A decision underlies phototaxis in an insect

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Background

Like a moth into the flame - Phototaxis is an iconic example for innate preferences. Such preferences likely reflect evolutionary adaptations to predictable situations and have traditionally been conceptualized as hard-wired stimulus-response links. Perhaps therefore, the century-old discovery of flexibility in *Drosophila* phototaxis has received little attention.

Results

Here we report that across several different behavioral tests, light/dark preference tested in walking is dependent on the flies' ability to fly. If we temporarily compromise flying ability, walking photopreference reverses concomitantly. Neuronal activity in circuits expressing dopamine and octopamine, respectively, plays a differential role in this case of behavioral flexibility.

Conclusions

We conclude that flies monitor their ability to fly, and that flying ability exerts a fundamental effect on action selection in *Drosophila*. This work suggests that even behaviors which appear simple and hard-wired comprise a value-driven decision-making stage, negotiating the external situation with the animal's internal state, before an action is selected.

Keywords

Behavioral Flexibility; Octopamine; Dopamine; Decision-making; Drosophila; invertebrates

Background

In their struggle for survival, animals need not just the capability to trigger behaviors at the appropriate time, but these behaviors need to be flexible in response to or anticipation of changes in environmental and internal conditions. What may be an appropriate response to a given stimulus when the animal is hungry may be maladaptive when the animal is seeking a mating partner, and *vice versa*. The relative values of extrinsic and intrinsic factors must be analyzed and weighed in order to shape the behavior to be adaptive in a particular situation. Across animal phyla, biogenic amines have been found to be part of a complex network involved in such value-driven processes. In invertebrates, Dopamine (DA) and Octopamine (OA) are two important modulators of behavior. OA, the invertebrate counterpart of the adrenergic vertebrate system, has been implicated in state-dependent changes in visual-processing [1,2], experience-dependent modulation of aggression [3], social decision-making [4], and reward [5]. DA is also known for its countless roles in physiological and behavioral processes across animal phyla such as reward [5–7], motivation [8,9] and value-based or goal-directed decision-making [8,10–14]. Complementing such flexible behaviors are simple, innate responses such as escape responses, taxis/kinesis behaviors, or fixed action patterns. They are commonly thought to be less flexible and more automatic, but with the advantage of either being especially efficient, fast, or with only a low cognitive demand. However, recent research has shown that many of these behaviors are either more complex than initially imagined [15–18] or liable to exploitation [19]. Due to observations like these, the general concept of behaviors as responses to external stimuli ('sensorimotor hypothesis') has come under ever more critical scrutiny in the last decade. Studying what can arguably be perceived as the most iconoclastic of stereotypic insect responses, the approach of a bright light (phototaxis), we provide evidence that the simple input-output relationships long assumed to underlie most if not all behaviors, may not even exist.

Drosophila melanogaster phototactic behavior has been studied for at least one hundred years. As most flying insects, flies move towards a light source after being startled, showing positive

phototaxis. This innate preference for light appears to be species- and strain-specific and has been described as part of a fly's personality [20]. Interestingly, experiments described by McEwen in 1918 and Benzer in 1967 demonstrated that wing defects affect phototaxis also in walking flies. These early works showed that flies with clipped wings did not display the phototactic response to light, whereas cutting the wings from mutants with deformed wings did not decrease their already low response to light any further [21,22]. The fact that manipulating an unrelated organ, such as wings, affects phototaxis contradicts the assumed hard-wired organization of this behavior, suggesting that it may not be a simple matter of stimulus and rigid, innate response, but that it contains at least a certain element of flexibility. In this work, we systematically address the factors involved in this behavioral flexibility and begin to explore the neurobiological mechanisms behind it.

Methods

Strains and fly rearing.

Flies were reared and maintained at 25°C in vials containing standard cornmeal agar medium [23] under 12h light/dark cycles with 60% humidity, except for experiments involving UAS-*trpA1* or UAS-*shibire*^{TS}, in which parental crosses and their offspring were maintained at 18°C under 12h light/dark cycles with 60% humidity.

Stocks obtained from the Bloomington Drosophila Stock Center (RRID:SCR_006457**;** NIH P40OD018537) were used in this study: *UAS-TrpA1* (26263), *th*-GAL4 (8848), *tdc2*-GAL4 (9313), and *PKC*$^{\Delta}$ (18258).

The sources of other stocks are detailed here:

w^{1118}, w^{1118}*; hs*-Gal4 (heat shock inducible GAL4), and *UAS-PKCi* (inhibitory pseudosubstrate of protein kinase C) were provided by Henrike Scholz (University of Cologne, Germany).

WTB is a Wild-type Berlin strain from our stock in Regensburg.

CS^{RE} is a *CS* strain bred in our lab in Regensburg.

CS^{TZ} and *FoxP*^{3955} were provided by Troy Zars (University of Missouri, USA).

104 *rsh*^{1} was provided by B. van Swinderen (The University of Queensland, Australia).

105 *rut*^{2080}, *mb247*-GAL4 and *UAS-CNT-E* were provided by Martin Heisenberg (Rudolf Virchow
106 Center, Germany).

107 *act88F*-Gal4 was provided by Juan A. Navarro (University of Regensburg, Germany).

108 *A9*-GAL4 and *UAS-baboo*^{QD} were provided by Florian Bayersdorfer (University of Regensburg,
109 Germany).

110 **Mechanical manipulations**

111 Unless described otherwise, 24h before the experiment 2-5 d old flies were briefly anesthetized
112 under CO_2. In the standard wing-clipping procedure, the distal two thirds from both wings were
113 clipped from half of the individuals (Fig. 1A). At least 30 flies with clipped wings and 30 flies with
114 intact wings were placed in the same vial until the experiment was performed, in which they
115 were tested together. For other manipulations, one of the different treatments (see Fig. 1) was
116 applied to half of the flies of a given group. At least sixty flies (half of them with injury) were
117 placed in vials for a 24h recovery period and tested together. Flies with abdominal injury were
118 not mixed with intact flies to avoid mistakes during the evaluation of the experiment due to the
119 inconspicuous nature of the injury.

120 Haltere removal was performed by pulling each haltere with forceps, while the antennal damage
121 was produced by clipping the third segment of the antenna (funiculus). The abdominal injury was
122 performed with a sharpened needle, and was always made ventrally in one side of the fourth
123 abdominal segment.

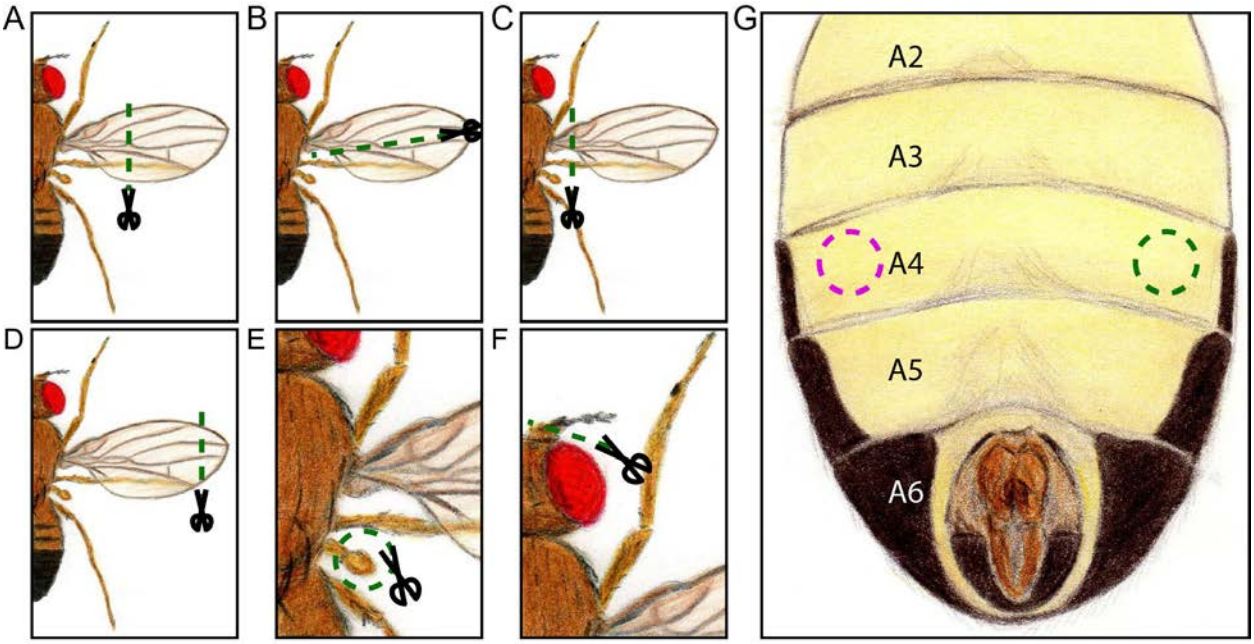


Figure 1. Schematic representation of the different injuries made to the flies. *A*, This was the standard procedure, where the distal two thirds from both wings were removed. ***B*,** Longitudinal cut. Half of the wing was removed. It was applied to both wings in experiments of Fig. 4*A*,*B*. ***C*,** Whole wing cut. It was used in Fig. 4*C*,*D* to remove only one wing (the side was randomly selected), and in Fig. 4*E*,*F* to remove both wings. ***D*,** End of the wing cut. Around 20% of each wing was removed. It was used in Fig. 4*E,F*. ***E*,** Haltere removal. Both halteres were removed and the effect on photopreference is presented in Fig. 4*G,H*. ***F*,** Antennal damage. The third segment of both antennae was cut. This treatment was used for experiments in Fig. 4*I,J.* ***G*,** Abdominal injury. Flies were stabbed on one side of the ventral fourth abdominal segment (the side was randomly selected). The results of the effect of this injury in phototaxis are depicted in Fig. 4*K,L*.

