A value-based behavioural choice underlies phototaxis in *Drosophila*

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Abstract

Like a moth into the flame - phototaxis is commonly thought of as the iconic example of hard-wired input-output relationships in insect brains. Perhaps therefore, the century-old discovery of flexibility in *Drosophila* phototaxis has received little attention. Here we report that across several different behavioural tests, light/dark preference is dependent on the flies' ability to fly. If we temporarily compromise flying ability, phototaxis reverses concomitantly. Neuronal activity in circuits expressing dopamine and octopamine, respectively, plays a differential role in this case of behavioral flexibility. We conclude that flies constantly monitor their ability to fly, and that flying ability exerts a fundamental effect on action selection in *Drosophila*. This work suggests that even behaviours which appear simple and hard-wired comprise a value-driven decision-making stage, negotiating external stimuli with the animal's internal state, before an action is selected.

Introduction

In their struggle for survival, animals need not just the capability to trigger behaviours at the appropriate time, but these behaviours 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 value of extrinsic and intrinsic factors must be analysed in order to shape the behaviour to be adaptive in a particular situation. Across animal phyla, biogenic amines have been shown 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 behaviour. 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 behavioural 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 behaviour 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. Interestingly, experiments described by McEwen in 1918 and Benzer in 1967 demonstrated that wing defects affect phototaxis 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 [20,21]. The fact that manipulating an unrelated organ, such as wings, affects positive phototaxis contradicts the assumed hard-wired organisation of this behaviour, 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. Data from experiments with mechanical and genetic manipulations of flying ability suggest that flies are constantly monitoring their flying capability and adjust their phototactic preference accordingly. Consistent with the hypothesis that phototaxis is an inherently value driven decision-making process, we found that the neuromodulators DA and OA are implicated in this adjustment of phototactic preference with distinctive roles in different conditions. Our results demonstrate that even such a straightforward 'response' as insect phototaxis is not a reflex-like reaction to a stimulus, but rather a more complex decision-making process involving the concurrent valuation of internal state (or goal) and external stimuli.

Results

Wing-clipping effect is absent in flightless flies.

Motivated by the findings of McEwen and Benzer, we decided to explore the nature of the phototactic change observed in wingless flies. First, we wondered if the wing-clipping effect on phototaxis could be observed in other genetic backgrounds. Therefore, flies with and without wings from two Canton-S strains inbred in different labs (CS^{TZ} *and* CS^{RE}) and from Wild Type Berlin (*WTB*) line were tested in Benzer's Countercurrent Paradigm (BCP). In accordance with

the published observations, the three lines showed a significant reduction in BCP performance index (PI) when the wings were cut (Fig. 1a). This reduction was apparent despite large variations between the three lines in the PI levels from intact flies, showing that the reduction in phototaxis due to wing-clipping is independent from wild type genetic background and associated differences in baseline levels of performance.

Original experiments from McEwen, and then Benzer, showed that mutant flies with deformed wings displayed a lower positive phototaxis than wild types [20,21] and a diminished wing-clipping effect [21]. We wondered whether this simultaneous low phototaxis and absence of wing-clipping effect was due to a specific effect of these mutations or a general consequence of both manipulations altering the flies' ability to fly. In order to answer this question we tested three lines with flight impairments, the flightless *PKC*$^{\Delta}$ mutant, the wings of which are indistinguishable from wild type wings (Fig S1), 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}, [22]). Again replicating previous experiments, *CyO* flies showed a reduced PI that remained unchanged in wing-clipped animals (Fig. 1a). Similarly, *A9>babo*^{QD} showed less attraction to light and no significant wing-clipping effect (Fig. 1b), while all genetic controls behaved similar to wild type flies. Remarkably, *PKC*$^{\Delta}$ mutants exhibited the same behavioural characteristics as *CyO* flies (Fig. 1a). Hence, we conclude that the reduction in phototaxis is not dependent on the origin of wing damage or the damage itself, but probably to wing utility.

The behavioural change is immediate

If flies were constantly monitoring their flying ability, wing-clipping should have an almost instantaneous effect on the behaviour. Thus, to find out when the behavioural switch 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 anaesthesia on phototactic behaviour [23], we only used CO_2 anaesthesia for recovery times longer than 30min, and cold anaesthesia for 0 and 5min recoveries. We found that the PI reduction could be observed in all tested groups (Fig. 1c). Moreover, the difference between intact and clipped flies increased with longer recovery phases, probably due to the vanishing of the anaesthesia 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 [24,25]. Even if flies were placed in BCP right after surgery and let to recover from anaesthesia only during the acclimation phase (0min group), it was possible to see

a significant decrease in the phototactic response. These results are consistent with the hypothesis that flies continually monitor their ability to fly.

