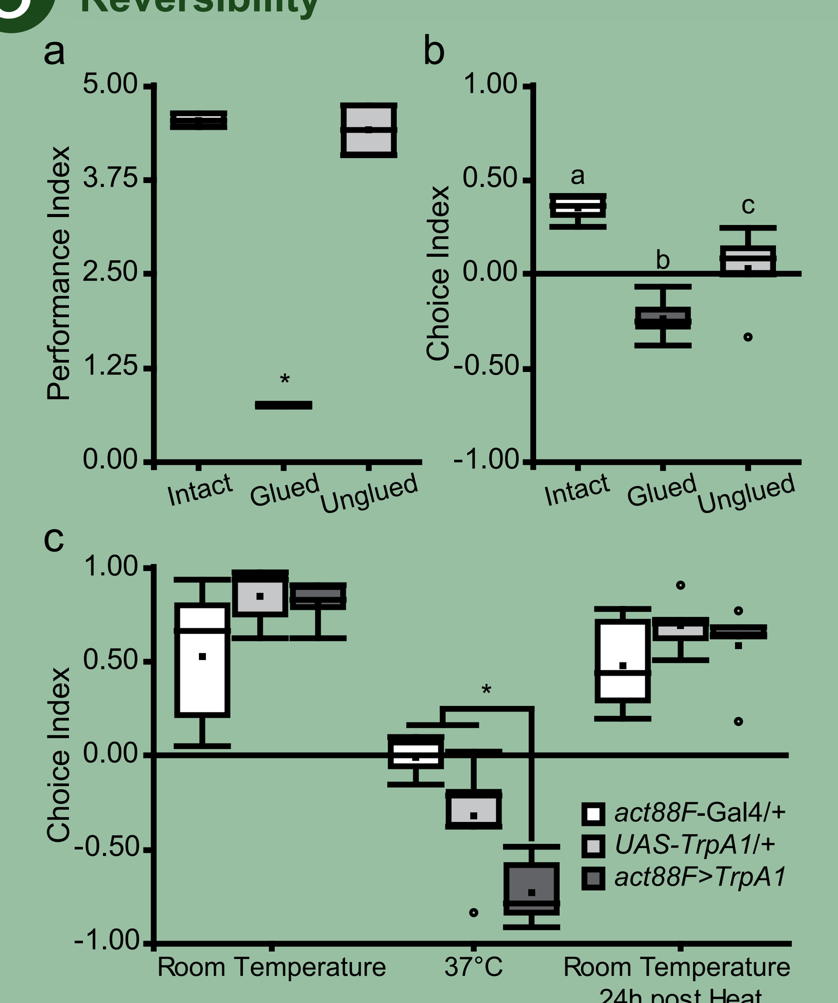


Only injuries affecting flying capability promote a behavioural switch. **a, c, e, g, i, k,** BCP Performance Index from *WTB* flies with and without different injuries. **b, d, f, h, j, l,** T-Maze Choice Index from *WTB* flies with and without different injuries. **a, b,** Longitudinal cut of the wings. N=7, a: p<0.0001, b: p<0.0001. **c, d,** Only one wing cut. N=7, a: p<0.0001, b: p=0.0001. **e, f,** Wing clipped at different lengths. Randomize Blocked ANOVA; N=6; e: Block p= 0.0939, Interaction Wings Integrity (intact or clipped) vs Degree of Injury (without wing or end of the wing cut): p=0.0868, Wings Integrity: p<0.0001, Degree of Injury: p=0.7971; f: Block p= 0.2378, Interaction Wings Integrity vs Degree of Injury: p=0.0071, simple effects: end cut vs intact: p<0.0004, without wings vs intact: p<0.0001, end cut vs without wings: p=0.0007, intact (control from end cut) vs intact (control from without wings): p=0.8648. **g, h,** Both halteres pulled out. g: N=5, p=0.0001, h: N=7, p=0.0001. **i, j,** Both antennas damaged. i: N=6, p=0.0004, j: N=7, p=0.0403. **k, l,** abdominal wound. k: N=6, p=0.3765, l: N=6, p=0.5517. **a, b, c, d, g, h, i, j, k, l,** Paired T-Test. See figure 1 for detailed graph information.