Wing gluing

Flies were cold anesthetized using a custom made cold air station and their wings were glued together in their natural relaxed posture using a 3M sucrose solution. To unglue the wings flies were cold anesthetized and their abdomen gently submerged in water to dissolve the sucrose. After each process flies were left to recover overnight. Flies were discarded from the analysis if their wings were damaged because of the treatments or unglued by chance.

Countercurrent Apparatus

Phototactic preference was evaluated using Benzer's Countercurrent Apparatus [21] (http://dx.doi.org/10.17504/protocols.io.c8gztv). The apparatus was completely transparent and consisted of two acrylic parts, a lower one with 6 parallel tubes (an initial tube + 5), and a movable upper part with 5 parallel test tubes. Each plastic tube had a length of 6.8 cm, an inner diameter of 1.5 cm, and an outer diameter of 1.7 cm. The test group was placed in the initial tube and was left in darkness to acclimate for 10 min, with the apparatus placed horizontally. Thereafter, flies were startled by tapping the apparatus, making all of them end up at the bottom of the tube. The apparatus was placed horizontally and the upper part shifted, making the initial tube face the first test tube for 15 seconds, allowing the flies to move towards the light if the test tube was facing it, or away from it if the initial tube was facing the light. Then, the upper part was shifted again and flies that moved to the test tube were transferred to the next tube of the lower part by tapping the apparatus, and the same test was repeated 4 more times. The light source was always placed at 30 cm from the apparatus and consisted of a fluorescent warm white tube (OSRAM 18W/827), which delivers 1630 lux at that distance.

The Performance Index was calculated using the formula:

$$PI = \frac{(\#F_5 \times 5) + (\#F_4 \times 4) + (\#F_3 \times 3) + (\#F_2 \times 2) + (\#F_1 \times 1) + (\#F_0 \times 0)}{\#F_T}$$

where $\#F_n$ was the number of flies in the tube n (being 0 the initial tube and 5 the last test tube), and $\#F_T$ was the total number of flies. If the test tubes were on the bright side a higher index meant a more positive phototaxis. In each experiment a PI was calculated for the wingless flies and other for the intact flies. The tubes were cleaned thoroughly after each test.

In figures 3A and 6A the Effect Size was calculated using Glass Δ estimator.

T-Maze

Light/Darkness choice was measured in a custom build PVC opaque T-Maze with only one transparent (acrylic) choice tube (http://dx.doi.org/10.17504/protocols.io.c8azsd). Flies were placed in an initial dark tube (10 cm long, 1.5 cm inner diameter, and 2.5 cm outer diameter) and

were left to dark adapt for 10 min. Then, they were transferred to the elevator chamber (1.5 cm diameter, 1.5 cm height) by gently tapping the apparatus, where they remained for 30s. Next, the elevator was placed between the dark and the bright tube (both 20 cm long, 1.5 cm inner diameter, and 2.5 cm outer diameter), and flies were allowed to choose for 30s. As the source of light, the same fluorescent tube as for Benzer's Countercurrent Apparatus was used, and placed 31.5 cm above the base of the T-Maze.

The Choice Index was calculated using the formula:

$$CI = \frac{(\#F_L \times 1) + (\#F_D \times -1) + (\#F_E \times 0)}{\#F_T}$$

where $\#F_L$meant the number of flies in the transparent tube, $\#F_D$ was the number of flies in the opaque tube, and $\#F_E$ was the number of flies that remained in the elevator. A *CI* of 1 meant all the flies chose the light, while an index of -1 meant a dark photopreference. The tubes were cleaned thoroughly after each round.

Buridan

Locomotion towards dark objects was evaluated using Buridan's paradigm as explained in Colomb *et al.* [24]. Briefly, 3-6d old flies were selected and half of them had their wings clipped under CO_2 anesthesia (http://dx.doi.org/10.17504/protocols.io.c7vzn5). They were left to recover overnight within individual containers, with access to water and sugar (local store) before being transferred to the experimental setup. The setup consists of a round platform (117 mm in diameter) surrounded by a water-filled moat placed at the bottom of a uniformly illuminated white cylinder (313 mm in height) with 2 stripes of black cardboard (30mm wide, 313 mm high and 1 mm thick) placed 148.5 cm from the platform center one in front of the other. Flies were prevented from escaping by a transparent lid over the platform. The experiment duration was set to 900 seconds. Data were analyzed using BuriTrack and CeTrAn [24] (RRID:SCR_006331), both available at http://buridan.sourceforge.net.

Genetic manipulation of flying ability and neuronal activity

For the experiments involving *TrpA1 and the act88f-GAL4* driver, experimental flies and their respective controls were raised at 18°C. 3-5d old flies were tested at room temperature (RT) and recovered for 5-6h at 18°C. Then, they were transferred to a 37°C climate room where they were placed in an acclimation vial for 15min. Next they were transferred to the first tube of the T-maze placed in the 37°C climate room, and the experiment proceeded as explained above. The choice step was reduced to 15s to compensate for the increased activity that flies showed in pilot experiments. After counting the flies, they were transferred to fresh vials and placed at 18°C for 24h. After this recovery phase, they were tested again at RT. We noticed that the CI obtained for wild types could differ between chambers at 37°C .

In the case of manipulation of dopaminergic and octopaminergic neural activity with shi^{TS} or *TrpA1* the same protocol was applied but instead of 37°C, 32°C were used and the choice step was 30s long.

Statistical Analysis

Statistical analyses were performed with InfoStat, version 2013 (Grupo InfoStat, Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Córdoba, Argentina) and R (http://www.r-project.org/). Number of replicates in each experiment was adjusted to provide a statistical power of at least 80% using pilot experiments. As dictated by the experimental design and data composition, a paired T-test, a Randomized Block Design ANOVA or an ANOVA were performed. Normality was tested using Shapiro–Wilks test, and the homogeneity of variance was assessed with Levene's test. A value of $p<0.05$ was considered statistically significant. After ANOVA, a Tukey least-significant difference or an orthogonal contrasts test was performed. If an interaction between factors was significant in two-way ANOVAs, simple effects were performed, and p values were informed. In figures 1A, 3B-E and H, and 7C and D, homogeneity of variance was violated. In figures 1A, and 3B-E and H a Wilcoxon test was used, while in figures 7C and D

215 Kruskal-Wallis test was employed for multiple comparisons. The alpha value was corrected
216 using Bonferroni's correction.

217 **Availability of data and materials**

218 The datasets supporting the conclusions of this article are available in the FigShare repository,
219 http://dx.doi.org/10.6084/m9.figshare.1502427.

220

221 **Results**

222 **Wing-clipping effect is absent in flightless flies.**

223 Motivated by the findings of McEwen and Benzer, we decided to explore the nature of the
224 phototactic change observed in wingless flies. After replicating Seymour Benzer's original results
225 on wild type flies and mutant flies with deformed wings (Fig. 2A), we wondered if the wing-
226 clipping effect on phototaxis could be also observed in other genetic backgrounds. Therefore,
227 flies with and without wings from two Canton-S strains inbred in different laboratories (CS^{TZ} *and*
228 CS^{RE}) and from the Wild Type Berlin (*WTB*) line were tested in Benzer's Countercurrent
229 Paradigm (BCP). All three lines showed a significant reduction in BCP performance index (PI)
230 when the wings were cut (Fig. 2B). This reduction was apparent despite large variations
231 between the three lines in the PI levels from intact flies, showing that the reduction in phototaxis
232 due to wing-clipping can be observed across laboratory strains, with its magnitude dependent on
233 genetic background and/or associated differences in baseline levels of phototactic performance.
234 Original experiments from McEwen, and then Benzer, showed that mutant flies with deformed
235 wings displayed a lower positive phototaxis than wild types [21,22] and a diminished wing-
236 clipping effect [22] (replicated in Fig. 2A). We wondered whether this simultaneous low
237 phototaxis and absence of wing-clipping effect was due to a specific effect of these mutations or
238 a general consequence of both manipulations altering the flies' ability to fly. In order to tackle this
239 question, we tested three lines with flight impairments, the flightless PKC^{Δ} mutant, the wings of

which are indistinguishable from wild type wings (Fig. 2*D*), the *CyO* balancer line with curly wings, and a transgenic line in which the wings were deformed due to an overexpression of a constitutively active form of the *baboon* receptor in wing imaginal discs (*A9>babo*^{QD}, [25]). Again replicating previous experiments, *CyO* flies showed a reduced PI that remained unchanged in wing-clipped animals (Fig. 2B). Similarly, *A9>babo*^{QD} showed less attraction to light and no significant wing-clipping effect (Fig. 2*C*), while all genetic controls behaved similar to wild type flies. Remarkably, *PKC*$^{\Delta}$ mutants exhibited the same behavioral characteristics as *CyO* flies (Fig. 2B). Hence, we conclude that the reduction in phototaxis is not dependent on the origin of wing damage or the damage itself, but probably on wing utility.

