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Animal Behaviour

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

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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 that 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 and 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 [1]. This reflex, which is known as the dorsal light response (DLR), has also been described in detail in fish [2]. 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 [3], which has been assessed in freely flying hoverflies based on free fall experiments [4], contrary to the well-known negative gravitaxy behaviour observed in walking *Drosophila* [5,6]. 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* [7,8] and has not been studied so far during free flight.

In this 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 and bottom lighting). The position of the light source was found to be a crucial

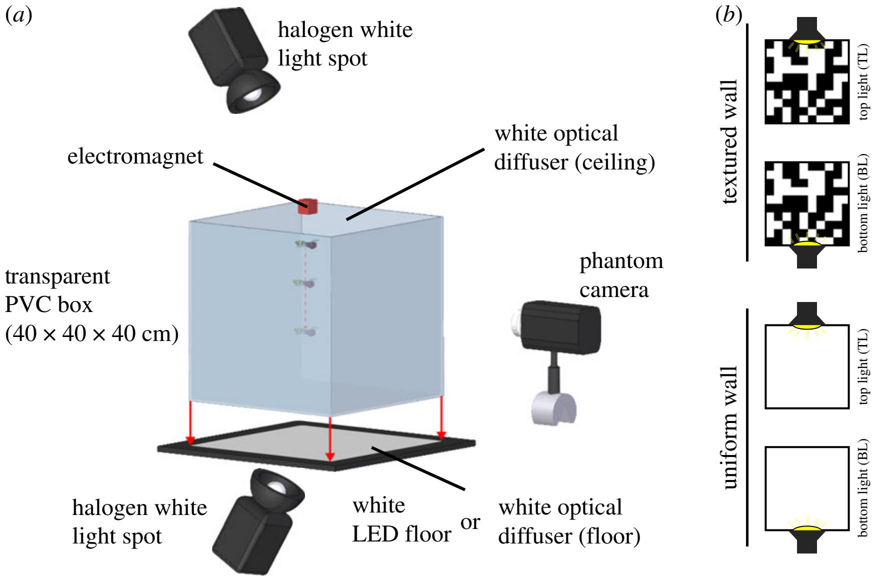

Figure 1. Experimental set-up. (*a*) The set-up used in this study was first presented in [4]. In this version, a white backlit LED panel was added to illuminate the box from below. (*b*) Four environments were tested: Contrasting textured lateral walls with top lighting (CT), contrasting textured lateral walls with bottom lighting (CB), uniform lateral walls with top lighting (UT), and uniform lateral walls with bottom lighting (UB). (Online version in colour.)

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 two-dimensional chequerboard patterns generating optic flow (OF) may be involved in hoverflies' attitude and lift control processes [9,10]. 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.

2. Methods

(a) Animals

Hoverfly pupae (*Episyrphus balteatus*) were purchased from Katz Biotech AG, Baruth, Germany. To allow us to magnetically maintain the animals in the resting position (see figure 1*a*), a piece of entomological pin approximately 5 mm long was glued to the dorsal part of the animals' thorax, perpendicularly to their longitudinal axis: the pin (approx. 5 mg) weighed approximately 15% of the hoverfly's mass (approx. 35 mg). The insects' flight ability was checked in the breeding cages throughout the experiments. Thirty-nine hoverflies (19 in halogen/LED experiments and 20 in the control experiments) aged from 3 to 28 days were tested (nine males and 10 females in the halogen/LED experiments and two males and 18 females in the control experiments).

(b) Experimental procedure

Hoverflies were subjected to free fall conditions in a modified version of the set-up previously presented in [4]. In this version, the box was illuminated alternatively from above (top lighting: TL) with a white halogen light (Kaiser Studiolight H) or from below (bottom lighting: BL) with a white backlit LED panel (Phlox, 50 × 50 cm^2) featuring a uniformity as high as 95% and two peaks (one at 450 nm and one at approx. 550 nm) that match the spectral sensitivity of the hoverfly's (*Erisalis tenax*) photoreceptor cells [11]).