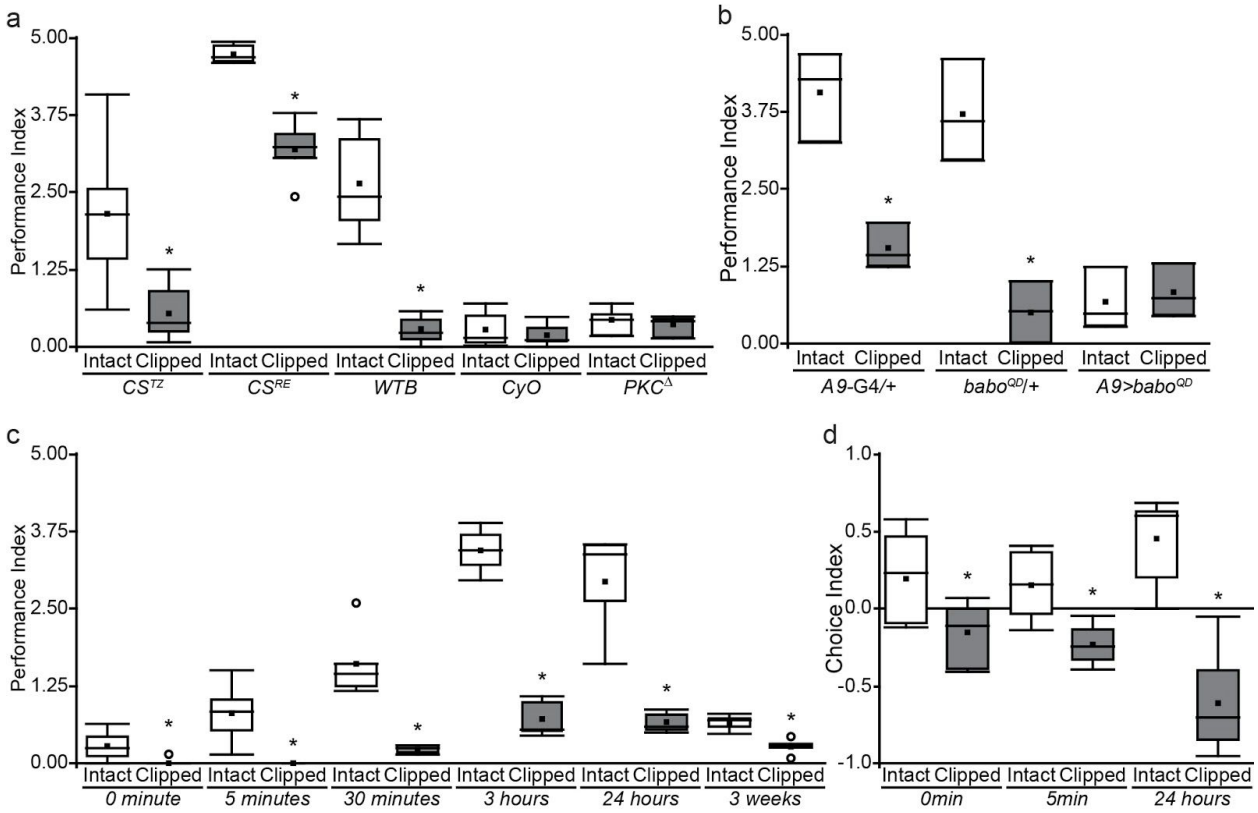

Figure 1. Flies without wings become negatively phototactic. **a**, BCP Performance Index from three wild type strains and two flightless mutants with intact and clipped wings. Paired T-test; CS^{TZ}: N=6, p=0.0036; CS^{RE}: N=5, p=0.0003; *WTB*: N=12, p<0.0001; *CyO*: N=14, p=0.0663; PKC^{Δ}: N=4, p=0.4130. **b**, 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<0.0001, 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).

Wingless flies become negatively phototactic

One hypothetical evolutionary explanation for the reduced phototaxis is that flies unable to fly may gain an advantage from hiding from predators, rather than to seek, in vain, to escape by flight. One prediction of this hypothesis is that the flightless flies should exhibit negative

phototactic behaviour, when given the choice between a bright and a dark location. To test this hypothesis, we used a T-Maze to study choice behavior in flightless and flying flies. In the T-maze, flies were allowed to choose between a bright or a dark tube, respectively. As in BCP, we selected different recovery times (0min, 5min or 24h). As predicted, intact flies showed a positive phototactic *Choice Index* (CI), while wing-clipped flies switched to negative phototaxis immediately after their wings were cut (Fig. 1d). We conclude that the wing-clipping effect on phototaxis is not just a decrease in the response towards light as proposed by McEwen (1918), but instead comprises a more radical change in the behaviour: flightless flies actively avoid brightness, having reversed their light/dark preference.

Only injuries affecting flight ability promote a behavioural switch

To further confirm that the switch in light/dark preference was in fact affected by the lack of flying ability, we tested the effects of a series of injuries (Supplementary Fig. 1), only some of which affecting 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, phototactic behaviour switched in those flies with injuries on their wings (Fig. 2 a-d). Either flies with longitudinally cut wings (Fig. 2a,b) or one wing removed (Fig. 2c,d) exhibited diminished PI values in BCP and a pronounced negative phototaxis 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 wing-injuries with a smaller impact on flight would lead to less pronounced behavioural changes. Thus, we next compared the phototactic behaviour of flies, whose wings were completely removed, with those with only the end of the wings cut (Supplementary Fig. 1). It is worth to mention that McEwen also attempted to test if the decrease in positive phototaxis was directly proportional to the amount of wing removed, but the lower number of replicates, the use of ether as an anaesthetic, and a different setup, prompted us to obtain our own data (the same is true for antenna experiments –see below-).

Remarkably, in both cases, injured flies showed a statistically significant reduction in PI and CI, but both indexes were higher in flies with only the end of the wing cut (Fig. 2e,f). In fact, the behaviour from both types of injured flies was significantly different in the T-Maze paradigm (Fig 2f). Therefore, we conclude that behavioural change depends on the degree of the injury, and how much it affects flying ability.

So far, all injuries tested affected the wings. Hence, to test if the behavioural switch is dependent on flight ability and not on injury in general, 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 the halteres were removed, in another the distal segments of the antennae (funiculus and arista), while a small needle was used to carefully puncture the abdomen of the flies belonging to a third group (Supplementary Fig. 2). Consistent with our hypothesis, only injuries affecting flying ability led to a switch in phototactic behaviour (Fig. 2g-j), while a wound in the abdomen did not produce any detectable phototactic modification (Fig. 2k,l).

