

**Visually guided landing behaviour in the housefly, *Musca Domestica***



A thesis submitted towards partial fulfilment of  
BS-MS Dual Degree Programme

by

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under the guidance of

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## **Certificate**

This is to certify that this dissertation entitled “Visually guided landing behaviour in the housefly, *Musca Domestica*” towards the partial fulfilment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents original research carried out by “Sujay B at National Centre for Biological Sciences, Bangalore” under the supervision of “Dr. Sanjay Sane, Associate Professor, Department of Neurobiology” during the academic year 2016-2017.

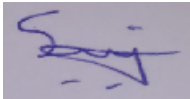


**Signature of the Supervisor**

**Date:** 30.3.2017

## **Declaration**

I hereby declare that the matter embodied in the report entitled “Visually guided landing behaviour in the housefly, *Musca Domestica*” are the results of the investigations carried out by me at the Department of Neurobiology, National Centre for Biological Sciences, Bangalore, under the supervision of Dr. Sanjay Sane and the same has not been submitted elsewhere for any other degree.



**Signature of the Student**

**Date:** 30.3.2017

## **Abstract:**

**Landing is a critical aspect of insect flight. The landing behaviour comprises of multiple components like deceleration, leg extension and orientation of the body towards the landing surface. We have used the housefly (*Musca domestica*) as a model system to study the initiation and control of the landing behaviour on plane surfaces at two different orientations: 1) vertical 2) inverted (ceiling). Nearly half of all flies performing inverted landings collided their head with the ceiling, despite extending their legs before touchdown. For the flies performing smooth inverted landings and vertical landings, the points of onset of deceleration, leg extension and body orientation were functions of both the flight velocity and distance from the landing surface. We observed greater variability in the order of initiation of the various components during vertical landings. Leg extension occurred before deceleration in a majority of the flies performing inverted landings. These flies also contacted the landing surface at greater velocities. Decelerating flies reduced their velocity as a logarithmic function of the distance from the landing surface while landing on both the surfaces. Our experiments suggest that flies utilize the rate of expansion of the landing object to initiate and control landings, and that the inverted landing behaviour can be more variable and error prone. Using a mathematical model we show that measuring the rate of expansions of either the entire landing target or a point on the landing surface, is not sufficient to initiate landings on all kinds of plane surfaces.**

## **List of tables**

<b>Table no.</b>	<b>Title</b>	<b>Page</b>
1	Distribution of smooth landings and bumps in the inverted landing trials	20
2	Order of initiation of the components of the landing behaviour during inverted landings	34
3	Order of initiation of the components of the landing behaviour during vertical landings	34

## List of figures

Figure no.	Title	Page
1	Leg extension response in tethered flies	7
2	Experimental setup	10
3	Digitized points	12
4	Flight variables	13
5	Velocity profiles	18
6	Onsets of deceleration for vertical landings	19
7	Onsets of deceleration for inverted landings	21
8	A “bump”	22
9	Time to contact of landings and bumps	22
10	Control of deceleration for vertical landings	23
11	$R^2$ of logarithmic and linear fits to the deceleration profiles of vertical landings	24
12	Control of deceleration for inverted landings	24-25
13	$R^2$ of logarithmic and linear fits to the deceleration profiles of inverted landings	25
14	Initiation of leg extension during vertical landings	26-27
15	Initiation of leg extension during inverted landings	27
16	Initiation of a reduction in body orientation	29-30
17	Time to contacts of the points of onset of a decrease in body orientation	30
18	Perpendicular velocities at touchdown	31
19	Time taken for the wings to come to rest after touchdown	32
20	Distributions of stop times for vertical and inverted landings	33
21	Order of initiation of the components of landing	33
22	Variables used to test the RREV and REV models	36
23	Predictions of RREV model for different thresholds	37
24	Predictions of REV model for different thresholds	39
25	Dependence of the RREV and REV models on object size	40
26	Expansion of a point on the landing object	42

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## **Introduction:**

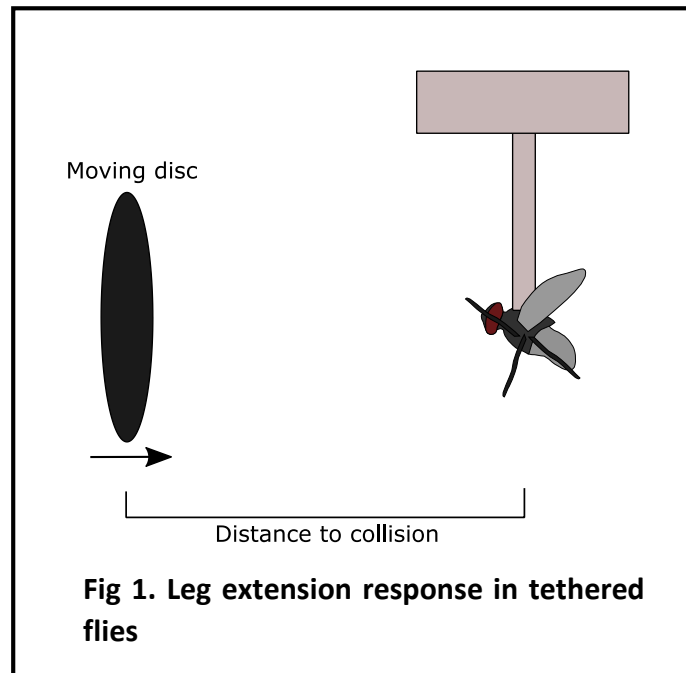
Insects are the most diverse and abundant taxa in the animal kingdom. The ability to fly is likely to have been a significant contributor to the evolutionary success of this class of organisms. One of the most crucial aspects of flight behaviour is the ability to perform controlled landings. The landing behaviour consists of multiple components. The insect must move towards a particular target, decelerate, orient itself parallel to the landing surface, extend its legs and smoothly contact the target. These set of landing related behaviours are collectively called the 'landing response'. Insects can land on a wide variety of substrates, which differ significantly in texture, size, inclinations, etc. How do insects manage to land safely on different kinds of surfaces, despite having a multi-faceted landing response? There has been a considerable amount of research on the cues used to initiate and control the landing response. Previous researchers have performed experiments both on tethered insects and freely flying insects, primarily on bees or flies.

