A decision underlies phototaxis in an insect

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26 **Background**

27 Like a moth into the flame - Phototaxis is an iconic example for innate preferences. Such

28 preferences likely reflect evolutionary adaptations to predictable situations and have traditionally

29 been conceptualized as hard-wired stimulus-response links. Perhaps therefore, the century-old

30 discovery of flexibility in *Drosophila* phototaxis has received little attention.

31 **Results**

32 Here we report that across several different behavioral tests, light/dark preference tested in

33 walking is dependent on the flies' ability to fly. If we temporarily compromise flying ability,

34 walking photopreference reverses concomitantly. Neuronal activity in circuits expressing

35 dopamine and octopamine, respectively, plays a differential role in this case of behavioral

36 flexibility.

37 **Conclusions**

38 We conclude that flies monitor their ability to fly, and that flying ability exerts a fundamental

39 effect on action selection in *Drosophila*. This work suggests that even behaviors which appear

40 simple and hard-wired comprise a value-driven decision-making stage, negotiating the external

41 situation with the animal's internal state, before an action is selected.

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43 **Keywords**

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

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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

78 phototaxis. This innate preference for light appears to be species- and strain-specific and has

79 been described as part of a fly's personality [20]. Interestingly, experiments described by

80 McEwen in 1918 and Benzer in 1967 demonstrated that wing defects affect phototaxis also in

81 walking flies. These early works showed that flies with clipped wings did not display the

82 phototactic response to light, whereas cutting the wings from mutants with deformed wings did

83 not decrease their already low response to light any further [21,22]. The fact that manipulating

84 an unrelated organ, such as wings, affects phototaxis contradicts the assumed hard-wired

85 organization of this behavior, suggesting that it may not be a simple matter of stimulus and rigid,

86 innate response, but that it contains at least a certain element of flexibility. In this work, we

87 systematically address the factors involved in this behavioral flexibility and begin to explore the

88 neurobiological mechanisms behind it.

89 **Methods**

90 **Strains and fly rearing.**

91 Flies were reared and maintained at 25°C in vials containing standard cornmeal agar medium

92 [23] under 12h light/dark cycles with 60% humidity, except for experiments involving UAS-*trpA1*

93 or UAS-*shibire^{TS}*, in which parental crosses and their offspring were maintained at 18°C under

94 12h light/dark cycles with 60% humidity.

95 Stocks obtained from the Bloomington Drosophila Stock Center (RRID:SCR_006457; NIH

96 P40OD018537) were used in this study: *UAS-TrpA1* (26263), *th*-GAL4 (8848), *tdc2*-GAL4

97 (9313), and *PKC^Δ* (18258).

98 The sources of other stocks are detailed here:

99 *w^{1118}*, *w^{1118}; hs*-Gal4 (heat shock inducible GAL4), and *UAS-PKCi* (inhibitory pseudosubstrate of

100 protein kinase C) were provided by Henrike Scholz (University of Cologne, Germany).

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

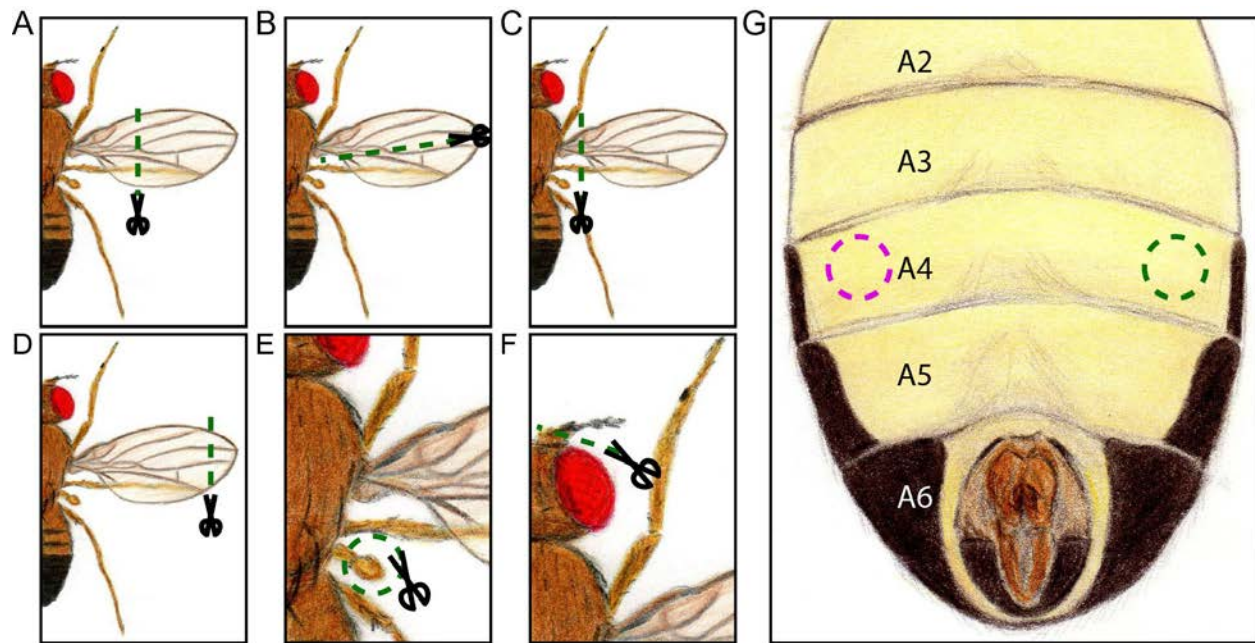102 *CS^{RE}* is a *CS* strain bred in our lab in Regensburg.

103 *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.

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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

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142   Phototactic preference was evaluated using Benzer's Countercurrent Apparatus [21]

143   (http://dx.doi.org/10.17504/protocols.io.c8gztv). The apparatus was completely transparent and

144   consisted of two acrylic parts, a lower one with 6 parallel tubes (an initial tube + 5), and a

145   movable upper part with 5 parallel test tubes. Each plastic tube had a length of 6.8 cm, an inner

146   diameter of 1.5 cm, and an outer diameter of 1.7 cm. The test group was placed in the initial

147   tube and was left in darkness to acclimate for 10 min, with the apparatus placed horizontally.

148   Thereafter, flies were startled by tapping the apparatus, making all of them end up at the bottom

149   of the tube. The apparatus was placed horizontally and the upper part shifted, making the initial

150   tube face the first test tube for 15 seconds, allowing the flies to move towards the light if the test

151   tube was facing it, or away from it if the initial tube was facing the light. Then, the upper part was

152   shifted again and flies that moved to the test tube were transferred to the next tube of the lower

153   part by tapping the apparatus, and the same test was repeated 4 more times. The light source

154   was always placed at 30 cm from the apparatus and consisted of a fluorescent warm white tube

155   (OSRAM 18W/827), which delivers 1630 lux at that distance.