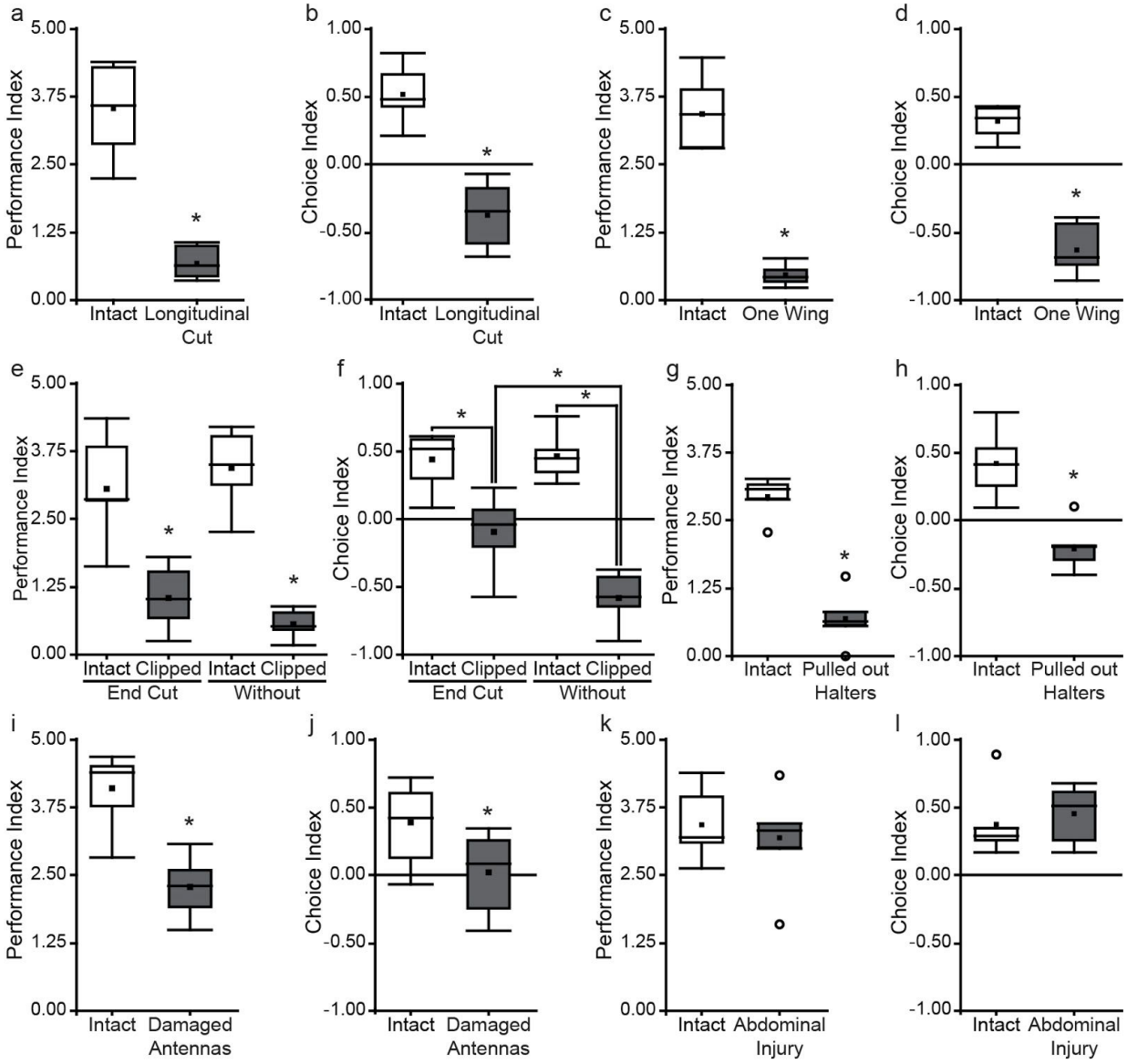

Figure 2. Only flight-disabling injuries affect phototactic preference. a, c, e, g, i, k, BCP Performance Index from WTB flies with and without different injuries. **b, d, f, h, j, l,** T-Maze Choice Index from WTB flies with and without different injuries. **a**, **b**, Longitudinal cut of the wings. 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. Randomized Block Design ANOVA; N=6; e: Block p= 0.0939, Interaction Wings Integrity (intact or clipped) vs Degree of Injury (without wings or end of the wings 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 removed. g: N=5, p=0.0001, h: N=7, p=0.0001. **i**, **j**, Both antennae 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.

The phototactic switch is fully reversible and traces flying ability.

If flies were constantly monitoring their flying ability and changing their phototactic behaviour accordingly, one would expect that transient impairments in flying ability would cause transient changes in phototactic preference. To examine the reversibility of the behavioural switch, 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, evidenced by a clear reduction of the PI and CI (Fig. 3a,b), showing again that the phototactic switch is independent from the cause of the flightlessness. Remarkably, positive phototaxis was completely restored after cleaning the wings of the tested flies (Fig. 3a,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 [26] driver. At room temperature, experimental flies tested in a 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 negative phototaxis that fully recovered when they were tested back at room temperature on the following day (Fig. 3c). It is worth to mention that 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 phototactic behaviour in accordance with their flying ability. Moreover, these changes are immediate and fully reversible.

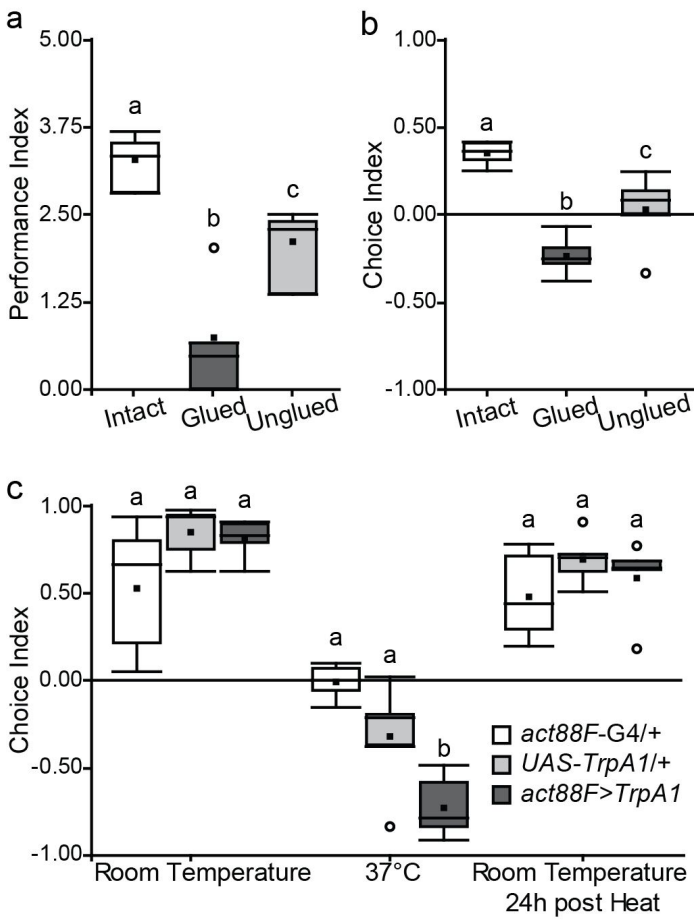

Figure 3. Phototactic preference changes together with flying ability in a reversible manner. a, **b**, Phototaxis assay from WTB flies before and after gluing their wings, and after ungluing them. **a,** BCP. Randomized Block Design ANOVA, N=4, Block p=0.0908, ANOVA p=0.0008, Tukey's *post hoc* test ($p<0.05$; least-significant difference=1.0257). **b**, T-Maze. Randomized Block Design ANOVA, N=5, Block p=0.1728, ANOVA p=0.0003, 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=0.1522, Interaction Genotype vs Temperature: p= 0.0003, simple effects with Tukey's *post hoc* test ($p<0.05$): least-significant difference=0.3490, Room Temperature: p=0.0730, 37°C: $p<0.0001$, Room Temperature 24h post heat: p=0.3441. Same letter indicates no significant differences. See figure 1 for detailed graph information.

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

Our phototaxis experiments may be interpreted as a specific instantiation of a measurement of the flies' more general preference for bright vs. dark objects or places. To test this interpretation, we compared intact and wingless flies from three lines (*WTB*, *CyO*, *PKC*$^{\Delta}$) in a modified version of Buridan's paradigm [27,28], where a roof prevents flies from escaping. In this experiment, flies

walk on a water-surrounded circular platform with two opposing vertical black stripes on the walls of a round panorama illuminated in bright white light from behind. Interestingly, paralleling the BCP and T-Maze experiments, *WTB* flies with clipped wings showed a stronger fixation of the black stripes than flies with intact wings (Fig. 4a). Moreover, wing-clipping of flightless *CyO* mutants did not show an effect in this paradigm (Fig. 4a). Similarly, wing clipping effect was also absent in PKC^{Δ} mutants. However, the behavior of intact and clipped PKC^{Δ} flies, was indistinguishable from a random walk, perhaps due to the many roles of *PKC*. Nevertheless, these results imply that phototaxis assays may be specific instances where flies can exhibit their light/dark preference, rather than a case where flies respond to packets of light reaching their retinae with an innate approach.

