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Role of the light source position in freely falling hoverflies' stabilization performances

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Abstract

The stabilization of plummeting hoverflies was filmed and analysed in terms of their wingbeat initiation times as well as the crash and stabilization rates. The flies experienced near-weightlessness for a period of time which depended on their ability to counteract the free fall by triggering their wingbeats. In this paradigm, hoverflies' flight stabilization strategies were investigated here for the first time under two different positions of the light source (overhead or bottom lighting). The crash rates were higher in bottom lighting conditions than with top lighting. In addition, adding a texture to the walls reduced the crash rates only in the overhead lighting condition. The position of the lighting also significantly affected both the stabilization rates and the time taken by the flies to stabilize, which decreased and increased under bottom lighting conditions, respectively, whereas textured walls increased the stabilization rates under both lighting conditions. These results support the idea that flies may mainly base their flight control strategy on visual cues and particularly, that the light distribution in the visual field may provide reliable, efficient cues for estimating their orientation with respect to an allocentric reference frame. In addition, the finding that the hoverflies' optic flow-based motion detection ability is affected by the position of the light source in their visual field suggests the occurrence of interactions between movement perception and this visual vertical perception process.

1 Introduction

Several flying insects including dipterans are known to keep their dorsal surface oriented upwards by holding the brightest part of their environment, which usually shines from above, in a constant position in their visual field [21]. This reflex, which is known as the Dorsal Light Response (DLR) has also been described in detail in fish [5]. The importance of the orientation of an artificial horizon in blowflies' head roll orientation processes has also been previously established, as well as the fact that these insects probably do not use gravity information to perform this task [10], which has been assessed in freely flying hoverflies based on free fall experiments [8], contrary to the well-known negative gravitaxy behaviour observed in walking *Drosophila* [18, 17]. These results suggest that visual processes predominate over gravity-based ones in the strategies used by flying flies to stabilize their flight, and support the idea that there exists some kind of vertical reference frame in flies' brains based on the DLR. However, although this reflex had been found to be closely linked to the head roll steering mechanism, this situation has been established only in tethered *Calliphora* and *Episyrphus* [12, 7] and has not been studied so far during free flight.

In the present study, it was therefore proposed to assess the effects of the change in the light source position on freely flying dipterans' stabilization performances. These performances were tested using a free fall procedure under four different visual conditions in which two differently textured lateral walls (uniform and textured) were combined with two different lighting sources (overhead or bottom lighting). The position of the light source was found to be a crucial factor for hoverflies to be able to regain a suitable flight attitude after a free fall. In addition, the results obtained in this study support the idea that the use of lateral visual cues such as 2-D chequerboard patterns generating optic flow (OF) may be involved in hoverflies' attitude and lift control processes [4, 9]. This study clearly shows not only that the light gradient perception and OF-based control processes are interlinked, but also that the static cues consisting of the light source position predominate over the insects' OF-based control processes.