Four different conditions were tested (figure 1*b*): 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). In addition to these conditions, two control experiments were conducted in which the CT condition was compared with a CB condition with white halogen bottom lighting instead of the LED panel to check whether the LED lighting condition affected the hoverflies' performances. The texture on the walls consisted of a randomly generated chequerboard (20 × 20 squares 4 cm^2 in size). The irradiance was measured in both illumination conditions with an ILT1700 radiometer (International Light Technologies) under both experimental conditions (with textured walls: CT and CB conditions) by orienting the light probe (SED033, visual field 3°) of the radiometer towards either the illuminated side or the opposite side. The irradiance was 1.12×10^{-8}/3.15×10^{-9} W cm^{-2} measured in the CT condition (direct/indirect measurements) and 1.10×10^{-9}/2.33×10^{-10} W cm^{-2} in the CB condition with the LED light. In the control experiments with two halogen lights, the irradiance was set at the same value (direct measurements) (1.24×10^{-8} W cm^{-2}).

A total number of 262 falls were conducted among the four different conditions (figure 1*b*), and 91 additional falls were conducted in the control experiments. At each experimental session, a hoverfly was exposed to the four environments consecutively in random order. Each hoverfly could undergo several experimental sessions, but no more than once a day in order to prevent the occurrence of any habituation or fatigue effects. We always checked between experimental sessions whether the hoverflies equipped with their glued pin were able to fly in the breeding cages.

(c) Image analysis

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

(d) Statistical analysis

Data were analysed statistically using a generalized linear mixed-effects model procedure ('glmer' in R v. 3.2.3) and selected using the Akaike information criterion ([12]).

