

1 **Mechanisms of selection for the control of action in *Drosophila***  
2 ***melanogaster***

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13 **Running title:** Mechanism of selection in *Drosophila*

14 **Key words:** Action selection, selective attention, motor re-orientation, model organism,  
15 fixation, visuo-motor integration, *Drosophila melanogaster*

16 **Summary statement:** In this study we investigated adult fly locomotor behaviour in  
17 response to distracting stimuli during free walking. Kinematic data reveal an interesting  
18 phenomenon of motor re-orientation.

19

## 20 **ABSTRACT**

21 In the last few years several studies have investigated the neural mechanisms underlying  
22 spatial orientation in *Drosophila melanogaster*. Convergent results suggest that this  
23 mechanism is associated with specific neural circuits located within the Central Complex  
24 (CC). Furthermore such circuits appear to be associated with visual attention, specifically  
25 with selective attention processes implicated in the control of action. Our aim was to  
26 understand how wild-type flies react to the abrupt appearance of a visual distractor during  
27 an ongoing locomotor action. Thus, we adapted the well-known 'Buridan paradigm', used  
28 to study walking behaviour in flies, so we could specifically address the mechanisms  
29 involved in action selection. We found that flies tended to react in one of two ways when  
30 confronted with a visual distractor during ongoing locomotion. Flies either: (i) committed to  
31 a new path situated midway between the original target and the distractor, consistent with  
32 a novelty effect; or (ii) remained on the original trajectory with a slight deviation in direction  
33 of the distractor. We believe that these results provide the first indication of how flies react,  
34 from the motor point of view, in a bi-stable context requiring the presence of selection-for-  
35 action mechanisms. Some considerations on the neural circuits underlying such  
36 behavioural responses are advanced.

## 37 **INTRODUCTION**

38 Living organisms have evolved neural information processing systems to allow interaction with the  
39 environment so as to maximize the probability of survival and reproduction. To reach this goal,  
40 appropriate information about the environment has to be extracted by perceptual systems in a form  
41 that can be used to guide actions (Tipper et al., 1998; Castiello, 1999). Visual attention systems  
42 appear to operate by mapping out relevant perceptual aspects of the environment and translating  
43 them into appropriate action control parameters. *Drosophila melanogaster* also seems to employ  
44 such mechanisms, for instance, in order to avoid predator attacks, to prevent collisions with  
45 obstacles or to head efficiently towards salient visual stimuli (Card and Dickinson, 2008; van  
46 Breugel and Dickinson, 2012; Maimon et al., 2008). Therefore, it is reasonable to assume that the  
47 presence of efficient action selection mechanisms constitute an evolutionarily conserved  
48 characteristic (Strausfeld and Hirth, 2013; Grillner and Robertson, 2016). The putative neural  
49 substrate of an action selection system in flies is thought to be contained within a doughnut-shaped  
50 structure called the ellipsoid body (EB) (Fiore et al., 2015), which is part of a wider ensemble of  
51 modular neuropils involved in locomotor behaviour termed the central complex (CC), (Strauss and

52 Heisenberg, 1993; Martin et al., 1999; Pfeiffer and Homberg, 2014). Recently, using a two-photon  
53 calcium imaging technique, it has been shown that a class of CC neurons termed E-PG neurons –  
54 having their dendritic tree in the EB and their axonal branches in the Protocerebral Bridge (PB) and  
55 Gall brain regions – are involved in tracking the orientation of a visual landmark and, to a lesser  
56 extent also the direction of body motion (Seelig and Jayaraman, 2015). The neurons of this circuit  
57 are arranged in a toroidal pattern, functionally subdividable into wedges. Each wedge responds to a  
58 particular direction of navigation through a mechanism involving a ring attractor dynamic model  
59 which explains how information concerning visual landmarks is integrated with self-motion in  
60 order to allow navigation (Turner-Evans et al., 2017; Heinze, 2017). Furthermore, this circuit is  
61 thought to be the neural centre for visual attention since it is characterized by a discrete single  
62 ‘bump’ of activity following the presentation of multiple visual stimuli (de Bivort and van  
63 Swinderen, 2016). This is reminiscent of a sort of attentional focus (Castiello and Umiltà, 1990;  
64 Castiello and Umiltà, 1992) and suggests a unified neurophysiological phenomenon which could  
65 form the basis of selection for the programming of locomotion direction.

66         Despite the above mentioned neurophysiological findings underlying landmark selection,  
67 little is known regarding the heading control in free moving adult flies. Horn and Wehner (1975)  
68 showed that walking flies faced with two stripes presented concomitantly and separated by an  
69 angular distance of less than 60 deg, preferred to move along the direction determined by the  
70 bisector of the angle between the two objects (Horn and Wehner, 1975). Conversely, when angles  
71 greater than 75 deg were considered, the flies showed a distribution of orientations with two  
72 maxima directed toward either of the two stripes. This behaviour has been described in terms of a  
73 superposition of two turning-tendency functions, which are phase shifted according to the angle  
74 subtended by the landmarks (Poggio and Reichardt, 1973; Horn and Wehner, 1975). In the light of  
75 recent findings, suggesting that the E-PG neurons operate according to ring attractor dynamics  
76 (Kim et al., 2017), it might be speculated that, in the case of Horn and Wehner’s experiment (1975),  
77 the ‘compass needle’ of the ring attractor points in a direction which is midway between the two  
78 landmarks. According to this idea, it has been observed that on some occasions E-PG activity  
79 transitioned from one offset to another relative to the two landmarks, indicating that this ambiguity  
80 may lead the fly to adopt an intermediate orientation (Seelig and Jayaraman, 2015). Thus, the  
81 turning tendency underlying fixation behaviour and the ring attractor model of the CC could be two  
82 sides of the same coin (Bahl et al., 2013; Seelig and Jayaraman, 2013).

83         With this in mind, we tested how the abrupt presentation of a visual stimulus to flies which  
84 are already engaged in locomotion (walking) toward a pre-existing visual target, would determine  
85 the activation of selection-for-action mechanisms which are then deployed in movement

86 kinematics. To this end, we capitalized on an innate fly behaviour (i.e. unconditioned) in which flies  
87 continue to freely run back and forth between two opposing stripes inside a circular open arena (i.e.  
88 ‘Buridan's paradigm’; Götz, 1980; Bühlhoff et al., 1982; Strauss and Heisenberg, 1993; Strauss and  
89 Pichler, 1998). In our modified ‘Buridan paradigm’ a second stripe (with respect to the fly’s visual  
90 field) was presented while the fly was already moving towards the pre-existing stimulus. We  
91 hypothesize that the appearance of the extra stripe might determine three possible scenarios: i) if the  
92 presence of the second stripe does not alter the originally programmed direction of locomotion, then  
93 the fly's movement should proceed in the direction of the first stripe, with no apparent changes  
94 along the path of the locomotion trajectory; ii) if the presence of the second stripe has a distracting  
95 effect, and therefore needs to be inhibited in order for the fly to proceed in the originally planned  
96 direction, then some evidence of this inhibitory process might be detectable in the form of slight  
97 perturbations in the locomotion trajectory; iii) if the presence of the second stripe determines the  
98 insurgence of an alternative motor program, which has the power to override the original one, then  
99 a dramatic change in direction toward the novel stripe should be evident. Surprisingly, the  
100 appearance of the novel target seemed to produce a tendency in the flies to turn towards a point  
101 midway between the two targets, as already shown by Horn and Wehner (1975). However, a more  
102 in depth analysis of the trajectories led us, in fact, to the identification of two alternative specific  
103 locomotor behaviours, namely that flies either: (i) committed to a new path situated midway  
104 between the original target and the distractor, consistent with a novelty effect; or (ii) presented a  
105 slight deviation of the original trajectory in the direction of the distractor. This in turn allows for  
106 interesting considerations regarding the nature of the selection-for-action mechanism in *Drosophila*  
107 *melanogaster*. In particular, the first type of response implies the abortion of the ‘old’ motor  
108 program in favour of a new one, while the second type of response suggests the deployment of an  
109 inhibitory mechanism operationalized in the form of slight trajectory changes.