Wing-clipping effect is not related to memory and learning processes

An alternative hypothesis to a constant monitoring of flying ability, may be a near-instantaneous learning mechanism by which the animals attempt flight and immediately learn about the futility of their attempt. To test this hypothesis, we screened a selection of mutant/transgenic fly lines with a variety of known learning and memory impairments using BCP. We selected lines known to affect classical olfactory conditioning/operant world-learning, operant self-learning, or any other Mushroom Body-dependent learning processes. In order to avoid differences related to specific locomotor characteristics from the different lines, the wing-clipping effect was assessed as the proportion of behavioural change and a *Change Index* was computed (see Materials and Methods). Remarkably, all lines tested showed a clear behavioural change after wing-clipping, evidenced by a decrease in their PI of around 50%, irrespective of the baseline value (Fig. 4b). While 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 behavioral switch after wing-clipping. These results corroborate the findings above, that the switch is instantaneous and does not require thorough training or learning from repeated attempts to fly.

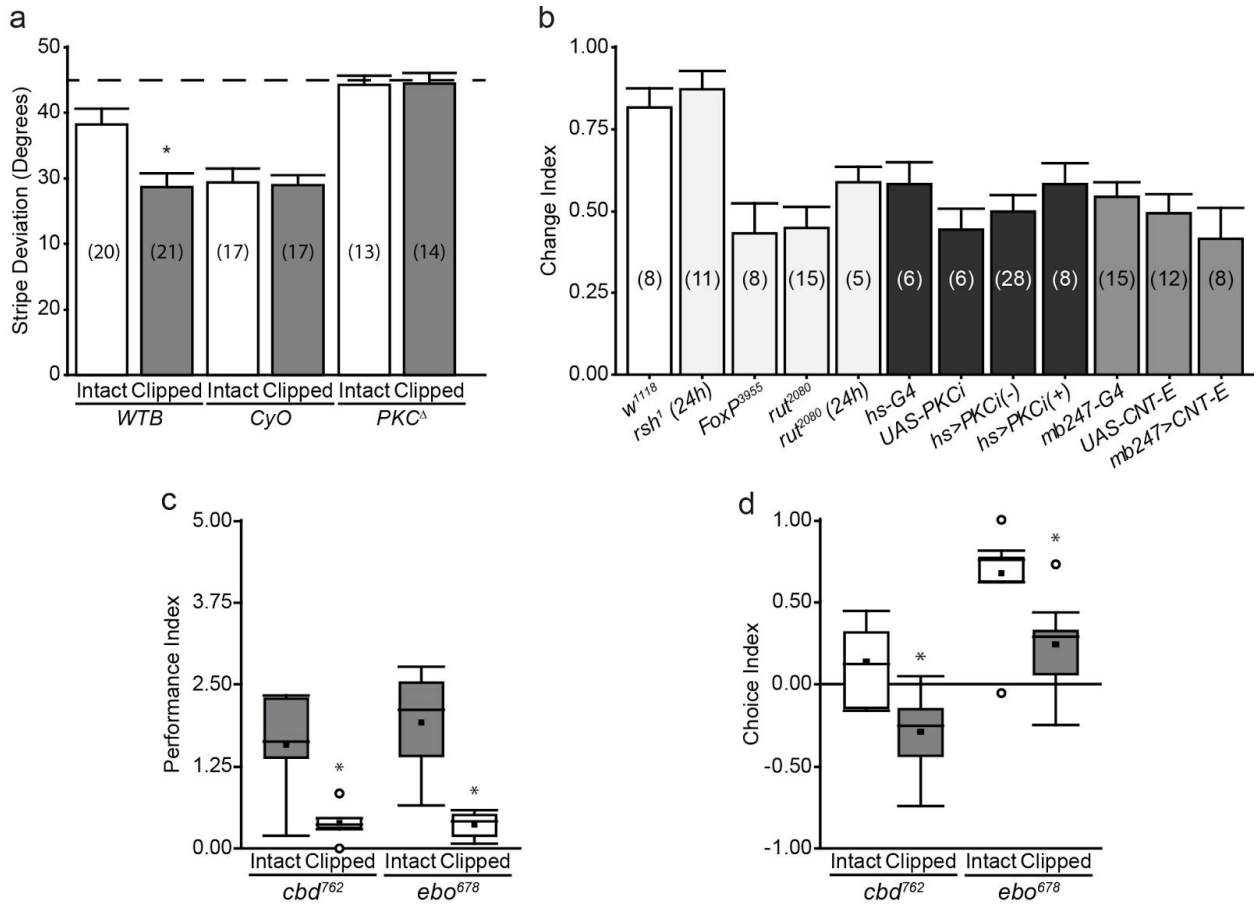

Figure 4. Flying ability also affects black object preference, and the wing-clipping effect on phototaxis is independent from known learning/memory processes or Central Complex integrity. a, Stripe deviation in Buridan's Paradigm from a *WTB*, *CyO* and *PKC*$^{\Delta}$ with and without wings. Two way 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. Numbers in brackets indicate the sample size, dotted line indicate 44° which is the score of random walk. **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.

The behavioural switch is not central complex-dependent

The central complex is a higher-order neuropil related to locomotion [29,30], visual information processing [31], orientation [32], visual pattern [33,34] and spatial working memory [35]. 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

phototactic preference measured either in BCP or T-Maze (Fig. 4c,d). It is worth to mention that, although *ebo*^{678} wingless flies still showed a positive phototaxis, their PI was dramatically decreased in comparison with intact *ebo*^{678} flies. Hence, if the central complex plays a role in this process, it is likely not a crucial one.

DA and OA differently modulate intact and wingless fly behaviour

Biogenic amines have long been known for their role in mediating the processing and assignment of value [4,9–12,14,36–43]. If indeed it is the preference of light vs. dark that is switched when a fly's flying ability is altered, it is straightforward to hypothesize that the two biogenic amines involved in valuation in *Drosophila*, octopamine (OA) and dopamine (DA), are involved also in this instance of value-based decision-making as well. Moreover, flies depleted of DA show reduced phototaxis in BCP[42] further motivating the manipulation of this amine pathway. Finally, flies without OA show a pronounced impairment in flight performance and maintenance [44], making OA an interesting candidate for phototaxis as well.

To evaluate the involvement of DA and OA for phototaxis, we acutely disrupted synaptic output from two separate groups of neurons by expressing the temperature-sensitive form of dynamin (*Shibire; shi*^{TS}, [45]) 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. We found a genotype-independent and long-lasting effect of temperature switch on the flies' PI in the BCP. Hence, we show results from T-Maze here and the BCP results in the supplementary information. 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 phototaxis, while wing-clipped flies showed negative phototaxis (Fig. 5a,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 became negatively phototactic (Fig. 5a). Conversely, blocking synaptic output from octopaminergic neurons only affects wingless flies, which now preferred the bright arm of the maze (Fig. 5b). Replicating the reversibility described above, after a 24h recovery phase, flies tested at room temperature showed wild type behavior, meaning positive phototaxis for intact flies and negative phototaxis for wing-clipped flies (Fig. 5a,b). The conventional interpretation of these results is that synaptic

transmission from octopaminergic/tyraminergic (OA/TA) neurons is necessary for the negative phototaxis of flightless flies, while synaptic transmission from DA neurons is necessary for the positive phototaxis of intact flies.