156   The Performance Index was calculated using the formula:

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

157   where $\#F_n$ was the number of flies in the tube n (being 0 the initial tube and 5 the last test tube),

158   and $\#F_T$ was the total number of flies. If the test tubes were on the bright side a higher index

159   meant a more positive phototaxis. In each experiment a PI was calculated for the wingless flies

160   and other for the intact flies. The tubes were cleaned thoroughly after each test.

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

162   **T-Maze**

163   Light/Darkness choice was measured in a custom build PVC opaque T-Maze with only one

164   transparent (acrylic) choice tube (http://dx.doi.org/10.17504/protocols.io.c8azsd). Flies were

165   placed in an initial dark tube (10 cm long, 1.5 cm inner diameter, and 2.5 cm outer diameter) and

166   were left to dark adapt for 10 min. Then, they were transferred to the elevator chamber (1.5 cm

167   diameter, 1.5 cm height) by gently tapping the apparatus, where they remained for 30s. Next,

168   the elevator was placed between the dark and the bright tube (both 20 cm long, 1.5 cm inner

169   diameter, and 2.5 cm outer diameter), and flies were allowed to choose for 30s. As the source of

170   light, the same fluorescent tube as for Benzer's Countercurrent Apparatus was used, and placed

171   31.5 cm above the base of the T-Maze.

172   The Choice Index was calculated using the formula:

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

173   where $\#F_L$ meant the number of flies in the transparent tube, $\#F_D$ was the number of flies in the

174   opaque tube, and $\#F_E$ was the number of flies that remained in the elevator. A *CI* of 1 meant all

175   the flies chose the light, while an index of -1 meant a dark photopreference. The tubes were

176   cleaned thoroughly after each round.

177   **Buridan**

178   Locomotion towards dark objects was evaluated using Buridan's paradigm as explained in

179   Colomb *et al.* [24]. Briefly, 3-6d old flies were selected and half of them had their wings clipped

180   under CO_2 anesthesia (http://dx.doi.org/10.17504/protocols.io.c7vzn5). They were left to recover

181   overnight within individual containers, with access to water and sugar (local store) before being

182   transferred to the experimental setup. The setup consists of a round platform (117 mm in

183   diameter) surrounded by a water-filled moat placed at the bottom of a uniformly illuminated white

184   cylinder (313 mm in height) with 2 stripes of black cardboard (30mm wide, 313 mm high and 1

185   mm thick) placed 148.5 cm from the platform center one in front of the other. Flies were

186   prevented from escaping by a transparent lid over the platform. The experiment duration was set

187   to 900 seconds. Data were analyzed using BuriTrack and CeTrAn [24] (RRID:SCR_006331),

188   both available at http://buridan.sourceforge.net.

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190 **Genetic manipulation of flying ability and neuronal activity**

191 For the experiments involving *TrpA1 and the act88f-GAL4* driver, experimental flies and their

192 respective controls were raised at 18°C. 3-5d old flies were tested at room temperature (RT) and

193 recovered for 5-6h at 18°C. Then, they were transferred to a 37°C climate room where they were

194 placed in an acclimation vial for 15min. Next they were transferred to the first tube of the T-maze

195 placed in the 37°C climate room, and the experiment proceeded as explained above. The choice

196 step was reduced to 15s to compensate for the increased activity that flies showed in pilot

197 experiments. After counting the flies, they were transferred to fresh vials and placed at 18°C for

198 24h. After this recovery phase, they were tested again at RT. We noticed that the CI obtained for

199 wild types could differ between chambers at 37°C .

200 In the case of manipulation of dopaminergic and octopaminergic neural activity with shi^{TS} or

201 *TrpA1* the same protocol was applied but instead of 37°C, 32°C were used and the choice step

202 was 30s long.

203 **Statistical Analysis**

204 Statistical analyses were performed with InfoStat, version 2013 (Grupo InfoStat, Facultad de

205 Ciencias Agropecuarias, Universidad Nacional de Córdoba, Córdoba, Argentina) and R

206 (http://www.r-project.org/). Number of replicates in each experiment was adjusted to provide a

207 statistical power of at least 80% using pilot experiments. As dictated by the experimental design

208 and data composition, a paired T-test, a Randomized Block Design ANOVA or an ANOVA were

209 performed. Normality was tested using Shapiro–Wilks test, and the homogeneity of variance was

210 assessed with Levene's test. A value of $p<0.05$ was considered statistically significant. After

211 ANOVA, a Tukey least-significant difference or an orthogonal contrasts test was performed. If an

212 interaction between factors was significant in two-way ANOVAs, simple effects were performed,

213 and p values were informed. In figures 1A, 3B-E and H, and 7C and D, homogeneity of variance

214 was violated. In figures 1A, and 3B-E and H a Wilcoxon test was used, while in figures 7C and D

8

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

240 which are indistinguishable from wild type wings (Fig. 2D), the *CyO* balancer line with curly

241 wings, and a transgenic line in which the wings were deformed due to an overexpression of a

242 constitutively active form of the *baboon* receptor in wing imaginal discs (*A9>babo^{QD}*, [25]). Again

243 replicating previous experiments, *CyO* flies showed a reduced PI that remained unchanged in

244 wing-clipped animals (Fig. 2B). Similarly, *A9>babo^{QD}* showed less attraction to light and no

245 significant wing-clipping effect (Fig. 2C), while all genetic controls behaved similar to wild type

246 flies. Remarkably, *PKC$^{\Delta}$* mutants exhibited the same behavioral characteristics as *CyO* flies (Fig.

247 2B). Hence, we conclude that the reduction in phototaxis is not dependent on the origin of wing

248 damage or the damage itself, but probably on wing utility.

