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Hide if you cannot fly? Behavioral plasticity in flightless *Drosophila*

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1. Introduction

About one hundred years ago, T.H. Morgan suggested to his student to cut the wings of flies and test their response to light. The student observed that flies with cut wings showed no response to light. Shortly afterwards, Robert McEwen continued to work on the subject (McEwen, journal of experimental Zoology, 1918). In his study, he showed that the effect was specific to the clipping of the wings and not other appendages. He also tested some mutants with non-functional wings and found that cutting their wings did not decrease the already low response ot light. In 1963, Chiang was the first to correlate phototaxis behavior with flying abilities, by looking at the development of both traits in juvenile imagos: young non-flying flies prefer shaded areas to brightly lit ones, and both traits change concomitantly at about 7h after emergence. Finally, in 1967, Benzer presented his counter-current apparatus that allows multiple testing of phototaxis behavior and confirmed that flies without wings do not walk towards the light.

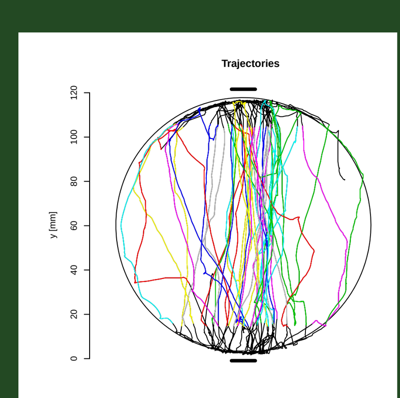
2. Methods

Benzer counter-current apparatus. Wings were manipulated under CO_2 anaesthesia in groups of 100 flies (50 were manipulated and 50 were left intact. The 100 flies were loaded into the first source tube of the Benzer counter-current apparatus, consisting of five target and six source tubes (see figure). Flies were tested in three different setting: with the light towards the target tubes, away from the target tubes or above the apparatus. A phototaxis run lasted 15s. After 5 runs the experiment was ended and the flies were counted. From the number of flies in each tube, a performance index was calculated:

$$PI=[(0*F0)+(1*F1)+(2*F2)+(3*F3)+(4*F4)+(5*F5)]/\Sigma$$

The relative effect size of the wing manipulation was calculated from the PIs of manipulated and intact flies for each experiment:

Buridan's Paradigm. Individual flies walk for 15 min in the arena. Two black bars are positioned on opposite sides. A roof is put on the arena, such that flies are prevented from flying away. The presence of the roof alters the behavior of the flies: they seem to be less attracted by the bars (data not shown).