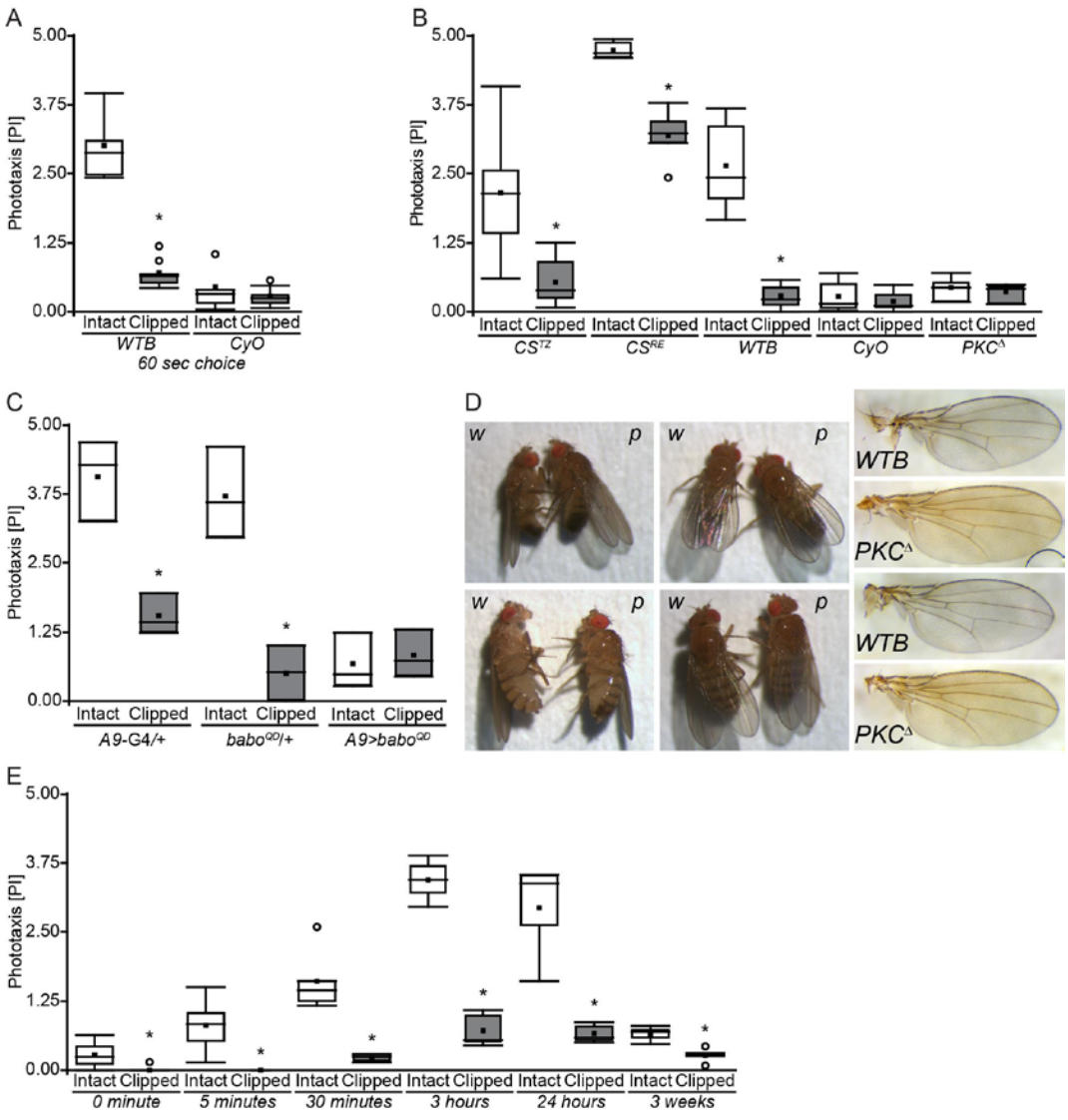


Figure 2. The wing-clipping effect is observable across genetic backgrounds and throughout adult lifespan, but is absent in flightless flies. ***A***, Replication of the original BCP experiments using 60s of time in which the animals were allowed to walk towards the light. Wilcoxon test; *WTB*: N=8, p<0.001; *CyO*: N=8, p=0.505 ***B***, BCP Performance Index (15s choice time) from three wild type strains and two flightless mutants with intact and clipped wings. Paired T-test; CS^{TZ}: N=6, p=0.003; CS^{RE}: N=5, p<0.001; *WTB*: N=12, p<0.001; *CyO*: N=14, p=0.066; PKC^{Δ}: N=4, p=0.413. ***C***, BCP Performance Index from flies with a genetic manipulation of wing development (*A9*>$babo^{QD}$) and their genetic control groups (*A9*-G4/+, $babo^{QD}$/+). Randomized Block Design ANOVA; N=3; Block p<0.001, Interaction Genotype vs Wings Integrity: p<0.001, simple effect Genotype: *A9*-G4/+: p<0.001, $babo^{QD}$/+: p<0.001, *A9*>$babo^{QD}$: p=0.401. ***D***, Lateral and dorsal view of wing posture of *WTB* (*w*) and PKC^{Δ} (*p*) males (upper panels) and females (lower panels). Right panels: Examples of wing anatomy from *WTB* flies and PKC^{Δ} mutant flies. ***E***, BCP Performance Index of WTB flies after different recovery time lengths. Paired T-Test, 0 minutes: N=6, p=0.023; 5 minutes: N=6, p=0.008; 30 minutes: N=5 , p=0.007; 3 hours: N=5, p<0.001 ; 24 hours: N=5, p=0.005 ; 3 weeks: N=5 , p=0.004. * indicates significant differences. Box plot show quantiles 0.05, 0.25, 0.75 and 0.95, median, mean (black square), and outliers (circle).

The behavioral change is immediate

If flies were able to assess wing utility, wing-clipping might have an almost instantaneous effect on the behavior. Thus, to find out when the behavioral change takes place, we assessed wing-clipped *WTB* flies at different time points after the injury was made. Flies from different groups were tested either 3 weeks, 24h, 3h, 30min, 5min or immediately after the surgery. To diminish the effects of anesthesia on phototactic behavior [26], we only used CO_2 anesthesia for recovery times longer than 30min, and cold anesthesia for 0 and 5min recoveries. We found that the reduction in phototaxis could be observed in all tested groups (Fig. 2*A*). Moreover, the difference between intact and clipped flies increased with longer recovery phases, probably due to the vanishing of the anesthesia effect, only to decrease again in aged flies, perhaps due to a combination of a deteriorated locomotor activity and a decreased response to light in old flies

277 [27,28]. Even if flies were placed in BCP right after surgery and let to recover from anesthesia
278 only during the acclimation phase (0min group), it was possible to see a significant decrease in
279 phototaxis. These results are consistent with the hypothesis that flies continually (or at relatively
280 short intervals) monitor their ability to fly.

281 **Wingless and untreated flies do not differ in their locomotor activity**

282 A potential explanation for the reduction in phototaxis is a possible reduction in locomotor activity
283 in treated flies. We tested this hypothesis by placing the light source not only in front of the
284 horizontal tubes of the BCP, but also above them, with the light shining perpendicular to the
285 trajectory of the flies. In addition, we tested for negative phototaxis by placing the light source on
286 the same side of the starting tube, such that we were able to count the flies with negative
287 phototaxis. This tripartite experimental design allowed us to directly compare all three situations:
288 light source on the opposite side of the starting tube (positive phototaxis), light source on top of
289 the BCP (no taxis; locomotor activity control), and light source on the same side as the starting
290 tube (negative phototaxis). In order to facilitate direct comparison of the behavioral
291 consequences of wing-clipping in the three situations, we assessed the proportion of behavioral
292 change with the *Glass Δ Effect Size* (ES). A positive ES in positive phototaxis indicates a
293 reduction in positive phototaxis after wing-clipping. A positive ES in the no-taxis situation
294 indicates a decrease in locomotor activity after wing-clipping, a negative ES an increase. A
295 negative ES in the negative phototaxis situation indicates an increase in negative phototaxis
296 after wing-clipping. We could not find any evidence for a reduced locomotor activity in these
297 experiments. If anything, there was a small tendency of wing-clipped flies, instead of reducing
298 their locomotor activity to actively avoid the light source (Fig. 3A).

