



HAL
open science

To crash or not to crash: how do hoverflies cope with free-fall situations and weightlessness?

Roman Goulard, Jean-Louis Vercher, Stéphane Viollet

► To cite this version:

Roman Goulard, Jean-Louis Vercher, Stéphane Viollet. To crash or not to crash: how do hoverflies cope with free-fall situations and weightlessness?. *Journal of Experimental Biology*, 2016, 219 (16), pp.2497-2503. 10.1242/jeb.141150 . hal-01436016

HAL Id: hal-01436016

<https://hal.science/hal-01436016>

Submitted on 27 Nov 2017

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

RESEARCH ARTICLE

To crash or not to crash: how do hoverflies cope with free-fall situations and weightlessness?

Roman Goulard, Jean-Louis Vercher and Stéphane Viollet*

ABSTRACT

Insects' aptitude to perform hovering, automatic landing and tracking tasks involves accurately controlling their head and body roll and pitch movements, but how this attitude control depends on an internal estimation of gravity orientation is still an open question. Gravity perception in flying insects has mainly been studied in terms of grounded animals' tactile orientation responses, but it has not yet been established whether hoverflies use gravity perception cues to detect a nearly weightless state at an early stage. Ground-based microgravity simulators provide biologists with useful tools for studying the effects of changes in gravity. However, in view of the cost and the complexity of these set-ups, an alternative Earth-based free-fall procedure was developed with which flying insects can be briefly exposed to microgravity under various visual conditions. Hoverflies frequently initiated wingbeats in response to an imposed free fall in all the conditions tested, but managed to avoid crashing only in variably structured visual environments, and only episodically in darkness. Our results reveal that the crash-avoidance performance of these insects in various visual environments suggests the existence of a multisensory control system based mainly on vision rather than gravity perception.

KEY WORDS: Flight stabilization, Free fall, Gravity perception, Vision, Dipterous, Insect

INTRODUCTION

The ability of flying insects such as flies, hoverflies, wasps and dragonflies to perform highly demanding flight manoeuvres (Collett and Land, 1975; Schilstra and van Hateren, 1998; Zeil et al., 2008; Wijngaard, 2010; Viollet and Zeil, 2013; Mischiati et al., 2015) is known to involve the accurate control of their body's roll and pitch movements as well as a precise stabilization of their gaze. However, the question as to whether these insects use gravity orientation cues to stabilize their attitude and hence their flight remains to be answered (Bender and Frye, 2009). In crickets and flies placed on tilted surfaces or a Styrofoam ball, gravity perception processes based on gravity-sensitive sensillae (Horn and Föller, 1998), leg load cues (Horn and Knapp, 1984; Hengstenberg, 1993; Kress and Egelhaaf, 2012; Mendes et al., 2014) and antennal receptors (Horn and Kessler, 1975; Horn and Bischof, 1983; Kamikouchi et al., 2009) enable insects to stabilize their gaze and compensate for their body tilt, but it has not yet been established whether they use gravity perception processes during flight. In the cockroach, the tricholiths – pendulous gravity-sensitive sensilla located on the cerci (Walthall

and Hartman, 1981; Hartman et al., 1987) – were reported to be involved in contralateral wingbeating and possibly in flight equilibrium (Fraser, 1977). Apart from cockroaches' responses, Taylor and Krapp (2007) have suggested that a static sense of gravity would be largely irrelevant in view of the fast accelerations performed by many insects during flight. However, the perfect hovering performances observed in many insect species such as *Episyrphus* might be largely due to robust vertical references such as gravity cues. In this case, a specialized gravity organ serving as a linear accelerometer (i.e. analogous to the inner ear in mammals), like the devices used in aerial vehicles (aircraft, helicopters and unmanned aerial vehicles), would therefore usefully complement the halteres, which serve as dipterans' rate gyros. Once placed in a free-fall condition, an accelerometer measuring the physical acceleration (also called the proper acceleration) will yield a value equal to zero (or almost zero). This state, which is also known as 'zero gravity' or 'zero-g', always produces a sensation of weightlessness in falling subjects. This means that, during the free fall, graviception (which can be used by falling subjects to determine their orientation with respect to gravity) is no longer available. However, the occurrence of a transition from a proper acceleration equal to g , due to a static position, to zero is a reliable indicator of a free-fall state.

The effects of changes in gravity on living organisms have mainly been studied by performing complex, expensive experiments on Earth and in space (Ishay and Sadeh, 1975; Hill et al., 2012; Herranz et al., 2013). In view of the heavy investments required to perform experiments on insects on these lines, we decided to develop a low-cost platform. With the original inexpensive setup presented here, insects can be briefly subjected to near-weightless conditions in an Earth-based laboratory. To address this question in the case of dipterans, we investigated the following: (i) how do tethered insects react when a free-fall situation is suddenly triggered?; (ii) do hoverflies use gravity perception cues to detect a near-weightless state and stabilize their flight?

In a structured visual environment lined with a pattern of horizontal stripes, hoverflies consistently produced wingbeats in response to an imposed free fall. These insects' crash-avoidance abilities and the response times recorded when they were exposed to free-fall situations in various visual environments suggest the existence of a multisensory control process based mainly on vision as well as other sensory inputs, such as those based on airflow-sensing processes.

MATERIALS AND METHODS

Animals

Hoverfly pupae, *Episyrphus balteatus* (De Geer 1776), were purchased (Katz Biotech AG, Baruth, Germany) and reared until hatching in a cage measuring 53×29×29 cm, which was subjected to a 12 h light:12 h dark cycle at a temperature of 25±2.5°C. Newly hatched adults had *ad libitum* access to a pollen–sugar mixture and

Aix-Marseille Université, CNRS, Institute of Movement Science, UMR 7287, Marseille 13288, France.