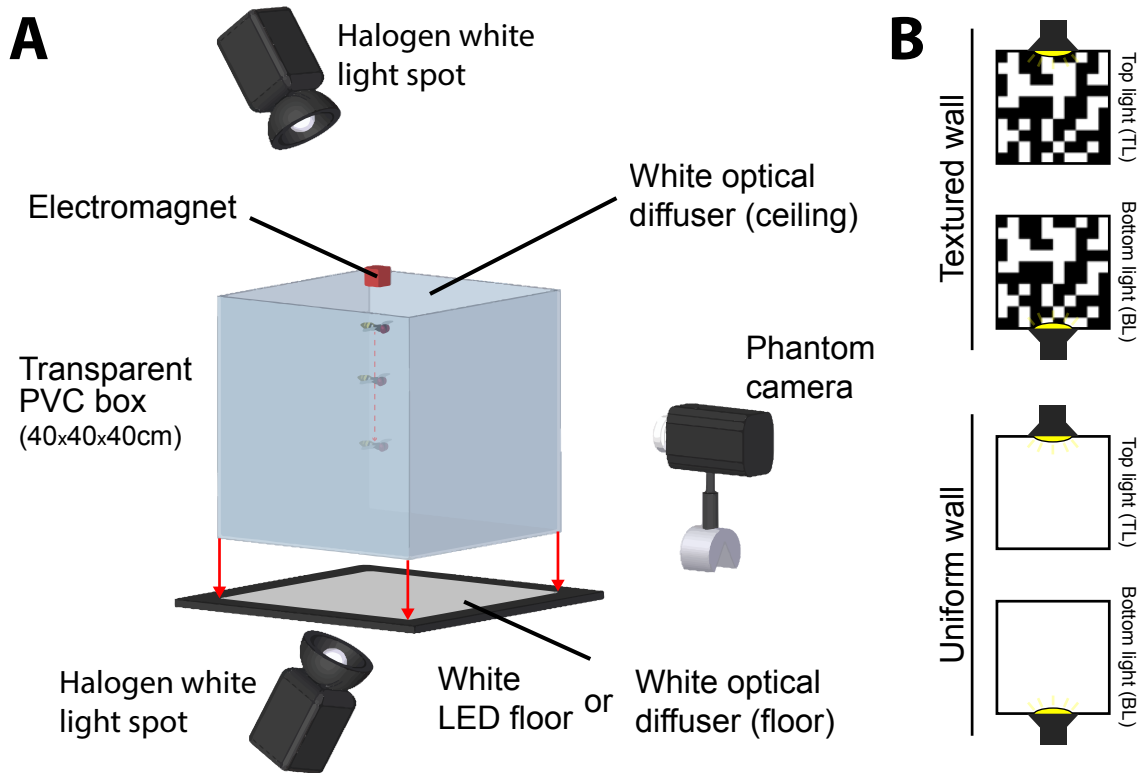


Figure 1: **Experimental setup.** (A) The setup used in this study was first presented in [8]. In the present version, a white backlit LED panel was added to illuminate the box from below. (B) Four environments were tested: Contrasting textured lateral walls with Top lighting (CT), Contrasting textured lateral walls with Bottom lighting (CB), Uniform lateral walls with Top lighting (UT), and Uniform lateral walls with Bottom lighting (UB).

28 2 Methods

29 Animals

30 Hoverfly pupae (*Episyrphus balteatus*) were purchased from Katz Biotech AG, Baruth, Ger-
 31 many. To magnetically maintain the animals in resting position (see in figure 1A), a piece
 32 of entomological pin approximately 5 mm long was glued to the dorsal part of the animals'
 33 thorax, perpendicularly to their longitudinal axis: the pin ($\approx 5mg$) weighed approximately
 34 15% of the hoverfly's mass ($\approx 35mg$). The insects' flight ability was checked in the breeding
 35 cages throughout the experiments. 39 hoverflies (19 in Halogen/LED experiments and 20 in
 36 the control experiments) aged from 3 to 28 days were tested (9 males and 10 females in the
 37 Halogen/LED experiments and 2 males and 18 females in the control experiments).

38 **Experimental procedure**

39 Hoverflies were subjected to free fall conditions in a modified version of the setup previously
40 presented in [8]. In the present version, the box was illuminated alternately from above (Top
41 Lighting: TL) with a white halogen light (Kaiser Studioliight H) and from below (Bottom
42 Lighting: BL) with a white backlit LED panel (Phlox, $50 \times 50 \text{ cm}^2$) featuring a uniformity as high
43 as 95% and two peaks (450nm and one at around 550nm) that match the spectral sensitivity
44 of the hoverfly's (*Erisalis tenax*) photoreceptor cells [15]).

45 Four different conditions were tested (fig 1B): Contrasting textured lateral walls with Top
46 lighting (CT), Contrasting textured lateral walls with Bottom lighting (CB), Uniform lateral
47 walls with Top lighting (UT) and Uniform lateral walls with Bottom lighting (UB). In addi-
48 tion to these conditions, two control experiments were conducted in which the CT condition
49 was compared with a CB condition with white halogen bottom lighting instead of the LED
50 panel to check whether the LED lighting condition affected the hoverflies' performances. The
51 texture on the walls consisted of a randomly generated chequerboard (20x20 squares 4 cm^2
52 in size). The irradiance was measured in both illumination conditions with an ILT1700 ra-
53 diometer (International Light Technologies) under both experimental conditions (with tex-
54 tured walls: CT and CB conditions) by orienting the light probe (SED033, visual field 3°) of
55 the radiometer towards either the illuminated side or the opposite side. The irradiance mea-
56 sured in the CT condition (direct/indirect measurements) was $1.12 \cdot 10^{-8} / 3.15 \cdot 10^{-9} \text{ W.cm}^{-2}$
57 and $1.10 \cdot 10^{-9} / 2.33 \cdot 10^{-10} \text{ W.cm}^{-2}$ in the CB condition with the LED light. In the control
58 experiments with two halogen lights, the irradiance was set at the same value (direct measure-
59 ments) ($1.24 \cdot 10^{-8} \text{ W.cm}^{-2}$).