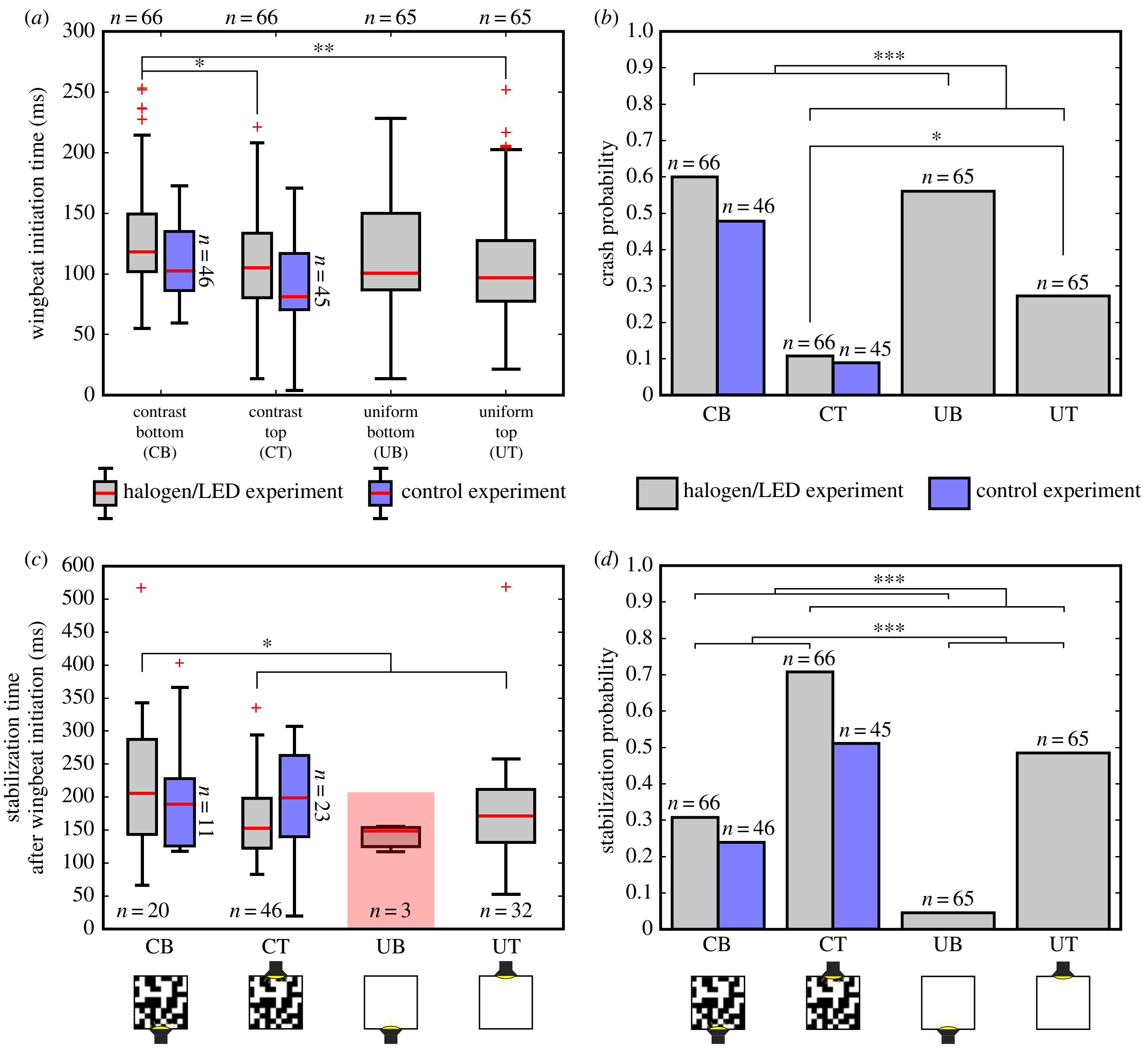

Figure 2. (*a*) Boxplot of the wingbeat triggering times (ms). (*b*) Bar plot of the crash rates. (*c*) Boxplot of the stabilization times (ms). (*d*) Bar plot of the stabilized flight rates. Boxes are composed of first, second and third quartiles, and whiskers correspond to extreme data, amounting to no more than 1.5 times the interquartile distance. Significance code, *p*-value: $0 < {***} < 0.001 < {**} < 0.01 < {*} < 0.05$. All data used are summarized in the supplementary material. (Online version in colour.)

3. Results

As observed previously [4], hoverflies subjected to free falls initiated their flight after approximately 100 ms in both uniform and contrasting wall texture conditions ($p = 0.12$; $F = 2.4727$; figure 2*a*). However, top lighting conditions significantly decreased the reaction wingbeat triggering) times ($p < 0.01$; $F = 9.4352$). The flies' performances in the CB condition differed significantly from those observed in the CT ($p < 0.05$; $z = -2.863$) and UT ($p < 0.01$; $z = -3.294$) conditions, but the differences in the mean times did not exceed 20 ms ($\bar{\Delta}_{WB}$ (ms): CB = 131.2500; CT = 107.7500; UB = 116.5152; UT = 104.3087). It is worth noting that during our control experiments, the wingbeat triggering times (figure 2*a*) were significantly shorter by around 20 ms ($p < 0.001$; $F = 19.6268$), but that this did not significantly reduce the difference in the effects observed between CB and CT ($p = 0.39$; $F = 0.7303$).

Bottom lighting conditions induced a much larger number of touchdowns on the floor (figure 2*b*), amounting to approximately 60% of all the trials, than under overhead lighting conditions ($p < 0.001$; $\chi^2 = 35.8369$), which enabled the hoverflies to avoid crashing in 70–75% (UT) to 90% (CT) of the flights. It is worth noting that the crash rates were quite similar between halogen/LED and control experiments (effect of LED light: $p = 0.68$; $\chi^2 = 0.1694$; interaction with light position effect: $p = 0.35$; $\chi^2 = 0.8571$), which confirms the validity of using an LED panel to stimulate light-dependent stabilization behaviour. In addition, the crash rates were not significantly affected by the presence of textured walls ($p = 0.18$; $\chi^2 = 1.7578$), whereas a significant interaction was found to occur between the lighting and texture conditions ($p = 0.01$; $\chi^2 = 6.5524$). The presence of a two-dimensional chequerboard pattern on the walls significantly decreased the crash rates under overhead lighting conditions (*post hoc* Tukey contrast, CT versus UT: $p < 0.05$; $z = 2.838$) but not under bottom lighting conditions (*post hoc* Tukey contrast, CB versus UB: $p = 0.95$; $z = -0.525$).

In the subsequent analysis, stabilized flight was taken to occur whenever the fly adopted a positive vertical speed, corresponding to a rising flight, without subsequently crashing onto the floor (figure 2*c*). As the number of stabilized flights observed in the case of UB was very small ($n = 3$), no definite conclusions could be reached about the effects of this condition on the stabilization times, and these data were therefore removed from the statistical analysis. The lighting conditions significantly affected the stabilization times ($p < 0.05$; $F = 5.5942$): the mean stabilization time was approximately 50 ms longer in the CB environment than that recorded in the two conditions with overhead lighting ($\bar{\Delta}_{Stab}$ (ms): CB =222.75; TL (UT and CT)=169.0465).