*Author for correspondence (stephane.viollet@univ-amu.fr)

 S.V., 0000-0003-1585-9822

water, as well as to real flowers to stimulate flying behaviour. A piece of entomological pin approximately 5 mm in length was glued to the dorsal part of the animals' thorax, perpendicular to their longitudinal axis (Fig. 1A): the pin (~5 mg) weighed approximately 15% of the hoverfly's mass (~35 mg). The animals' flight and hovering abilities were then checked in the breeding cages. In the subsequent experiments, 24 hoverflies were tested (12 males and 12 females; in darkness: $N=18$, in a striped environment: $N=21$, in a uniformly white environment: $N=23$). Animals were aged from 3 to 21 days during the experiments.

Procedure

Hoverflies were exposed to microgravity by subjecting them to free-fall conditions in the following original set-up. An electromagnet (TEAC RL-1615) was used to suspend the insects with their legs dangling from the ceiling of a 40×40×40 cm box (see Fig. 1B). The box was covered with a white diffuser (PMMA WH02, 3 mm thickness) and illuminated from above by a halogen light (Kaiser Studiolight H). Hoverflies were filmed through a two-way mirror with a fast camera (Phantom Miro M110) at a rate of 1600 frames s^{-1} at full resolution (1280×800 pixels). Flies were then released to make them fall by switching off the magnetic field. Until initiating their wingbeats, the flies experienced near-weightlessness for a period of up to 290 ms (see Fig. 2).

A total number of 313 falls were conducted in the box in which three visual environments were presented (Fig. 1C): total darkness, a nearly uniform white environment and a visual environment in which two sides of the box were lined with horizontal black and white stripes (2.8 cm width; see Movie 1). Complete darkness was obtained by switching off the experimental room light, covering the box with a large black cloth and placing two additional black panels on the sides of the box. Recordings in darkness are obtained by using two infrared LED projectors (BLANKO, wavelength of 850 nm). Three consecutive falls in the same experimental condition were conducted at each run; in a given experimental condition, individuals were subjected to several runs on different days. We always checked whether the hoverflies could fly with their glued pin in the breeding cages before and after each experiment to confirm that their flight ability was not affected by eventually crashing on the floor. Only flying animals were randomly selected to perform experiments on each day. The horizontal and vertical 2D positions of the hoverflies' centre of mass moving over a uniform background were recorded using a custom-made image-processing program running under MATLAB.

Statistical analysis

All the results presented here were analysed statistically using R free-ware with a generalized linear mixed-effects model procedure (glmer) and selected using the Akaike information criterion (AIC),