## 110 **MATERIALS AND METHODS**

### 111 **Animals**

112 The experiments were performed on adult wild-type fruit flies (*Drosophila melanogaster*; Oregon-  
113 R strain). All flies were reared on standard cornmeal-sucrose-yeast medium at 22°C in a 12 h  
114 light/12 h dark cycle at 60% relative humidity. Fly crowding was also controlled (20-30 flies each  
115 vial) to avoid competition for food. Only individual 2-5 day-old male flies were used. Flies were  
116 kept in their food vials until the beginning of the experiment. Thus for the experiment flies were not

117 starved nor were their wings clipped. All experiments were conducted between zeitgeber time 2 and  
118 4 at room temperature 22-23°C.

## 119 **Experimental setup**

120 To test how flies respond to the sudden appearance of new visual stimulus (distractor) during free  
121 walking toward a fixed visual stimulus (block) we employed a cylindrical led-emitting-diode (LED)  
122 modular display (Reiser and Dickinson, 2008) positioned around the fly (Fig. 1), and consisting in  
123 48 (12 x 4) LED panels (each panel made by an 8 x 8 array of LEDs) (IO Rodeo Inc, Pasadena, CA,  
124 USA). A custom-designed transparent arena made of 3D-printed resin (iMaterialise HQ, Leuven,  
125 BE, EU) was placed within the cylindrical LED display. The cylindrical LED display and the  
126 transparent arena were in turn mounted on solid stainless steel brackets which were fixed to an  
127 aluminium breadboard (Thorlabs Inc, Newton, NJ, USA). The setup was thus positioned on an anti-  
128 vibration table, protected by a Faraday cage and covered with heavy black fabric. The arena  
129 (maximum height at the centre = 3.5 mm; diameter = 109 mm) was designed so as to i) confine flies  
130 in 2D space, ii) not allow the flies to reach the edge of the arena and iii) to impede flight by means  
131 of a glass ‘ceiling’ (Simon and Dickinson, 2010). The arena was backlit by an infrared (IR) LED  
132 array source (LIU850A, Thorlabs Inc, Newton, NJ, USA) and the IR light was diffused using paper  
133 diffuser films placed between the IR light source and the arena. A CCD camera (Chameleon 3,  
134 FLIR System Inc, Wilsonville, OR, USA) with 1288 x 964 pixel resolution, fitted with a 2.8-8 mm  
135 varifocal lens (Fujifilm, Tokyo, JP) and an 850 nm band pass filter (MidOpt Inc, Woodwork Lane  
136 Palatine, IL, USA) was mounted 36 cm above the arena in order to record fly activity. Videos of  
137 flies moving in the arena were recorded at 21 frames s<sup>-1</sup>, following selection of a 700 x 700 pixel  
138 region of interest which included the entire arena. In order to allow the experimenter to visually  
139 observe all events occurring within the arena (including whether visual patterns were being  
140 correctly displayed) an HD webcam (C310, Logitech, Lausanne, CH, EU) was also mounted  
141 alongside the infrared camera.

## 142 **Procedure**

143 Flies were individually loaded into the arena with a mouth aspirator and were left to adapt in  
144 complete darkness for at least 5 min. Individuals were then subjected to a ‘Buridan’s paradigm’, by  
145 illuminating two opposed bright stripes of 4 x 16 LEDs (width x height) each one covering 15 deg  
146 of the fly’s visual field when observed from the centre of the chamber. The classical interpretation  
147 of the phenomenon underlying this paradigm refers to the alternation between fixation and anti-  
148 fixation of attractive landmarks represented by black stripes on a bright background (Bülthoff et al.,

149 1982). Apparently, bright stripes on a dark background show no difference in terms of fixation  
150 (Horn and Wehner, 1975; Seelig and Jayaraman, 2013). Preliminary experiments in our  
151 experimental setup showed a more robust response to the ‘Buridan’s paradigm’ in individuals tested  
152 with bright stripes on dark background, and, therefore, we decided to run our experiments with this  
153 setup. In our experiments, individual fly locomotion in ‘Buridan’s paradigm’, consisting in the fly  
154 continuously running to and fro between two opposing bright targets, was initially recorded for  
155 200s (see Movie 1 in supplementary materials). Flies which did not exhibit this behaviour (i.e.  
156 remained still or roamed at random) were not further considered (Kain et al., 2012). This allowed to  
157 distinguish between flies that adopted a behaviour termed ‘quantum search action’ (i.e. a fixation  
158 and anti-fixation strategy) from those which did not manifest such behaviour. In other words, this  
159 procedure was aimed at selecting the ‘searcher’ phenotype considered for the following part of the  
160 experiment (Bülthoff et al., 1982). At the end of this selection phase, the behavioural task-proper  
161 was initiated. While the fly was still performing the ‘Buridan paradigm’, a second visual target  
162 (distractor) was presented the moment the fly crossed the virtual midline of the arena while moving  
163 between the two opposing bright stripes (a modified detour paradigm; Neuser et al., 2008).  
164 Therefore, our task consisted in a classical ‘Buridan paradigm’ performed under two alternative  
165 conditions. A distraction condition in which a single distracting-signal (chosen randomly among  
166 four alternative signals) was presented concomitantly with the ‘Buridan paradigm’ stimuli,  
167 whenever the individual crossed a virtual central window (27 mm width x 3.6 mm depth; see Fig. 2)  
168 along the chosen path. From this point on we shall refer to this condition as the ‘distractor’  
169 condition. Distractors consisted in bright stripes of the same dimensions as the Buridan stripes (i.e.  
170 15 deg of the fly’s visual field when viewed from the center of the arena). The distractors appeared  
171 randomly to the right or left of the fly at an angle of either 30 or 60 deg with respect to an ideal line  
172 connecting the opposing Buridan stripes. Each time a fly crossed the virtual central window, the  
173 distractor appeared for a 3 s period. During this period the two opposing Buridan stripes were  
174 always present. A ‘block’ condition, instead, consisted in the presentation of the Buridan stripes  
175 without any distractor. The experiment ended when the ‘block’ and the ‘distractor’ conditions had  
176 been presented seven times (with an average experiment lasting 30 min).

## 177 **Software and management**

178 The cylindrical LED display was controlled using available MATLAB (MathWork Inc, Natick,  
179 MA, USA) scripts (Reiser and Dickinson, 2008). The MATLAB Image Acquisition Toolbox was  
180 used to implement the system for video recording. Furthermore, in order to detect the position of  
181 the fly’s head in a specific spatial location (i.e. inside the virtual central window within the circular

182 arena) and activate the necessary visual patterns on the LED panels accordingly, we implemented a  
183 system for real time tracking using the FAST (Features from Accelerated Segment Test) method  
184 (Rosten and Drummond, 2006) provided by the MATLAB Computer Vision System Toolbox.  
185 Online tracking analysis, video recording and control of the LED arena were integrated into a single  
186 custom GUI (Graphical Unit Interface), thus providing us with a unified software environment to  
187 manage all experimental variables. All the scheduled events involved in each experiment were  
188 automatically controlled by means of a custom script.

### 189 **Off-line tracking**

190 To obtain a greater definition of the fly's 2D position and body orientation we decided to track the  
191 fly's trial recordings off-line using the CTRAX open source software (Branson et al., 2009). Errors  
192 occurring during tracking were fixed manually using appropriate available MATLAB scripts  
193 (CTRAX, FixErrors Toolbox) (Branson et al., 2009). Finally, other available MATLAB scripts  
194 (CTRAX, Behavioral Microarray Toolbox) were used to compute a suite of speed and acceleration  
195 properties (Branson et al., 2009).

### 196 **Data pre-processing**

197 The files obtained following the off-line tracking analysis pipeline described above were  
198 transformed into .txt files, and imported into the R software (R Development Core Team, 2017)  
199 environment for data pre-processing and an initial exploratory analysis by means of custom scripts.  
200 For the trajectory analysis, only data from tracks in which single flies were directed towards the  
201 target were selected (i.e. all tracks in the opposite direction were removed). The minimum track  
202 length considered for analysis was 9 mm (i.e. 50 pixels; spatial resolution was 5.5 pixels per mm).  
203 Using this data frame (see Table 1 and Table 2) we performed track-centering. This operation  
204 proved necessary due to the fact that, in order to trigger the appearance of the distractor and to start  
205 the video recording, the flies had to cross a virtual central window within the circular arena. Given  
206 the dimensions of this virtual window, the tracks showed scattered starting-points along the x-axis  
207 (width of the window), depending upon the point at which the fly entered the virtual window.  
208 Therefore, since we were interested in evaluating the deviation of the fly locomotion paths caused  
209 by the different distractors and since the body orientations of the flies were uniform among  
210 conditions (Fig. 3A), we centered the starting point of all tracks at  $x = 0$ . Due to the limited depth of  
211 the triggering window the starting y values appeared to be more homogeneously distributed among  
212 the experimental conditions (Fig. 3B). Nonetheless, for uniformity, tracks were also centered at  $y =$   
213 0.