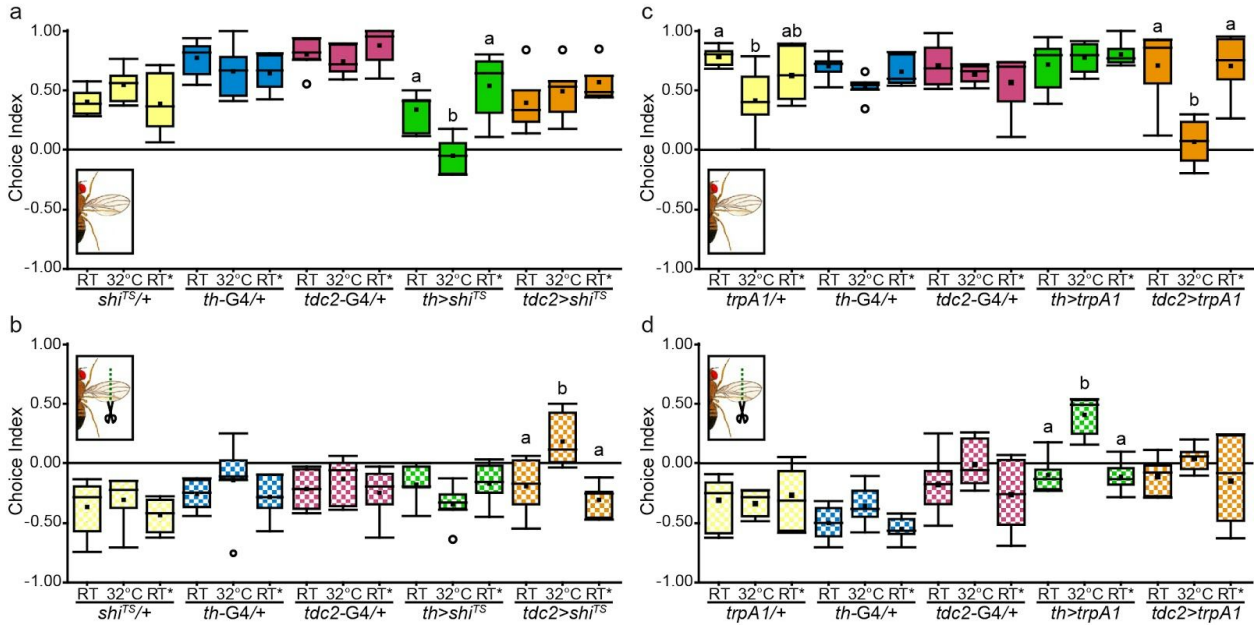

Figure 5. Dopamine and Octopamine are necessary and sufficient to modulate phototactic behaviour, but with opposite effects. a, b, Choice Index from flies with (a) and without (b) wings before, during and after DA or OA neuron silencing. **a,** Randomized Block Design ANOVA, Block p=0.0197, Interaction 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,** Randomized Block Design 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 DA or OA neuron activation. **c,** Kruskal-Wallis for temperature factor comparison within genotypes (alpha after correction=0.0125): *trpA1*/+ p=0.0120, *th*-GAL4/+ p=0.0691, *tdc2*-GAL4/+ p=0.6673, *th>trpA1* p=0.9697, *tdc2>trpA1* p=0.0035. *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=0.0125): *trpA1*/+ p=0.8338, *th*-GAL4/+ p=0.1495, *tdc2*-GAL4/+ p=0.1257, *th>trpA1* p=0.0046, *tdc2>trpA1* p=0.4150. *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 1 for detailed graph information.

We also transiently activated OA/TA and DA neurons, respectively, using the temperature sensitive *TrpA1* channel [46], while testing the flies for their light/dark preference. Again, at room temperature, when the channel is closed, flies with and without wings behaved similar to wild type animals (Fig. 5c,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 behaviour. Flies with clipped wings and activated DA neurons now preferred the bright arm of the maze, with no effect on intact flies (Fig. 5d). Conversely, activating OA/TA neurons only had an effect on flies with intact wings, which now chose the dark arm of the maze, while no effect could be observed in wing-clipped flies (Fig. 5c). Again, when tested back at room temperature 24h later, wild type behaviour was restored. The conventional interpretation of these results is that active OA/TA neurons are sufficient for negative phototaxis, while the activation of DA neurons is sufficient for positive phototaxis.

In summary, we conclude from our data that flies constantly monitor their flying ability and changes in it have a broad impact on the flies' valuation of external stimuli, specifically brightness and darkness. This valuation process appears to be mediated by a concerted action of octopaminergic/tyraminergic and dopaminergic circuits.

Discussion

McEwen's discovery captured our attention because of its implications for the supposed rigidity of simple behaviors in simple animals. We designed our experiments with the intent to test various hypotheses potentially explaining the reduction in phototaxis observed by McEwen. A straightforward explanation for a lower response to light are unspecific motor deficits in the flightless flies. However, flightless flies actively move towards darkness in the T-Maze experiments (Fig. 1d). We also excluded that the effect may only be a peculiar deficit in a particular strain of *Drosophila*, as the wing-clipping effect was present in all control lines tested in the different experiments. Another hypothesis was that injury may trigger negative phototaxis as a general escape response to bodily harm. This explanation appears unlikely, as flies with injured abdomen show intact phototaxis (Fig. 2). In this respect, it is worth to mention that removing the halteres seemed to have a greater effect than damaging the antennae. This difference may be explained by their respective roles during flight. Halteres are comparable to biological gyroscopes and are important for rapid flying manoeuvres [47–49], while antennae contain mechanoreceptors, whose information, combined with visual information, is important

for visually-guided steering and achieving a stable flight speed [50,51]. Moreover, even without injury, flightless flies show negative phototaxis, irrespective of the method used to render them flightless (Figs. 1a, b, 3, 5). We can also exclude that the trauma of manipulating the flies triggered a persistent shock-response, as the effect is reversible (Fig. 3). It also seems unlikely that damage to or removal of the sensory organs in the wings directly triggers negative phototaxis, as flies still change their light/dark preference even in our experiments where the wings remained intact (Fig. 3). We also tentatively conclude that the process does not require any of the known learning processes (Fig. 4b) and is (near) instantaneous (Fig. 1c, d). The observation that none of our hypotheses withstood our experimental tests together with the double dissociation of two neuromodulators known for their involvement in valuation on phototaxis, prompted our current hypothesis that a value-based decision-making process underlies phototaxis in *Drosophila* (more information in supplementary discussion). This conclusion extends and corroborates recent evidence raising doubts as to the general validity of the sensorimotor hypothesis [38,52,53].

Methods

Strains and fly rearing.

Flies were reared and maintained at 25°C in vials containing standard cornmeal agar medium [54] 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 (NIH P40OD018537) were used in this study: *UAS-TrpA1* (26263), *th-GAL4* (8848), *tdc2-GAL4* (9313), and *PKC*$^{\Delta}$ (18258).