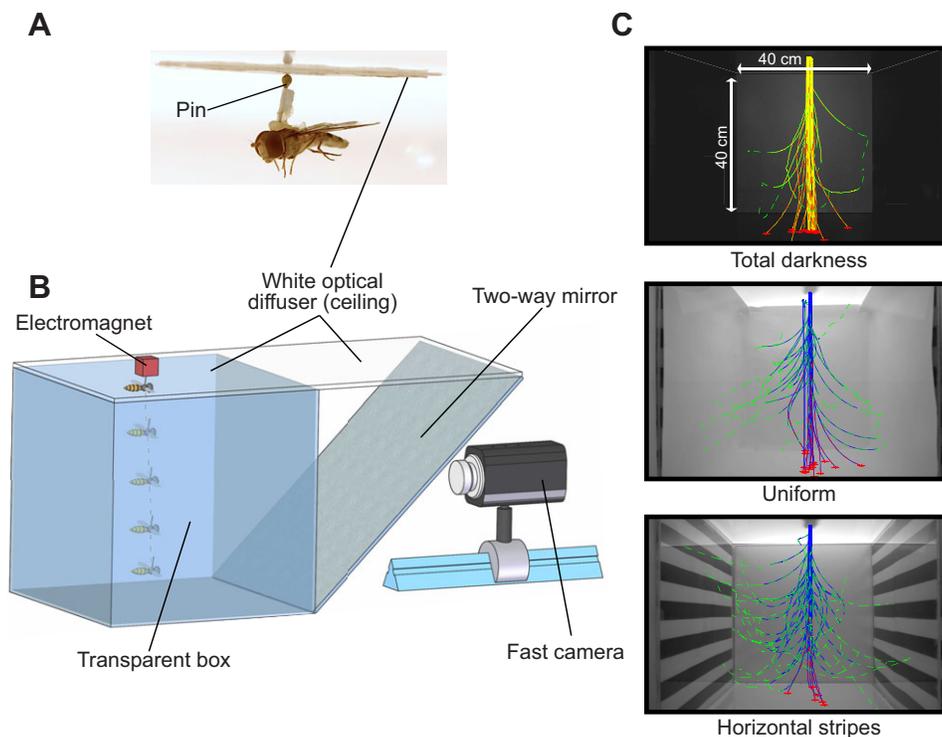


Fig. 1. Experimental set-up used to analyse hoverflies' reactions to free-fall conditions. (A) Hoverflies were tethered by waxing an entomological pin (mass 5 mg, diameter 0.25 mm, length 5 mm) onto their thorax perpendicular to their longitudinal axis. The pin was aligned perpendicular to the ceiling (a white diffuser) by means of an electromagnet. The vertical orientation of the pin in the magnetic field prevented the hoverflies from touching the ceiling of the box with their legs. (B) Set-up consisting of a 40×40×40 cm transparent PVC box illuminated from above by a halogen light. Hoverflies were filmed in the box with a fast camera through a two-way mirror at a rate of 1600 frames s^{-1} in full resolution (1280×800 pixels). The mirror was tilted at an angle of 45 deg to make the hoverfly see a uniform white wall (i.e. the reflection of the white light-diffusing ceiling). A manual switch was used to trigger the camera and simultaneously turn off the power to the electromagnet, thus releasing the resting fly and causing it to fall. (C) Hoverflies were exposed during these experiments to three different visual scenes: a completely dark environment, a uniformly illuminated white box, and a box in which two sides were lined with a regular pattern of black and white stripes. Typical trajectories of free-falling hoverflies exposed to the three visual conditions are presented (yellow and blue lines). Dotted green trajectories correspond to wing flapping resulting in a successful flight, i.e. in no crashing of the flies onto the floor, whereas dotted red curves correspond to crashes due to late wingbeat onset or no wingbeats at all. Hoverflies were said to have crashed when they touched the floor, as indicated by red asterisks.

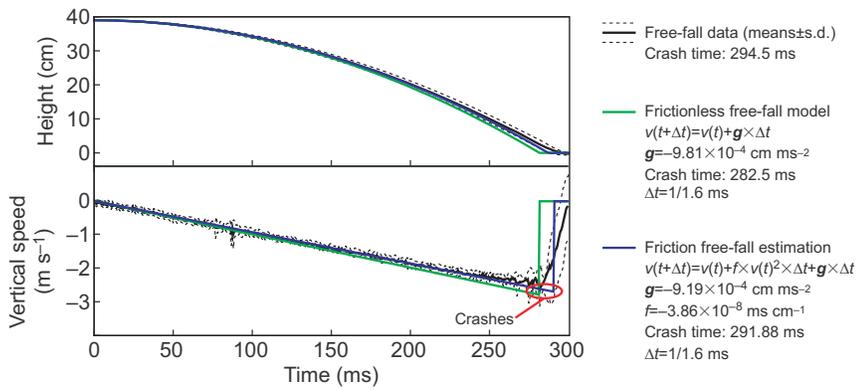


Fig. 2. Kinematic model for the trajectory of the free-falling fly. Comparison between average non-flying individuals' vertical free-fall trajectories and the free-fall model with or without friction. In the model with friction, the gravity acceleration g and the friction coefficient f were estimated based on the first half of the mean data. v , velocity; Δt , sampling period.

mathematically defined for a given model as a trade-off between likelihood and number of parameters:

$$\text{AIC} = 2k - 2\ln(L), \quad (1)$$

where k is the number of parameters and L is the likelihood of the model; the model with the smallest AIC was selected (see Akaike, 1974).

In addition to the visual conditions (fixed effect), other factors such as individual (random effect), repeated experimental runs (random effect), date of experiment (random effect), sex (fixed effect) and, in some cases, wingbeat initiation time (fixed effect) were tested. Statistical significance of the effects observed was subsequently calculated by performing ANOVAs for deviance variation on each of the models selected. Only significant fixed effects are discussed in the paper.

RESULTS

Free-fall detection

Fig. 1A,B shows the set-up used in this study to impose free-fall conditions on hoverflies, and Fig. 1C shows a set of trajectories recorded in the three different visual conditions. Falling in darkness resulted in a large number of touch-downs (referred to here as crashes), whereas in the presence of stripes on the walls, all the hoverflies initiated wingbeats and often generated a large enough thrust to compensate for their weight without crashing (see Table S1). In addition, most of the hoverflies were able to reach the upper part of the box when its walls were lined with stripes, whereas their trajectories mostly ended in the lower part of the box

when it was uniformly white or placed in darkness (Fig. 1C). All the data recorded in all the conditions from individuals that initiated no wingbeats (which we have called non-flying individuals) were grouped together in order to check the conformity of the descent height and speed with classical kinematic models. As shown in Fig. 2, the average height and descent speed of the flies versus time diverged slightly from the frictionless model, but showed a very close fit with the model for a freely falling object subjected to drag. However, with an identified friction coefficient as small as 3.86×10^{-8} , the drag force can presumably be neglected and the non-flying individuals can therefore be assumed to have been briefly subjected to near-weightless conditions. This is a crucial point in our procedure because under weightless free-fall conditions, a linear accelerometer will give a proper acceleration value equal to zero, whereas it will measure g at rest. Therefore, our hypothesis that insects may be endowed with an accelerometer-like organ sensitive to gravity could be checked by testing its ability to detect the transition between resting and free-fall conditions.

The presence of even quite sparse visual cues (in the striped and uniformly white boxes) enabled the hoverflies to initiate their wingbeats during almost every fall, whereas only 80% of the falls in darkness ($P < 0.001$, $F = 32.5426$) resulted in flight initiation (Fig. 3A). In the presence of horizontal stripes, only 10% of the falls ended in crashes, compared with 70% of the falls in complete darkness (χ^2 : $P < 0.001$, deviance = 20.743; see Fig. 3B), although 70% of the crashes in darkness happened when flight was initiated (red dotted lines in Fig. 1C). Despite the hoverflies' near-perfect ability to detect free-fall conditions in the uniformly white