### ***Tethered flight experiments***

Tethering an insect allows the experimenter to exercise control over the sensory stimuli presented to the insects, which may not be fully possible in freely flying insects. Studies of landing responses in tethered insects involve the presentation of real or virtual visual expansion stimuli to the insects. Changes in the wing kinematics or leg movements or both are quantified.

When a frontal expansion stimulus is provided to tethered flies, the prothoracic legs extend forwards, whereas the mesothoracic legs are lowered and extended sideways. The response of the metathoracic legs is variable (Borst, 1986; Goodman, 1960). Leg extension is a fixed action pattern, which does not require further presentation of the stimulus once the behaviour is initiated (Borst, 1986). The latency of the leg extension response varies depending on stimulus conditions. Once elicited however, the pattern and time duration of the leg extension response remains the same (Borst, 1986). Apart from leg extension, a looming object elicits a forward shift in the wing stroke envelope and an increase in the wing beat frequency. In free flight, such a change occurs during a pitch maneuver, which results in an increase in the vertical component of flight velocity and a decrease in the horizontal component (Borst and Bahde, 1988; Tammero and Dickinson, 2002) .

The cues governing the initiation of the leg extension response have been rigorously studied by Borst and Bahde, 1988. They observed that houseflies extend their legs at a greater distance to collision (distance of the point of onset of leg extension from the approaching target), when a disc is moved towards them at higher velocities (See fig 1). For a given approach velocity of the disc, the distance to collision decreases with the increase in diameter of the disc. The distance to collision is greater when a visually structured disc (a disc with concentric black and white rings) is moved towards a tethered fly, when compared to moving a black disc towards the fly with the same velocity. The distance to collision is also affected by the start point of approach of the disc. It is greater when the disc is moved towards the fly from a larger distance, provided that the approach velocity is the same. Presentation of a front to back optic flow stimulus along with a looming disc increases the distance to collision, whereas a back to front stimulus decreases the distance to collision. Borst and Bahde, 1988 also observed that the latencies of the wing responses (changes in wing stroke envelope and wing beat frequencies) to various looming stimuli are positively correlated with the latencies of the leg extension response during the presentation of the same stimulus.



A fly experiencing an expansion stimulus for a short duration does not initiate a landing response. However, a virtual object expanding at the same rate can induce a landing response if presented for a longer duration. When the short duration stimulus is presented to the fly before the long duration stimulus, the latency of the landing response reduces. The magnitude of decrease in latency is higher if the time interval between the short duration and long duration stimulus is shorter (Borst, 1989).

The leg extension response can also be triggered by a reduction in light intensity (Borst, 1986; Goodman, 1960). The pattern and duration of the leg extension response is the same as the landing responses to looming targets. However, the latency of the response is shorter (Borst, 1986).

A spatio-temporal integration model (STIM) was proposed to explain how the landing response in flying insects is triggered by the underlying motion detectors in the eye. The model has several components. First, motion of the visual field is extracted by a 2-D array of local motion detectors of the correlation type. Second, the output from the local detectors is spatially integrated by large field units. Front-to-back motion stimuli provide excitatory inputs and back-to-front motion stimuli provide inhibitory inputs to the large field units. The signals from the large field units feed into a leaky temporal integrator. Third, if the summed signals in the temporal integrator cross a threshold value, the landing response is triggered. Fourth, the latency of the landing response is lower if the threshold is reached sooner (Borst, 1989, 1990; Borst and Bahde, 1988).

This model explains the landing responses of flying insects to looming objects in tethered flight. However, it does not explain the triggering of the leg extension response by a reduction in the light intensity.

An expanding stimulus in the frontal portion of a fly's visual field results in both a wing response and a leg extension response. However, an expanding stimulus in the lateral portion of the visual field results in an increase in the stroke amplitude of one wing and a decrease in the stroke amplitude of the other wing. In free flight this occurs when a fly turns away from an object and is termed the "collision avoidance response" (Tammero and Dickinson, 2002). The latency of the landing response is greater than that of the collision avoidance response, for expanding stimuli of the same magnitude. The latency of the collision avoidance response does not depend on the rate of expansion of the stimulus. However, for the landing response, it decreases with an increase in the expansion rate of the stimulus. These results suggest that both the landing response and collision avoidance response are mediated via separate neural pathways (Tammero and Dickinson, 2002).

### ***Free flight experiments***

Despite the critical nature of the landing behaviour, studies on landing in freely flying insects are rare, perhaps because of the difficulties inherent in ensuring that insects land on a specific target. In such experiments, although the experimenter has less control of the sensory stimuli experienced by each animal, they are closer to natural conditions.

The final moments of the landing behaviour have been documented in houseflies and bees. When a housefly approaches the underside of a horizontal surface, it extends its front legs upward and the meso and metathoracic legs sideways. It contacts the ceiling with its front legs and the body rotates towards the surface, bringing the meso and metathoracic legs in contact with it. These observations were qualitative in nature (Dalton, 1975; Hyzer, 1962). When honeybees approach a surface, they hover before landing. The hover distance is independent of the inclination of the surface. The angle between the body and the vertical axis increases with the increase in inclination of the surface. It implies that the honeybees orient themselves towards the surface before landing. The appendage closest to the surface makes the first contact with the surface, typically the hind legs for landings on horizontal or sloped surfaces and the forelegs



or antennae for landings on vertical surfaces (Evangelista et al., 2010). Similar observations have been made in bumblebees (Reber et al., 2016).

The cues used to trigger the landing response have also been studied in freely flying insects. Houseflies landing on a sphere initiate deceleration at a critical value of the relative retinal expansion velocity (RREV) of the landing target (Wagner, 1982).