The source 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).

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

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

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

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

Wing clipping

Unless described otherwise, 24h before the experiment 2-5 d old flies were briefly anesthetized under CO_2, and the distal two thirds from both wings were clipped from half of the individuals. At least 30 flies with clipped wings and 30 flies with intact wings were placed in the same vial until the experiment was performed, in which they were tested together.

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.

Antennal damage, Halteres removal, and Abdominal Injury

Flies (2-5d old) were anesthetized with CO2 and one of the different treatments (see supplementary Fig. 2) was applied to half of them. At least sixty flies (half of them with injury) were placed in vials for 24h recovery and tested together. Flies with abdominal injury were not mixed with intact flies to avoid mistakes during the evaluation of the experiment due to the inconspicuous nature of the injury.

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

Countercurrent Apparatus

Phototactic preference was evaluated using Benzer's Countercurrent Apparatus [20]. The test group was placed in the initial tube and was left to acclimate for 10 min. 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 initial tube faced 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. Flies that moved to the test tube were transferred to the next tube, and the same test was repeated 4 more times. The Preference Index was calculated using the formula:

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

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.

The change index in figure 4b was calculated as the proportion of change in the PI between flies with and without wings: 1 - ($PI_{clipped}$/PI_{intact}).

T-Maze

Light/Darkness choice was measured in a custom build PVC opaque T-Maze with only one transparent choice tube (acrylic). Flies were placed in an initial dark tube and were left to dark adapt for 10 min. Then, they were transferred to the elevator chamber by gently tapping the apparatus, where they remained for 30s. Next, the elevator was placed between the dark and the bright tube, and flies were allowed to choose for 30s.

The Choice Index was calculated using the formula:

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

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 negative phototaxis and 0 meant no choice. The tubes were cleaned thoroughly after each round.

Buridan

Locomotion towards dark objects was evaluated using Buridan's paradigm as explained in [28]. Briefly, 3-6d old flies were selected and half of them had their wings clipped under CO_2 anaesthesia. 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 analysed using BuriTrack and CeTrAn[28], 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. Three to five days old flies were tested at room temperature (RT) and recovered for 5-6h at 18°C. Then, they were transferred to 37°C climate room where they were placed in an acclimation vial for 15min. Next they were transferred to the first tube from a 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 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.

In the case of manipulation of dopaminergic or 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 experiments were 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 figure 5c and d, homogeneity of variance was violated and a Kruskal-Wallis test was employed for multiple comparisons. The alpha value was corrected using Bonferroni's correction. Raw data are available at http://dx.doi.org/10.6084/m9.figshare.1502427.

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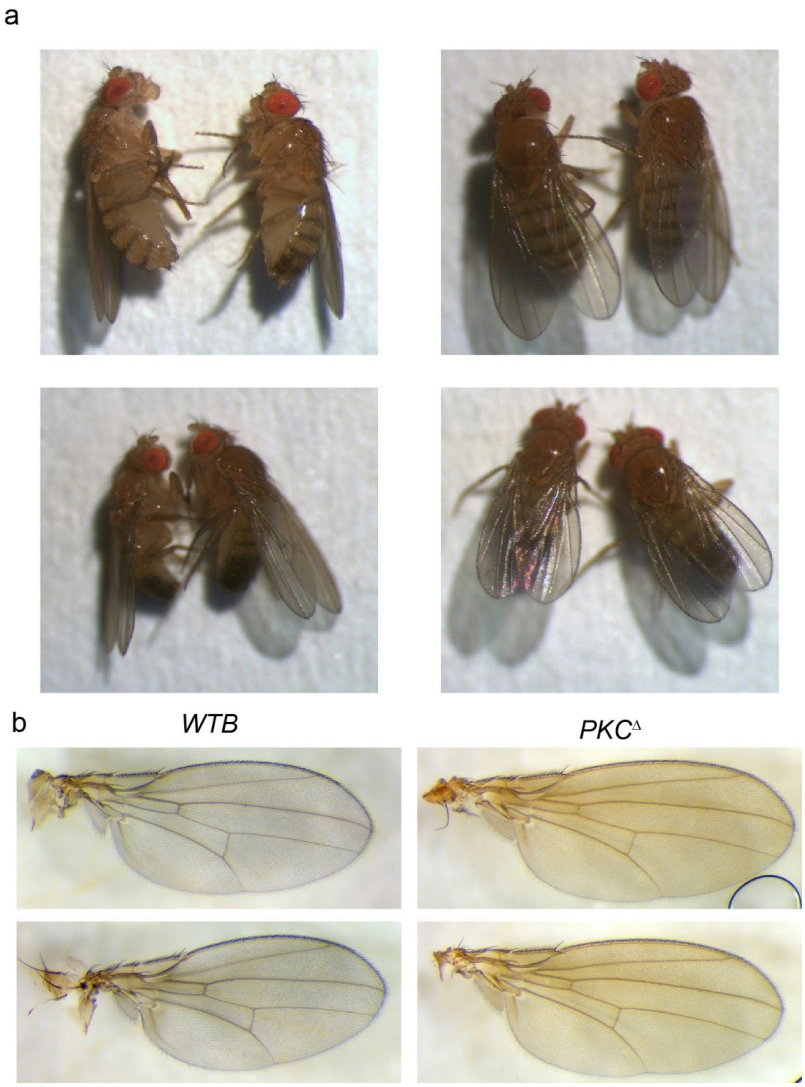
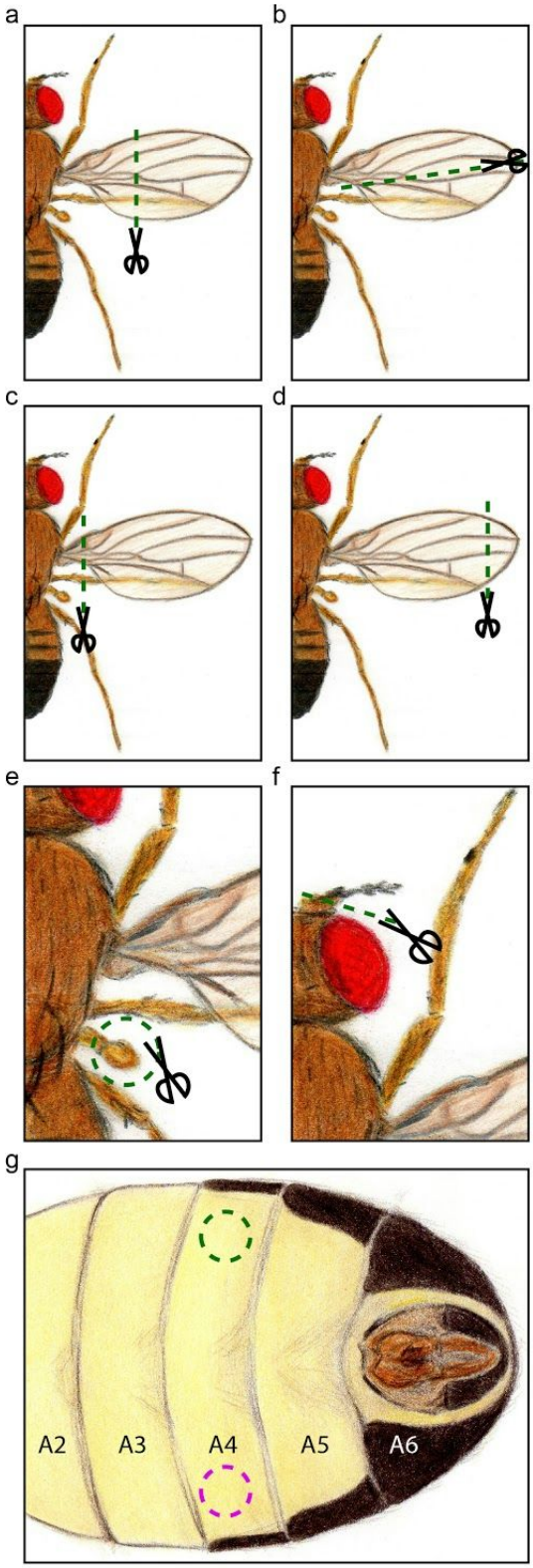
Supplementary Figure 1