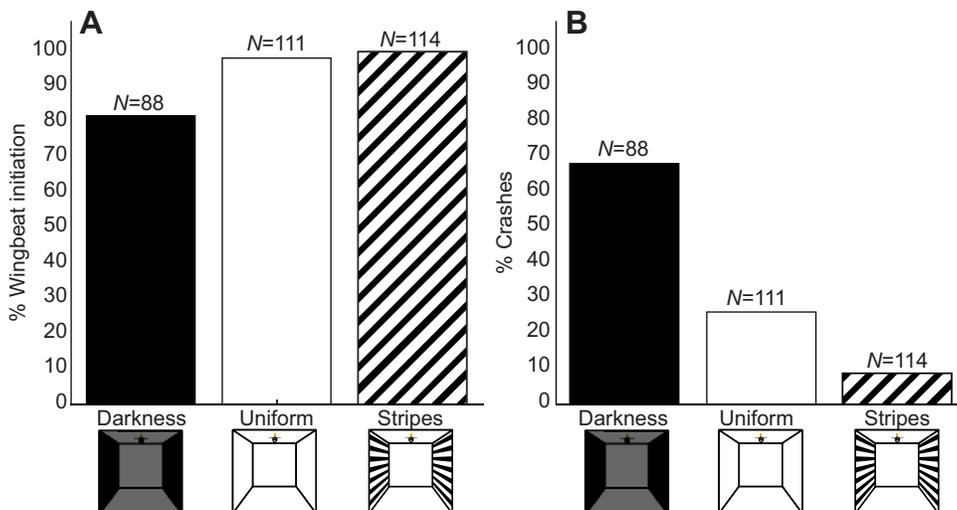


Fig. 3. Reactions to free-fall conditions and crash-avoidance performance.

(A) Hoverflies' wingbeat initiation rates in each visual condition. Total darkness induced a slight decrease in wingbeat initiation compared with the all-white and striped conditions ($P < 0.001$, $F = 32.5426$). (B) Hoverflies' crash rate in each visual condition. The presence of horizontal stripes in an illuminated environment drastically improved the flies' ability to avoid crashing (χ^2 : $P < 0.001$, deviance = 20.743). N , total number of trials in each condition.

environment, a larger number of crashes occurred than in the striped environment. As shown in Fig. 4B, hoverflies placed in darkness responded to free-fall conditions more belatedly [$P < 0.001$, $F = 58.9701$; median time elapsing before the onset of wingbeats: 186 ± 52 ms (\pm quantile deviation)] compared with the striped and uniformly white environments, where flight was triggered at around 112 ± 23 and 116 ± 37 ms, respectively, after the beginning of the fall.

Flight stabilization

Hoverflies' fail-safe braking performances are due to their ability to produce a sufficiently strong and appropriately oriented thrust to compensate for their weight and thus stop falling. We therefore analysed the trajectories and annotated those in which the insects started slowing down their fall and performed rising vertical flight before landing on a wall or crashing onto the floor. As the insects' stabilization can be assumed to depend mainly on the speed and pitch conditions at the onset of wingbeats, data were subdivided into four groups depending on the wingbeat initiation time (0–100, 100–150, 150–200 and >200 ms after the beginning of the fall). In the striped visual environment, hoverflies easily managed to stabilize their flight by cancelling the vertical falling speed (Fig. 4A,C), and a later wingbeat initiation had relatively little effect on their fail-safe braking rates (these rates decreased by 5% and 10% after a delay of 50 and 100 ms, respectively, χ^2 : $P = 0.0274$, deviance = 4.8651). The fail-safe braking rates gradually decreased at intervals of up to 200 ms between the start of falls and the onset of wingbeats, beyond which any flight initiation was followed by crashing (apart from one

case, in which flight was triggered after 208 ms). As confirmed by the average trajectories shown in Fig. 4A, in all the visual conditions tested, flights initiated after 200 ms of free falling were almost never successful, probably because the limits of the stabilization ability of *E. balteatus* were reached in this particular set-up. When falling in a uniformly white environment, triggering wingbeats within 100 ms of the onset of the fall greatly improved the hoverflies' ability to compensate for their weight (85% rate), but the success rate dropped greatly to 60% after 100 ms and 45% after 150 ms ($P = 0.0792$, $F = 3.1713$; see Fig. 4C). In darkness, the fail-safe braking rate was relatively high when wingbeat triggering occurred less than 100 ms after the insects' release (75%) but decreased sharply to less than 40% after delays ranging between 100 and 150 ms, and to 20% after 150 ms (χ^2 : $P = 0.02810$, deviance = 4.822, see Fig. 4C). The stabilization performance recorded in degraded visual surroundings (i.e. uniform white and darkness conditions) was significantly lower compared with that for the striped box (χ^2 : $P < 0.001$, deviance = 38.625). It is worth noting that late wingbeats initiated 150–200 ms after the beginning of the fall in the striped environment resulted in similar fail-safe braking rates to those recorded when the wingbeats were initiated within 100 ms in the uniform white environment and in darkness (see Fig. 4C).

DISCUSSION

The main aim of this study was to develop and test a new experimental platform for studying the sensory processes involved in flies' perception of gravity during flight. Being tethered to the