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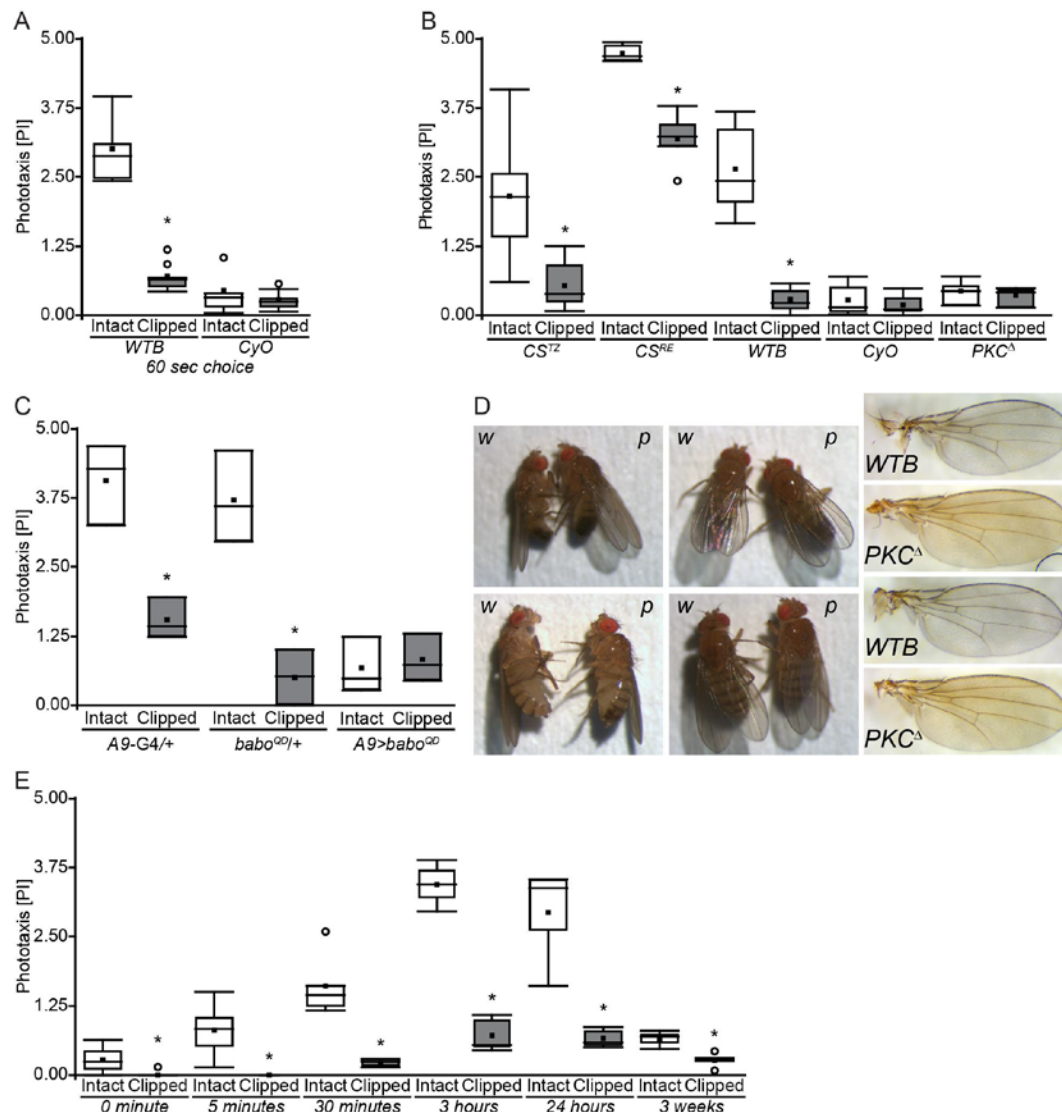
250 **Figure 2. The wing-clipping effect is observable across genetic backgrounds and throughout adult**

251 **lifespan, but is absent in flightless flies**. *A*, Replication of the original BCP experiments using 60s of

252 time in which the animals were allowed to walk towards the light. Wilcoxon test; *WTB*: N=8, p<0.001; *CyO*:

253 N=8, p=0.505 *B*, BCP Performance Index (15s choice time) from three wild type strains and two flightless

254 mutants with intact and clipped wings. Paired T-test; CS^{TZ}: N=6, p=0.003; CS^{RE}: N=5, p<0.001; *WTB*:

255 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

256 genetic manipulation of wing development ($A9>babo^{QD}$) and their genetic control groups ($A9$-G4/+,

257 $babo^{QD}$/+). Randomized Block Design ANOVA; N=3; Block p<0.001, Interaction Genotype vs Wings

258 Integrity: p<0.001, simple effect Genotype: $A9$-G4/+: p<0.001, $babo^{QD}$/+: p<0.001, $A9>babo^{QD}$: p=0.401.

259 *D*, Lateral and dorsal view of wing posture of *WTB* (*w*) and PKC^{Δ} (*p*) males (upper panels) and females

260 (lower panels). Right panels: Examples of wing anatomy from *WTB* flies and PKC^{Δ} mutant flies. *E*, BCP

261 Performance Index of WTB flies after different recovery time lengths. Paired T-Test, 0 minutes: N=6,

262 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,

263 p=0.005 ; 3 weeks: N=5 , p=0.004. * indicates significant differences. Box plot show quantiles 0.05, 0.25,

264 0.75 and 0.95, median, mean (black square), and outliers (circle).

265

266 **The behavioral change is immediate**

267 If flies were able to assess wing utility, wing-clipping might have an almost instantaneous effect

268 on the behavior. Thus, to find out when the behavioral change takes place, we assessed wing-

269 clipped *WTB* flies at different time points after the injury was made. Flies from different groups

270 were tested either 3 weeks, 24h, 3h, 30min, 5min or immediately after the surgery. To diminish

271 the effects of anesthesia on phototactic behavior [26], we only used CO_2 anesthesia for recovery

272 times longer than 30min, and cold anesthesia for 0 and 5min recoveries. We found that the

273 reduction in phototaxis could be observed in all tested groups (Fig. 2*A*). Moreover, the difference

274 between intact and clipped flies increased with longer recovery phases, probably due to the

275 vanishing of the anesthesia effect, only to decrease again in aged flies, perhaps due to a

276 combination of a deteriorated locomotor activity and a decreased response to light in old flies

11

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

303   several decades [24,29]. We compared total activity time, walking speed, and pause duration in

304   intact and wingless flies from three lines (*WTB*, *CyO*, *PKC*^Δ) in a modified version of Buridan's

305   Paradigm, where a roof prevents the flies from escaping. The results show only occasional small

306   differences with the overall tendency of wingless flies exhibiting, if anything, slightly higher

307   general activity than intact flies (Fig. 3B, C, D).