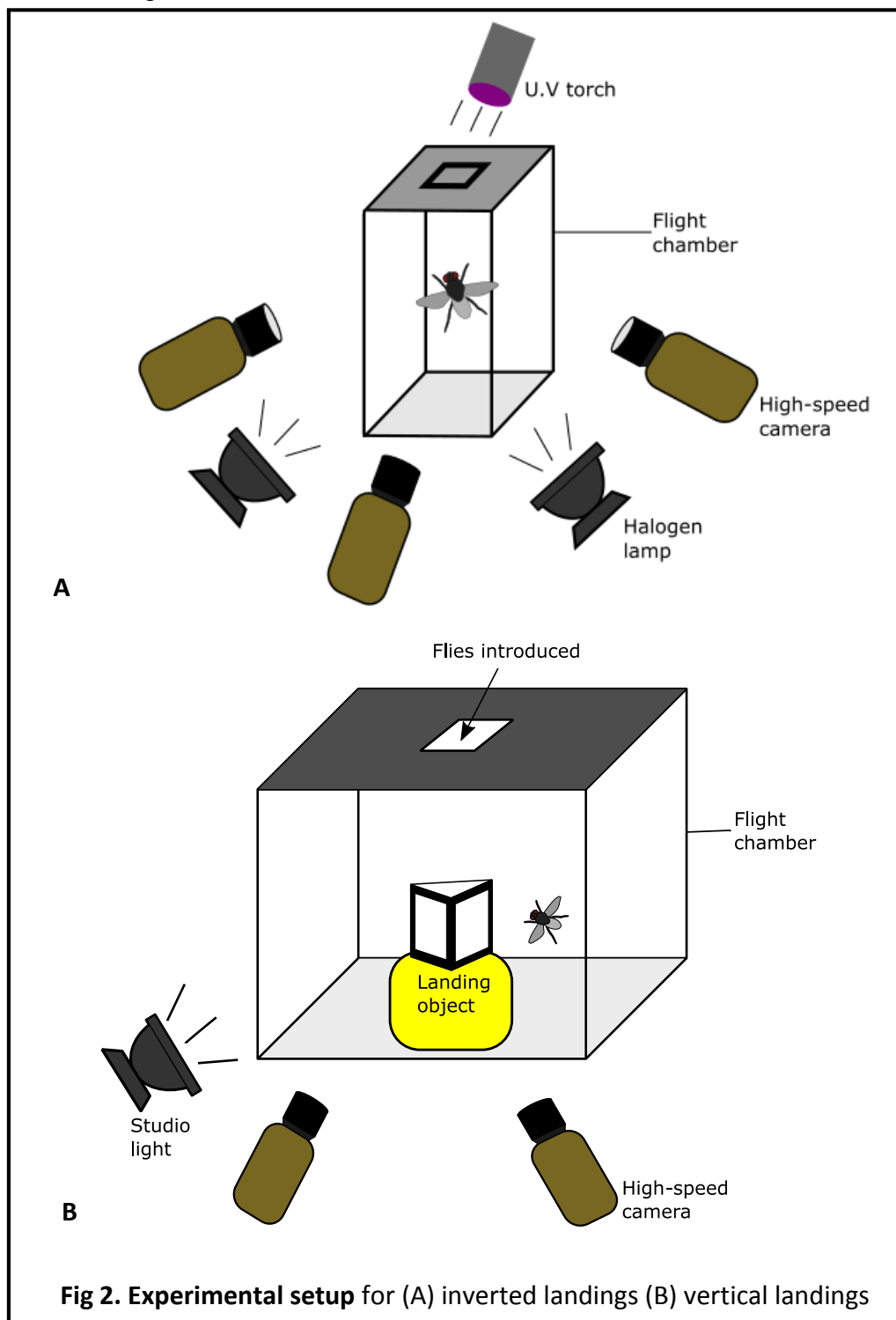
$$\text{RREV} = \frac{\text{Rate of expansion of the image of the landing target on the retina}}{\text{Angular size of the image of the landing target on the retina}}$$

*Drosophila* landing on a cylindrical object also initiate deceleration at a threshold value of RREV. However, they extend their legs when the angular size of the landing post crosses a critical value (van Breugel and Dickinson, 2012). These results suggest that the various components of the landing behaviour could be triggered by different cues.

Once initiated, the landing behaviour must be controlled to achieve smooth landings. How do flying insects control their flight speeds as they approach the landing surface? Honeybees approaching a horizontal surface to land maintain the angular velocity of the image of the ground at a constant value, which results in a linear decrease in the horizontal flight speed. The descent speed is maintained proportional to the horizontal speed resulting in a linear decrease in the descent speed with a decrease in height. This strategy ensures that both the horizontal and descent speeds reach a value of close to zero at touchdown (Srinivasan et al., 2000). Once honeybees flying towards a vertically inclined disc initiate deceleration, they control it by maintaining the rate of expansion of the disc in a given viewing direction at a constant value. This strategy can be used to control landings on any arbitrary surface, and the authors propose that it may be universal (Baird et al., 2013).

Dipterans seem to initiate the landing response at a critical value of RREV, when landing on both cylindrical and spherical surfaces. Do flies follow a similar strategy when landing on plane surfaces, as they do when landing on spherical or cylindrical surfaces, or surfaces with different inclinations? Do flies use the same mechanism as bees to control landings? In this study, we have examined in houseflies (*Musca domestica*), the initiation and control of the landing behaviour on two kinds of landing objects, 1) A vertically oriented surface (vertical landing) 2) The underside of a horizontal surface (inverted landing). We studied how flight velocity and distance from the landing surface affects the initiation of three components of the landing response:

deceleration, leg extension and the orientation of the fly in the direction of the landing plane. The approach velocity has been plotted as a function of the distance from the landing plane, to determine the cues used to control deceleration. We find that the points of onset of the components of the landing response are functions of both flight velocity and the distance from the landing surface. Flies appear to respond to cues from the expansion of the landing object to initiate and control the landing response. The variability in the initiation of the landing response was considerably higher for inverted landings.