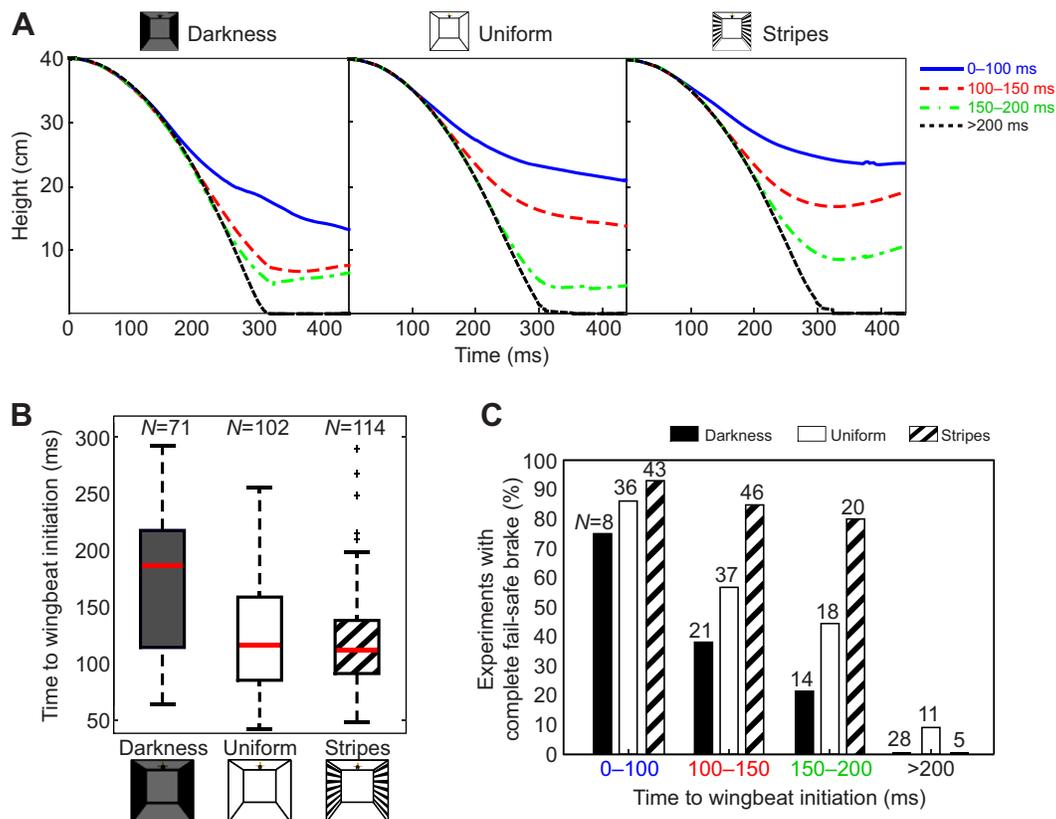


Fig. 4. Reaction times and stabilization performance. (A) Average vertical trajectories versus time recorded in each visual condition in four wingbeat initiation time ranges: 0–100 ms, 100–150 ms, 150–200 ms and >200 ms. (B) Boxplot of the time elapsed from the onset of the free fall to the onset of wingbeats. The absence of light greatly increased the time taken to initiate wingbeats ($P < 0.001$, $F = 10.1414$). (C) Percentage of experiments in which hoverflies braked their initial descent by initiating wingbeats in each condition. These percentages were subdivided into four groups, depending on the wingbeat initiation times: 0–100 ms, 100–150 ms, 150–200 ms and >200 ms.

ceiling with their legs dangling at the start of each trial certainly corresponded to a highly unnatural situation for this animal, but the high rate of positive reactions obtained even in darkness confirmed the hoverflies' ability to respond suitably to the free-fall situations. The existence of this reflex therefore confirms the validity of the present paradigm as a means of studying the mechanisms involved in dipterans' anti-crash responses. Alternatively, the existence of such a reflex would be useful to trigger flight in an insect in which observing spontaneous flying behaviour is unexpected, with non-functional halteres for example, to allow the study of free-flight stabilization performance.

It has been established that vertebrates are endowed with otolith organs, which estimate the orientation of the vertical and detect any changes in the gravity conditions (Angelaki and Cullen, 2008). However, very few studies have focused so far on assessing the time required to elicit a muscle response to free-fall conditions. For example, in humans suspended by their hands, it has been reported that the contraction of the gastrocnemius muscle occurs 75 ms after the onset of an unexpected vertical fall (Melvill Jones and Watt, 1971): this response time is similar to those recorded in the muscles of cats subjected to free-fall conditions (Watt, 1976). In monkeys undergoing a sudden free fall, egomotion cues perceived by the visual system contribute mainly within 60–120 ms to the animals' muscle reactions (Vidal et al., 1979), whereas in baboons, the latency of the splenius muscle response (EMG) to a fall at an acceleration of 8.8 m s^{-2} was reported to be 29 ms in darkness and 22 ms under normal visual conditions (Lacour et al., 1981), which suggests that visual cues contribute to faster responses. In arthropods, pendulous sensilla, which may act like an inner ear and are located in the ventral part of the cerci, have been identified in the cockroach (Walthall and Hartman, 1981; Hartman et al., 1987), but as far as we know, the use of gravity perception has only been confirmed by testing the insects' tactile responses and varying the orientation of the ground (Horn and Kessler, 1975; Horn and Bischof, 1983; Horn and Knapp, 1984; Hengstenberg, 1993; Horn and Föllner, 1998; Kamikouchi et al., 2009). The response latencies recorded here were much longer than those involved in the mechanosensory responses elicited, for example, by stimulated halteres within only 5 ms via a feedforward control pathway (Sandeman and Markl, 1980). In addition, fruit-flies took only 45 ms to reject a yaw perturbation via a heading feedback loop (Ristoph et al., 2010), 5 ms to detect a roll disturbance and elicit counter-torque movements and 30 ms to reject the perturbation (Beatus et al., 2015). If an early gravity perception process was involved in the insects' responses to free-fall situations, similar wingbeat initiation rates could be expected to occur in darkness, and faster mean reaction times would therefore have been recorded than those measured here in the most favourable visual condition (around 110 ms in the presence of stripes), contradicting the existence of an accelerometer-like organ in dipteran.