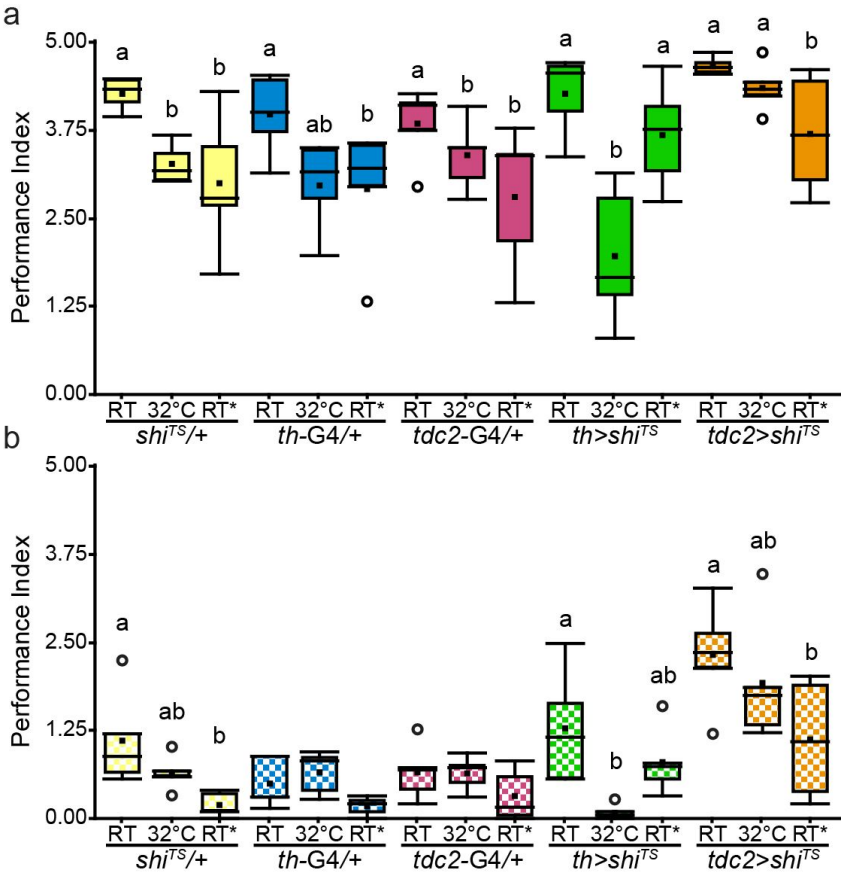
Supplementary Figure 1. *PKC*$^{\Delta}$ mutants have wings with normal shape. a, Wing posture of females (upper panels) and males (bottom panels), lateral view (left panels), dorsal view (right panels). In each panel the fly on the left side is a *WTB* and the fly on the right is a *PKC*$^{\Delta}$ mutant. **b,** Examples of wing anatomy from *WTB* flies and *PKC*$^{\Delta}$ mutant flies.

Supplementary Figure 2



Supplementary Figure 2. Schematic representation of the different injuries made to the flies. a, Wing-cut used in most of the experiments, 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 figure 2a and 2b. **c,** Whole wing cut. This injury was used in figure 2c and 2d to remove only one wing (the side was randomly selected), and in figure 2e and 2f to remove both wings. **d,** End of the wing cut. Around 20% of each wing was removed. It was used in figure 2e and 2f. **e,** Halteres removal. Both halteres were removed and the effect on phototaxis is presented in figure 2g and 2h. **f,** Antennal damage. The third segment of both antennae was cut. This treatment was used for experiments in figure 2i and 2j. **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 figure 2k and 2l.

Supplementary Figure 3



Supplementary Figure 3. BCP Performance Index from flies with (a) and without (b) wings before, during and after Dopamine or Octopamine neurons silencing. Flies showed a lower and more variable PI after 32°C exposure. Temperature shock seems to induce a long lasting effect on the behaviour in the BCP. **a)** Randomized Block Design ANOVA, N=5, Block p<0.0001, Interaction Genotype vs Temperature p=0.0003, simple effects with Tukey's *post hoc* test (p<0.05): least-significant difference= 0.6343, *shi*^{ts}/+ p<0.0001, *th*-GAL4/+ p<0.0002, *tdc2*-GAL4/+ p=0.001, *th>shi*^{ts} p=0.0002, *tdc2>shi*^{ts} p=0.0018. **b)** Randomized Block Design ANOVA, N=5, Block p<0.0001, Interaction Genotype vs Temperature p=0.0012, simple effects with Tukey's *post hoc* test (p<0.05): least-significant difference= 0.8426, *shi*^{ts}/+ p=0.0337, *th*-GAL4/+ p=0.3444, *tdc2*-GAL4/+ p=0.5395, *th>shi*^{ts} p=0.0035, *tdc2>shi*^{ts} p=0.0028. 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 1 for detailed graph information.

Supplementary Discussion

Today, it is widely acknowledged that the human brain is constantly active with external stimuli exerting only a modulatory effect (see, e.g., [1]). Also in invertebrates, the appeal of simple input-output concepts is now rapidly waning, despite already studying seemingly simple behaviors. Mechanosensory input, otherwise reliably triggering escape responses in leeches, ceases to be followed by a response when the feeding animal releases serotonin, blocking sensory transmission from the mechanosensory neurons [2]. Optomotor responses in flies, among the most reliably reproducible behaviors in animals, can vary in amplitude up to 40 fold, depending on state of the animal being either quiescent, walking or flying; the mechanisms of this modulation can be traced all the way from the sensory pathway to motor neurons and involve OA [3–8]. With such a multitude of amine-controlled complex negotiation processes even in relatively simple behaviors in relatively simple nervous systems, can one find even simpler behaviors to search for stimulus-response relationships? Moreover, in these previous preparations, the response was found to be only modulated in magnitude but not in its valence. Perhaps biogenic amines only modulate the strength of the stimulus-response coupling in these animals, leaving the concept essentially intact?