299 We tested the generality of these results in two additional experiments, Buridan's paradigm and
300 a T-maze. Buridan's Paradigm, where the flies walk on a water-surrounded circular platform with
301 two opposing vertical black stripes on the walls of a round panorama illuminated in bright white
302 light from behind, has been used as a standard test for walking speed and locomotor activity for

several decades [24,29]. We compared total activity time, walking speed, and pause duration in intact and wingless flies from three lines (*WTB*, *CyO*, *PKC*$^{\Delta}$) in a modified version of Buridan's Paradigm, where a roof prevents the flies from escaping. The results show only occasional small differences with the overall tendency of wingless flies exhibiting, if anything, slightly higher general activity than intact flies (Fig. 3B, C, D).

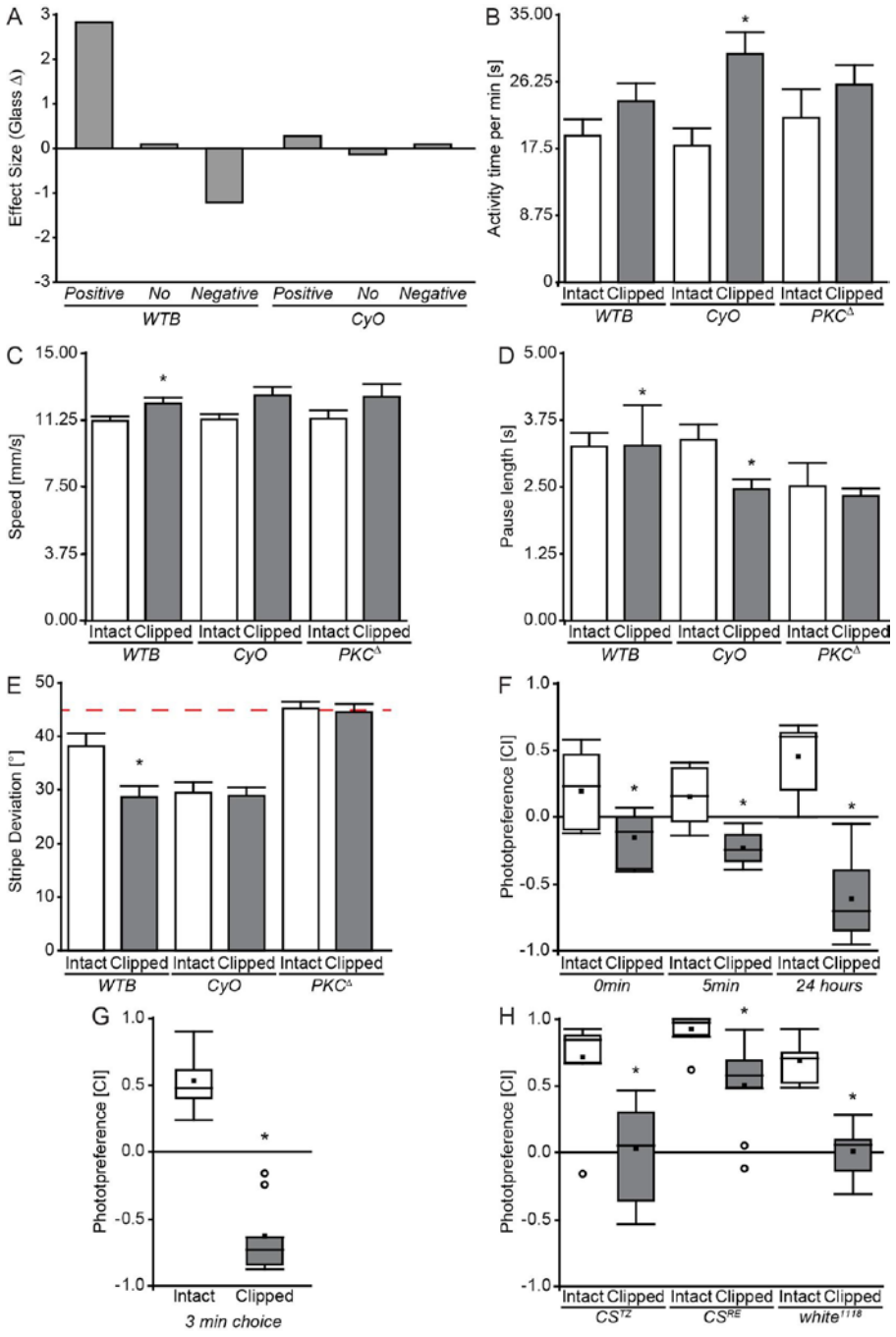


Figure 3. Flies without wings are not less active and prefer darker stimuli. ***A***, Effect Size of wing clipping on BCP with the light source on the opposite side of the starting tube (positive phototaxis -*Positive*-), light source on top of the BCP (no taxis -*No*-; locomotor activity control), and light source on the same side as the starting tube (negative phototaxis -*Negative*-). ***B-E,*** Buridan's paradigm. *WTB*: Intact, N=20; Clipped, N=21. *CyO*, N=17. PKC^{Δ}, N=13. Wilcoxon test. ***B,*** Activity time. *WTB*: p=0.151. *CyO*, p=0.002. PKC^{Δ}, p=0.526. ***C***, Speed. *WTB*: p=0.033. *CyO*, p=0.056. PKC^{Δ}, p=0.159. ***D***, Pause Length. *WTB*: p=0.022. *CyO*, p=0.002. PKC^{Δ}, p=0.426. ***E***, Stripe deviation. *WTB*: p=0.004. *CyO*, p=0.959. PKC^{Δ}, p=0.98. Dotted line indicates 45°, the mean value for computer-generated data. ***F***, T-Maze Choice Index after different recovery time lengths. Paired T-Test, 0 minutes: N=7, p=0.003; 5 minutes: N=6, p=0.026; 24 hours: N=6, p<0.001. ***G***, T-Maze Choice Index with 3 min choice step. Paired T-test; *WTB*: N=8, p<0.001. ***H***, Choice Index of CS^{TZ}, CS^{RE} and w^{1118} flies with intact and clipped wings. Wilcoxon test. CS^{TZ}, p=0.003. CS^{RE} p<0.001. w^{1118} p<0.001. See figure 2 for detailed graph information.

Black stripe fixation in Buridan's Paradigm is influenced by flying ability

Interestingly, the wing-clipped wild type flies also showed a stronger fixation of the black stripes in Buridan's Paradigm, compared to the intact flies, while the flightless flies did not show such a difference (Fig.3E). This result is consistent with the tendency of the wild type flies to show some negative phototaxis after wing clipping (Fig. 3A). One possible explanation for these two congruent observations in such disparate experiments is that the darker stimuli become more attractive after wing clipping in situations where the animals are faced with a choice of darker and brighter stimuli. One prediction of this hypothesis is that other experiments where the animals face a choice of bright and dark stimuli should also be affected by wing-clipping. To test the generality of the wing-clipping effect and to obtain a third independent test of general activity, we set out to develop a T-maze experiment, where the animals are forced to choose between a dark and a bright arm.

Wing-clipped flies can show negative photopreference in a T-maze

After several pilot experiments with a variety of different T-maze designs, we arrived at an experimental design where wing-clipped WTB flies would robustly avoid the transparent tube and approach the dark tube (see Material and Methods). As for the BCP, we selected different recovery times (0min, 5min or 24h). Congruent with the BCP results, intact flies showed a positive photopreference, while wing-clipped flies switched to light avoidance and a negative photopreference immediately after their wings were cut (Fig. 3*F*). These results hold even if the flies are allowed three minutes to choose between the two arms of the T-Maze (Fig. 3G). Also similar to the results in the BCP, we found that the magnitude of the baseline photopreference in intact flies and the wing-clipping effect varied with the genetic background. In the case of the T-Maze, the size of the effect determined whether or not the wing-clipped flies would show positive or negative photopreference (Fig. 3H).