60 A total number of 262 falls were conducted among the four different conditions (see fig-
61 ure 1B), and 91 additional falls were conducted in the control experiments. At each experi-
62 mental session, a hoverfly was exposed to the four environments consecutively in random order.
63 Each hoverfly could undergo several experimental sessions, but no more than once a day in or-
64 der to prevent the occurrence of any habituation or fatigue effects. We always checked between
65 experimental sessions whether the hoverflies equipped with their glued pin were able to fly in
66 the breeding cages.

67 **Image analysis**

68 The horizontal and vertical 2-D positions of the hoverflies' centre of mass moving over a uni-
69 form background were recorded using a custom-made image-processing program running under
70 MATLAB. The fly's speed was calculated from the positions recorded by applying a Savitzky-
71 Golay procedure (order 2, window: 51). Stabilization was determined automatically when the
72 fly reached a positive vertical speed without touching either a wall or the ground.

73 **Statistical analysis**

74 Data were analysed statistically using a generalized linear mixed-effects model procedure ('glmer'
75 in R v3.2.3) and selected using the Akaike Information Criterion ['AIC' [1]].

76 **3 Results**

77 As observed previously [8], hoverflies subjected to free falls initiated their flight after approxi-
78 mately 100ms in both uniform and contrasting wall texture conditions ($p = 0.12$; $F = 2.4727$;
79 Figure 2A). However, top lighting conditions significantly decreased the reaction wingbeat
80 triggering) times ($p < 0.01$; $F = 9.4352$). The flies' performances in the CB condition dif-
81 fered significantly from those observed in the CT ($p < 0.05$; $z = -2.863$) and UT ($p < 0.01$;
82 $z = -3.294$) conditions, but the differences in the mean times did not exceed 20ms ($\bar{\Delta}_{WB}$ (ms):
83 $CB = 131.2500$; $CT = 107.7500$; $UB = 116.5152$; $UT = 104.3087$). It is worth noting that
84 during our control experiments, the wingbeat triggering times (see Figure 2A) were significantly
85 shorter by around 20ms ($p < 0.001$; $F = 19.6268$), but that this did not significantly reduce the
86 difference in the effects observed between CB and CT ($p = 0.39$; $F = 0.7303$).

87 Bottom lighting conditions induced a much larger number of touchdowns on the floor (Fig-
88 ure 2B), amounting to approximately 60% of all the trials, than under overhead lighting con-
89 ditions ($p < 0.001$; $Chi^2 = 35.8369$), which enabled the hoverflies to avoid crashing in 70-75%
90 (UT) to 90% (CT) of the flights. It is worth noting that the crash rates were quite similar
91 between Halogen-LED and control experiments (Effect of LED light: $p = 0.68$; $Chi^2 = 0.1694$;
92 interaction with light position effect: $p = 0.35$; $Chi^2 = 0.8571$) which confirms the validity of
93 using a LED panel to stimulate light-dependent stabilization behaviour. In addition, the crash

94 rates were not significantly affected by the presence of textured walls ($p = 0.18$; $Chi^2 = 1.7578$),
95 whereas a significant interaction was found to occur between the lighting and texture conditions
96 ($p = 0.01$; $Chi^2 = 6.5524$). The presence of a 2-D chequerboard pattern on the walls signifi-
97 cantly decreased the crash rates under overhead lighting conditions (Post-hoc Tukey contrast,
98 CT vs UT: $p < 0.05$; $z = 2.838$) but not under bottom lighting conditions (Post-hoc Tukey
99 contrast, CB vs UB: $p = 0.95$; $z = -0.525$).

100 In the subsequent analysis, stabilized flight was taken to occur whenever the fly adopted
101 a positive vertical speed, corresponding to a rising flight, without subsequently crashing onto
102 the floor (Figure 2C). Since the number of stabilized flights observed in the case of UB was
103 very small ($n = 3$), no definite conclusions could be reached about the effects of this con-
104 dition on the stabilization times, and these data were therefore removed from the statisti-
105 cal analysis. The lighting conditions significantly affected the stabilization times ($p < 0.05$;
106 $F = 5.5942$): the mean stabilization time was approximately 50ms longer in the CB environ-
107 ment than that recorded in the two conditions with overhead lighting ($\bar{\Delta}_{Stab}$ (ms): $CB = 222.75$;
108 TL (UT & CT) = 169.0465).