Arguably, moving towards the light might be ranked among the least complex of behaviors, as plants manage phototropism without a nervous system and the larvae of the marine polychaete worm *Platynereis* show positive phototaxis with neither brain nor even inter- or motor neurons [9]. In insects, a positive phototactic response quickly becomes maladaptive in the case of the candle or street light at night or when the animal is trapped at a tilted window, intuitively suggesting a hardwired connection between light and approach. On the other hand, McEwen's and Benzer's observations about the impact of wing condition on *Drosophila* phototaxis, provided evidence that even this simple innate behaviour may be more than just a hardwired response. Our experiments showed that manipulating the ability to fly reversibly alters the flies' preference of light vs. dark not only in magnitude, but also in valence, and imply that the

biogenic amines DA and OA/TA are necessary and sufficient for the modulation of this preference. From these results, it is tempting to generalize that the internal state of an organism always modulates action selection, primarily via the action of biogenic amines, irrespective of the apparent complexity or simplicity of the task.

Nevertheless, it is still prudent to attempt to discuss our results with respect to the traditional stimulus-response perspective. In its conceptually simplest form, the phototactic sensorimotor pathway may be switched between light triggering attraction (positive phototaxis) or aversion (negative phototaxis) by the activity of two circuits, one dopaminergic and another one octopaminergic/tyraminergic. In flies with the ability to fly, dopaminergic neurons are tonically active (i.e, in an ON state), while octopaminergic/tyraminergic neurons are tonically inhibited (i.e, in an OFF state), leading to light triggering attraction. Conversely, in flightless flies, dopaminergic neurons are in the OFF state, while octopaminergic/tyraminergic neurons are in the ON state, leading to light triggering aversion. Thus, inhibition of one or the other pathway leads to different behavioural outputs [10]. In this scenario, the sensorimotor information is gated towards attraction or aversion in a state-dependent modulation of a branched stimulus-response chain. This perspective requires each possible sensory stimulus to be connected to a multitude of motor responses with many modulating circuits exerting their gating functions via their tonic ON/OFF states. Phrased differently, the intrinsic activity of the central modulatory pathways determine how behaviors are shaped by sensory stimuli. This formulation deviates drastically from the original stimulus-response concept, essentially conflating the distinction between a reflexive and an active concept of brain function (e.g.,[1]), onto an active concept. Interestingly, a recent report from a model organism whose connectome is dominated by feed-forward connections, the nematode worm *C. elegans*, found that olfactory stimuli only modulate ongoing ON/OFF fluctuations in a circuit controlling reversal behavior even in this animal with its mere 302 neurons [11].

Therefore, it is both conservative and consequential to discuss our results in terms of insect phototaxis being part of a spectrum of actions, selected after a central decision-making stage. Moreover, both OA and DA action is associated with decision-making mechanisms [12–14] as well as state-dependent changes [7,14–17]. There is also an extensive literature on the role of biogenic amines in value-based decision-making [2,12,13,15,18–27]. Our results that output from OA/TA and DA neurons, respectively, appears to be sufficient and necessary for phototaxis provide independent evidence that phototaxis involves a decision-making step, above and beyond the experiments where flying ability was manipulated by various means. Curiously, the function of these circuits appears to lie in their tonic, rather than their phasic firing properties: constant activation of these neurons throughout the test-phase caused the behavioral shift, irrespective of where the animal was looking. However, while it has been shown that neurons fire action potentials when trpA1 is activated, without recording from these neurons during the behavior, it cannot be excluded that the depolarization only serves to cancel out strong tonic inhibition. Therefore, we are currently designing single animal experiments where such recordings can be performed. It is tempting to predict that the tonic activity in the respective OA/TA and DA sub-populations will show spontaneous fluctuations which predict phototactic choice in individual flies.

Interestingly, DA and OA/TA seem to be necessary and sufficient eac^{28}h for a different aspect of the behaviour. Our results indicate that, while OA/TA is necessary and sufficient for shifting the light/dark preference away from light, DA is necessary and sufficient for shifting it towards light, compared to the respective opposite state. This finding complements emerging evidence currently revising the initial hypothesis that DA mediated aversive value while OA mediated attractive value. Instead, both seem to be involved in mediating certain aspects of value albeit in different modalities or domains. According to this most recent literature, OA mediates the attractive value of sweet taste [29,30], while DA mediates the attractive value of sugar reinforcement [31,32] and the aversive value of electric shock [33]. In binary choice situations like the

T-Maze, it is impossible to know which is the driving force, attraction, aversion or both. Hence, we do not yet know the precise role DA and OA play in mediating the attractive and aversive values of bright or dark situations.

After wing injury, the capability of flies to escape from a potential risk is drastically reduced. A new strategy is needed in order to survive. It is tempting to interpret that the observed change in light preference is reflecting the necessity of the fly to hide until the danger goes away or the flying ability returns (e.g., Fig. 3). This flexibility could be explained in terms of changes in outcome expectations [34]. For diurnal flying insects, light probably represents the possibility of finding food, a mate, and freedom, without entirely ruling out the danger of being caught by a predator. When flying ability is compromised, the value of the different consequences of moving towards light changes and the dangers become more prominent due to the difficulties to escape, hence the flies choose to hide. In this view, the alteration in flying ability promotes a shift in the outcome expectation, which finally drives the selection of an alternative, more adaptive action. The observation that each fly, when it is freshly eclosed from the pupal case and the wings are not yet expanded, goes through a phase of reduced phototaxis until its wings render it capable of flying [35], supports such an adaptive interpretation of this behavioral flexibility.

In addition to developmental reversals of phototaxis [35], parasitism is also well-known to alter phototactic behavior of arthropods (e.g., [36–39]). The amino acid precursor of both DA and OA, Tyrosine, was found to be associated with nematomorph parasite infection in crickets [37]. Conspicuously, parasite infection appears to reverse the normally negative phototaxis in these crickets. While OA was not tested, DA did not show any difference in infected, vs. non-infected animals. It is thus conceivable that manipulation of host OA signalling by the parasite is one mechanism by which the parasite is altering host phototaxis behavior. Similarly, in crustaceans, administering another biogenic amine, Serotonin, was found to reverse phototaxis [37].

Regardless of the theoretical context within which the behavioural flexibility may be discussed, our findings demonstrate that even innate preferences, such as phototaxis, are not completely hard-wired, and depend on the animal's state and other factors. This gives the animal the possibility to decide, for example, when it is better to move towards the light or hide in the shadows. Moreover, the fact that flies adapt their phototactic choice behaviour in accordance with their flying ability shows that flies have the cognitive tools required to evaluate the capability to perform an action and to let that evaluation impact other actions - an observation reminiscent of meta-cognition.

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