214

Table 1. Velocity and Distance with respect to the experimental condition

| Condition | No. trajectories | Velocity (mm s <sup>-1</sup> )<br>( $\mu \pm \sigma$ ) | Distance (mm)<br>( $\mu \pm \sigma$ ) |
|-----------|------------------|--|---------------------------------------|
| block     | 123              | 18.83±13.62  | 46.64±13.32                           |
| 30°R      | 137              | 18.43±13.60  | 48.23±12.74                           |
| 30°L      | 135              | 18.50±13.87  | 48.80±13.09                           |
| 60°R      | 127              | 18.19±13.68  | 47.41±14.33                           |
| 60°L      | 131              | 18.44±13.54  | 49.88±14.39                           |

215

Table 2. Number of trials by fly

| Fly ID | No. trajectories | Fly ID | No. trajectories | Fly ID | No. trajectories |
|--------|------------------|--------|------------------|--------|------------------|
| Fly_1  | 32               | Fly_8  | 30               | Fly_15 | 32               |
| Fly_2  | 34               | Fly_9  | 34               | Fly_16 | 29               |
| Fly_3  | 27               | Fly_10 | 32               | Fly_17 | 30               |
| Fly_4  | 30               | Fly_11 | 33               | Fly_18 | 27               |
| Fly_5  | 31               | Fly_12 | 34               | Fly_19 | 32               |
| Fly_6  | 33               | Fly_13 | 30               | Fly_20 | 31               |
| Fly_7  | 34               | Fly_14 | 31               | Fly_21 | 27               |

216

## 217 **Statistical approach**

218 In order to understand how the presence of distractors explained the orientation and the trajectories  
219 taken by the flies we tested a series of Linear Mixed Effects (LME) models using the R package  
220 *lme4* (Bates et al., 2014). We used LME because such models allow to adjust estimates for repeated  
221 sampling (i.e. more than one observation arises from the same fly) and for imbalance in sampling  
222 (i.e. some flies are sampled more than others). LME also allow to take into account the  
223 experimental variation (i.e. variation among flies or among other groupings within the data) and to  
224 avoid the harmful effects of averaging, since this tends to remove variation (McElreath, 2016).  
225 Subsequently, the LMEs were compared in order to select the best model (i.e. the best fit to the  
226 data). For model selection we used the Bayesian Information Criterion (BIC) also known as the  
227 Schwarz information criterion or Schwarz's BIC (Schwarz, 1978), an index that measures the  
228 efficiency of the model in terms of data forecasting. Since BIC tends to favour models with fewer



229 parameters, we further conducted a Bayes Factor (BF) analysis with a method based on the  
230 multivariate generalizations of Cauchy priors (JZS method, see Liang et al., 2008) using the R  
231 package *BayesFactor* (Morey and Rouder, 2015). We used this parameterization because it allows  
232 BF to have excellent statistical properties independently of the phenomenon under study (a method  
233 also known as ‘objective Bayesian’, see Wagenmakers, 2007). The Bayes Factor expresses the ratio  
234 between the plausibility of observed data under M1 (our best model) and the plausibility of  
235 observed data under M0 (the null model). We compared different models, and the one with the  
236 highest Bayes Factor (greatest plausibility) was selected. With the *BayesFactor* package, which  
237 inherits the MCMC (Markov Chain Monte Carlo) sampling algorithm from the R package *coda*  
238 (Plummer et al., 2006), we were also able to compute the posterior distributions of parameters (with  
239 10000 iterations). This approach to estimating parameters enabled us to take maximum advantage  
240 of LME modelling, which provided the direct probability of an effect (i.e. posterior probability) as  
241 well as the computation of the evidence for the results. Analysis of variance (ANOVA) and non-  
242 parametric Mann-Whitney-Wilcoxon tests were also used, under the null hypothesis that the sample  
243 distributions belonged to the same population.

## 244 RESULTS

### 245 Orientation effects

246 As a first step we investigated the body orientation adopted by the flies at the time the second visual  
247 stimulus (i.e. the distractor) was presented. Before proceeding with this analysis we ascertained  
248 whether the flies maintained comparable velocity amplitudes across all conditions (*conditions* refers  
249 to the presence or absence of one of the four possible distractors). This was done in order to avoid  
250 any bias due to variations in velocity determined by the experimental conditions. We found no  
251 evidence for differences in velocity amplitude across conditions (Fig. 4A, B). Next, a series of LME  
252 models were fitted to the fly trajectory data (first two seconds following the presentation of the  
253 distractor) in order to obtain the best-fit model explaining the spatial orientation of flies as a  
254 function of time. The best fitting model (the one having the lowest BIC) was the following:

$$255 \quad Y = \beta_1 X_1 + \beta_2 X_1 X_2 + \beta_3 X_2 + \lambda_i + \epsilon,$$

256  $Y$  represents the predicted orientation,  $\beta_1$  and  $\beta_3$  are the intercepts of regressions represented by the  
257 condition and time variables, respectively, while  $\beta_2$  is the slope that represents the interaction  
258 between conditions and time. Finally,  $\lambda_i$  represents the random effect which results in variation of  
259 the regression intercepts among trials within flies, while  $\epsilon$  represents the error component. At a first  
260 glance, the linear regressions relating to the fixed effects (i.e. the orientation of flies in relation to

261 the experimental condition) show that flies tend to orient consistently towards the distractor (Fig.  
262 4C) suggesting an influence of the distractor on the orientation of the flies. However, as the data in  
263 Fig. 4C also suggest, flies did not tend to turn fully towards the distractor. This can be more clearly  
264 appreciated by evaluating a summary-measure of the orientation predicted by the LME model, that  
265 is, the sum of the two coefficients  $\beta_1$  and  $\beta_2$  which in the LME model both refer to the experimental  
266 condition effects (i.e. condition and condition-time interaction, respectively). This provides a more  
267 direct and global representation of the change in orientation of flies following the presentation of  
268 the distractors – showing that the orientation of flies does not precisely match the expected  
269 orientation based on the position of the distractor (Fig. 4D). Rather, the model predicts that the  
270 orientation of flies, following distractor presentation, is intermediate between the orientation of the  
271 original trajectory and that of the distractor-influenced trajectory. Fig. 4E represents the distribution  
272 of the random effects. Given the significant length of each experimental session (i.e. approx. 30  
273 min) we also evaluated the possibility that flies could show signs of fatigue across trials, which in  
274 turn might affect the re-orientation behaviour. Inspection of the average velocity profiles with  
275 respect to time for each trial does not suggest the onset of fatigue, which would presumably result  
276 in a systematic decrease in velocity as a function of time (supplementary material Fig. S1).