**Fig 2. Experimental setup for (A) inverted landings (B) vertical landings**

## **Materials and methods:**

### ***Animals:***

Houseflies (*Musca domestica*) were captured from the wild. The flies were stored in a container with access to both sugar and water. Around 10-12 hours before the start of the inverted landing experiments, the flies were subjected to a cold treatment of  $-30^{\circ}\text{C}$  for a duration of 3 minutes to immobilize them. They were transferred to 50 ml Falcons (Thermo Fischer Scientific Inc.), with each Falcon housing 3-6 flies. A wet piece of cotton was placed in each Falcon to ensure that the flies do not dehydrate. Before filming, the flies were further subjected to a cold treatment of  $-30^{\circ}\text{C}$  for 2.5 minutes. The immobilized flies were placed in the filming chamber and allowed to recover for 10 minutes. The flies used for the vertical landing experiments were not subjected to any cold treatment.

### ***Flight chamber:***

For the inverted landing experiments, a glass box (5 cm  $\times$  5 cm  $\times$  10 cm) with a translucent ceiling made of filter paper was used as the filming chamber. A black square outline of dimensions 1.5 cm  $\times$  1.5 cm was drawn on the centre of the ceiling. The square was drawn for two reasons. First, flies are known to orient towards high contrast stripes in tethered conditions (for example, Poggio and Reichardt, 1973; Virsik and Reichardt, 1976). Therefore, the rectangle serves as a fly attractant, and increases the probability of obtaining a landing. Second, the image of the rectangle expands on the retina as the fly approaches the ceiling, allowing the fly to measure the expansion of the ceiling. We used an ultraviolet lamp that shone from behind the translucent ceiling to attract flies. The flight chamber was illuminated using two 150 watt halogen lamps, and two nikon SMZ25 stereomicroscope lights, to  $\sim 30000$  lux (measured using a center 337 luxmeter, Center Technology Corp.). Three high speed cameras, filming at 4000 frames  $\text{s}^{-1}$  (two phantom v7.3 and one phantom v611, Vision Research Inc.) were focused on the filming chamber (Fig 2 A)

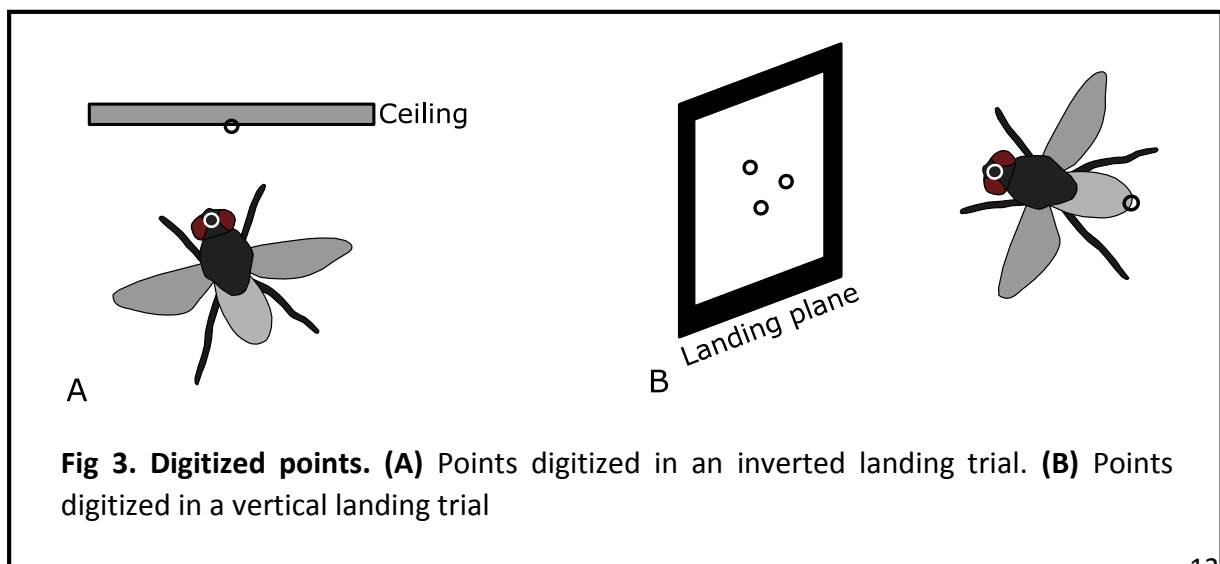
For the vertical landing experiments, a transparent plexiglass box (28 cm  $\times$  28 cm  $\times$  28 cm) was used as the filming chamber. A prism shaped landing object was placed in the centre of the chamber. The object consisted of three 4.5 cm  $\times$  4.5 cm  $\times$  4.5 cm pieces of chart paper attached to each other. Each piece was white in the central region with a black outline. The chamber was lit by a studio light (Simpex Compact