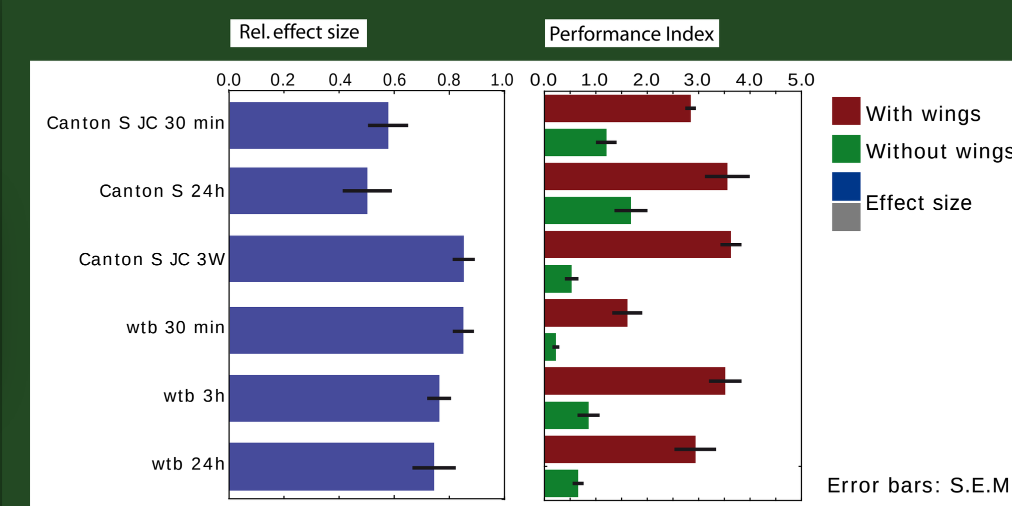


3. Results

Phototaxis

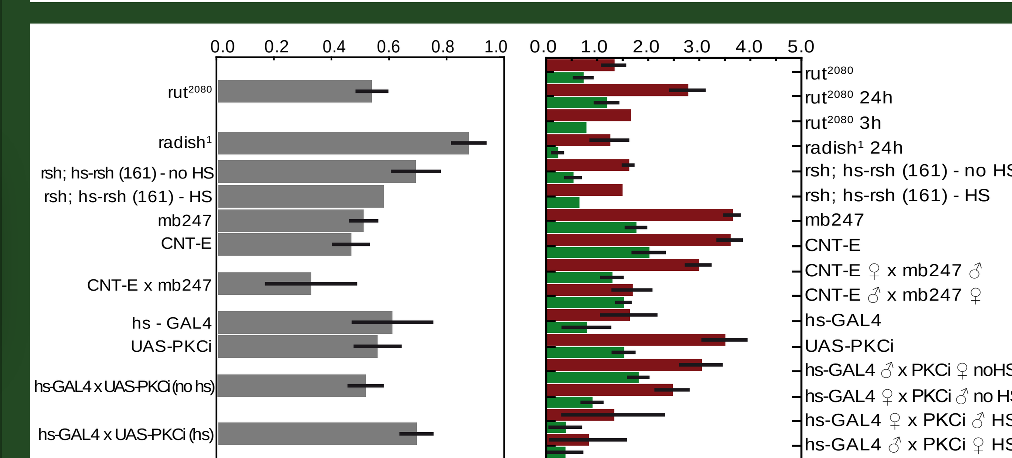
a) Instantaneous

The effect of clipping flies' wings on their behavior is independent of recovery time after the manipulation. Relative effect size in both photo- and geotaxis varies little with recovery time.



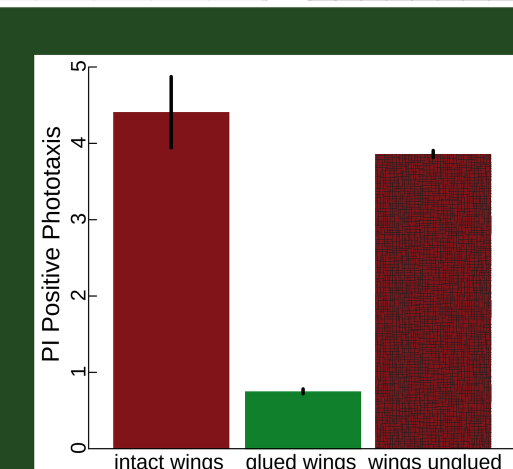
b) Independent of learning

Manipulations of different processes involved in learning and memory have only little impact on the wing-clipping effect. There may be a quantitative contribution, but this remains to be confirmed



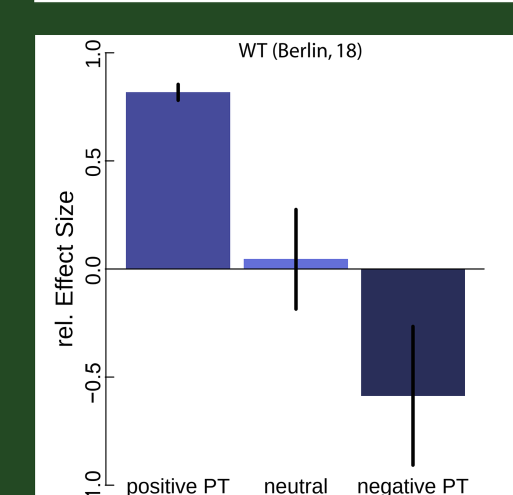
c) Reversible

The wing-clipping effect can be mimicked without wing damage by gluing the wings together using a sucrose solution. Cleaning the wings after the experiment and then re-testing the flies, restores the original response to light.



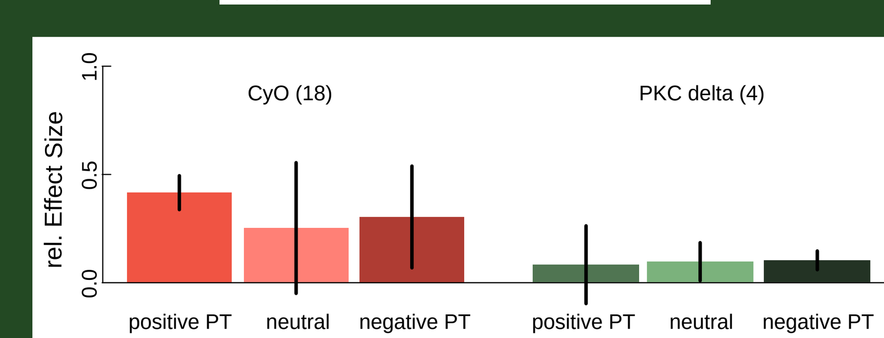
d) Specific

The wing-clipping effect is specific to directional light. It decreases positive phototaxis (left) and increases negative phototaxis (right), leaving general locomotor activity intact (middle).