## 277 **Spatial trajectories**

278 Considering the idea that distractors could act on flies through a novelty effect, as a measure of the  
279 flies' commitment to move towards the stimuli we explored the displacement of flies along the x-  
280 axis at mid-path (i.e. after the flies had travelled 24 mm following the presentation of the  
281 distractor). We hypothesized that, given the premise, there might be a reduction in the shift of the  
282 flies' trajectory towards the new target whenever the target presented was (randomly) preceded by  
283 one of the same kind (i.e. on the same side and at the same angle, in which case it would not be  
284 interpreted by the fly as a novel stimulus). Interestingly, a tendency consistent with this idea could  
285 in fact be observed (Fig. 5A). As a corollary, distinct left and right shifts (depending on the type of  
286 distractor presented) were evident at the end of the paths (Fig. 5B), meaning that flies not only re-  
287 oriented toward the distractor but that in so doing, they also committed to a new path (for individual  
288 tracks see supplementary material Fig. S2). In order to obtain a model of the flies' trajectories,  
289 which would provide an objective and quantitative evaluation of the strength and the extent of the  
290 tendency of flies to shift their trajectories towards the distractors, we tested seven LME models  
291 (Table 3). To this end we considered only trajectories at least 45 mm long, (which corresponds to  
292 the radius of the surface of the arena effectively explorable by flies), were considered. The best  
293 LME (i.e. the one with the lowest BIC), LME\_6, was a very parsimonious model consisting of only

294 one  $\beta_1$  interaction parameter (representing the interaction between distance (d) and ‘distractor’  
295 condition as a fixed effect, d:condition, Fig. 5C) in addition to a stochastic variation in the intercept  
296 among trials within flies (fly:trial, Fig. 5D) as a random effect:

$$297 \quad Y = \beta_1 X_1 X_2 + \lambda_i + \epsilon,$$

298 In this case,  $Y$  represents the displacement of flies along the x-axis. This implies that the best model  
299 represents effects as changes in the slope of the fitted line (which represents the interaction),  
300 according to the ‘distractor’ condition (Table 4). An estimate of the Confidence Intervals (CI) of the  
301 interaction parameters (Table 5) shows that none of them overlap which, in the classic frequentist  
302 perspective, implies a statistically significant difference between the effects of different conditions.  
303 The predictor ( $\beta_1$ ) can be converted into an angular measure by means of a simple trigonometric  
304 conversion:

$$305 \quad \beta_1 = \sin^{-1} \left\{ \beta_1 \cdot \left( \frac{180}{\pi} \right) \right\},$$

306 and in this way it is possible to highlight the direction of flies predicted by the model (Fig. 5E). As  
307 already seen in the case of the fly body orientations, albeit to a lesser extent, the trajectories of the  
308 flies also shifted coherently with the distractor position (i.e. the greater the angle of the distractor  
309 with respect to the original trajectory, the farther the flies’ path shifted in the direction of the  
310 distractor). None of the trajectories’ regression per condition seems to predict an angulation (with  
311 respect to the fly) superimposable to the real angle subtended for both the ‘block’ and the  
312 ‘distractor’ conditions. Flies ended between the two but closer to the original target, with a little  
313 difference between the 30 and the 60 deg conditions.

314

Table 3. BIC of LMEs

| Model | df | BIC      |
|-------|----|----------|
| LME_6 | 7  | 137673.5 |
| LME_5 | 8  | 137678.9 |
| LME_7 | 12 | 137716.4 |
| LME_4 | 13 | 137721.6 |
| LME_3 | 9  | 147580.7 |
| LME_1 | 12 | 164847.1 |
| LME_2 | 7  | 164885.5 |
| LME_0 | 8  | 168269.8 |

315

Table 4. Coefficients of the LME\_6

| Parameter         | Estimate   | Std. Error | t value    |
|-------------------|------------|------------|------------|
| d:condition block | 0.0312009  | 0.0044081  | 7.078053   |
| d:condition 30°R  | -0.1638399 | 0.0040951  | -40.008641 |
| d:condition 30°L  | 0.2085999  | 0.0042346  | 49.261415  |
| d:condition 60°R  | -0.2733438 | 0.0045307  | -60.331208 |
| d:condition 60°L  | 0.3052126  | 0.0042944  | 71.072942  |

316

Table 5. Estimated C.I. of parameters

| Parameter                               | 2.5%       | 97.5%      |
|---|------------|------------|
| sd_(Intercept) fly:trial ( $\sigma_1$ ) | 5.7658491  | 6.5809188  |
| $\sigma$                                | 3.9055042  | 3.9762879  |
| d:condition block                       | 0.0225617  | 0.0398401  |
| d:condition 30°R                        | -0.1718659 | -0.1558140 |
| d:condition 30°L                        | 0.2003008  | 0.2168990  |
| d:condition 60°R                        | -0.2822242 | -0.2644633 |
| d:condition 60°L                        | 0.2967963  | 0.3136289  |

317

### 318 **Bayesian trajectories model**

319 The BF analysis highlighted a less parsimonious model with respect to the one which was selected  
 320 using the frequentist approach:

$$Y = \beta_1 X_1 + \beta_2 X_1 X_2 + \beta_3 X_2 + \lambda_i + \epsilon$$

321 This model, in addition to a  $\beta_2$  interaction term (d:condition), also presented the  $\beta_1$  and  $\beta_3$   
 322 parameters, which individually represent the effects of experimental condition and distance,  
 323 respectively. In the case of this model, the distribution of parameters and the goodness of fit were  
 324 evaluated (i.e. the standard error of residuals and the R-squared were estimated), in order to assess  
 325 the goodness of the model (Table 6). In this case, a “confidence interval” was computed, based on  
 326 the Highest Posterior Interval (HPI), using the R package *TeachingDemos* (Snow, 2016) (Table 7).  
 327 In practice, all points in an HPI region have a higher posterior density than points outside the  
 328 region. For this reason HPI is also called Highest Density Interval (HDI). Notwithstanding the  
 329 slightly more complex model produced by the BF analysis, this model provided essentially the same  
 330 general explanation for the experimental data as the LME model. Also in this case, none of the

331 parameters bound to the ‘experimental condition’ variable showed any overlap in the predicted  
 332 values in terms of HPI (Fig. 6A), suggesting that the distractors produced significant deviations of  
 333 the flies’ trajectories both in terms of shift and slope. The  $\beta_3$  parameter (i.e. distance) showed a  
 334 non-significant shift in the intercept of the regressions (Fig. 6B), while the  $\lambda_i$  random effect due to  
 335 the variation between flies appeared minimal (Fig. 6C). This analysis confirmed that flies respond  
 336 to distractors by shifting their locomotor trajectories essentially in accordance with the location of  
 337 the distractor, albeit not proportionately. In fact, with distractors presented at 30 deg flies tended to  
 338 adopt a heading of 10 deg, while with distractors presented at 60 deg flies adopted a heading of 16  
 339 deg.  
 340

Table 6. Model goodness of fit

| Residual-se | R-squared |
|-------------|-----------|
| 7.199331    | 0.4120791 |

341

Table 7. HPI of parameters

| Parameter         | 2.5%       | 97.5%      |
|-------------------|------------|------------|
| distance          | 0.0073964  | 0.0209496  |
| condition block   | -0.0433319 | 0.3353007  |
| condition 30°R    | -4.2541136 | -3.8968358 |
| condition 30°L    | 3.8712083  | 4.2320447  |
| condition 60°R    | -6.9712379 | -6.5951677 |
| condition 60°L    | 6.4814631  | 6.8356365  |
| d:condition block | -0.0303948 | -0.0031744 |
| d:condition 30°R  | -0.1868404 | -0.1609655 |
| d:condition 30°L  | 0.1671820  | 0.1942019  |
| d:condition 60°R  | -0.2949580 | -0.2669707 |
| d:condition 60°L  | 0.2775440  | 0.3045376  |
| $\sigma^2$        | 50.9114193 | 52.7319502 |

## 342 Kinematics indices

343 The ‘partial attraction’ effect determined by the appearance of distractors led us to hypothesise that  
 344 perhaps a high number of the trajectories used in the model construction and analysis were  
 345 trajectories of flies which remained on the original straight path (i.e. which essentially did not

346 respond to the distractor), impacting on the leverage of the model. Indeed, the raw distribution of  
347 the position of flies within the arena shows peaks which are consistent with the position of the  
348 original target (supplementary material Fig. S3). In order to clarify this issue we considered  
349 separately two situations: (i) the trials in which the distractor determined only a slight shift in the  
350 fly's trajectory in that direction, with the fly essentially maintaining the direction towards the  
351 original target (type 1); (ii) the trials in which the presence of the distractor determined a dramatic  
352 change in trajectory, with the fly abandoning the original direction in favour of the one dictated by  
353 the distractor (type 2). Type 2 trajectories were selected by considering a shift of at least 9 mm from  
354 an ideal straight line – orthogonal to the original target – at the time the individual crossed the  
355 middle of the path. This arbitrary procedure did not affect the balancing of the trials per condition in  
356 favour of one of the two types, maintaining a similar numerosity in the 'block' condition (Fig. 7A).  
357 Following this, a new parameter (i.e. shift) was introduced in the LME model as a third component  
358 of the interaction between distance and condition, thus increasing the values of the predictors (Fig.  
359 7B). This kind of manipulation allowed us to investigate possible changes in kinematics following  
360 the appearance of the distractor. During the first 21 frames (i.e. 1 s), the flies executed a fast turn in  
361 response to the distractor (Fig. 7C). In particular, around 250 ms the type 1 flies began to perform a  
362 body saccade in the contrary direction, while type 2 flies continued to maintain an orientation which  
363 was coherent with the distractor position (Fig. 7D). These fast turns did not affect the final  
364 trajectories of the flies (Fig. 7E).