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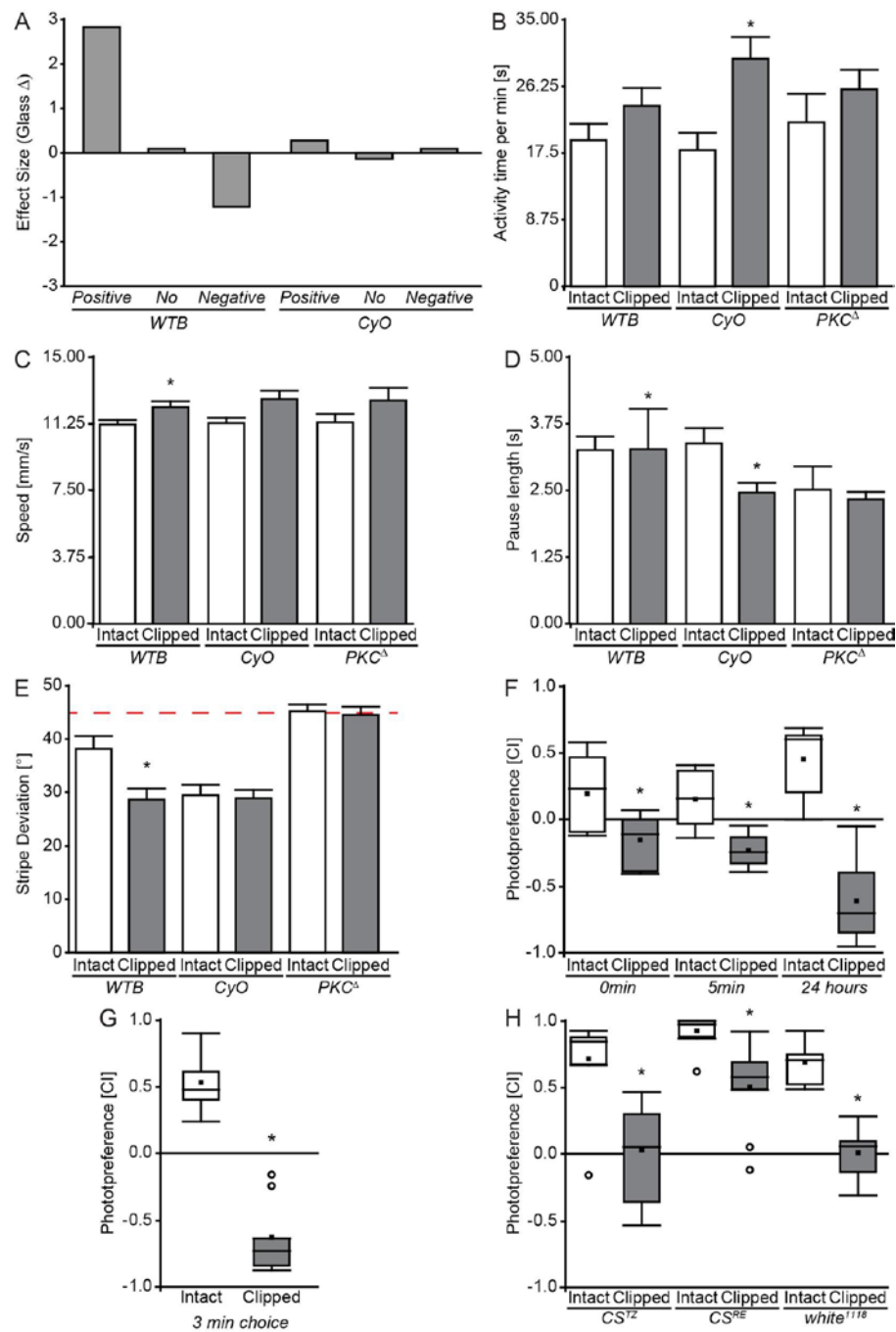
309   **Figure 3. Flies without wings are not less active and prefer darker stimuli.** *A*, Effect Size of wing

310   clipping on BCP with the light source on the opposite side of the starting tube (positive phototaxis -

311   *Positive*-), light source on top of the BCP (no taxis -*No*-; locomotor activity control), and light source on the

312   same side as the starting tube (negative phototaxis -*Negative*-). *B-E*, Buridan's paradigm. *WTB*: Intact,

313   N=20; Clipped, N=21. *CyO*, N=17. PKC^Δ, N=13. Wilcoxon test. *B*, Activity time. *WTB*: p=0.151. *CyO*,

314   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.

315   *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,

316   p=0.98. Dotted line indicates 45°, the mean value for computer-generated data. *F*, T-Maze Choice Index

317   after different recovery time lengths. Paired T-Test, 0 minutes: N=7, p=0.003; 5 minutes: N=6, p=0.026; 24

318   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.

319   *H*, Choice Index of CS^{TZ}, CS^{RE} and w^{1118} flies with intact and clipped wings. Wilcoxon test. CS^{TZ}, p=0.003.

320   CS^{RE} p<0.001. w^{1118} p<0.001. See figure 2 for detailed graph information.

321

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

323   Interestingly, the wing-clipped wild type flies also showed a stronger fixation of the black stripes

324   in Buridan's Paradigm, compared to the intact flies, while the flightless flies did not show such a

325   difference (Fig.3E). This result is consistent with the tendency of the wild type flies to show some

326   negative phototaxis after wing clipping (Fig. 3A). One possible explanation for these two

327   congruent observations in such disparate experiments is that the darker stimuli become more

328   attractive after wing clipping in situations where the animals are faced with a choice of darker

329   and brighter stimuli. One prediction of this hypothesis is that other experiments where the

330   animals face a choice of bright and dark stimuli should also be affected by wing-clipping. To test

331   the generality of the wing-clipping effect and to obtain a third independent test of general activity,

332   we set out to develop a T-maze experiment, where the animals are forced to choose between a

333   dark and a bright arm.

334

335

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

337 After several pilot experiments with a variety of different T-maze designs, we arrived at an

338 experimental design where wing-clipped WTB flies would robustly avoid the transparent tube

339 and approach the dark tube (see Material and Methods). As for the BCP, we selected different

340 recovery times (0min, 5min or 24h). Congruent with the BCP results, intact flies showed a

341 positive photopreference, while wing-clipped flies switched to light avoidance and a negative

342 photopreference immediately after their wings were cut (Fig. 3*F*). These results hold even if the

343 flies are allowed three minutes to choose between the two arms of the T-Maze (Fig. 3G). Also

344 similar to the results in the BCP, we found that the magnitude of the baseline photopreference in

345 intact flies and the wing-clipping effect varied with the genetic background. In the case of the T-

346 Maze, the size of the effect determined whether or not the wing-clipped flies would show positive

347 or negative photopreference (Fig. 3H).

348

349 **Only injuries affecting flight abilities promote a change in photopreference**

350 While the mutant or transgenic flies used so far may shift their photopreference due to unknown

351 side effects, the shift in wing-clipped flies could in principle be brought about either directly by

352 the injury or indirectly via a detection of flying ability. To distinguish between these two

353 hypotheses, we tested the effects of a series of injuries (see Materials and Methods, Fig. 1), only

354 some of which affecting some aspect of flight, in BCP and in the T-Maze. First, we evaluated

355 flies with a longitudinal cut through their wings and flies with only one of the two wings

356 completely removed (the side was randomly selected). Both manipulations cause flightlessness.

357 Again, we observed the same shift in photopreference as with standard wing-clipping (Fig. 4*A-*

358 *D*). Both flies with longitudinally cut wings (Fig. 4*A,B*) and one wing removed (Fig. 4*C,D*)

359 exhibited diminished phototaxis in BCP and a negative photopreference in the T-Maze. During

360 our pilot experiments, we observed that flies with different degrees of injuries on their wings

361 behaved differently. Therefore, we hypothesized that manipulations affecting only some aspects

362   of flight behavior, rather than abolishing flight completely, might lead to less pronounced

363   behavioral changes. Thus, we next compared the behavior of flies whose wings were completely

364   removed, with those where only the tip of the wings had been removed. Flies with partially

365   removed wings are still able to fly, but with reduced torque during turns and reduced lift/thrust

366   [30]. It is worth mentioning that McEwen also attempted to test if the decrease in positive

367   phototaxis was directly proportional to the amount of wing removed, but his low number of

368   replicates, the use of ether as an anesthetic, and his different setup, prompted us to obtain our

369   own data (the same for antenna experiments –see below–).