Only injuries affecting flight abilities promote a change in photopreference

While the mutant or transgenic flies used so far may shift their photopreference due to unknown side effects, the shift in wing-clipped flies could in principle be brought about either directly by the injury or indirectly via a detection of flying ability. To distinguish between these two hypotheses, we tested the effects of a series of injuries (see Materials and Methods, Fig. 1), only some of which affecting some aspect of flight, in BCP and in the T-Maze. First, we evaluated flies with a longitudinal cut through their wings and flies with only one of the two wings completely removed (the side was randomly selected). Both manipulations cause flightlessness. Again, we observed the same shift in photopreference as with standard wing-clipping (Fig. 4*A-D*). Both flies with longitudinally cut wings (Fig. 4*A,B*) and one wing removed (Fig. 4*C,D*) exhibited diminished phototaxis in BCP and a negative photopreference in the T-Maze. During our pilot experiments, we observed that flies with different degrees of injuries on their wings behaved differently. Therefore, we hypothesized that manipulations affecting only some aspects

of flight behavior, rather than abolishing flight completely, might lead to less pronounced behavioral changes. Thus, we next compared the behavior of flies whose wings were completely removed, with those where only the tip of the wings had been removed. Flies with partially removed wings are still able to fly, but with reduced torque during turns and reduced lift/thrust [30]. It is worth mentioning that McEwen also attempted to test if the decrease in positive phototaxis was directly proportional to the amount of wing removed, but his low number of replicates, the use of ether as an anesthetic, and his different setup, prompted us to obtain our own data (the same for antenna experiments –see below–).

In both cases (complete and partial removal), injured flies showed a statistically significant reduction in BCP phototaxis and T-Maze photopreference, but both indices were higher in flies with only the end of the wing cut (Fig. 4*E,F*). In fact, the behavior from both types of injured flies was significantly different from one another in the T-Maze paradigm (Fig. 4*F*). Therefore, we conclude that behavioral change depends to some extent on the degree of the injury, and on which aspects of flight behavior it affects. To test yet other aspects of flight behavior, we administered injuries that did not affect the wings, in two organs related to flight (halteres and antennae) and one unrelated to flight (the abdomen). In one group of flies, we removed the gyroscopic halteres, mechanosensors involved in sensing body rotation and necessary for free flight [31–33]. In another, we removed the distal segments of the antennae (funiculus and arista), depriving the flies of their most important mechanosensor for airspeed and wind direction [34–36]. The two different treatments both significantly decreased photopreference values (Fig. 4G-J). However, only the manipulation abolishing free flight completely, haltere removal, also led to negative photopreference in the T-Maze (Fig. 4H). Affecting flight stabilization and speed by removing parts of the antennae renders the flies indifferent to the light, on average (Fig. 4J). Thus, when flies are still able to fly, but individual aspects of flight behavior are disrupted such as stabilization, torque, speed or lift/thrust, their photopreference is less severely affected than when flight is abolished completely. To test whether any injury, even one that does not affect any

aspect of flight at all, can affect photopreference, we used a small needle to carefully puncture the abdomen of the flies. Consistent with the results so far, a wound in the abdomen did not produce any detectable shift in photopreference (Fig. 4*K,L*).

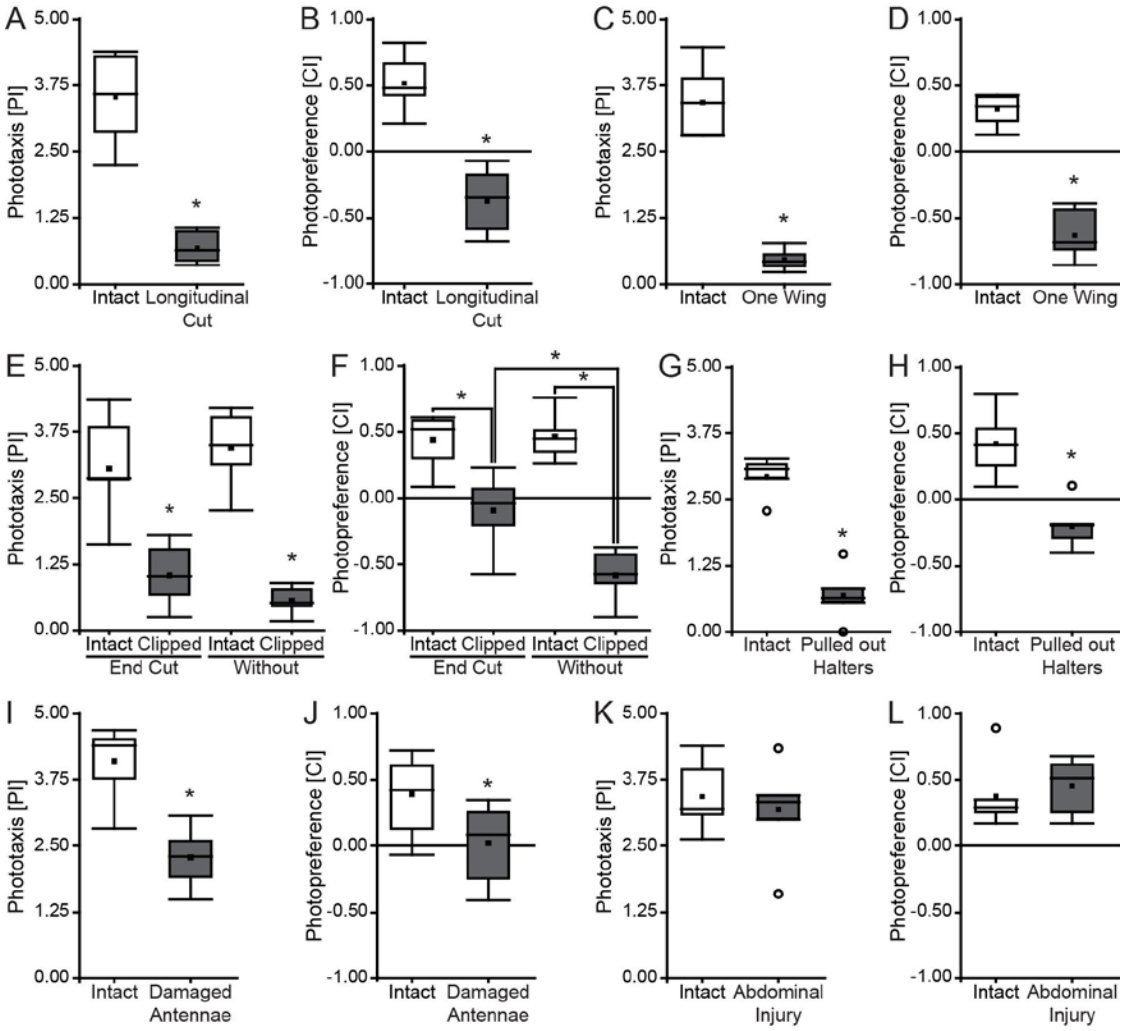


Figure 4. Only flight-affecting injuries change photopreference. ***A, C, E, G, I, K,*** BCP Performance Index from WTB flies with and without different injuries. ***B, D, F, H, J, L,*** T-Maze Choice Index from WTB flies with and without different injuries. ***A, B***, Longitudinal cut of the wings. N=7, *A*: p<0.001, *B*: p<0.001. ***C, D***, Only one wing cut. N=7, *C*: p<0.001, *D*: p<0.001. ***E, F***, Wing clipped at different lengths. Randomized Block Design ANOVA; N=6; *E*: Block p= 0.094, Interaction Wings Integrity (intact or clipped) vs Degree of Injury (without wings or end of the wings cut): p=0.087, Wings Integrity: p<0.001, Degree of Injury: p=0.797; *F*: Block p= 0.238, Interaction Wings Integrity vs Degree of Injury: p=0.007, simple effects: end cut vs intact: p<0.001, without wings vs intact: p<0.001, end cut vs without wings: p<0.001, intact

(control from end cut) vs intact (control from without wings): p=0.865. ***G, H***, Both halteres removed. *G*: N=5, p<0.001, *H*: N=7, p<0.001. ***I, J***, Both antennae damaged. *I*: N=6, p<0.001, j: N=7, p=0.04. ***K, L***, abdominal wound. *K*: N=6, p=0.377, *L*: N=6, p=0.552. *A, B, C, D, G, H, I, J, K, L,* Paired T-Test. See figure 1 for detailed graph information.

The shift in photopreference is reversible and traces flying ability.

If flies were monitoring the different aspects of their flying abilities and changing their photopreference accordingly, one would expect that transient impairments in flying ability would cause transient changes in photopreference. To examine the reversibility of the behavioral shift, we designed two complementary experiments. In the first, we tested *WTB* flies in BCP and T-Maze before and after gluing, as well as after ungluing their wings. Wing gluing perfectly reproduced the wing-clipping effect, evinced by a clear reduction of the PI and CI (Fig. 5*A,B*), showing again that the shift in photopreference is independent from the cause of the flightlessness. Remarkably, normal photopreference was restored after cleaning the wings of the tested flies (Fig. 5*A,B*).

In our complementary approach, we manipulated flying ability by reversibly altering Indirect Flight Muscle (IFM) contraction, expressing the temperature-sensitive *TrpA1* channel under the promoter of the IFM-specific gene *actin 88F* (*act88F*), using the *act88F*-GAL4 [37] driver. At room temperature, experimental flies tested in our T-Maze were indistinguishable from their genetic controls. However, at 37°C, when *TrpA1* caused a sustained IFM contraction disrupting wing movements, the same flies showed a marked preference for the dark arm of the maze that fully recovered when they were tested back at room temperature on the following day (Fig. 5*C*). The genetic controls also showed a CI decrease at 37°C, but it was less pronounced and significantly different from the experimental group. In sum, these results show that flies adjust their photopreference in accordance with their flying ability. Moreover, these changes are immediate and reversible.