## 365 **DISCUSSION**

366 The primary aim of this research was to evaluate in what way the abrupt presentation of different  
367 distracting visual stimuli to fruit flies which are already engaged in locomotion (walking) towards a  
368 pre-existing visual target, would influence the original locomotion action. Our results indicate that,  
369 following the presentation of a distractor, flies oriented their bodies according to a vector positioned  
370 midway between the original target and the distractor. Following the initial body orientation, flies  
371 then engaged in locomotion by committing to a new trajectory, essentially in one of two ways: (i)  
372 the presence of the distractor produced a slight perturbation in the original trajectory, but the  
373 ensuing movement then tended to proceed in the direction of the original target; (ii) the presence of  
374 the distractor determined the insurgence of an alternative motor program, which had the power to  
375 override the original one, leading to a dramatic change in the direction of the flies' motion.

## 376 **Buridan with light stripes**

377 First and foremost some considerations concerning the use of the type of stimuli used here are in  
378 order. By using a tethered flight simulator, it has been demonstrated that flies are usually attracted  
379 towards long vertical bright or dark stripes, as an ethological reflex which guides flies towards  
380 elements resembling vegetative perches (Maimon et al., 2008). Here we describe for the first time  
381 the free walking behaviour of flies consisting of recurrent orientation inversions (i.e. alternation  
382 between fixation and anti-fixation) between two diametrically opposed vertical bright stripes on a  
383 dark background. Pioneer studies had shown that recurrent inversion is maximized with vertical  
384 black stripes on a bright background (Bülthoff et al., 1982) and had considered the opposite contrast  
385 as a repellent configuration for flies (Heisenberg and Wolf, 1979). Notwithstanding these earlier  
386 observations, we observed a strong fixation response toward bright stripes in freely walking flies  
387 consistent with more recent studies using tethered flying flies (Reiser and Dickinson, 2008;  
388 Maimon et al., 2008). We are tempted to exclude that the recurrent direction inversions shown by  
389 the flies in our case was due to anti-fixation, because when presented with the distractor stripes flies  
390 were attracted to and maintained the distractor in front of them (suggesting fixation). Although the  
391 functional distinction between flying and walking paradigms, as well as differences in the  
392 experimental protocols, such as wing clipping (McEwen, 1918; Gorostiza et al., 2016), might be at  
393 the basis of these contrasting findings, it is difficult to draw a coherent explanatory picture, and the  
394 exact reason for the discrepancies remains as yet unknown. Rather, it is possible that the intensity of  
395 the light used may have played a role in determining the discrepancies concerned with anti-fixation  
396 behaviour of the flies, since in the case of LED displays (such as those used in the present study)  
397 the maximum luminance reachable is  $72 \text{ cd m}^{-2}$  ( $\text{cd m}^{-2} = \text{lux}$ ) (Reiser and Dickinson, 2008), while  
398 in the setups used in previous studies the luminance ranged between 300 and  $1910 \text{ cd m}^{-2}$  (Götz and  
399 Wenking, 1973; Bülthoff et al., 1982; Virsik and Reichardt, 1976), which is at least 4 times higher.  
400 This suggests that long vertical bars with high light intensities lead to avoidance, while long vertical  
401 bars of lower brightness (i.e. in the region of 72 lx) would represent an attracting stimulus, possibly  
402 because under these conditions the bar appears similar to the reflectance of natural vegetation posts.  
403 This hypothesis seems to be corroborated by a report of Heisenberg and Wolf (1984), in which a  
404 grey background makes bright stripes as attractive as black stripes on a white background, while  
405 bright stripes on a black background produce anti-fixation behaviour (Heisenberg and Wolf, 1984).

## 406 **Novelty effect**

407 One aspect of the present results suggests that in our experimental paradigm the appearance of the  
408 visual distractor stimuli produced a novelty effect in the flies. In particular this was manifested by  
409 the re-orienting behaviour of the flies immediately following the appearance of the distractors. A  
410 similar effect has been reported for tethered flying flies which showed a preference for a previously  
411 uncued side of the arena when faced with bilateral stimuli (Shiozaki and Kazama, 2017). In neural  
412 terms, it has been suggested that the *Drosophila* EB ring neurons (R4), are involved in determining  
413 the slow turning tendency (i.e. body re-orientation) associated with this kind of visual experience.  
414 Silencing of those neurons abolishes the innate behaviour for preferential orientation toward novel  
415 stimuli (i.e. previously uncued sides) (Shiozaki and Kazama, 2017).

416 In another study using calcium imaging, the authors found that visual responses in ring neurons are  
417 suppressed when competing stimuli are present in the contralateral visual field (Sun et al., 2017). In  
418 this respect, contralateral suppression is hypothesized to act as a mechanism for location-based  
419 stimulus selection by reducing the responses of ipsilateral stimuli in the presence of a second  
420 stimulus. Furthermore, this suppressive effect appears to dependent upon short-term stimulus  
421 history, specifically, ring neurons baseline activity showed a rebound after contralateral  
422 suppression, a phenomenon which could be involved in modulating the flies' subsequent visual  
423 responses to both ipsilateral and contralateral stimuli (Sun et al., 2017). Such evidence could  
424 partially explain our results, at least in terms of the novelty represented by the distractor.

425 The EB ring neurons – which innervate four concentric rings within the EB – appear to be  
426 retinotopically modulated by visual patterns but not by locomotor states (Seelig and Jayaraman,  
427 2013). These neurons are possibly upstream from the EB wedge neurons, and convey visual  
428 information to the integrator layer. In fact, some of these neurons (R4d and R3) have been  
429 implicated in visual working memory (Neuser et al., 2008) and others (R4 and R1) in space-  
430 learning linked to visual patterns (Ofstad et al., 2011) without affecting locomotor activity. Our  
431 findings add to this literature by showing that flies are attracted by a novel visual stimulus and that  
432 the attraction is manifested not only through a re-orienting of the body, but also by the ensuing  
433 commitment of the individual to a new locomotor path.

## 434 **Reactive turning tendency**

435 Our data are consistent with the 'reactive turning tendency' described by Horn and Wehner (1975),  
436 who noted that flies preferred to orient toward a position midway between two vertical stripes  
437 placed at an angular distance less than 60 deg (Horn and Wehner, 1975). In our paradigm, the



438 sudden appearance of the distractor added a ‘turning tendency’ of the body to the one already  
439 engaged by shifting the internal compass needle toward the distractor. Differently from what  
440 reported by Horn and Wehner (1975), we observed that the trajectories did not lie midway between  
441 the original stimulus and the distractor, but instead remained closer to the former, meaning that the  
442 original stimulus had acquired the status of a stronger landmark. In our opinion, this behaviour  
443 might be the indication of a well-established motor program which is relatively ‘impermeable’ to  
444 the possible perturbation determined by the appearance of the distractor. This is in line with the  
445 observation that the E-PG neurons show a persistent activity maintaining the compass needle  
446 information even when the animal is in total darkness (Seelig and Jayaraman, 2015). The activity of  
447 such neurons remains linked to the position of a single vertical stripe even in the presence of a  
448 second identical stripe. Furthermore, the activity of such neurons does not always shift  
449 instantaneously following the abrupt displacement of a single visual target (Seelig and Jayaraman,  
450 2015). Therefore, it would seem that the accomplishment of a coherent motor program requires  
451 locking on to a target.