370   In both cases (complete and partial removal), injured flies showed a statistically significant

371   reduction in BCP phototaxis and T-Maze photopreference, but both indices were higher in flies

372   with only the end of the wing cut (Fig. 4*E,F*). In fact, the behavior from both types of injured flies

373   was significantly different from one another in the T-Maze paradigm (Fig. 4*F*). Therefore, we

374   conclude that behavioral change depends to some extent on the degree of the injury, and on

375   which aspects of flight behavior it affects. To test yet other aspects of flight behavior, we

376   administered injuries that did not affect the wings, in two organs related to flight (halteres and

377   antennae) and one unrelated to flight (the abdomen). In one group of flies, we removed the

378   gyroscopic halteres, mechanosensors involved in sensing body rotation and necessary for free

379   flight [31–33]. In another, we removed the distal segments of the antennae (funiculus and arista),

380   depriving the flies of their most important mechanosensor for airspeed and wind direction [34–

381   36]. The two different treatments both significantly decreased photopreference values (Fig. 4G-

382   J). However, only the manipulation abolishing free flight completely, haltere removal, also led to

383   negative photopreference in the T-Maze (Fig. 4H). Affecting flight stabilization and speed by

384   removing parts of the antennae renders the flies indifferent to the light, on average (Fig. 4J).

385   Thus, when flies are still able to fly, but individual aspects of flight behavior are disrupted such

386   as stabilization, torque, speed or lift/thrust, their photopreference is less severely affected than

387   when flight is abolished completely. To test whether any injury, even one that does not affect any

16

388   aspect of flight at all, can affect photopreference, we used a small needle to carefully puncture

389   the abdomen of the flies. Consistent with the results so far, a wound in the abdomen did not

390   produce any detectable shift in photopreference (Fig. 4*K,L*).

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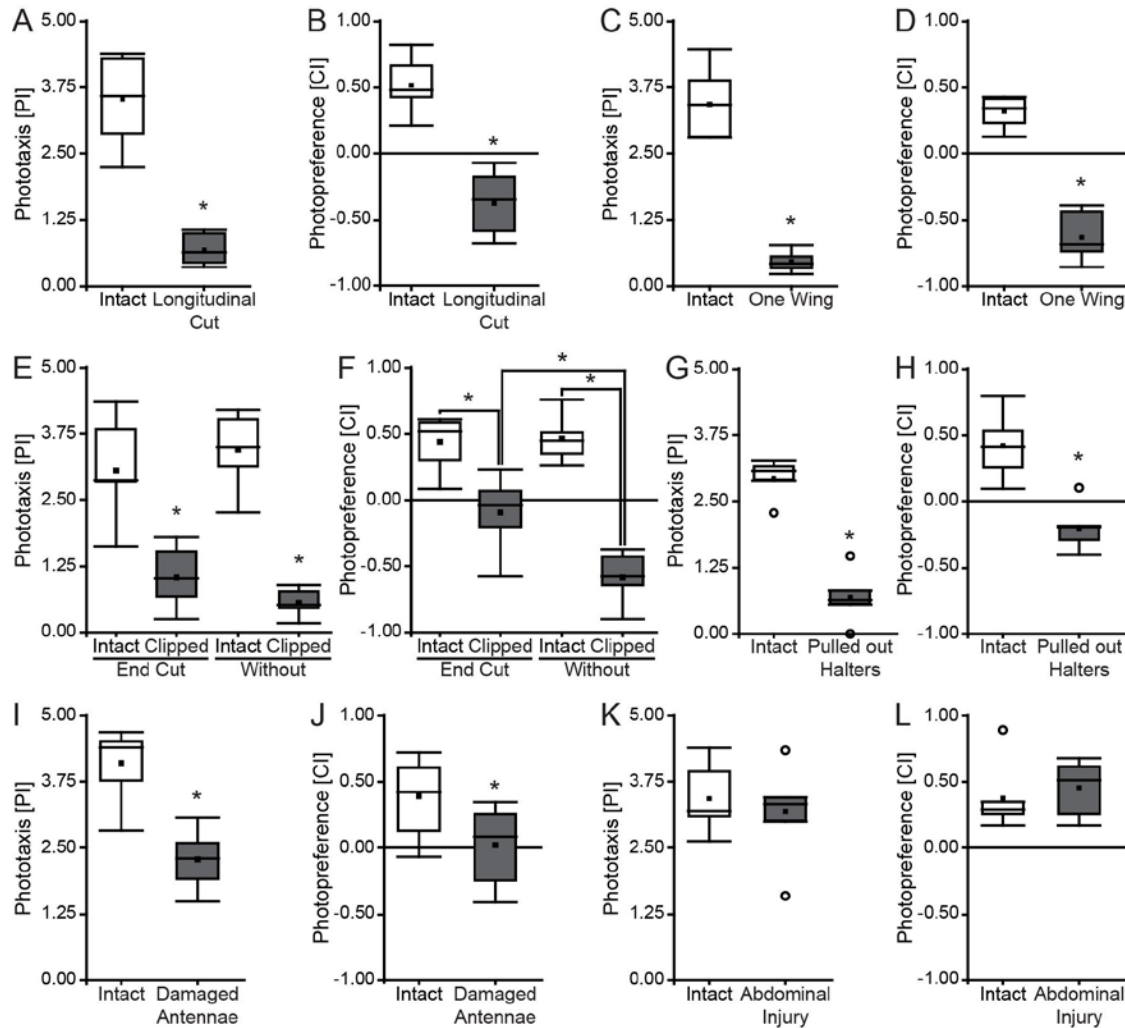
392   **Figure 4. Only flight-affecting injuries change photopreference.** *A, C, E, G, I, K,* BCP Performance

393   Index from WTB flies with and without different injuries. *B, D, F, H, J, L,* T-Maze Choice Index from WTB

394   flies with and without different injuries. *A*, *B*, Longitudinal cut of the wings. N=7, *A*: $p<0.001$, *B*: $p<0.001$.

395   *C, D*, Only one wing cut. N=7, *C*: $p<0.001$, *D*: $p<0.001$. *E, F*, Wing clipped at different lengths.