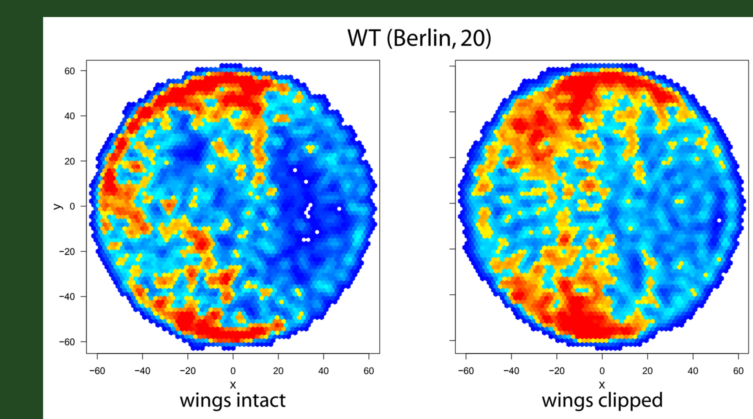


e) Flight ability-dependent

The wing-clipping effect is dependent on the ability to fly. Both flies with deformed wings (CyO mutants, left) and flies with intact wings but without flight ability (PKC delta mutants, right) show strongly reduced wing-clipping effects an all three light conditions.

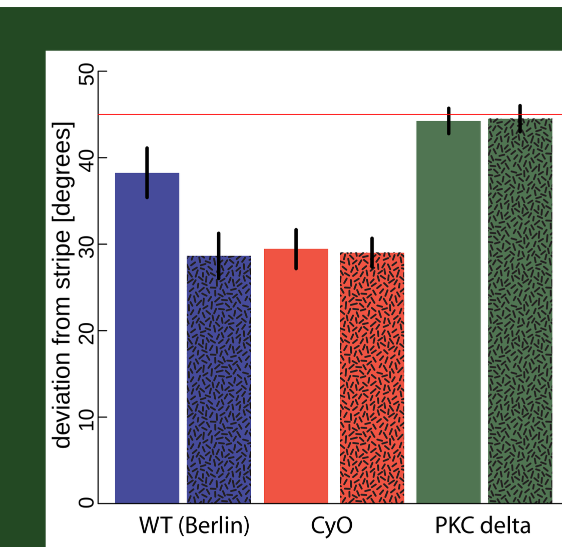


Buridan's



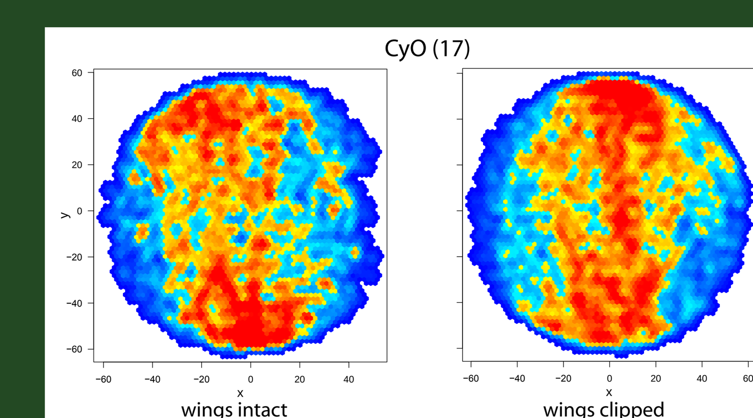
a) Stripe fixation enhanced

Clipping the wings of wild type flies increases the well-described, stereotypical fixation/antifixation behavior in walking flies.