#### 452 **Selection for action via inhibition**

453 We were interested in understanding how flies detected and reacted to an abrupt distraction during  
454 the execution of a motor program. According to our original hypothesis we expected the distractor  
455 stimuli to determine an inhibitory or attracting behaviour acting upon already programmed  
456 trajectories, similarly to the interference effect observed in human and non-human primates under  
457 analogous circumstances (Tipper et al., 1998; Sartori et al., 2014; Bulgheroni et al., 2017). In these  
458 studies, participants were instructed to initiate a reaching movement after two stimuli (a target and a  
459 distractor) were presented. When the investigators compared a condition in which the target was  
460 presented alone with that in which there was a distractor acting as an alternative potential target,  
461 they found that the reaching path was affected in the latter case with the arm trajectory deviating  
462 away from or nearer to the distractor. This was observed even with regard to distractor objects that  
463 were unlikely obstacles to the reaching action. As those objects are also included in the initial  
464 processing of the whole context in which the action will be carried out, the motor program  
465 appropriate to reaching them is also produced in parallel, thus producing trajectory changes (Tipper  
466 et al., 1992; Tipper et al., 1997; Bulgheroni et al., 2017). This effect has been explained in terms of  
467 selective attention mechanisms mediating the selection of objects for action, with a specific  
468 mechanism acting to inhibit competing internal representations of distractor objects (Tipper, 1985;  
469 Tipper et al., 1992; Meegan and Tipper, 1998). Put simply, the effects caused by the presence of  
470 nearby objects seem to reflect inhibitory mechanisms. When the target is identified, the reaching

471 movement towards the non-target is inhibited. But because there is an overlap between the target  
472 and the non-target(s), the act of reaching towards the target is affected by this non-target inhibition.  
473 Another crucial aspect of this model is that the amount of inhibition might be determined by the  
474 levels of activation of perceptual inputs. That is, inhibition is reactive such that its level is  
475 determined by the relative salience of the distractor. Thus distractors causing greater levels of  
476 neural excitation receive greater levels of inhibitory feedback. In the present circumstances our flies  
477 exhibited two kinds of behaviour in response to the distractor. The majority of flies fully espoused  
478 the new path dictated by the distractor. The remaining flies, maintained the original path with only a  
479 slight deviation toward the distractor. In both cases the flies acknowledged the presence of the  
480 distractor by making a fast saccade movement toward it within the first 250 ms from the onset of  
481 locomotion. This early fast saccade response could rely on the optomotor system, via the horizontal  
482 system neurons (HS; Bahl et al., 2013; Kim et al., 2015; Fujiwara et al., 2016). Nonetheless, to  
483 explain the present results our preferred idea is that inhibitory processes in *Drosophila*  
484 *melanogaster* occur at the level of the neuroanatomical structures involved in heading behaviour  
485 (Seelig and Jayaraman, 2015). This implies the involvement of the CC and in particular of the EB,  
486 with specific reference to the role played by dopamine in releasing and inhibiting motor programs.  
487 Similarly to what occurs in the mammalian brain (Grillner and Robertson, 2016), the signal  
488 involved in starting and halting an action sequence could be based on phasic dopamine release onto  
489 the EB in a manner similar to what is observed in the case of the nigrostriatal circuit of mice (Jin  
490 and Costa, 2010). The quantitative modulation of dopamine, via different receptors and/or perhaps  
491 through different types of neurons (Green et al., 2017), could engage and disengage the action  
492 programs, by respectively strengthening or weakening the inhibitory process. A high level of phasic  
493 release might enhance the specificity of action selection processes and movement initiation, while  
494 tonic release might inhibit the modules for action. This double mechanism would facilitate the  
495 emergence of motor responses from a repertoire of possible actions in order to readily cope with the  
496 sensory inputs determined by environmental variations. Fiore and collaborators (2015) suggest that  
497 a phasic dopamine release would allow the system to change the strength of the connections  
498 between sensory inputs and the EB, thus affecting the probability that the related motor action  
499 would be selected again. Conversely, a tonic release would not alter the connections' strength but  
500 would make the global system more stable (i.e. maintenance of selection) or unstable (i.e. sensitive  
501 to changes) depending on the receptor type involved (Fiore et al., 2015). However, it remains  
502 unclear how the system would differently weigh opposing pathways in order to regulate action  
503 selection. In this respect, our paradigm might provide a novel theoretical and methodological  
504 territory within which to classify and distinguish different mechanisms concerned with action

505 selection in flies. Further research, considering the manipulation of the neuroanatomical circuit  
506 discussed above, is needed in order to dissect the neural mechanism underlying the action selection.

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512 The authors declare that they have no conflicts of interest with respect to their authorship or the  
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517

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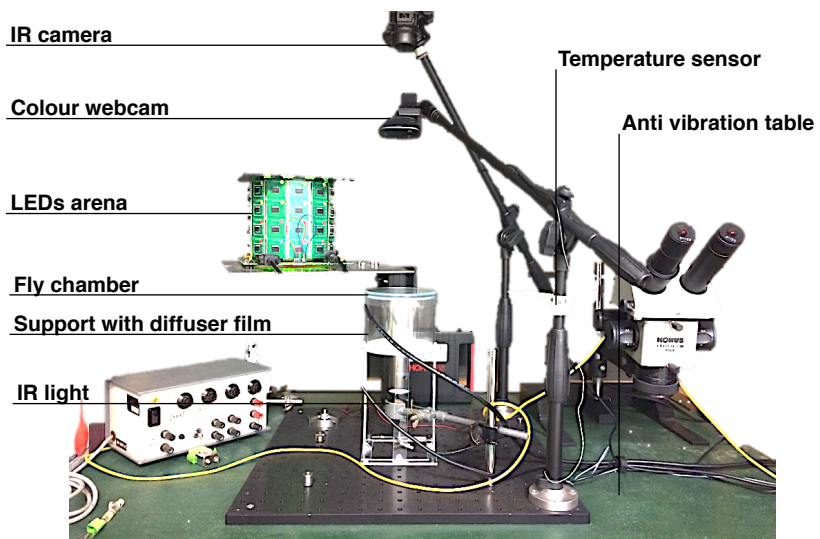
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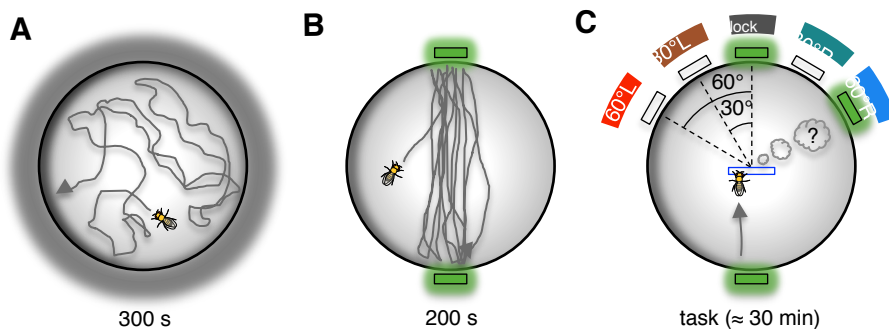
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**Fig. 1**

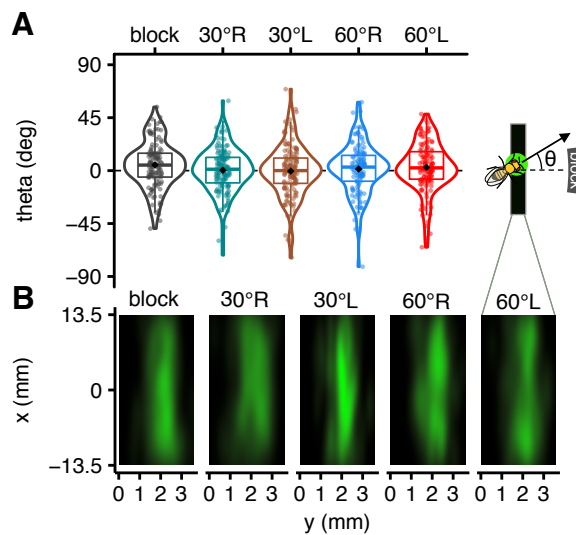


**Fig. 1. Experimental setup.** Image showing the main components of the setup utilized in the experiment described in the paper.