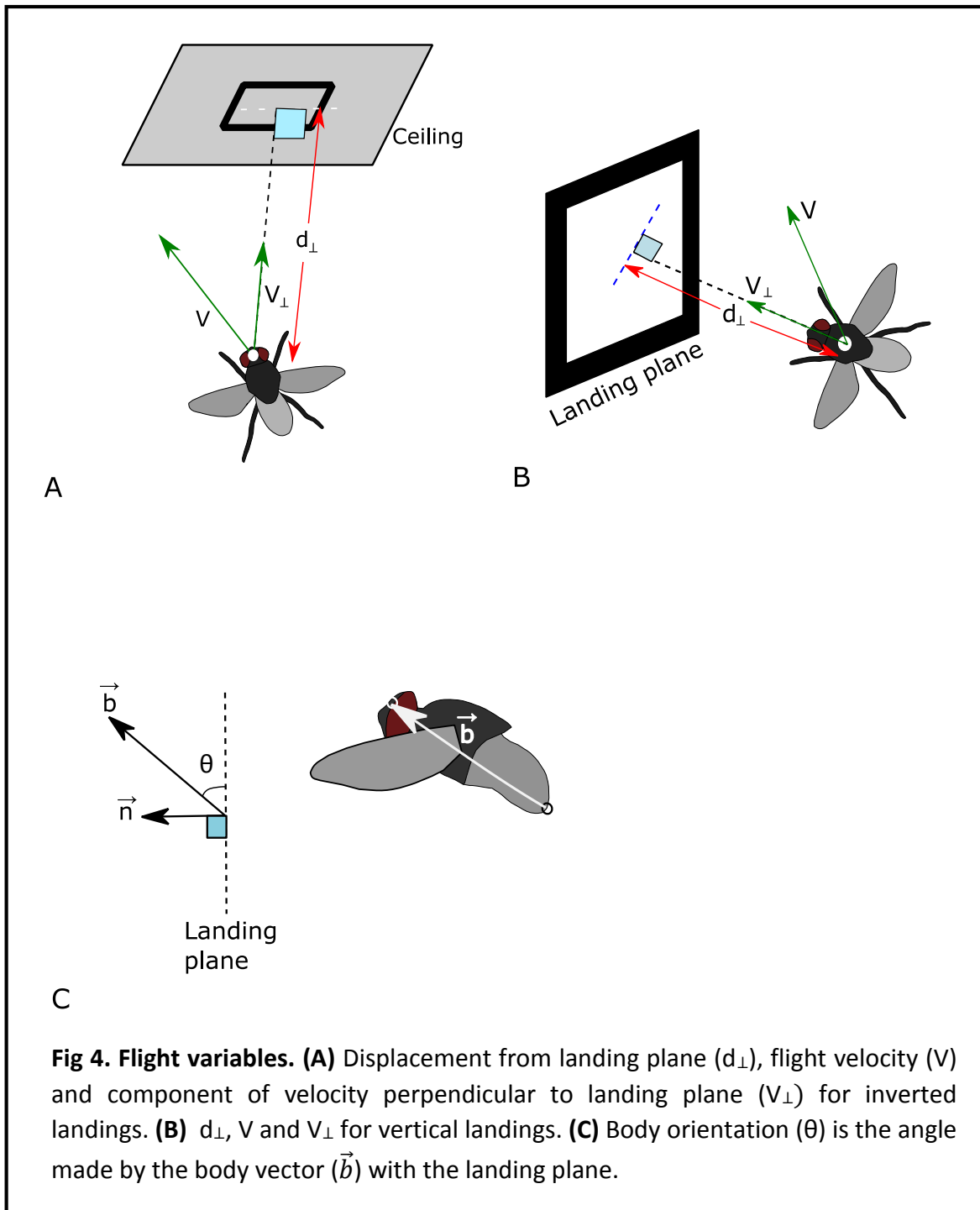
300) to ~3000 lux. 2 high speed cameras (Vision Research, phantom v7.3), filming at 3000 frames s<sup>-1</sup>, were focused on the landing object (Fig 2 B).

**Experimental protocol:**

The experiments were performed at 25°C. Before filming, the flight chambers were calibrated using objects with known dimensions. During each inverted landing trial, 3-6 cold anesthetized flies were placed in the filming chamber and allowed to recover for ten minutes. The flight chamber was then filmed for three minutes. The halogen lamps were switched on only during this duration, as they generate considerable heat. If a fly landed on the ceiling within three minutes, the landing was recorded. In a landing trial, the fly generally took off from a lateral wall, and rotated about its longitudinal axis (roll rotation) by almost 360°. The frame in which the roll visually seemed to end was chosen as the start point and the frame when the wings stopped beating was chosen as the end point of each video. One landing was recorded per batch of flies. This process ensured that no fly was recorded more than once. If no landings occurred in 3 minutes, the batch of flies were discarded and the next batch of anesthetized flies were placed in the chamber.

For the vertical landing experiments, flies were released into the chamber from the top. Landings on the object were recorded. We cannot rule out the possibility of the same fly being recorded in more than one landing trial. The flies generally flew around the box and performed a saccade towards the object before landing. The frame where the saccade visually seemed to end was chosen as the start point of each video. Similar to inverted landings, the frame at which the wings stopped beating was chosen as the end point.





### Analysis:

#### Computation of flight variables

The videos for both the inverted landing and vertical landing experiments were calibrated and digitized using custom MATLAB (MathWorks, Inc.) software (Hedrick, 2008). The tip of the head and a point on the ceiling were digitized and converted to 3-D coordinates for the inverted landing trials (Fig 3 A). The tips of the head and abdomen, and three points on the landing object were digitized and converted to 3-D coordinates for the vertical landing trials (Fig 3 B). From the digitized points, the

following flight variables were extracted using custom codes written in MATLAB (MathWorks, Inc.):

1. *Displacement from the landing plane ( $d_{\perp}$ ):* For the inverted landing trials, the coordinate system was chosen such that the z-axis is perpendicular to the ceiling (the landing plane). Therefore,

$$\mathbf{d}_{\perp} \text{ at the } i^{\text{th}} \text{ frame (for inverted landings)} = \mathbf{z}_i - \mathbf{z}_{\text{ceiling}} \quad (1), \text{ (Fig 4 A)}$$

Where,  $z_i$  = z coordinate of the head tip at the  $i^{\text{th}}$  frame

$z_{\text{ceiling}}$  = z coordinate of the digitized point on the ceiling

For the vertical landing trials, three points were digitized on the landing plane. The points were arbitrarily labelled 1, 2 and 3. Two vectors were obtained by subtracting the coordinates of point 2 from that of point 1, and the coordinates of point 3 from that of point 2. The direction of the cross product of these two vectors is the direction of the normal vector to the plane. For each frame, the midpoint of the line joining the head and tail tips was computed. The displacement between the midpoint and point 1 at the  $i^{\text{th}}$  frame is denoted by  $d_i'$ .

$$\mathbf{d}_{\perp} \text{ at the } i^{\text{th}} \text{ frame (for vertical landings)} = \mathbf{d}_i' \cdot \vec{n} \quad (2), \text{ (Fig 4 B)}$$

Where  $\vec{n}$  is the unit normal vector to the landing plane and the symbol ' $\cdot$ ' stands for dot product.