3 Reversibility



The phototactic change is fully reversible and accompanies flying ability. **a, b,** Gluing and ungluing the wings. **a,** BCP Performance Index from *WTB* flies with intact, glued and unglued wings. One way ANOVA, N=2, p=0.0014, Tukey's post hoc test (p<0.05; least-significant difference=1.169). **b,** T-Maze Choice Index from *WTB* flies before and after gluing their wings, and after ungluing them. Randomize Blocked ANOVA, N=5, Block p=0.1728, ANOVA p=0.0003, Tukey's *post hoc* test (p<0.05; least-significant difference=0.253). Different letters indicates significant differences. **c,** Genetic manipulation of Indirect Flight Muscles contraction and flying ability. T-Maze Choice Index before during and after 37°C exposure of experimental and control flies. Randomize blocked ANOVA, N=5, Block p=0.1522, Interaction Genotype vs Temperature: p= 0.0003, simple effects: Room Temperature: p=0.0730, 37°C: p<0.0001, Tukey's post hoc test (p<0.05; least-significant difference=0.3490), Room Temperature 24h post heat: p=0.3441. See figure 1 for detailed graph information.

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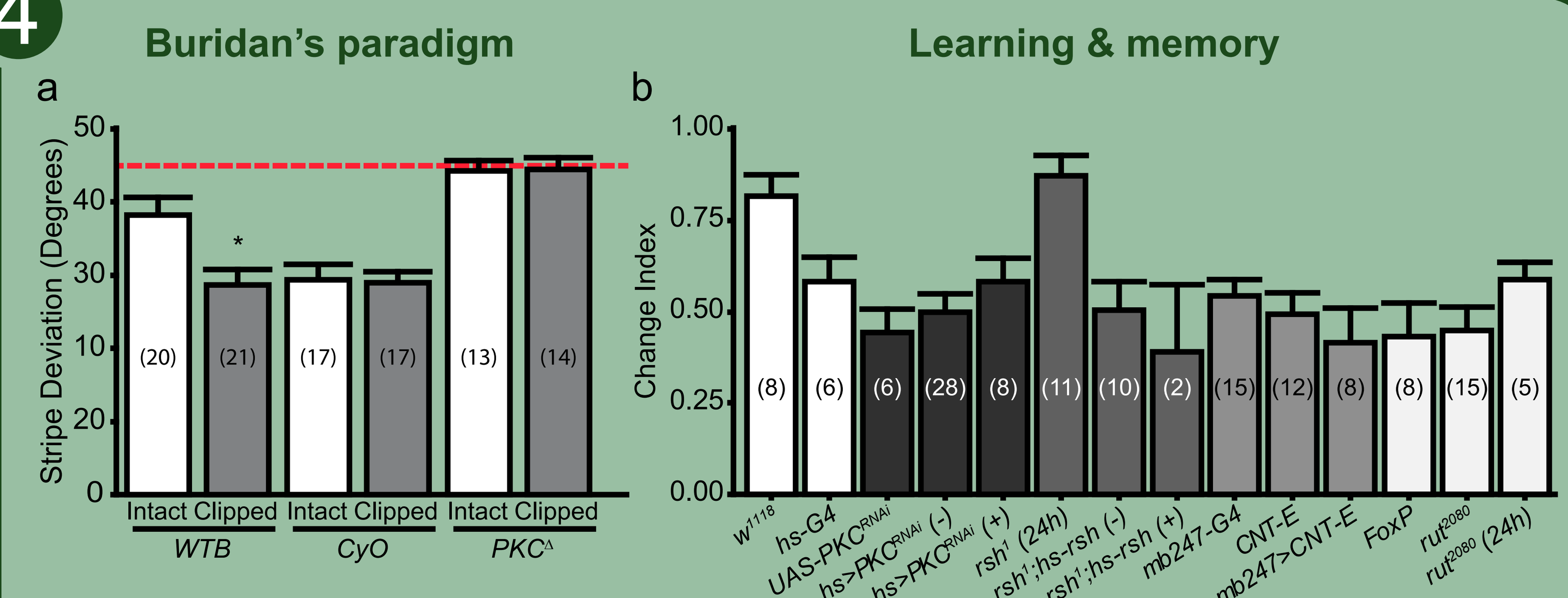