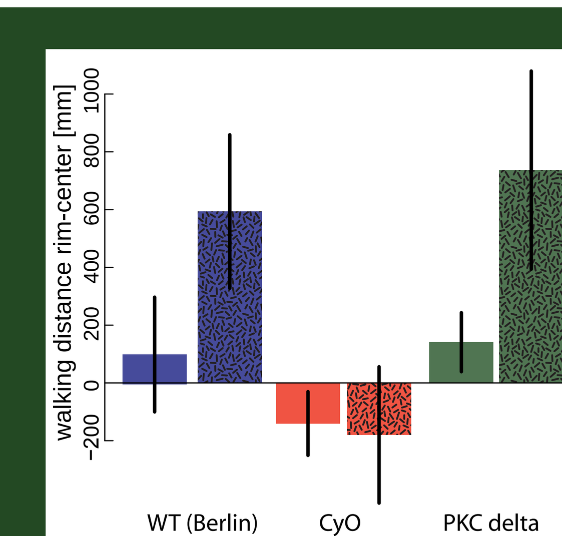
b) No effect in flightless flies

The increase in fixation observed in wild type flies after wing clipping cannot be observed in flightless mutants. Neither the wing-deformed CyO, nor the flightless but wing-intact PKC delta mutant flies increase fixation behavior after wing clipping.



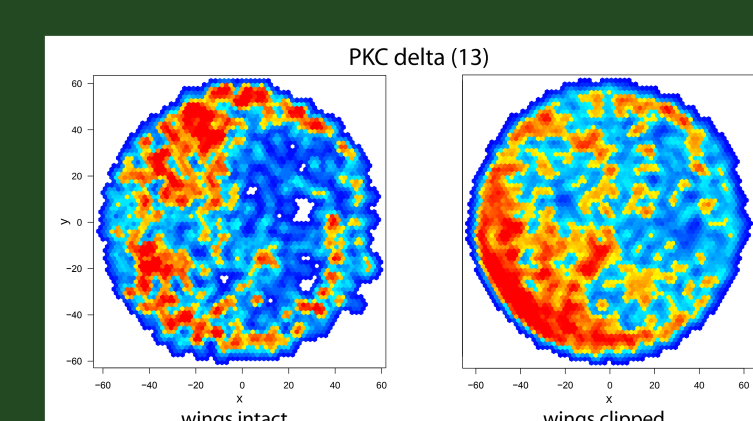
c) CyO mutants fixate well

The wing-deformed CyO mutants already fixate the stripes well before wing-clipping and do not seem to increase fixation behavior after wing-clipping.



d) Centrophobism enhanced

Wing-clipping enhances centrophobism in wild type and PKC delta mutant flies, but not in CyO flies. Thus, centrophobism appears to be modulated independently of flight-ability.



e) PKC delta centrophobism

The wing-clipping induced centrophobism in PKC delta flies is independent of the visual stimuli on the arena wall. Thus, stripe fixation but not centrophobism is specifically affected by manipulations of flight ability.

4. Conclusions

Plasticity means 'simple' behaviors are not so simple
Simple taxis behaviors are considered to be hard-wired input-output systems: the sensory input triggers motor output via developmentally determined neuronal connections. Examples of such simple behaviors include the photo- and stripe fixation tested here. However, even such simple behaviors show some degree of plasticity: walking flies whose wings have been cut show reduced positive phototaxis and increased stripe fixation compared to intact walking flies.

Immediate, robust plasticity
The wing clipping effect appeared as soon as the flies recovered from anaesthesia and lasted for the lifetime of the animals. We have tested a large number of different wildtype and transgenic strains for their reduction in phtototaxis after clipping of their wings. The only fly strains in which wing-clipping did not lead to a reduction in phototaxis were already flightless flies. These experiemnts suggest that the wing-clipping efect is unlikely to be due to learning effects

Plasticity affects stimulus valuation
The behavioral changes brought about by clipping the wings appear not to affect general walking behavior in the Benzer counter-current apparatus. Instead, walking towards the light (positive phototaxis) appears decreased, while walking away from the light (negative phototaxis) appears increased. A similar behavioral disposition has been reported for immature imagos, which cannot fly, yet. It appears as if wing-clipping modifies the valuation of visual stimuli: light becomes less attractive and darkness becomes more attractive.
The experiments in Buridan's paradigm support the interpretation that flightless flies show strikingly different behavioral responses to light/dark stimuli, compared with flies which are able to fly. Clipping the wings of these flightless flies does not alter their behavior with regard to light/dark stimuli any further.

Co-opting behavioral dispositions?
The results so far prompt us to formulate the following working hypothesis: Flies possess an online flight-ability monitor. The status of this monitor determines the attractiveness of light and darkness, respectively. It is tempting to speculate that this mechanism evolved to protect immature imagos by inducing hiding after eclosion and to facilitate dispersal and foraging once the cuticle has fully hardened. Sufficiently frequent wing damage could have kept this mechanism active also in mature flies.

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