2. *Perpendicular velocity ( $V_{\perp}$ ):* It is the component of velocity perpendicular to the landing plane.

$$\mathbf{Flight \ velocity \ at \ the \ (i+1)^{\text{th}} \ frame, \ for \ inverted \ landings \ (V)} = \frac{\mathbf{h}_{i+1} - \mathbf{h}_i}{\tau} \quad (3), \text{ (Fig 4 A)}$$

Where,  $h_i, h_{i+1}$  = coordinates of the head tip at the  $i^{\text{th}}$  and  $(i+1)^{\text{th}}$  frames

$\tau$  = time duration of 1 frame (0.25 ms)

$$\mathbf{V}_{\perp} \text{ at the } (i+1)^{\text{th}} \ \text{frame (for inverted landings)} = V_z, \ \text{the z coordinate of V.}$$

$$\mathbf{Flight \ velocity \ at \ the \ (i+1)^{\text{th}} \ frame \ for \ vertical \ landings \ (V)} = \frac{\mathbf{m}_{i+1} - \mathbf{m}_i}{\tau} \quad (4), \text{ (Fig 4 B)}$$

Where,  $m_i, m_{i+1}$  = coordinates of the midpoint of the line joining the head and tail tip  
at the  $i^{\text{th}}$  and  $(i+1)^{\text{th}}$  frames

$\tau$  = time duration of 1 frame ( $\frac{1}{3}$  ms)

$$\mathbf{V}_{\perp} \text{ at the } (i+1)^{\text{th}} \text{ frame (for vertical landings)} = \mathbf{V} \cdot \vec{n} \quad (5), \text{ (Fig 4 B)}$$

3. *Body orientation* ( $\theta$ ): This quantity was computed solely for the vertical landing experiments. For each frame, the body vector ( $\vec{b}$ ) was computed by subtracting the coordinates of the head tip from the tail tip. Body orientation, is the angle made by the body vector with the landing plane.

$$\text{Body orientation } (\theta) = \sin^{-1} \left( \frac{|\vec{n} \cdot \vec{b}|}{|\vec{b}|} \right) \quad (6), \text{ (Fig 4 C)}$$

Where the notation  $|a|$  means “the absolute value of quantity a”.

4. *Time to contact*: It is the time taken to contact the landing surface if the fly continues to fly towards it at the current  $V_{\perp}$ . Time to contact at any frame is therefore the ratio of  $d_{\perp}$  to  $V_{\perp}$  at that frame.

Before calculating the flight variables, the 3-D coordinates of all the points were smoothed using a Butterworth filter of order 4, to reduce digitization errors. The magnitude and phase response was compared for each order of Butterworth filter. A fourth order filter was chosen as it maximized the passband and minimized the ripple effect. We calculated the turning frequency ( $\gamma$ ) for all the videos.

$$\gamma = \frac{\text{Total number of observed turns in a video}}{\text{Time duration of the video}}$$

The maximum  $\gamma$  for the inverted landing videos was 62.5 Hz. For vertical landings, it was  $\approx 21.9$  Hz. The maximum turning frequencies were chosen as the cutoff frequencies for the Butterworth filters.

#### *Onsets of the components of the landing behaviour*

In a majority of the trials, the fly began to decelerate at a point and continued to do so until touchdown (Fig 5 A, B). This point was marked as the point of onset of deceleration. In a few cases, the fly stopped decelerating at a point before touchdown.

$V_{\perp}$  increased slightly after this point. In such cases, the point where the decrease in  $V_{\perp}$  began was marked as the point of onset of deceleration. The point of onset of a reduction in body orientation was chosen using the same rule, from plots of  $\theta$  versus time (Fig 16 A). The frame of onset of leg extension was chosen to be the first frame in which the extension of any of the legs were visually detectable (the front legs forward, the other legs sideways).

### *Statistics:*

Intuitively, one would expect flying insects to initiate the landing response earlier when flying towards the landing surface at greater velocities, to avoid crash landings. Therefore, we calculated the Pearson's correlation coefficients ( $R$ ) between  $V_{\perp}$  and  $d_{\perp}$  at the points of initiation of the components of the landing response. We also fit linear equations to the  $V_{\perp}$  versus  $d_{\perp}$  plots, and computed the coefficient of determination ( $R^2$ ) of the best fit lines (for example, Fig 6 A). Once initiated, the  $V_{\perp}$  appeared to decrease as a logarithmic function of  $d_{\perp}$ . To test this, we fit a logarithmic equation to these plots (Fig 10 B, 12 B). The values of  $R^2$  were computed. Baird et al., 2013, propose a model which predicts a linear relationship between  $V_{\perp}$  and  $d_{\perp}$  after the initiation of deceleration (see "How is deceleration controlled?" section of the discussion). Therefore, we also fit linear equations to the decelerating portion of the  $V_{\perp}$  versus  $d_{\perp}$  plots (Fig 10 B, 12 B). The regression analysis, calculation of  $R$  and  $R^2$  were performed using Microsoft Excel. The sample sizes of both inverted landings ( $n=17$ ) and vertical landings ( $n=18$ ) are small. Therefore, we used non-parametric tests to establish differences between the flight variables for inverted and vertical landings, as well as between flies which land smoothly and those that crash their head onto the landing surface. The Wilcoxon signed rank test was used to compare paired samples (Fig 11, 13). The Mann Whitney U test was used to compare unpaired samples (for example, Fig 9, 18). The non-parametric tests were executed in MATLAB (Mathworks Inc.). To test whether flies initiate the landing response at a constant value of time to contact, we computed in Microsoft Excel, the coefficient of variation of the distributions of time to contact, at the points of onset of the landing components.