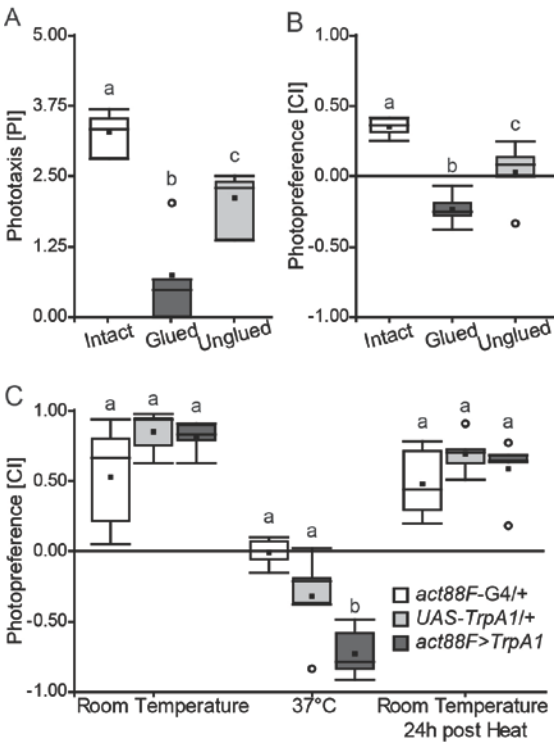
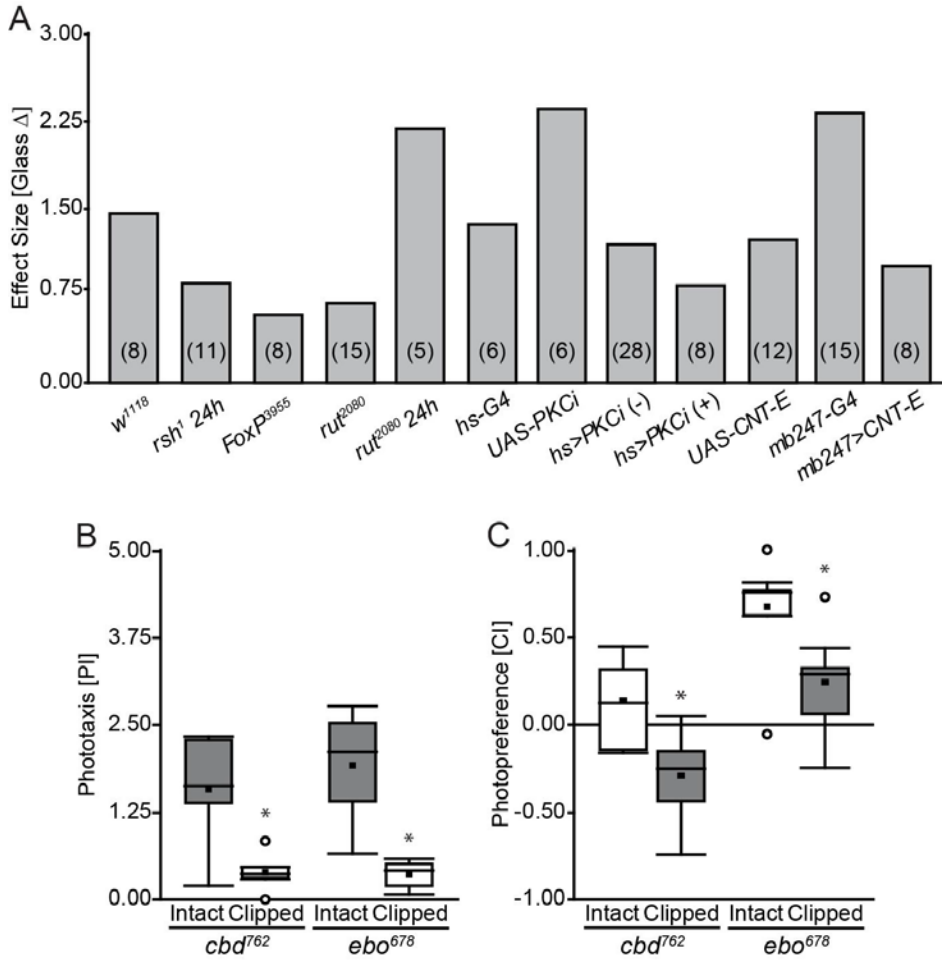


Figure 5. Photopreference changes together with flying ability in a reversible manner. A, BCP tests in flies before, during and after their wings had been rendered useless by applying (and then removing) sucrose solution. Randomized Block Design ANOVA, N=4, Block p=0.091, ANOVA p<0.001, Tukey's *post hoc* test (p<0.05; least-significant difference=1.0257). ***B***, T-Maze. Randomized Block Design ANOVA, N=5, Block p=0.173, ANOVA p<0.001, Tukey's *post hoc* test (p<0.05; least-significant difference=0.232). ***C***, Genetic manipulation of IFM contraction and flying ability. T-Maze Choice Index before, during and after 37°C exposure of experimental and control flies. Randomized Block Design ANOVA, N=5, Block p=0.152, Interaction Genotype vs Temperature: p<0.001, simple effects with Tukey's *post hoc* test (p<0.05): least-significant difference=0.349, Room Temperature: p=0.073, 37°C: p<0.001, Room Temperature 24h post heat: p=0.344. Same letter indicates no significant differences. See figure 1 for detailed graph information.

Wing-clipping effect is not dependent on known learning and memory processes

The reversibility of the shift in photopreference is reminiscent of a learning process where the animal may evaluate its flight capabilities at one point and then remember this outcome until the next evaluation. For instance, the animals may attempt flight and immediately learn about the

442 futility of their attempt. Until the next attempt, the flies remember this state and shift their
443 photopreference accordingly. To test this hypothesis, we screened a selection of
444 mutant/transgenic fly lines with a variety of known learning and memory impairments using BCP.
445 We selected lines known to affect classical olfactory conditioning/operant world-learning, operant
446 self-learning, or any Mushroom Body-dependent learning processes. In order to avoid
447 differences related to specific locomotor characteristics from the different lines, here again the
448 wing-clipping effect was assessed with the *Effect Size*. Remarkably, all lines tested showed a
449 clear behavioral change after wing-clipping, evidenced by a decrease in their PI with and *Effect*
450 *Size* around 0.6 or more, irrespective of the baseline value (Fig. 6*A*).



451

452 **Figure 6. The wing-clipping effect is independent from known learning/memory processes or**
453 **neuropil areas associated with learning. *A*,** 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,*** behavioral 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=0.005; *ebo*^{678}, N= 6, p=0.004. d, *cbd*^{762}, N= 8, p=0.002, *ebo*^{678}, N= 7, p<0.001. See figure 1 for detailed graph information.

The behavioral switch is not central complex-dependent

The central complex is a higher-order neuropil related to locomotion [38,39], visual information processing [40], orientation [41], visual pattern recognition [42,43] and spatial working memory [44]. As many of these functions may be important for either phototaxis or its flexibility, we tested two structural mutants of this neuropil, Central Body Defect (*cbd*^{762}) and Ellipsoid Body Open (*ebo*^{678}). However, wing-clipped *cbd*^{762} as well as *ebo*^{678} flies both showed a clear significant change in their photopreference measured either in BCP or T-Maze (Fig. 6*B*,*C*). We note that, although *ebo*^{678} wingless flies still showed a preference for the bright tube in the T-Maze, their PI was significantly decreased in comparison with intact *ebo*^{678} flies. While more sophisticated manipulations of central complex function are clearly warranted, we tentatively conclude that if the central complex plays a role in this process, it is likely not a crucial one, or one that does not require an anatomically intact central complex.