109 A large number of stabilized flights occurred with overhead lighting and either textured or uni-
110 form lateral walls (Figure 2D). In the BL conditions, hoverflies produced poorer stabilization
111 performances than in the TL conditions ($p < 0.001$; $Chi^2 = 46.144$), and they were almost un-
112 able to prevent themselves from continuing to fall in the uniform environment (UB). Hoverflies
113 surrounded by textured lateral walls, i.e., in conditions CT and CB, achieved better perfor-
114 mances than under the same lighting condition with uniform walls, i.e., in conditions UT and
115 UB, respectively ($p < 0.001$; $Chi^2 = 23.463$). It was also observed that the stabilization rates
116 were similar between Halogen-LED and control experiments (Effect of LED light: $p = 0.11$;
117 $Chi^2 = 2.5727$; interaction with light position: $p = 0.27$; $Chi^2 = 1.2127$) whereas the effects of
118 light position on stabilization time depended on the kind of light (Effect of LED light: $p = 0.49$;
119 $Chi^2 = 0.4730$; interaction with light position: $p < 0.01$; $Chi^2 = 7.52$). It can be seen from
120 figure 2C that this effect was mainly observed on the CT condition, where no differences seemed
121 likely to occur. The differences were probably due to the fact that different populations were
122 tested in the control and Halogen/Led experiments.