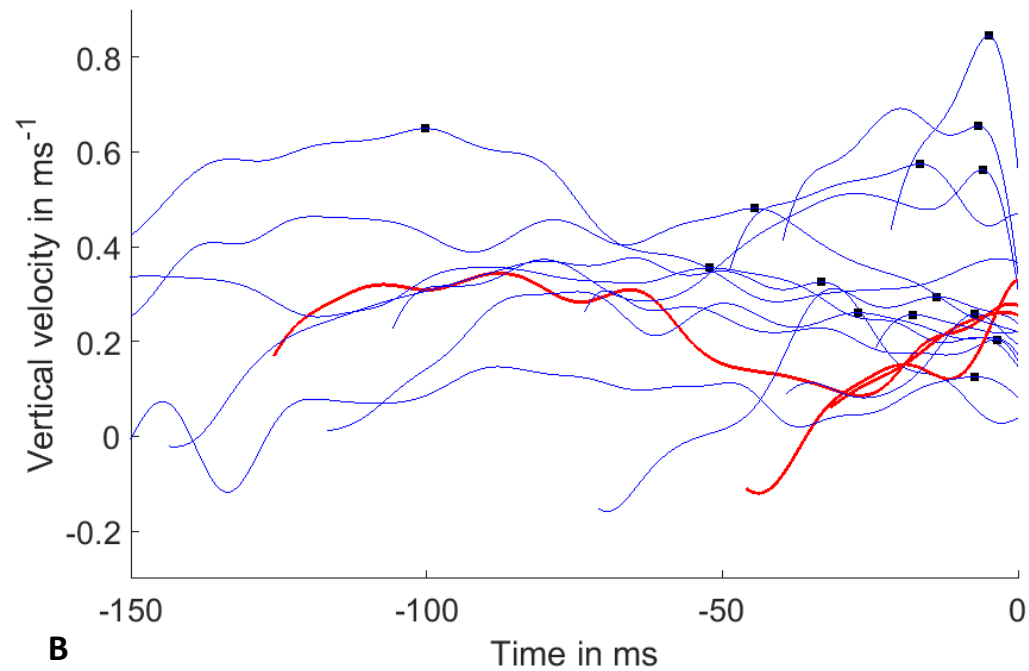
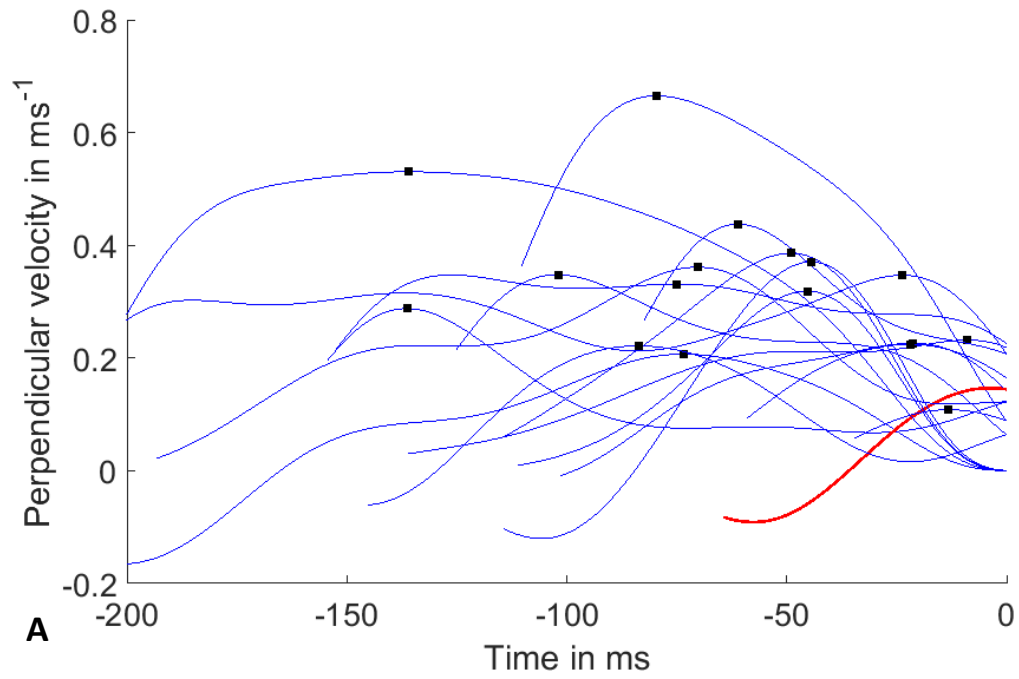
## Results:

### **1. Modulation of flight velocities as the flies approach the landing target**

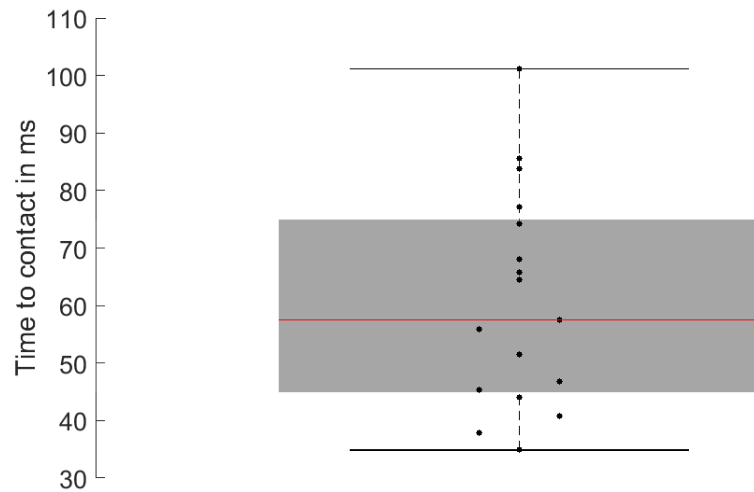
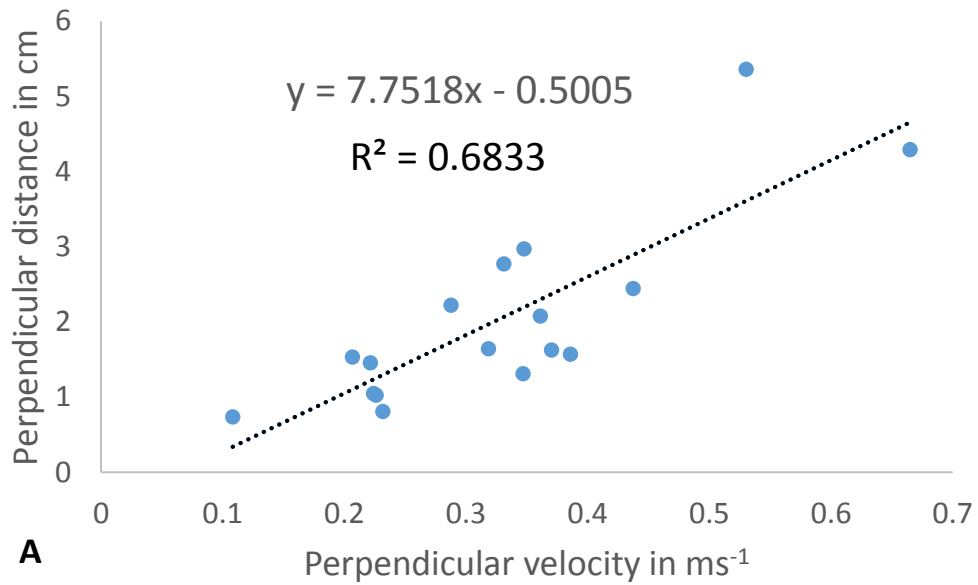
#### *a) Initiation of deceleration:*