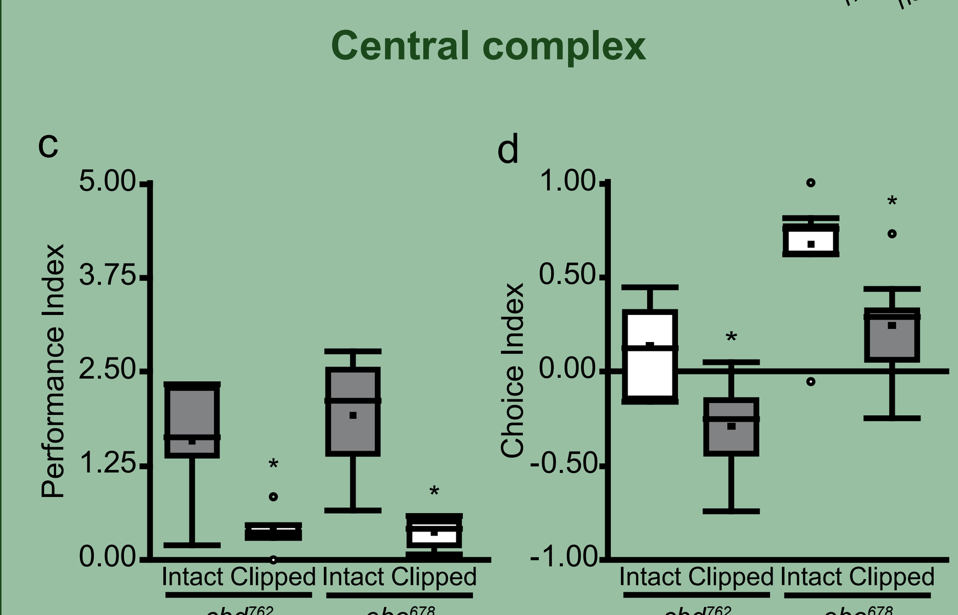

Flight disability also affects black object preference, and the effect on phototaxis is independent from Memory and Learning processes, and Central Complex integrity. **a,** Stripe deviation in Buridan's Paradigm from a *WTB*, *CyO* and *PKC*$^{\Delta}$ with and without wings. Two ways ANOVA, Interaction Genotype vs Wing Integrity: p= 0.0213, orthogonal contrasts: *CyO* Intact vs Clipped p=0.8812, *PKC*$^{\Delta}$ Intact vs Clipped p=0.9353, *WTB* Intact vs Clipped p=0.0004. N=Numbers in brackets . **b,** Proportion of change in Performance Index after wing clipping (Change Index) from several lines with learning and memory impairments and their controls. N=Numbers in brackets . **c, d,** Behavioural performance from two structural Central Complex mutants with intact and clipped wings on BCP (c) and T-Maze (d). Paired T-Test. **c,** *cbd*^{762}, N= 6, p=0.0050, *ebo*^{678}, N= 6, p=0.0038. **d,** *cbd*^{762}, N= 8, p=0.0016, *ebo*^{678}, N= 7, p=0.0009. See figure 1 for detailed graph information.