123

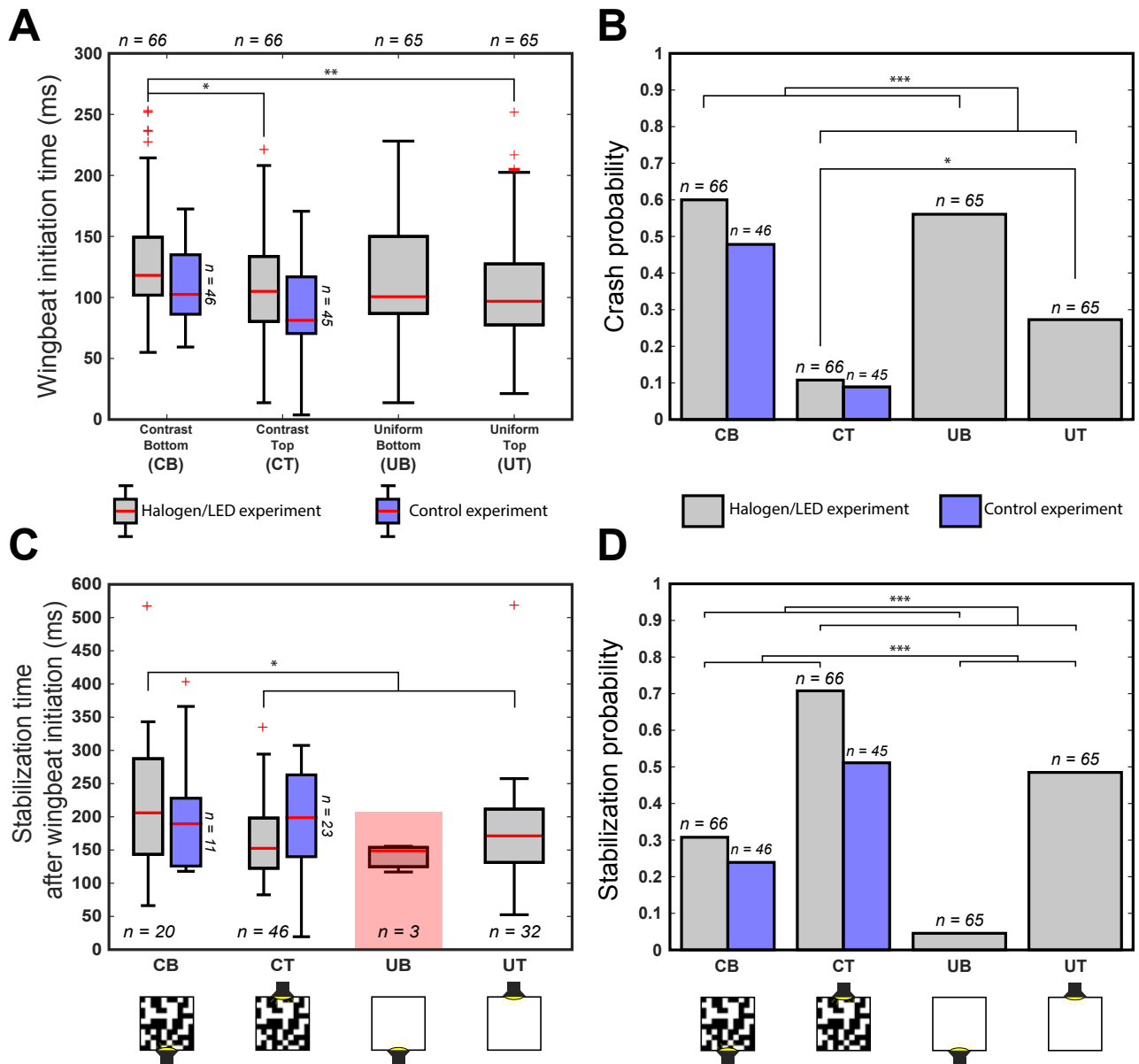


Figure 2: (A) Boxplot of the wingbeat triggering times (ms). (B) Bar plot of the crash rates. (C) Boxplot of the stabilization times (ms). (D) Bar plot of the stabilized flight rates. Boxes are composed of 1st, 2nd and 3rd quartiles, and whiskers correspond to extreme data, amounting to no more than 1.5 times the interquartile distance. Significance code, p value: $0 < *** < 0.001 < ** < 0.01 < * < 0.05$

124 4 Discussion

125 In this study, it was attempted for the first time to investigate the impact of the light source
126 position in hoverflies' visual field on their ability to stabilize their flight. In previous studies,
127 it was suggested that the light gradient generated by an artificial horizon may impact insects'
128 attitude perception processes via a mechanism called the Dorsal Light Response [12, 7]. Using
129 a free fall procedure, we reported that hoverflies starting to fly in an unsteady initial state
130 were found to be able to recover stabilized flight efficiently only in situations where the light
131 came from above. In addition to the crucial position of the light source, the optic flow infor-
132 mation generated during a free fall may also be used by hoverflies to ultimately avoid crashing
133 [11, 4, 27, 9], but these cues probably do not suffice for stabilization purposes. The light gra-
134 dient probably provides hoverflies with a means of estimating their absolute orientation in the
135 environment in order to control their attitude, as found to occur in locusts [6]. This static cue
136 providing a subjective vertical reference value about of the external world [10] would certainly
137 require robust visual processing integrating the lighting information over the whole or most of
138 the spherical field of view. To investigate in greater detail the extent to which the light gradient
139 is actually involved, LED panels might be a useful means of finely controlling the homogeneity
140 of the illumination generated and the amplitude of the light gradient, and generating dynamic
141 changes in the lighting conditions.

142

143 One of the main hypotheses put forward in previous studies on flies' sensorimotor reflexes
144 is that they may depend only on movement perception processes and compensatory reflexes
145 [28]. The inputs originating from both visual structures, the compound eyes and the ocelli
146 [19, 3, 23, 24], and from the halteres [25, 22, 13, 2], which are fused together non-linearly [16],
147 may compensate for a large range of disturbances [26]. A system of this kind is liable, however,
148 to be subject to accumulated errors during flight, resulting in a drift in the attitude control
149 process and eventually in crashing. The results obtained here therefore indicate that the DLR
150 may play an important role by providing a reliable time-invariant vertical reference frame which
151 may be used to complement the insects' motion-based reflexes. However, the initial position
152 of hoverflies with their legs dangling may have decreased the ability of the chordotonal organs
153 (organs acting as pressure sensors [30] linked to postural reflex in insects [14, 20] which were

154 stimulated in this situation only by the legs' weight) to estimate their orientation with respect
155 to the gravity experienced prior to the fall and we therefore cannot rule out the latter hypothesis.

156

157 In conclusion, this initial study shows that the position of the light source plays an impor-
158 tant role in hoverflies' flight stabilization processes. The results presented here suggest that
159 both static (light source, DLR) and movement (optic flow) cues are probably involved [10]. A
160 further question which arises here is how these two visual processes (the DLR and OF-based
161 processes) are fused together to ensure robust flight stabilization under natural conditions, as
162 previously suggested in the case of optomotor responses [29]. Future studies in which conflict-
163 ing situations are generated would probably help to understand how these different sensory
164 processes are combined in dipterans' brains.

165

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172 Retina for Innovative Sensing) ANR-12- INSE-0009].

173 **Ethics**

174 No ethical assessments for animal research purposes or permission to carry out fieldwork were
175 required for this study.

176 **Data accessibility**

177 Data are provided in Excel tables in the supplementary files (Data_total.xls).

178 **Competing interests**

179 We have no competing interests to declare

180 **Authors' contributions**

181 RG designed and conducted the experiments, analysed the data, carried out the statistical anal-
182 yses and drafted the manuscript; AV conducted experiments and helped to draft the manuscript;
183 JLV helped with the statistical analyses and with the drafting of the manuscript. SV designed
184 the experiment, coordinated the study and helped to draft the manuscript. All the authors
185 gave their final approval for publication of the manuscript and agree to be held accountable for
186 the work performed herein.

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