396   Randomized Block Design ANOVA; N=6; *E*: Block $p= 0.094$, Interaction Wings Integrity (intact or clipped)

397   vs Degree of Injury (without wings or end of the wings cut): $p=0.087$, Wings Integrity: $p<0.001$, Degree of

398   Injury: $p=0.797$; *F*: Block $p= 0.238$, Interaction Wings Integrity vs Degree of Injury: $p=0.007$, simple effects:

399   end cut vs intact: $p<0.001$, without wings vs intact: $p<0.001$, end cut vs without wings: $p<0.001$, intact

400   (control from end cut) vs intact (control from without wings): p=0.865. *G, H*, Both halteres removed. *G*:

401   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*,

402   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

403   1 for detailed graph information.

404

405   **The shift in photopreference is reversible and traces flying ability.**

406   If flies were monitoring the different aspects of their flying abilities and changing their

407   photopreference accordingly, one would expect that transient impairments in flying ability would

408   cause transient changes in photopreference. To examine the reversibility of the behavioral shift,

409   we designed two complementary experiments. In the first, we tested *WTB* flies in BCP and T-

410   Maze before and after gluing, as well as after ungluing their wings. Wing gluing perfectly

411   reproduced the wing-clipping effect, evinced by a clear reduction of the PI and CI (Fig. 5*A,B*),

412   showing again that the shift in photopreference is independent from the cause of the

413   flightlessness. Remarkably, normal photopreference was restored after cleaning the wings of the

414   tested flies (Fig. 5*A,B*).

415   In our complementary approach, we manipulated flying ability by reversibly altering Indirect

416   Flight Muscle (IFM) contraction, expressing the temperature-sensitive *TrpA1* channel under the

417   promoter of the IFM-specific gene *actin 88F* (*act88F*), using the *act88F*-GAL4 [37] driver. At

418   room temperature, experimental flies tested in our T-Maze were indistinguishable from their

419   genetic controls. However, at 37°C, when *TrpA1* caused a sustained IFM contraction disrupting

420   wing movements, the same flies showed a marked preference for the dark arm of the maze that

421   fully recovered when they were tested back at room temperature on the following day (Fig. 5*C*).

422   The genetic controls also showed a CI decrease at 37°C, but it was less pronounced and

423   significantly different from the experimental group. In sum, these results show that flies adjust

424   their photopreference in accordance with their flying ability. Moreover, these changes are

425   immediate and reversible.

18

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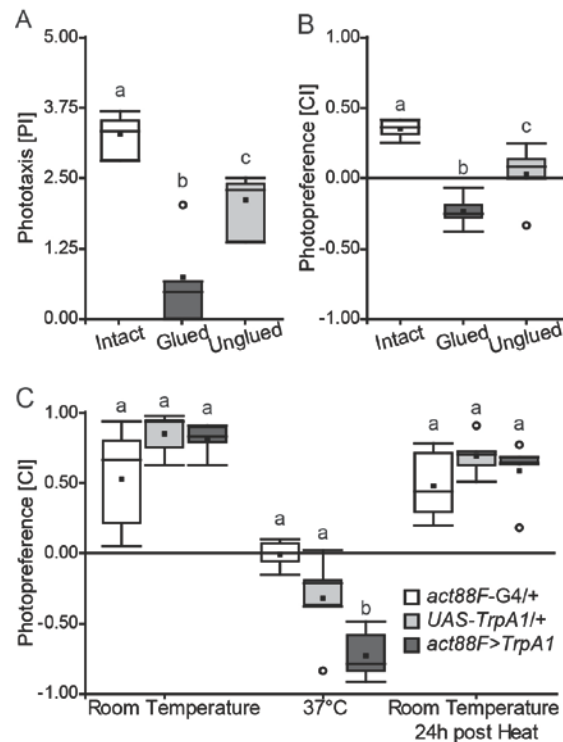
Figure 5. Photopreference changes together with flying ability in a reversible manner. A, BCP tests in flies before, during and after their wings had been rendered useless by applying (and then removing) sucrose solution. Randomized Block Design ANOVA, N=4, Block p=0.091, ANOVA p<0.001, Tukey's *post 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

19

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*).

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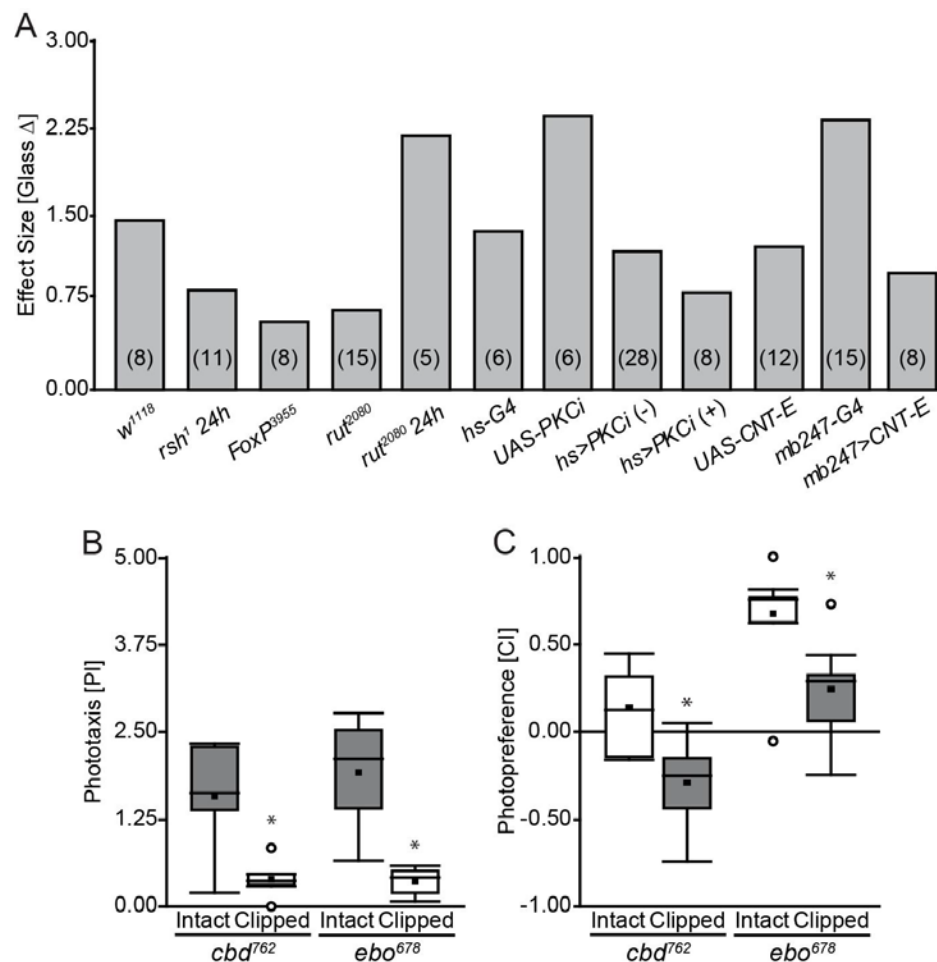
452   **Figure 6. The wing-clipping effect is independent from known learning/memory processes or**

453   **neuropil areas associated with learning. *A,*** Proportion of change in Performance Index after wing-

454   clipping (Change Index) from several lines with learning and memory impairments and their controls.