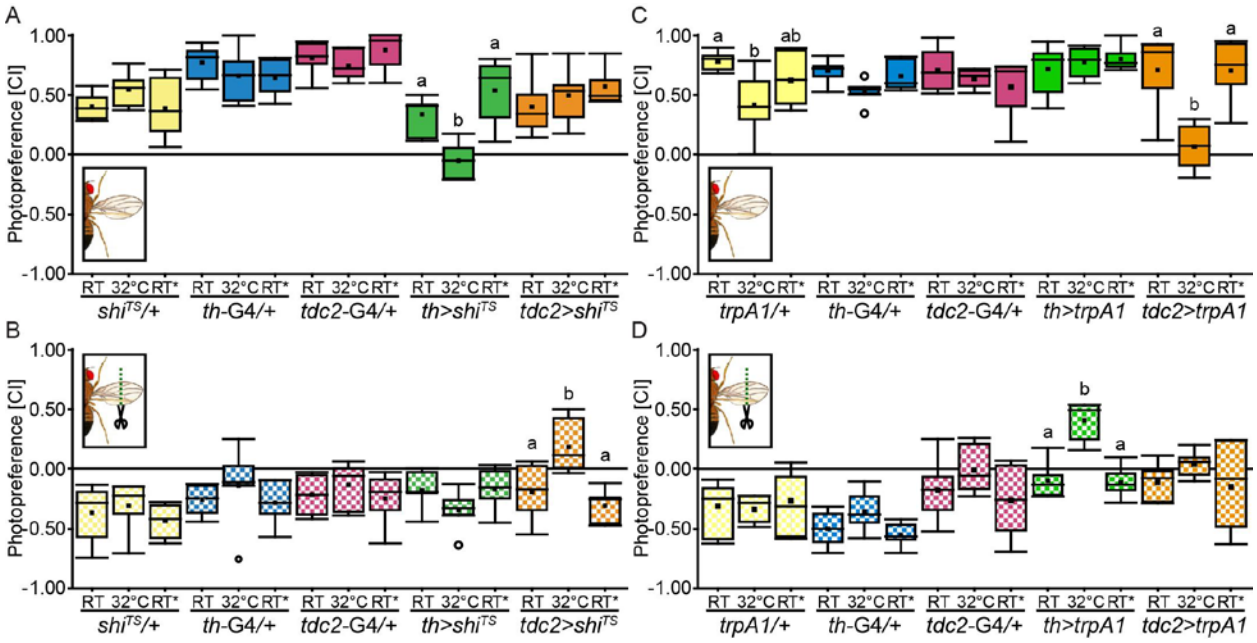
DA and OA differently modulate intact and wingless fly behavior

In the absence of any evidence that any of the known learning processes or neuropils known to be relevant for learning or other aspects of orientation/choice behaviors are involved in the shift in photopreference, we explored the hypothesis that any unknown learning mechanism as well as an unknown constant monitoring of flying ability may rely on a re-valuation of sensory input after wing manipulation. That is, whether or not any memory is involved, the consequence of being rendered flightless may be identical: a re-valuation of sensory input, such that previously attractive stimuli become more aversive and previously aversive stimuli become more attractive.

Biogenic amines have long been known for their role in mediating the processing and assignment of value [4,9–12,14,45–52]. If indeed it is the photopreference that is shifted when a fly's flying ability is altered, it is straightforward to hypothesize that the two biogenic amines most known for being involved in valuation in *Drosophila*, octopamine (OA) and dopamine (DA), may be involved in this instance of value-based decision-making as well. Moreover, mutant flies that lack tyrosine hydroxylase (*th*) only in the nervous system, i.e. neuronal specific DA-deficients, show reduced phototaxis in BCP [51] further motivating the manipulation of this amine pathway. Finally, flies without OA show a pronounced impairment in flight performance and maintenance [53], making OA an interesting candidate for testing photopreference as well.

To evaluate the involvement of DA and OA neurons for photopreference, we acutely disrupted synaptic output from two separate groups of neurons by expressing the temperature-sensitive form of dynamin (*Shibire; shi^{TS}, [54]*) either under control of the *th*-GAL4 driver (driving in dopaminergic neurons) or under control of the *tdc2*-GAL4 driver (driving in octopaminergic, as well as tyraminergic, neurons). We tested the resulting transgenic flies with and without wings in BCP and T-Maze. Although BCP and T-Maze results tended to agree, we only obtained clear results in our T-Maze experiments. The reason for the less clear results in the BCP was a genotype-independent and long-lasting effect of the temperature switch on the flies' PI in the BCP. Hence, we show the results from the T-Maze experiments here and the BCP results are available for download with the rest of the raw data. In the T-Maze at permissive room temperature, when dynamin is in its wild type conformation, in all tested groups, flies with intact wings showed positive CIs, while wing-clipped flies showed negative CIs (Fig. 7*A,B*). In contrast, when the same experiment was performed at the restrictive 32°C (i.e., blocking synaptic activity), we found opposite effects in flies with dopaminergic, and octopaminergic/tyraminergic neurons blocked, respectively. While disrupting synaptic output from dopaminergic neurons appeared to have little if any effect on clipped animals, flies with intact wings shifted their preference to the dark tube (Fig. 7*A*), rendering their CI indistinguishable from that of their

506 wingless siblings with which they were tested (Fig. 7B). Conversely, blocking synaptic output
507 from octopaminergic neurons only affected wingless flies, which now preferred the bright arm of
508 the maze (Fig. 7*B*), similar to their siblings capable of flight with which they were tested (Fig.
509 7A). Replicating the reversibility described above, after a 24h recovery phase, flies tested at
510 room temperature showed wild type behavior, meaning positive photopreference for intact flies
511 and negative photopreference for wing-clipped flies (Fig. 7*A,B*). The conventional interpretation
512 of these results is that synaptic transmission from octopaminergic/tyraminergic (OA/TA) neurons
513 is necessary for shifting the photopreference towards darkness in flightless flies, while synaptic
514 transmission from DA neurons is necessary for setting the preference of intact flies towards the
515 bright arm.



516

517 **Figure 7. Dopamine and Octopamine are necessary and sufficient to modulate phototactic**
518 **behavior, but with opposite effects. *A, B*,** Choice Index from flies with (a) and without (b) wings before,
519 during and after DA or OA neuron silencing. ***A*,** Randomized Block Design ANOVA, Block p=0.026,
520 Interaction Genotype vs Temperature p<0.001, simple effects with Tukey's *post hoc* test (p<0.05, least-
521 significant difference=0.24, *tdc2>shi*^{ts} least-significant difference= 0.263): *shi*^{ts}/+ p=0.208, *th*-GAL4/+
522 p=0.417, *tdc2*-GAL4/+ p=0.428, *th>shi*^{ts} p<0.001, *tdc2>shi*^{ts} p=0.242. N=6 except for *tdc2>shi*^{ts} RT* (N=5).

***B*,** Randomized Block Design ANOVA, Block p=0.006, Interaction Genotype vs Temperature p=0.02, simple effects with Tukey's *post hoc* test (p<0.05, least-significant difference=0.278, *tdc2>shi^{ts}* least-significant difference= 0.288): *shi^{ts}*/+ p=0.533, *th*-GAL4/+ p=0.394, *tdc2*-GAL4/+ p=0.6, *th>shi^{ts}* p=0.262, *tdc2>shi^{ts}* p<0.001. N=6 except for *tdc2>shi^{ts}* RT* (N=5). ***C*, *D*,** Choice Index from flies with (a) and without (b) wings before, during and after DA or OA neuron activation. ***C*,** Kruskal-Wallis for temperature factor comparison within genotypes (alpha after correction=0.013): *trpA1*/+ p=0.012, *th*-GAL4/+ p=0.069, *tdc2*-GAL4/+ p=0.667, *th>trpA1* p=0.97, *tdc2>trpA1* p=0.004. *th*-GAL4/+ and *th>trpA1*, N=6; *trpA1*/+, *tdc2*-GAL4/+ *tdc2>trpA1*, N=7. ***D*,** Kruskal-Wallis for temperature factor comparison within genotypes (alpha after correction=0.013): *trpA1*/+ p=0.834, *th*-GAL4/+ p=0.15, *tdc2*-GAL4/+ p=0.126, *th>trpA1* p=0.005, *tdc2>trpA1* p=0.415. *th*-GAL4/+ and *th>trpA1*, N=6; *trpA1*/+, *tdc2*-GAL4/+ *tdc2>trpA1*, N=7. Different letters indicate significant differences between temperatures for each genotype (only shown for genotypes where the factor temperature had a statistically significant effect). See figure 2 for detailed graph information.

We also transiently activated OA/TA and DA neurons, respectively, using the temperature sensitive *TrpA1* channel [55], while testing the flies for their photopreference. Again, at room temperature, when the channel is closed, flies with and without wings behaved similar to wild type animals (Fig. 6C,D). However, when tested in the same experiment at 32°C, where the *TrpA1* channel is open and depolarizes the neurons in which it is expressed, the flies showed a change in their behavior. Flies with clipped wings and activated DA neurons now preferred the bright arm of the maze, with no effect on intact flies (Fig. 7D). Conversely, activating OA/TA neurons only had an effect on flies with intact wings, abolishing their previous preference for the bright arm of the maze (Fig. 7C), rendering them indistinguishable from their wingless siblings with which they were tested, but which did not show any significant effect (Fig. 7D). Again, when tested back at room temperature 24h later, wild type behavior was restored. The conventional interpretation of these results is that active OA/TA neurons are sufficient for shifting