Comparisons between the performances recorded in the uniformly white box and the striped box in terms of similar wingbeat initiation rates and times (see Figs 3A and 4B) and greater crashing rate (Fig. 3B) show the existence of differences in the hoverflies' ability to perceive free-fall conditions and their subsequent ability to stabilize their flight. In the all-white box, hoverflies were probably able to detect the low-contrast patterns provided by contrasting edges between the sides and the ceiling and between the sides and the mirror. They may have been endowed with high contrast sensitivity vision (Collett and Land, 1975; O'Carroll et al., 1996), which would enable them to perceive the fall even in a poor visual environment, whereas achieving stabilization may have required a richer source of visual information, which was

only accessible in the striped box, to enable them to finely control the amplitude and orientation of their thrust. Although their fail-safe braking ability was certainly affected by the wingbeat initiation time, on which the initial speed and pitch depended, it can be seen from Fig. 4C that the insects in all the time groups braked less efficiently in darkness than in the striped box. In addition, contrary to the free-fall responses measured with stripes, the insects' braking ability was strongly dependent on the wingbeat initiation time, both in darkness and in the uniformly white environment. These data indicate that the hoverfly's ability to stop falling depends not only on its ability to quickly initiate wingbeats but also on the visual cues available, which means that vision was required to control the thrust and prevent the insect from crashing onto the floor. It has not yet been established whether dipterans use graviceptors or inclinometers to control their flight, which would still be at work in darkness. Bender and Frye (2009) have suggested that the halteres may serve as gravity sensors in flies. However, this means of estimating gravity would be negligible in comparison with processes based on the rotational acceleration applied to the halteres (Nalbach, 1993; Northrop, 2000) as a means of controlling the fast manoeuvres performed by flies during flight (Taylor and Krapp, 2007).

As the hoverflies' free fall perception and stabilization abilities were not completely absent in darkness, the possibility that they may have called on sensory modalities other than vision cannot be ruled out. The results obtained using the novel free-fall procedure presented here show that the cues generated by the sudden change in the microgravity load exerted on the hoverflies' dangling legs were probably not used by the insects to detect and stop themselves from falling. Alternatively, insects are sensitive to the air flow generated by self-motion, as observed by Fuller et al. (2014) in studies of forward speed control in fruit flies, by Combes and Dudley (2009) in responses of neotropical bees to turbulent wind and in studies in which the optic flow streamlining responses of bees were modulated by air flow (Taylor et al., 2013). The high sensitivity of an arthropod hair to airflow (Barth, 2014) might explain the fast responses occasionally observed in the present study in darkness. It has been suggested in many studies that a flying insect's ability to detect frontal air flow may contribute to sustaining the flapping flight performance of tethered animals (Goodman, 1965; Hengstenberg, 1984, 1988; Hengstenberg et al., 1986; Hensler and Robert, 1990). The delayed responses observed in darkness in this study may have resulted from the time required for the hoverfly's head to pitch downward passively, probably due to the weight of the pin (around 5 mg) attached to its thorax. Air flow cues may therefore have been used to trigger wingbeats and generate thrust, but this reflex often failed to completely brake the insects' descent and prevent them from crashing, whereas visually induced responses involving motion-sensing neurons, especially those with a vertical preferred direction of motion, may have occurred before the air flow reached a useful threshold. The shorter reaction times and greatly improved stabilization performance observed in the presence of stripes indicate that the thrust initiation and control were driven mainly by the presence of visual cues. Comparisons between the flies' crash rates in three different visual environments confirmed that vision certainly played a crucial role in the free-fall detection process. As suggested by previous findings on tethered flies and bumblebees (Wehrhahn and Reichardt, 1975; Tanaka and Kawachi, 2006), the lift response dynamic seems to be largely controlled by the image slip provided by periodically spaced vertical gratings. An optic flow-based process compensating for vertical motion is supported by observation of fruit flies (Straw et al., 2010) and houseflies (Wehrhahn and Reichardt, 1975; Wehrhahn, 1978) responding to

vertical grating motion. However, it is not clear in our experiment whether optic flow was the only type of visual cue available: lighting from above may well have enhanced the insects' attitude and thrust control system via the dorsal light reflex, for example (Goodman, 1965; Hengstenberg, 1993; Goulard et al., 2015). Motion-sensitive neurons provide flies with a crucial means of perceiving their egomotion via the optic flow generated by their flight; for example, hoverflies *Volucella* can perceive optic flows ranging from 0.2 to about 2000 deg s⁻¹ (O'Carroll et al., 1996). In the present study, the optic flow generated during the hoverflies' free fall reached a maximum value of approximately 775 deg s⁻¹ (in the case of a hoverfly falling at a constant mean acceleration of -981 cm s⁻² for up to ~290 ms at a distance of approximately 20 cm from each side of the box). This new picture of how insects may estimate and control their attitude differs from what is usually thought to occur in vertebrates, where inertial vertical perception is generally taken to result from a combination of tilt and rotational speed measurements (Merfeld, 1995), as well as differing from the strategies often used in avionics to stabilize aerial vehicles, based on the use of devices such as accelerometers (Mahony et al., 2012).

In conclusion, we have developed a totally new, non-expensive, easy means of studying insect behaviour during free-fall situations, which could be further used to study the flight stabilization reflexes involved in other contexts. The data obtained using this set-up show that the perception of falling and the compensatory stabilizing reflex induced in hoverflies certainly involved multisensory processes, including those based on the perception of the air flow and the vertical optic flow generated by the vertical slip speed of contrasts on the hoverflies' eyes rather than gravity perception processes. We propose to test these hypotheses in future experiments using the free-fall procedure presented here in order to determine the respective contributions of each of the sensory channels discussed above.

Acknowledgements

We are most thankful to Julien Dipéri for his contribution to building the experimental set-up, to Marc Boyron for developing the electronics on which all the work presented in this paper was based, and to Jessica Blanc for correcting and improving the English in the manuscript. We are grateful to the reviewers for their useful comments.