455   N=Numbers in brackets. *C, D,* behavioral performance from two structural Central Complex mutants with

456   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,

457   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.

458

459   **The behavioral switch is not central complex-dependent**

460   The central complex is a higher-order neuropil related to locomotion [38,39], visual information

461   processing [40], orientation [41], visual pattern recognition [42,43] and spatial working memory

462   [44]. As many of these functions may be important for either phototaxis or its flexibility, we tested

463   two structural mutants of this neuropil, Central Body Defect (*cbd*^762^) and Ellipsoid Body Open

464   (*ebo*^678^). However, wing-clipped *cbd*^762^ as well as *ebo*^678^ flies both showed a clear significant

465   change in their photopreference measured either in BCP or T-Maze (Fig. 6*B*,*C*). We note that,

466   although *ebo*^678^ wingless flies still showed a preference for the bright tube in the T-Maze, their PI

467   was significantly decreased in comparison with intact *ebo*^678^ flies. While more sophisticated

468   manipulations of central complex function are clearly warranted, we tentatively conclude that if

469   the central complex plays a role in this process, it is likely not a crucial one, or one that does not

470   require an anatomically intact central complex.

471

472   **DA and OA differently modulate intact and wingless fly behavior**

473   In the absence of any evidence that any of the known learning processes or neuropils known to

474   be relevant for learning or other aspects of orientation/choice behaviors are involved in the shift

475   in photopreference, we explored the hypothesis that any unknown learning mechanism as well

476   as an unknown constant monitoring of flying ability may rely on a re-valuation of sensory input

477   after wing manipulation. That is, whether or not any memory is involved, the consequence of

478   being rendered flightless may be identical: a re-valuation of sensory input, such that previously

479   attractive stimuli become more aversive and previously aversive stimuli become more attractive.

480   Biogenic amines have long been known for their role in mediating the processing and

481   assignment of value [4,9–12,14,45–52]. If indeed it is the photopreference that is shifted when a

482   fly's flying ability is altered, it is straightforward to hypothesize that the two biogenic amines most

483   known for being involved in valuation in *Drosophila*, octopamine (OA) and dopamine (DA), may

484   be involved in this instance of value-based decision-making as well. Moreover, mutant flies that

485   lack tyrosine hydroxylase (*th*) only in the nervous system, i.e. neuronal specific DA-deficients,

486   show reduced phototaxis in BCP [51] further motivating the manipulation of this amine pathway.

487   Finally, flies without OA show a pronounced impairment in flight performance and maintenance

488   [53], making OA an interesting candidate for testing photopreference as well.

489   To evaluate the involvement of DA and OA neurons for photopreference, we acutely disrupted

490   synaptic output from two separate groups of neurons by expressing the temperature-sensitive

491   form of dynamin (*Shibire; shi^{TS}, [54]*) either under control of the *th*-GAL4 driver (driving in

492   dopaminergic neurons) or under control of the *tdc2*-GAL4 driver (driving in octopaminergic, as

493   well as tyraminergic, neurons). We tested the resulting transgenic flies with and without wings in

494   BCP and T-Maze. Although BCP and T-Maze results tended to agree, we only obtained clear

495   results in our T-Maze experiments. The reason for the less clear results in the BCP was a

496   genotype-independent and long-lasting effect of the temperature switch on the flies' PI in the

497   BCP. Hence, we show the results from the T-Maze experiments here and the BCP results are

498   available for download with the rest of the raw data. In the T-Maze at permissive room

499   temperature, when dynamin is in its wild type conformation, in all tested groups, flies with intact

500   wings showed positive CIs, while wing-clipped flies showed negative CIs (Fig. 7*A,B*). In contrast,

501   when the same experiment was performed at the restrictive 32°C (i.e., blocking synaptic

502   activity), we found opposite effects in flies with dopaminergic, and octopaminergic/tyraminergic

503   neurons blocked, respectively. While disrupting synaptic output from dopaminergic neurons

504   appeared to have little if any effect on clipped animals, flies with intact wings shifted their

505   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.

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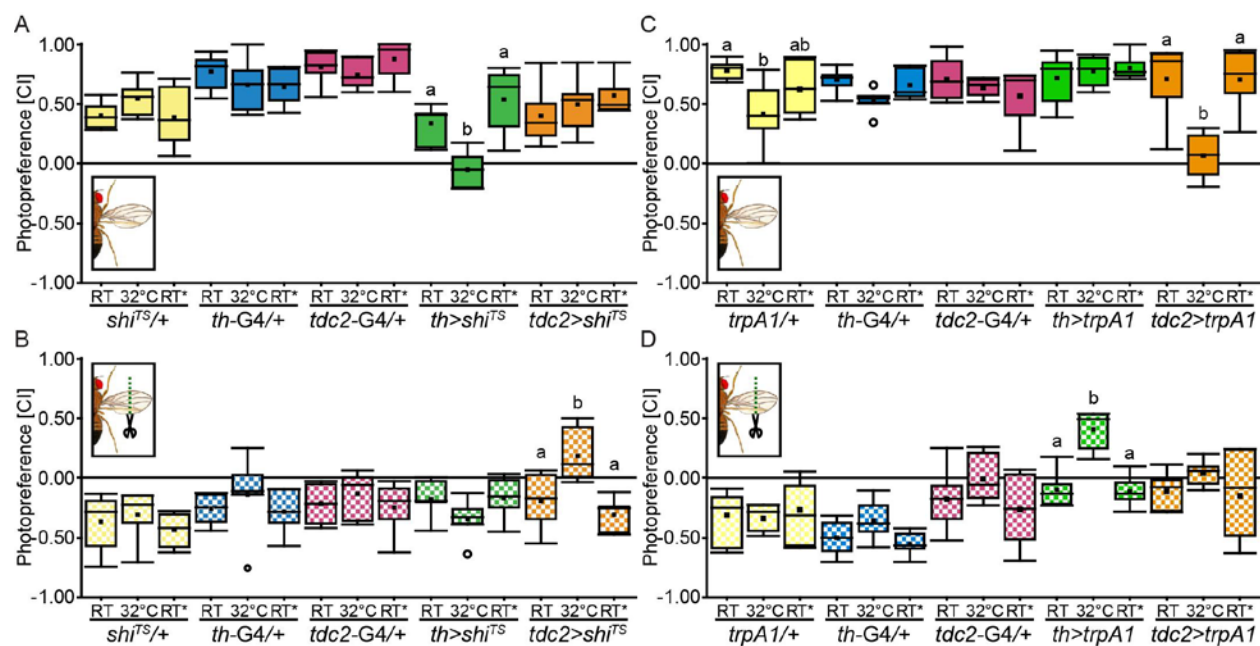
517 **Figure 7. Dopamine and Octopamine are necessary and sufficient to modulate phototactic**

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).