A large number of stabilized flights occurred with overhead lighting and either textured or uniform lateral walls (figure 2*d*). In the BL conditions, hoverflies produced poorer stabilization performances than in the TL conditions ($p < 0.001$; $\chi^2 = 46.144$), and they were almost unable to prevent themselves from continuing to fall in the uniform environment (UB). Hoverflies surrounded by textured lateral walls, i.e. in conditions CT and CB, achieved better performances than under the same lighting condition with uniform walls, i.e. in conditions UT and UB, respectively ($p < 0.001$; $\chi^2 = 23.463$). It was also observed that the stabilization rates were similar between halogen/LED and control experiments (effect of LED light: $p = 0.11$; $\chi^2 = 2.5727$; interaction with light position: $p = 0.27$; $\chi^2 = 1.2127$) whereas the effects of light position on stabilization time depended in the kind of light (effect of LED light: $p = 0.49$; $\chi^2 = 0.4730$; interaction with light position: $p < 0.01$; $\chi^2 = 7.52$). It can be seen from figure 2*c* that this effect was mainly observed in the CT condition, where no differences seemed likely to occur. The differences were probably due to the fact that different populations were tested in the control and halogen/LED experiments.

4. Discussion

In this study, it was attempted for the first time to our knowledge to investigate the impact of the light source position in hoverflies' visual field on their ability to stabilize their flight. In previous studies, it was suggested that the light gradient generated by an artificial horizon may impact insects' attitude perception processes via a mechanism called the DLR [7,8]. Using a free fall procedure, we reported that hoverflies starting to fly in an unsteady initial state were found to be able to recover stabilized flight efficiently only in situations where the light came from above. In addition to the crucial position of the light source, the OF information generated during a free fall may also be used by hoverflies to ultimately avoid crashing [9,10,13,14], but these cues probably do not suffice for stabilization purposes. The light gradient probably provides hoverflies with a means of estimating their absolute orientation in the environment in order to control their attitude, as found to occur in locusts [15]. This static cue providing a subjective vertical reference value about the external world [3] would certainly require robust visual processing integrating the lighting information over the whole or most of the spherical field of view. To investigate in greater detail the extent to which the light gradient is actually involved, LED panels might be a useful means of finely controlling the homogeneity of the illumination generated and the amplitude of the light gradient, and generating dynamic changes in the lighting conditions.

One of the main hypotheses put forward in previous studies on flies' sensorimotor reflexes is that they may depend only on movement perception processes and compensatory reflexes [16]. The inputs originating from both visual structures, the compound eyes and the ocelli [17–20], and from the halteres [21–24], which are fused together nonlinearly [25], may compensate for a large range of disturbances [26]. A system of this kind is liable, however, to be subject to accumulated errors during flight, resulting in a drift in the attitude control process and eventually in crashing. The results obtained here therefore indicate that the DLR may play an important role by providing a reliable time-invariant vertical reference frame which may be used to complement the insects' motion-based reflexes. However, the initial position of hoverflies with their legs dangling may have decreased the ability of the chordotonal organs (organs acting as pressure sensors [27] linked to postural reflex in insects [28,29], which were stimulated in this situation only by the legs' weight) to estimate their orientation with respect to the gravity experienced prior to the fall and we therefore cannot rule out the latter hypothesis.

In conclusion, this initial study shows that the position of the light source plays an important role in hoverflies' flight stabilization processes. The results presented here suggest that both static (light source, DLR) and movement (OF) cues are probably involved [3]. A further question that arises here is how these two visual processes (the DLR and OF-based processes) are fused together to ensure robust flight stabilization under natural conditions, as previously suggested in the case of optomotor responses [30]. Future studies in which conflicting situations are generated would probably help to understand how these different sensory processes are combined in dipterans' brains.

Data accessibility. Data are provided in Excel tables in the electronic supplementary material (Data_total.xls).

Author's contributions. R.G. designed and conducted the experiments, analysed the data, carried out the statistical analyses and drafted the manuscript; A.V. conducted experiments and helped to draft the manuscript; J.-L.V. helped with the statistical analyses and with the drafting of the manuscript. S.V. designed the experiment, coordinated the study and helped to draft the manuscript. All the authors gave their final approval for publication of the manuscript and agree to be held accountable for the work performed herein.

Competing interests. We have no competing interests to declare.

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