Competing interests

The authors declare no competing or financial interests.

Author contributions

R.G. and S.V. designed the experiments. R.G. and S.V. conceived the data analysis tools (tracking software). R.G. and S.V. conducted the experiments and interpreted the data. R.G., S.V. and J.-L.V. wrote the manuscript.

Funding

S.V. acknowledges support from the Centre National de la Recherche Scientifique (CNRS), Aix-Marseille Université and the Agence Nationale de la Recherche (ANR) [with the EVA project (Autonomous Flying Entomopter) and the IRIS project (Intelligent Retina for Innovative Sensing) ANR-12-INSE-0009].

Supplementary information

Supplementary information available online at <http://jeb.biologists.org/lookup/doi/10.1242/jeb.141150.supplemental>

References

- Akaike, H.** (1974). A new look at the statistical model identification. *IEEE Trans. Automat. Control* **19**, 716–723.
- Angelaki, D. E. and Cullen, K. E.** (2008). Vestibular system: the many facets of a multimodal sense. *Annu. Rev. Neurosci.* **31**, 125–150.
- Barth, F.** (2014). *Flow Sensing in Air and Water, chap. Chapter 7: The Slightest Whiff of Air: Airflow Sensing in Arthropods*, pp. 169–196. Berlin; Heidelberg: Springer-Verlag.
- Beatus, T., Guckenheimer, J. M. and Cohen, I.** (2015). Controlling roll perturbations in fruit flies. *J. R. Soc. Interface* **12**, 20150075.
- Bender, J. A. and Frye, M. A.** (2009). Invertebrate solutions for sensing gravity. *Curr. Biol.* **19**, R186–R190.
- Collett, T. S. and Land, M. F.** (1975). Visual control of flight behaviour in the hoverfly, *Syrnitta pipiens* L. *J. Comp. Physiol. A* **99**, 1–66.
- Combes, S. A. and Dudley, R.** (2009). Turbulence-driven instabilities limit insect flight performance. *Proc. Natl. Acad. Sci. USA* **106**, 9105–9108.
- Fraser, P. J.** (1977). Cercal ablation modifies tethered flight behaviour of cockroach. *Nature* **268**, 523–524.
- Fuller, S. B., Straw, A. D., Peek, M. Y., Murray, R. M. and Dickinson, M. H.** (2014). Flying *Drosophila* stabilize their vision-based velocity controller by sensing wind with their antennae. *Proc. Natl. Acad. Sci. USA* **111**, E1182–E1191.
- Goodman, L. J.** (1965). The role of certain optomotor reactions in regulating stability in the rolling plane during flight in the desert locust, *Schistocerca gregaria*. *J. Exp. Biol.* **42**, 385–407.
- Goulard, R., Julien-Laferrriere, A., Fleuriel, J., Vercher, J.-L. and Viollet, S.** (2015). Behavioural evidence for a visual and proprioceptive control of head roll in hoverflies (*Episyrphus balteatus*). *J. Exp. Biol.* **218**, 3777–3787.
- Hartman, H. B., Benett, L. P. and Moulton, B. A.** (1987). Anatomy of equilibrium receptors and cerci of the burrowing desert cockroach *Arenivaga* (Insecta, Blattodea). *Zoomorphology* **107**, 81–87.
- Hengstenberg, R.** (1984). *Localization and Orientation in Biology and Engineering, chap. Roll-Stabilization During Flight of the Blowfly's Head and Body by Mechanical and Visual Cues*, pp. 121–133. Lectures Notes in Computer Science. Berlin; Heidelberg: Springer-Verlag.
- Hengstenberg, R.** (1988). Mechanosensory control of compensatory head roll during flight in the blowfly *Calliphora erythrocephala* Meig. *J. Comp. Physiol. A* **163**, 151–165.
- Hengstenberg, R.** (1993). Multisensory control in insect oculomotor systems. In *Visual Motion and its Role in the Stabilization of Gaze* (ed. F. A. Miles and J. Wallman), pp. 285–298. Amsterdam: Elsevier Science.
- Hengstenberg, R., Sandeman, D. C. and Hengstenberg, B.** (1986). Compensatory head roll in the blowfly *Calliphora* during flight. *Proc. R. Soc. Lond. B Biol. Sci.* **227**, 455–482.
- Hensler, K. and Robert, D.** (1990). Compensatory head rolling during corrective flight steering in locusts. *J. Comp. Physiol. A* **166**, 685–693.
- Herranz, R., Anken, R., Boonstra, J., Braun, M., Christianen, P. C. M., de Geest, M., Hauslage, J., Hilbig, R., Hill, R. J. A., Lebert, M. et al.** (2013). Ground-based facilities for simulation of microgravity: organism-specific recommendations for their use, and recommended terminology. *Astrobiology* **13**, 1–17.
- Hill, R. J. A., Larkin, O. J., Dijkstra, C. E., Manzano, A. I., de Juan, E., Davey, M. R., Anthony, P., Eaves, L., Medina, F. J., Marco, R. et al.** (2012). Effect of magnetically simulated zero-gravity and enhanced gravity on the walk of the common fruitfly. *J. R. Soc. Interface* **9**, 1438–1449.
- Horn, E. and Bischof, H.-J.** (1983). Gravity reception in crickets: the influence of cereal and antennal afferences on the head position. *J. Comp. Physiol. A* **150**, 93–98.
- Horn, E. and Föller, W.** (1998). Induction of a gravity-related response by a single receptor cell in an insect. *Naturwissenschaften* **85**, 121–123.
- Horn, E. and Kessler, W.** (1975). The control of antennae lift movements and its importance on the gravity reception in the walking blowfly, *Calliphora erythrocephala*. *J. Comp. Physiol. A* **97**, 189–203.
- Horn, E. and Knapp, A.** (1984). On the invariance of visual stimulus efficacy with respect to variable spatial positions. *J. Comp. Physiol. A* **154**, 555–567.
- Ishay, J. and Sadeh, D.** (1975). Direction finding by hornets under gravitational and centrifugal forces. *Science* **190**, 802–804.
- Kamikouchi, A., Inagaki, H. K., Effertz, T., Hendrich, O., Fiala, A., Göpfert, M. C. and Ito, K.** (2009). The neural basis of *Drosophila* gravity-sensing and hearing. *Nature* **458**, 165–171.
- Kress, D. and Egelhaaf, M.** (2012). Head and body stabilization in blowflies walking on differently structured substrates. *J. Exp. Biol.* **215**, 1523–1532.
- Lacour, M., Vidal, P. P. and Xerri, C.** (1981). Visual influences on vestibulospinal reflexes during vertical linear motion in normal and hemilabyrinthectomized monkeys. *Exp. Brain Res.* **43**, 383–394.
- Mahony, R., Kumar, V. and Corke, P.** (2012). Multirotor aerial vehicles: modeling, estimation, and control of quadrotor. *IEEE Robot. Automat. Mag.* **19**, 20–32.
- Melville Jones, G. and Watt, D. G. D.** (1971). Muscular control of landing from unexpected falls in man. *J. Physiol.* **219**, 729–737.
- Mendes, C. S., Rajendren, S. V., Bartos, I., Márka, S. and Mann, R. S.** (2014). Kinematic responses to changes in walking orientation and gravitational load in *Drosophila melanogaster*. *PLoS ONE* **9**, e109204.
- Merfeld, D. M.** (1995). Modeling the vestibulo-ocular reflex of the squirrel monkey during eccentric rotation and roll tilt. *Exp. Brain Res.* **106**, 123–134.
- Mischiati, M., Lin, H.-T., Herold, P., Imler, E., Olberg, R. and Leonardo, A.** (2015). Internal models direct dragonfly interception steering. *Nature* **517**, 333–338.
- Nalbach, G.** (1993). The halteres of the blowfly *Calliphora*. *J. Comp. Physiol. A* **173**, 293–300.
- Northrop, R.** (2000). Selected examples of sensory receptors and small receptor arrays. In *Introduction to Dynamic Modeling of Neuro-Sensory Systems*, pp. 141–147. Boca Raton, FL: CRC Press.
- O'Carroll, D. C., Bidwell, N. J., Laughlin, S. B. and Warrant, E. J.** (1996). Insect motion detectors matched to visual ecology. *Nature* **382**, 63–66.
- Ristroph, L., Bergou, A. J., Ristroph, G., Coumes, K., Berman, G. J., Guckenheimer, J., Wang, Z. J. and Cohen, I.** (2010). Discovering the flight