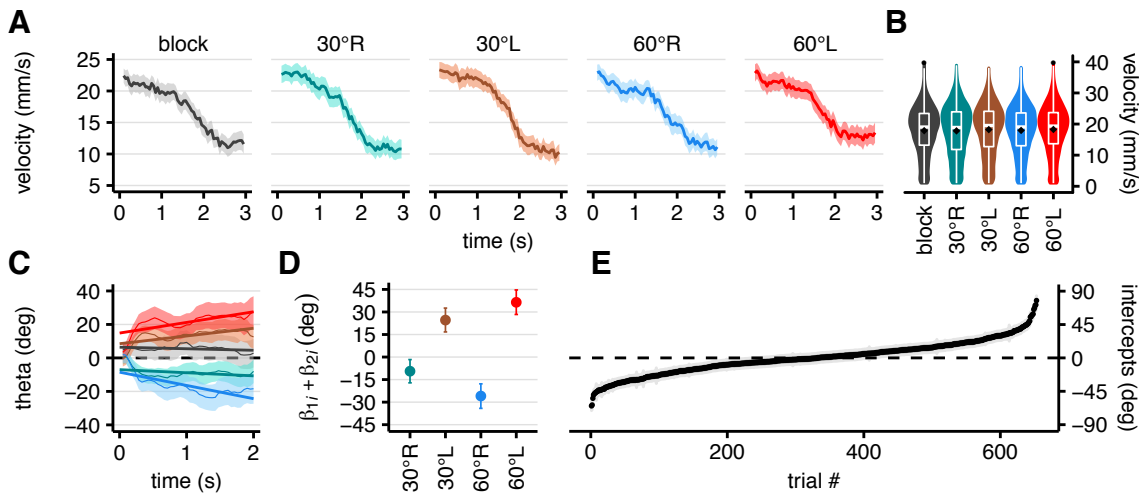


**Fig. 2**

**Fig. 2. Experimental procedure.** Cartoon showing the three phases involved in each experiment. (A) Acclimatization period in complete darkness for 300 s. (B) Two opposing bright green stripes were switched on and the behaviour was recorded for 200 s. (C) Behavioural task consisting in the random presentation of distracting visual stimuli (distractors) whenever the fly crossed a virtual central window (rectangle with blue borders). Behavioural task terminated when all five conditions were repeated seven times (about 30 min). Distractors are represented as: 'block' for no distraction, '30°R' and '60°R' for distraction at 30 or 60 deg on the right; '30°L' and '60°L' for distraction at 30 deg and 60 deg on the left.

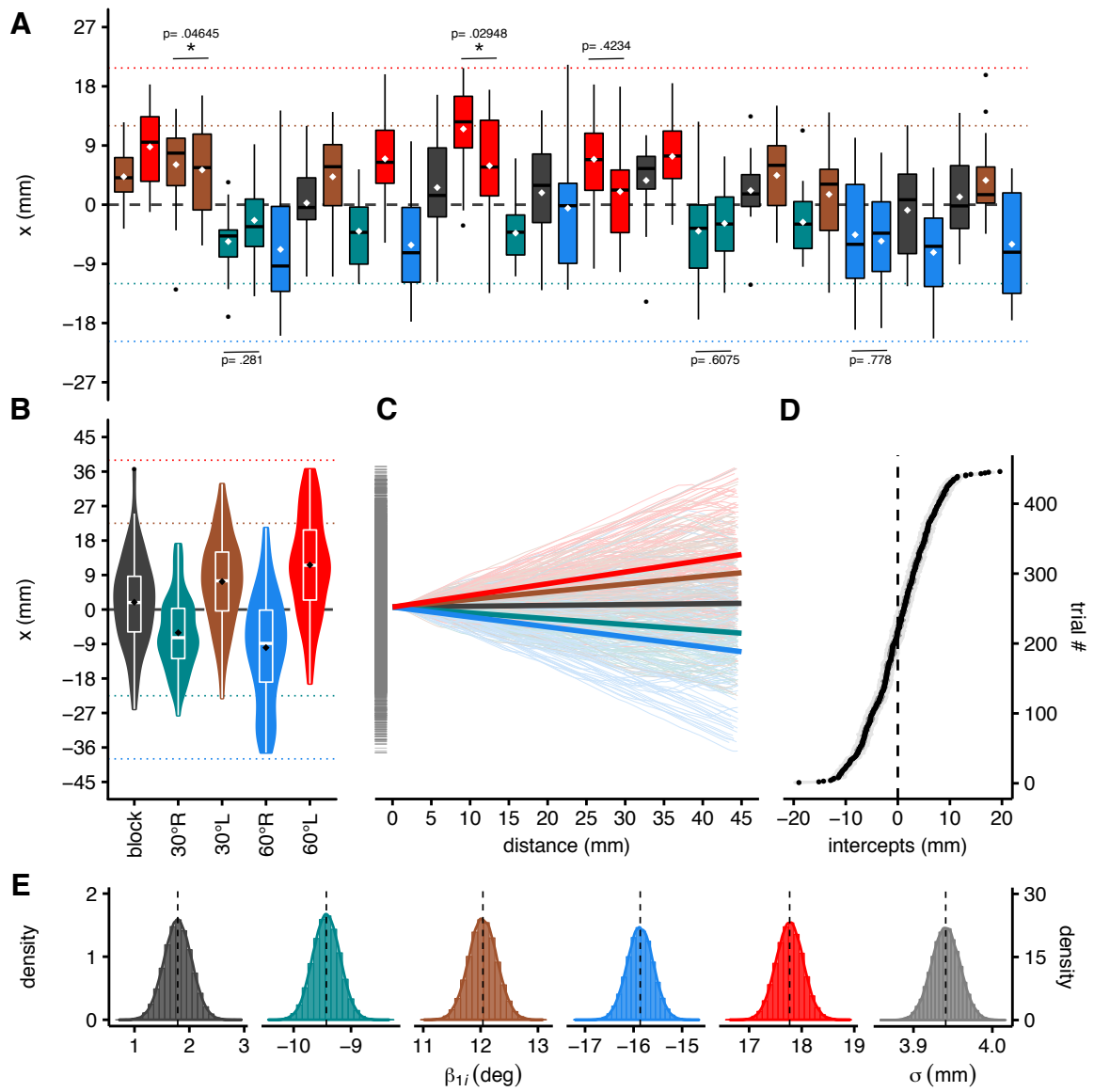
**Fig. 3**

**Fig. 3. Data inspection and trajectories centering.** (A) Box-violin plot (i.e. box plot plus data distribution) of flies orientations (theta; see inset) in degrees at the exact moment of distractor presentation. Plots show that flies orientations when faced with the trials do not differ consistently among different conditions and are approximately normally distributed. Colour coding: black correspond to the absence of distractors (block); green to distractor at 30 deg on the right side (30°R); brown to distractor at 30 deg on the left (30°L); blue to distractor at 60 deg on the right (60°R) and red to distractor at 60 deg on the left (60°L). Negative theta values refer to right-hand turns, while the positive ones to left-hand turns. The box-violin plot shows two measures of central tendency, the median in the box plot, and the mean of the data represented by the black square dot. (B) Heat map showing a density plot of all flies positions in the virtual rectangle when the distractor is presented. x and y-axis are in mm.

**Fig. 4**

**Fig. 4. Plots of orientations.** (A) Velocity profiles from  $t = 0$  s (crossing of the virtual central window; see Fig. 2C) to  $t = 3$  s in the five conditions during the task. Shaded regions represent the s.e.m. (B) Box-violin plot (i.e. box plot plus the data distribution) of the velocity values in the five conditions. One-way ANOVA provides no evidenced of differences between mean velocities among the five conditions ( $F_{(4, 31)} = .22$ ,  $R^2 = .53$ ,  $p = .93$ ). (C) Plot of the mean orientation (theta) from  $t = 0$  to  $t = 2$  s in the five conditions. Shaded regions represent s.e.m. Thick lines are regression lines for each condition. (D) Sum of the two coefficients  $\beta_1$  and  $\beta_2$  both referred to the condition effects (i.e. intercept and interaction with time), which allows to grasp the amount of change in orientation. (E) Random effect plot for each trial nested within flies (fly:trial). Dots represent the conditional means (also known as BLUPs, Best Linear Unbiased Predictions) while the shaded region (grey) corresponds to the standard deviations. In all images of the panel, the color-coding is as previously described (see Fig. 3).