23

523   ***B,*** Randomized Block Design ANOVA, Block p=0.006, Interaction Genotype vs Temperature p=0.02,

524   simple effects with Tukey's *post hoc* test (p<0.05, least-significant difference=0.278, *tdc2>shi^{ts}* least-

525   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,

526   *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

527   (b) wings before, during and after DA or OA neuron activation. ***C,*** Kruskal-Wallis for temperature factor

528   comparison within genotypes (alpha after correction=0.013): *trpA1*/+ p=0.012, *th*-GAL4/+ p=0.069, *tdc2*-

529   GAL4/+ p=0.667, *th>trpA1* p=0.97, *tdc2>trpA1* p=0.004. *th*-GAL4/+ and *th>trpA1*, N=6; *trpA1*/+, *tdc2*-

530   GAL4/+ *tdc2>trpA1*, N=7. ***D,*** Kruskal-Wallis for temperature factor comparison within genotypes (alpha

531   after correction=0.013): *trpA1*/+ p=0.834, *th*-GAL4/+ p=0.15, *tdc2*-GAL4/+ p=0.126, *th>trpA1* p=0.005,

532   *tdc2>trpA1* p=0.415. *th*-GAL4/+ and *th>trpA1*, N=6; *trpA1*/+, *tdc2*-GAL4/+ *tdc2>trpA1*, N=7. Different

533   letters indicate significant differences between temperatures for each genotype (only shown for genotypes

534   where the factor temperature had a statistically significant effect). See figure 2 for detailed graph

535   information.

536

537   We also transiently activated OA/TA and DA neurons, respectively, using the temperature

538   sensitive *TrpA1* channel [55], while testing the flies for their photopreference. Again, at room

539   temperature, when the channel is closed, flies with and without wings behaved similar to wild

540   type animals (Fig. 6C,D). However, when tested in the same experiment at 32°C, where the

541   *TrpA1* channel is open and depolarizes the neurons in which it is expressed, the flies showed a

542   change in their behavior. Flies with clipped wings and activated DA neurons now preferred the

543   bright arm of the maze, with no effect on intact flies (Fig. 7D). Conversely, activating OA/TA

544   neurons only had an effect on flies with intact wings, abolishing their previous preference for the

545   bright arm of the maze (Fig. 7C), rendering them indistinguishable from their wingless siblings

546   with which they were tested, but which did not show any significant effect (Fig. 7D). Again, when

547   tested back at room temperature 24h later, wild type behavior was restored. The conventional

548   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

25

575 found a reduction of phototaxis and never negative phototaxis (unpublished observation). In fact,

576 in these pilot experiments we have observed every possible difference between flying and

577 manipulated flies. In the end, we chose the experimental design that yielded positive and

578 negative scores, respectively, in WTB flies purely for practical reasons. Other wild type strains,

579 such as some Canton S substrains, do not show a negative photopreference in the T-Maze after

580 wing clipping (Fig. 3H). Taken together, these lines of evidence strongly suggest that

581 photopreference in *Drosophila* is a strain-specific continuum where experimental design assigns

582 more or less arbitrary values along the spectrum. In some special cases, this photopreference

583 manifests itself as phototaxis. If that were the case, phototaxis would constitute an example of a

584 class of experiments not entailing a class of behaviors.

585 This insight entails that different manipulations of flying ability ought to affect this continuum in

586 different ways. Complete loss of flight ought to have more severe effects than manipulations

587 affecting merely individual aspects of flight behavior, such as wing beat amplitude/frequency

588 (i.e., lift/thrust), torque, flight initiation, flight maintenance, proprioception or motion sensation.

589 We have found some evidence to support this expectation. For instance, clipping only the tips of

590 the wings does not eliminate flight, but affects torque as well as lift/thrust [30]. Flies with the tips

591 of their wings cut behaved indifferently in the T-Maze and do not avoid the bright tube (Fig. 4F).

592 Flies without antennae are reluctant to fly and have lost their main sense of air speed detection

593 [34–36], but they are still able to fly. Also these flies do not become light averse in the T-Maze

594 after the manipulation, but indifferent (Fig. 4I). Flies with removed gyroscopic halteres, on the

595 other hand, are severely affected in their detection of rotations and usually do not fly [31–33].

596 These flies avoid the bright arm of the T-Maze. Finally, injuries to flight-unrelated parts of the

597 fly's body did not affect photopreference (Fig. 4K, L), ruling out the change in photopreference

598 being a direct escape response to bodily harm. Further research is required to establish a

599 quantitative link between the many different aspects of flight behavior and their relation to

600 photopreference.

26

601   The generality of the wing-manipulation effect across experiments (Fig. 3) as well as the missing

602   direct connection between bodily harm and photopreference (Fig. 4) suggests that the change in

603   photopreference may be due to indirect effects via the common property of all our manipulations

604   affecting flying ability. This conclusion entails that the flies monitor their ability to fly and update

605   their photopreference accordingly. This process is reversible and reminiscent of a learning

606   process (Fig. 5): at one time point, the flies make the experience that they cannot fly and at a

607   later time point, they use this experience when making a decision that does not involve flying.

608   Once flying ability is restored, the same choice situation is solved with a different decision in the

609   absence of flight behavior. How the flies accomplish this learning task is yet unknown, but we

610   tentatively conclude that it is unlikely that any of the known learning pathways or areas involved

611   in different forms of learning play more than a contributing role (Fig. 6). While the molecular

612   learning mechanism remains unidentified, the process appears to be (near) instantaneous (Figs.

613   2, 3). Even though we cannot rule out that an unknown learning mechanism exists which is

614   unaccounted for in our screen, we conclude that at least none of the known learning

615   mechanisms suffices to explain the complete effect size of the shift in photopreference. These

616   results corroborate the findings above, that the switch is instantaneous and does not require

617   thorough training or learning from repeated attempts to fly. They do not rule out smaller

618   contributions due to these known learning processes or an unknown, fast, episodic-like learning

619   process. It is also possible, that the flies constantly monitor their flying ability and hence do not

620   have to remember their flight status. Despite these ambiguities, we have been able to elucidate

621   some of the underlying neurobiological mechanisms. Much as in other forms of learning and

622   valuation, neurons expressing the biogenic amine neuromodulators OA and DA appear to have

623   opposite functions in the modulation of photopreference (Fig. 7).

624   Although both DA and OA play some role in different aspects of flight behavior [53,56–58], these

625   cannot explain our results. In general, our biogenic amine neuron manipulated flies escape their

626   vial via flight if granted the opportunity. Thus, flight is not abolished in any of our transgenic lines

27

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

28

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

29

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