- autostabilizer of fruit flies by inducing aerial stumbles. *Proc. Natl. Acad. Sci. USA* **107**, 4820-4824.
- Sandeman, D. and Markl, H.** (1980). Head movements in flies (*Calliphora*) produced by deflexion of the halteres. *J. Exp. Biol.* **85**, 43-60.
- Schilstra, C. and van Hateren, J. H.** (1998). Stabilizing gaze in flying blowflies. *Nature* **395**, 654.
- Straw, A. D., Lee, S. and Dickinson, M. H.** (2010). Visual control of altitude in flying *Drosophila*. *Curr. Biol.* **20**, 1550-1556.
- Tanaka, K. and Kawachi, K.** (2006). Response characteristics of visual altitude control system in *Bombus terrestris*. *J. Exp. Biol.* **209**, 4533-4545.
- Taylor, G. K. and Krapp, H. G.** (2007). Sensory systems and flight stability: what do insects measure and why? *Adv. Insect Physiol.* **34**, 231-316.
- Taylor, G. J., Luu, T., Ball, D. and Srinivasan, M. V.** (2013). Vision and air flow combine to streamline flying honeybees. *Sci. Rep.* **3**, 2614.
- Vidal, P. P., Lacour, M. and Berthoz, A.** (1979). Contribution of vision to muscle responses in monkey during free-fall: visual stabilization decreases vestibular-dependent responses. *Exp. Brain Res.* **37**, 241-252.
- Viollet, S. and Zeil, J.** (2013). Feed-forward and visual feedback control of head roll orientation in wasps (*Polistes humilis*, Vespidae, Hymenoptera). *J. Exp. Biol.* **216**, 1280-1291.
- Walthall, W. W. and Hartman, H. B.** (1981). Receptors and giant interneurons signaling gravity orientation information in the cockroach *Arenivaga*. *J. Comp. Physiol. A* **142**, 359-369.
- Watt, D.** (1976). Response of cats to sudden falls: an otolith-originating reflex assisting landing. *J. Neurophysiol.* **39**, 257-265.
- Wehrhahn, C.** (1978). The angular orientation of the movement detectors acting on the flight lift response in flies. *Biol. Cybern.* **31**, 169-173.
- Wehrhahn, C. and Reichardt, W.** (1975). Visually induced height orientation of the fly *Musca domestica*. *Biol. Cybern.* **20**, 37-50.
- Wijngaard, W.** (2010). Accuracy of insect position control as revealed by hovering male *Eristalis nemorum*. *Proc. Neth. Entomol. Soc. Meet.* **21**, 75-84.
- Zeil, J., Boeddeker, N. and Hemmi, J. M.** (2008). Vision and the organization of behaviour. *Curr. Biol.* **18**, R320-R323.