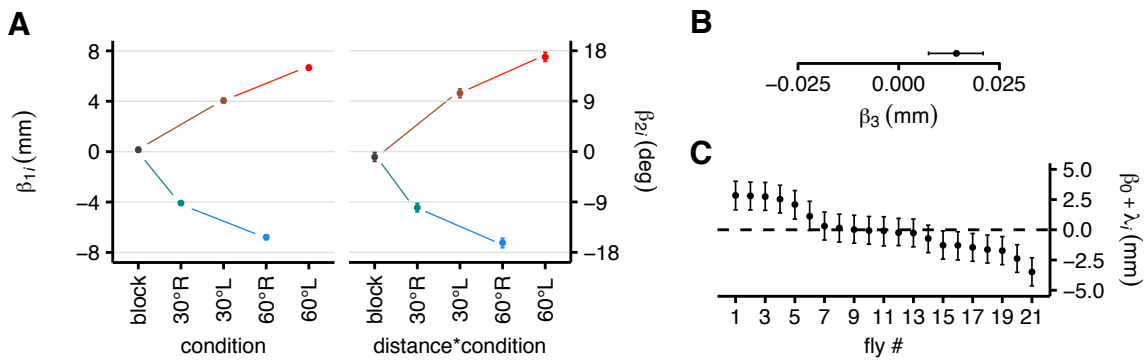
**Fig. 5**



## Fig. 5

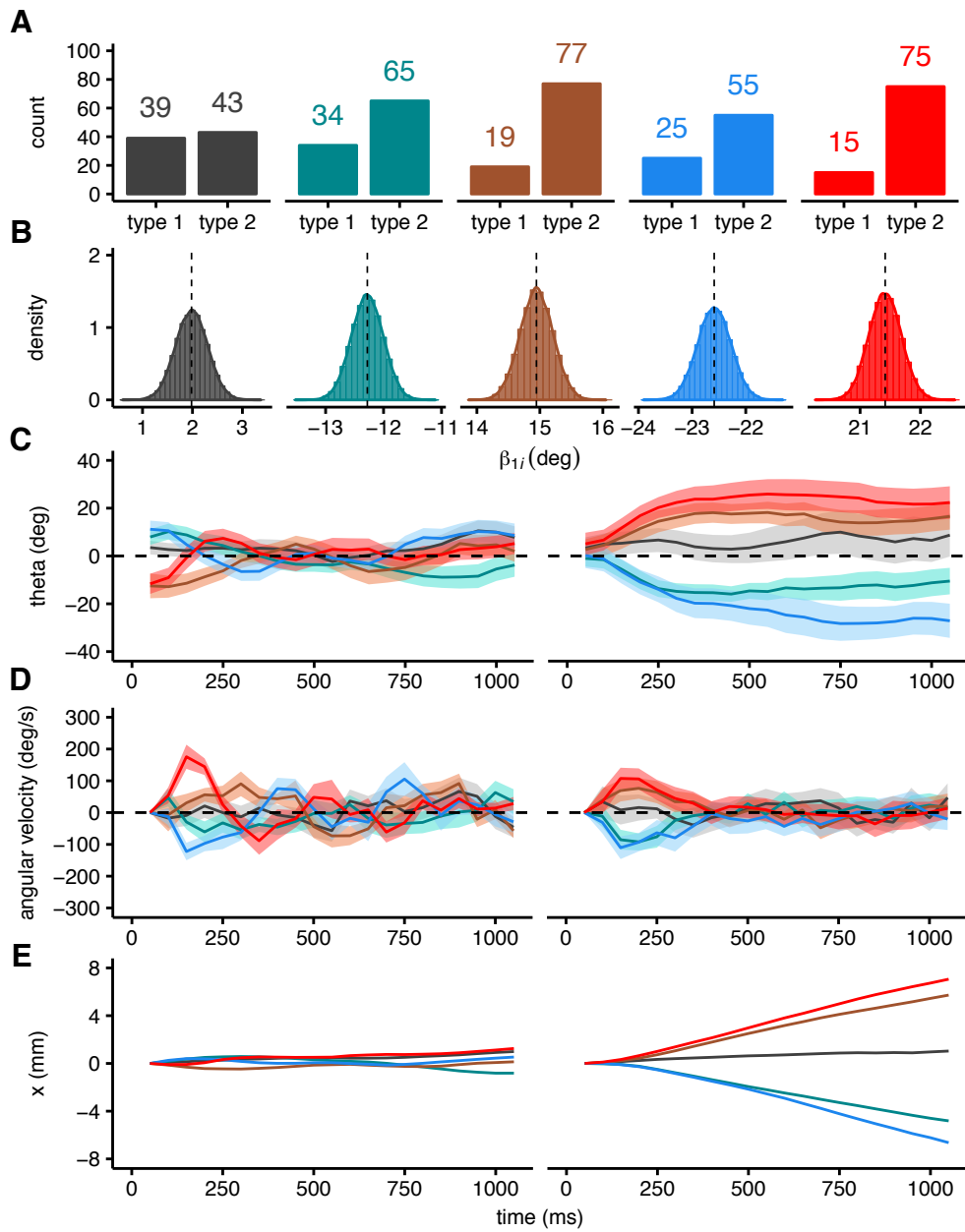
**Fig. 5. Plots of trajectories.** (A) Box and whiskers plot of the displacement of flies along the x-axis at mid-path (i.e. after the flies had travelled 24 mm following the presentation of the distractor). Each trial corresponds to a specific condition and is presented across time from left to right. This allows to appreciate the horizontal shift of the trajectories, at midway, between trials. This graph shows two measures of the central tendency, the median as a black horizontal line inside the box plot and the mean represented by a white squared dot. The vertical extension of each box represents the interquartile range (IQ). The whiskers extending from each box represent the extension of the data (i.e. max. and min. of the data within 1.5 times the IQ), while isolated black dots represent outliers. It can be observed that when the same distractor is immediately re-presented (which can only occur occasionally, due to the randomness of distractor presentation), the shift along the x-axis is usually smaller than the shift observed when the distractor is presented for the first time or has not been presented recently. Only on two occasions out of the six, did the mean displacement values between two successive presentations of the same distractor differ significantly ( $p = .04645$  and  $p = .02948$ ). Statistical comparisons were done using the non-parametric Mann-Whitney-Wilcoxon test. (B) Box-violin plot (i.e. box plot plus the data distribution) of the displacement of flies along the x-axis for each condition when flies have travelled for 45 mm (i.e. along the axis connecting the two fixed stimuli) by condition. (C) Plot of the regression lines (thicker lines) for each condition with the intercept fixed at  $x = 0$  for all trajectories (thinner lines). (D) Plot of conditional modes of the random effects of the LME\_6 model. Dots represent the conditional means (also known as BLUPs, Best Linear Unbiased Predictions) while the shaded region (grey) corresponds to the standard deviations. This represents the difference between the average predicted response for a given condition and the response predicted for a particular individual. (E) Approximate density profile of the probability density function for the sampling distribution for each parameter. The six distributions show the likelihoods of the five interaction parameters (between distance and condition), with  $\sigma$  representing the residual standard deviation. In all images of the panel the color-coding is as previously described (see Fig. 3).

**Fig. 6**



**Fig. 6. HPI plot of parameters.** (A) Fixed effects of condition parameter (on the left) and interaction parameter (on the right) between distance and condition with their 97.5% Highest Posterior Intervals (HPI). (B) Fixed effects of distance parameters with their 97.5% HPI. (C) Random effects plot of the model represented for each fly. Colours encode conditions as previously described (see Fig. 3).

**Fig. 7**



## Fig. 7

**Fig. 7. Trajectories split.** (A) Count of the trials falling into the two types of trajectories by condition. Type 1 represents the trajectories in which the shift of the flies was at least 9 mm from an ideal straight line uniting the two Buridan stimuli when flies reached the middle of the path, while type 2 represents the trajectories for which the shift was less than 9 mm. (B) Approximate density profile of the probability density function for the sampling distribution for the five conditions. Distributions show the likelihoods of the interaction parameters (between distance, condition and type). (C) Mean orientation of the flies during the first second by condition in the two types of trajectories. On the left are shown type 1 trajectories while on the right type 2 trajectories. The shaded region represents the s.e.m. (D) Mean of the angular velocity of the flies during the first 1000 milliseconds by condition in the two types of trajectories. Type 1 on the left, type 2 on the right. The shaded region represents the s.e.m. (E) Regression lines of the trajectories with the LOWESS (LOcally WEighted Scatterplot Smoothing) method during the first 1000 milliseconds by condition for the two types of trajectories. Type 1 on the left, type 2 on the right. In all images of the panel the color-coding is as previously described (see Fig. 3).