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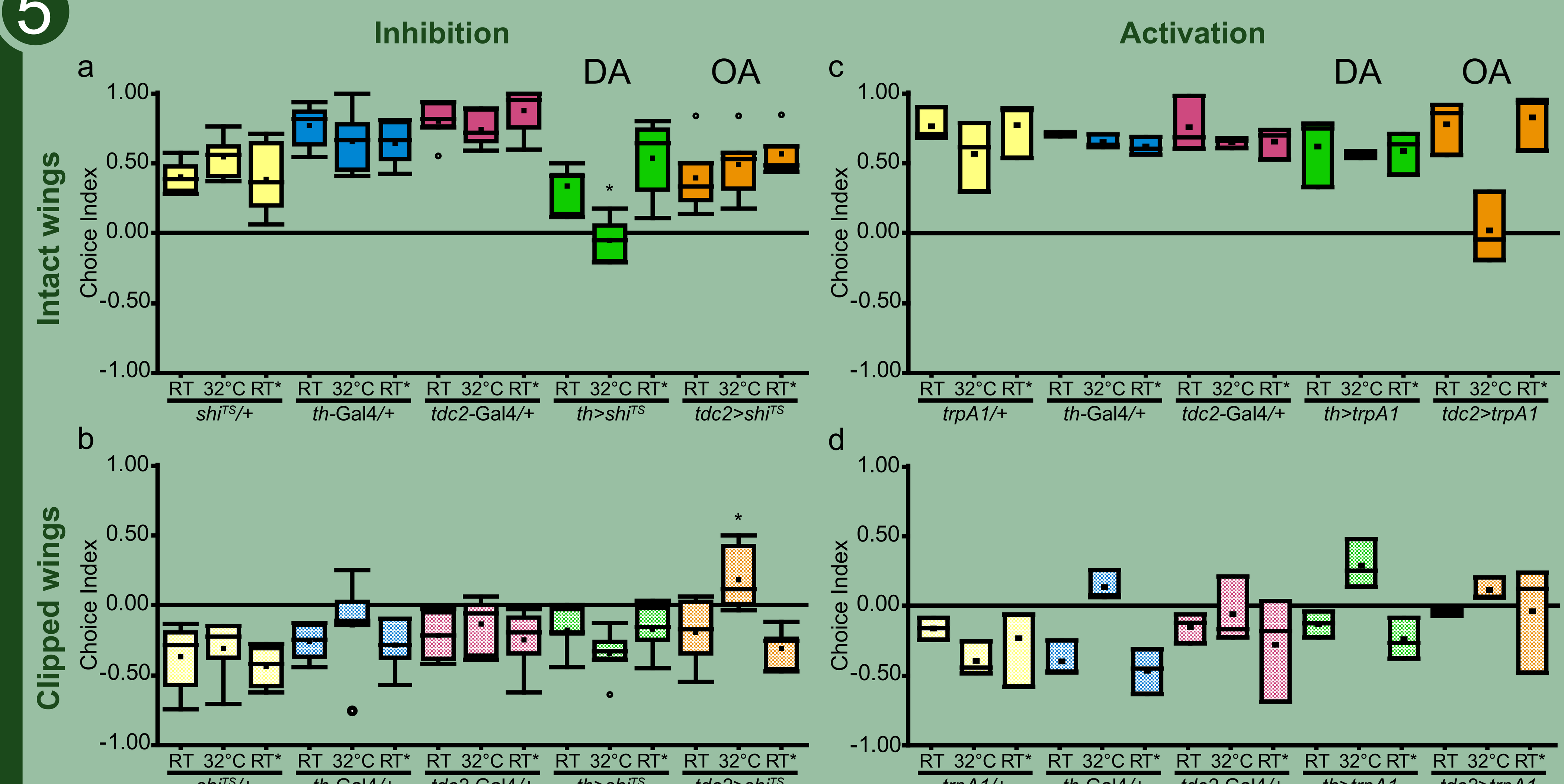

Dopamine and Octopamine are necessary and sufficient to modulate phototactic behaviour, but with opposite effects. **a, b,** Choice Index from flies with (a) and without (b) wings before, during and after Dopamine or Octopamine neurons silencing. **a,** Randomize Blocked ANOVA, Block p=0.0197, Interaction Genotype vs Temperature p=0.0001, simple effects with Tukey's post hoc test (p<0.05): *shi*^{TS}/+ p=0.2082, *th*-GAL4/+ p=0.41681, *tdc2*-GAL4/+ p=0.4278, *th>shi*^{TS} p<0.0001, *tdc2>shi*^{TS} p=0.1567. *shi*^{TS}/+ , *th*-GAL4/+, *tdc2*-GAL4/+ and *th>shi*^{TS} N=6, least-significant difference=0.2404; *tdc2>shi*^{TS} N=5, least-significant difference= 0.2634. **b,** Randomize Blocked ANOVA, Block p=0.0069, Interaction Genotype vs Temperature p=0.0080, simple effects with Tukey's post hoc test (p<0.05): *shi*^{TS}/+ p=0.5331, *th*-GAL4/+ p=0.3938, *tdc2*-GAL4/+ p=0.5999, *th>shi*^{TS} p=0.2624, *tdc2>shi*^{TS} p=0.0001. *shi*^{TS}/+ , *th*-GAL4/+, *tdc2*-GAL4/+ and *th>shi*^{TS} N=6, least-significant difference=0.2776; *tdc2>shi*^{TS} N=5, least-significant difference= 0.3041. **c, d,** Choice Index from flies with (a) and without (b) wings before, during and after Dopamine or Octopamine neurons activation (ongoing experiment).

Conclusions

Manipulating the ability to fly exerts a fundamental effect on action selection in *Drosophila*. Specifically, the preference between light and dark is profoundly altered and this change manifests itself in several behavioral tests. The biogenic amines dopamine and octopamine, respectively, appear to be both necessary and sufficient for different aspects of the neurobiological processes underlying this change. This work shows that even behaviors previously assumed to be hard-wired and inflexible consist of a complex decision-making stage. This insight calls the general concept of brains as input-output devices into question.

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