549 photopreference towards the dark arm of the maze, while the activation of DA neurons is
550 sufficient to set the flies' preference towards brightness.
551
552 In summary, we conclude from our data that flies (either constantly or from time to time) monitor
553 their flying ability and changes in it have a broad impact on the flies' valuation of external stimuli,
554 specifically brightness and darkness. This valuation process appears to be mediated by a
555 concerted, mirror symmetric action of octopaminergic/tyraminergic and dopaminergic circuits.
556
557
558 **Discussion**
559 McEwen's discovery captured our attention because of its implications for the supposed rigidity
560 of simple behaviors. We first reproduced the findings of McEwen [22] and Benzer [21] that wing
561 manipulation leads to a decrease in *Drosophila* phototaxis (Fig. 2). Slightly altering the
562 conditions of the BCP and comparing performance between two additional experiments, we
563 found that the decrease in phototaxis is not due to hypoactivity of wing-manipulated flies, but to
564 a more general change in the flies' assessment of their environment (Fig. 3). We discovered
565 evidence that the BCP is just one of several experiments that can measure a fly's general
566 photopreference. Manipulating the wings modulated this preference in all of the selected
567 experiments such that compromised flying ability yielded a decreased preference for brightness
568 (bright stimuli) and an increased preference for darkness (dark stimuli) across the experiments
569 chosen (Fig. 3). However, of these experiments, only the BCP can be argued to test phototaxis
570 proper. In Buridan's Paradigm the flies walk between two unreachable black stripes; and in the
571 T-Maze, the flies choose between a dark tube and a bright one where the light is coming from an
572 angle perpendicular to their trajectory. Neither of the two paradigms is testing taxis to or away
573 from a light source. Interestingly, in our pilot experiments, we have tested phototaxis in different
574 variations of the T-maze with various LEDs placed at the end of one of two dark tubes and only

found a reduction of phototaxis and never negative phototaxis (unpublished observation). In fact, in these pilot experiments we have observed every possible difference between flying and manipulated flies. In the end, we chose the experimental design that yielded positive and negative scores, respectively, in WTB flies purely for practical reasons. Other wild type strains, such as some Canton S substrains, do not show a negative photopreference in the T-Maze after wing clipping (Fig. 3H). Taken together, these lines of evidence strongly suggest that photopreference in *Drosophila* is a strain-specific continuum where experimental design assigns more or less arbitrary values along the spectrum. In some special cases, this photopreference manifests itself as phototaxis. If that were the case, phototaxis would constitute an example of a class of experiments not entailing a class of behaviors.

This insight entails that different manipulations of flying ability ought to affect this continuum in different ways. Complete loss of flight ought to have more severe effects than manipulations affecting merely individual aspects of flight behavior, such as wing beat amplitude/frequency (i.e., lift/thrust), torque, flight initiation, flight maintenance, proprioception or motion sensation. We have found some evidence to support this expectation. For instance, clipping only the tips of the wings does not eliminate flight, but affects torque as well as lift/thrust [30]. Flies with the tips of their wings cut behaved indifferently in the T-Maze and do not avoid the bright tube (Fig. 4F). Flies without antennae are reluctant to fly and have lost their main sense of air speed detection [34–36], but they are still able to fly. Also these flies do not become light averse in the T-Maze after the manipulation, but indifferent (Fig. 4I). Flies with removed gyroscopic halteres, on the other hand, are severely affected in their detection of rotations and usually do not fly [31–33]. These flies avoid the bright arm of the T-Maze. Finally, injuries to flight-unrelated parts of the fly's body did not affect photopreference (Fig. 4K, L), ruling out the change in photopreference being a direct escape response to bodily harm. Further research is required to establish a quantitative link between the many different aspects of flight behavior and their relation to photopreference.

The generality of the wing-manipulation effect across experiments (Fig. 3) as well as the missing direct connection between bodily harm and photopreference (Fig. 4) suggests that the change in photopreference may be due to indirect effects via the common property of all our manipulations affecting flying ability. This conclusion entails that the flies monitor their ability to fly and update their photopreference accordingly. This process is reversible and reminiscent of a learning process (Fig. 5): at one time point, the flies make the experience that they cannot fly and at a later time point, they use this experience when making a decision that does not involve flying. Once flying ability is restored, the same choice situation is solved with a different decision in the absence of flight behavior. How the flies accomplish this learning task is yet unknown, but we tentatively conclude that it is unlikely that any of the known learning pathways or areas involved in different forms of learning play more than a contributing role (Fig. 6). While the molecular learning mechanism remains unidentified, the process appears to be (near) instantaneous (Figs. 2, 3). Even though we cannot rule out that an unknown learning mechanism exists which is unaccounted for in our screen, we conclude that at least none of the known learning mechanisms suffices to explain the complete effect size of the shift in photopreference. These results corroborate the findings above, that the switch is instantaneous and does not require thorough training or learning from repeated attempts to fly. They do not rule out smaller contributions due to these known learning processes or an unknown, fast, episodic-like learning process. It is also possible, that the flies constantly monitor their flying ability and hence do not have to remember their flight status. Despite these ambiguities, we have been able to elucidate some of the underlying neurobiological mechanisms. Much as in other forms of learning and valuation, neurons expressing the biogenic amine neuromodulators OA and DA appear to have opposite functions in the modulation of photopreference (Fig. 7).

Although both DA and OA play some role in different aspects of flight behavior [53,56–58], these cannot explain our results. In general, our biogenic amine neuron manipulated flies escape their vial via flight if granted the opportunity. Thus, flight is not abolished in any of our transgenic lines

627 affecting OA, TA or DA neurons. However, there may be more subtle deficits in less readily
628 perceived aspects of flight. Experiments performed with mutant flies lacking OA demonstrated
629 that OA is necessary for initiation and maintenance of flight [53]. However, in our paradigm,
630 silencing OA/TA neurons promoted approaching light, the opposite effect of what would be
631 expected for a flightless fly (Fig. 7 B). Activating these OA/TA neurons, however, rendered the
632 flies indifferent in the T-Maze. OA/TA appear to be involved in flight initiation and maintenance
633 via opponent processes [53]. Transient activation of OA/TA neurons may lead to a subtle
634 alteration of flight performance and reduce photopreference in these flies. Similarly, it has been
635 shown that altering the development of specific DA neurons results in flight deficits (reduction of
636 flight time or loss of flight, depending on the treatment [57,58]). Our manipulations lasted for
637 approximately 30 min during adulthood, ruling out such developmental defects. Work in the
638 laboratory of Gaiti Hasan has also found that silencing of three identified TH-positive
639 interneurons for several days in the adult animal compromises flight to some extent (wing
640 coordination defects during flight initiation and cessation [56]). Our much shorter manipulation
641 does not lead to any readily observable flight defect. However, one needs not discuss whether
642 or not our aminergic manipulations may have had subtle effects on some aspects of flight
643 behavior, as we can compare these flies to the wing-clipped siblings with which they were tested
644 simultaneously (i.e., the flies with the maximum shift in photopreference due to completely
645 abolished flight). Comparing the intact DA-inactivated flies and OA/TA-activated flies (Fig. 7 A,C)
646 with their respective wingless siblings (Fig. 7 B,D) reveals that the CIs of the pairs of groups
647 become essentially indistinguishable at the restrictive temperature. In other words, intact flies
648 where DA neurons have been inactivated or OA/TA neurons have been activated behave as if
649 their wings had been clipped and their flight capabilities abolished completely, despite them
650 being capable of at least some aspects of flight. Hence, even if there were some contribution of
651 some aspect of flight behavior being subtly affected by manipulating these aminergic neurons,
652 there is a contribution of activity in these neurons that goes beyond these hypothetical flight

653 deficits. Therefore, we conclude that neither the OA/TA, nor the DA effects can be explained
654 only by subtle defects in one or the other aspect of flight behavior in the manipulated flies.
655 The precise neurobiological consequences of manipulating OA/TA and DA neurons,
656 respectively, are less certain, however. Not only are the two driver lines (*th*-GAL4 and *tdc2*-
657 GAL4) only imperfectly mimicking the expression patterns of the genes from which they were
658 derived. Our effectors, moreover, only manipulated the activity of the labeled neurons. One
659 manipulation (shi^{TS}) prevents vesicle recycling and likely affects different vesicle pools
660 differentially, depending on their respective release probabilities and recycling rates. The other
661 effector (*TrpA1*) depolarizes neurons. It is commonly not known if the labelled neurons may not
662 be co-releasing several different transmitters and/or modulators in the case of supra-threshold
663 depolarization. Hence, without further research, we can only state the involvement of the
664 labelled neurons, which as populations are likely to be distinct mainly by containing either DA or
665 TA/OA, respectively. If it is indeed the release of these biogenic amines or rather the (co-
666)release of yet unknown factors in these neuronal populations remains to be discovered.
667 In conclusion, our findings demonstrate that even innate preferences, such as those expressed
668 in classic phototaxis experiments, are not completely hard-wired, but depend on the animal's
669 state and many other factors. This endows the animal with the possibility to decide, for example,
670 when it is better to move towards the light or hide in the shadows. Moreover, the fact that flies
671 adapt their photopreference in accordance with their flying ability evinces that flies have the
672 cognitive tools required to evaluate the capability to perform an action and to let that evaluation
673 impact other actions - an observation reminiscent of meta-cognition.
674

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