Flies need to decelerate before landing to minimize the impact at touchdown. Fig 5 A shows the relationship between the component of flight velocity perpendicular to the landing plane ( $V_{\perp}$ ) and time, for all the vertical landing trials. The flies contact the landing surface at time 0 ms. It is clear that the flies decelerate before touchdown. The black squares represent the points of onset of deceleration. Out of a total of 18 trials, deceleration was observed in all but 1 trial (marked in blue). The relationship between the vertical velocity ( $V_{\perp}$ ) and time for flies landing on the inverted surface is depicted in fig 5 B. Deceleration was observed in 14 out the 17 trials (coloured in blue). The 3 trials where deceleration was not observed are marked in red. Black squares indicate the points of onset of deceleration. Therefore, a majority of the flies decelerate before touchdown while flying towards either vertically oriented or inverted surfaces.

Is the onset of deceleration dependent on flight velocity and distance from the landing plane? While approaching vertical surfaces, flies decelerated at a point further from the plane of landing if they were flying at greater velocities (Fig 6 A). The correlation between the 2 variables is high ( $R \approx 0.83$ ). A linear equation can fit the data points ( $R^2 \approx 0.68$ ). The implications of a linear relation is that the flies can estimate the time to contact to the landing surface at every frame, and extend their legs when the time to contact reaches a threshold value. We computed the values of the ratio of  $d_{\perp}$  to  $V_{\perp}$  at the frame of onset of deceleration, which is the value of time to contact to the landing target at that frame. The distribution of the calculated time to contact values at the frame of onset of deceleration is in fig 6 B. The time to contact falls in a range from ~35 to ~100 ms. The coefficient of variation of the distribution (ratio of standard deviation to the mean) is ~ 30%. Thus, the flies probably do not initiate deceleration at a threshold value of time to contact, but rather when time to collision falls within a certain range.



**Fig 5. Velocity profiles.** (A) The component of velocity perpendicular to the landing plane ( $V_{\perp}$ ) plotted against time for a total of 18 vertical landing trials. The black squares represent the points of onset of deceleration for each trial. In the trials marked red, the flies do not decelerate before touchdown. The rest of the trials (marked in blue) show a clear deceleration trend before touchdown. (B)  $V_{\perp}$  is plotted against time for a total of 17 inverted landing trials. The colour coding is same as in (A).



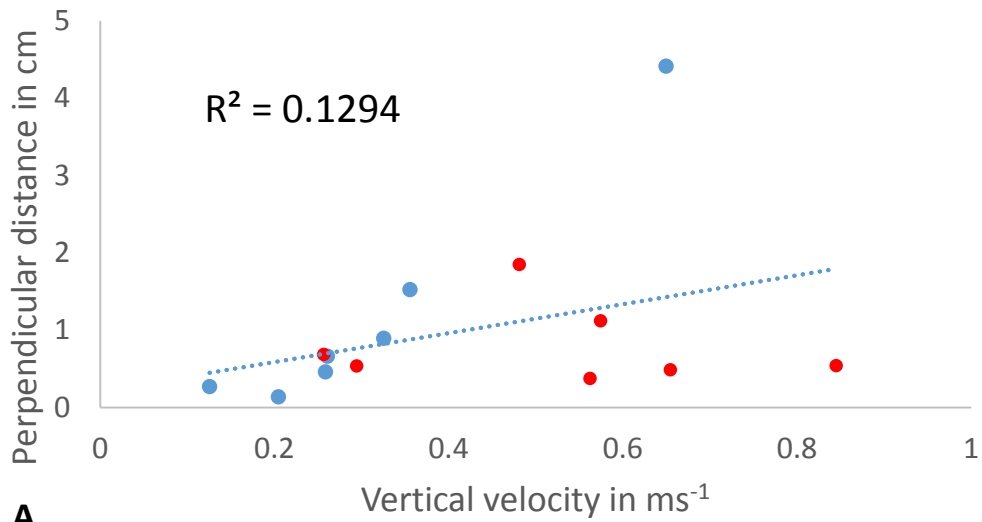
**Fig 6. Onsets of deceleration for vertical landings. (A)** When flies land on the vertical surface, there is a positive correlation between the displacement from the landing plane ( $d_{\perp}$ ) and the instantaneous perpendicular velocity ( $V_{\perp}$ ) at the frame of onset of deceleration ( $R \approx 0.83$ ). The  $R^2$  value of the best fit linear equation is  $\approx 0.68$ . The equation of the best fit line is displayed on the graph. **(B)** Boxplot of the calculated time to contact values. The red line is the median. The interquartile range between the 25<sup>th</sup> and 75<sup>th</sup> quartiles is represented by the y axes limits of the box. The whiskers extend up to 1.5 times the interquartile range. Subsequent boxplots will follow the same convention.

The relationship between  $d_{\perp}$  and  $V_{\perp}$  at the frame of onset of deceleration for flies landing on an inverted surface is shown in fig 7 A. There is a weak correlation between  $d_{\perp}$  and  $V_{\perp}$  ( $R \approx 0.35$ ) and a linear equation poorly fits the data ( $R^2 \approx 0.1294$ ). We obtained

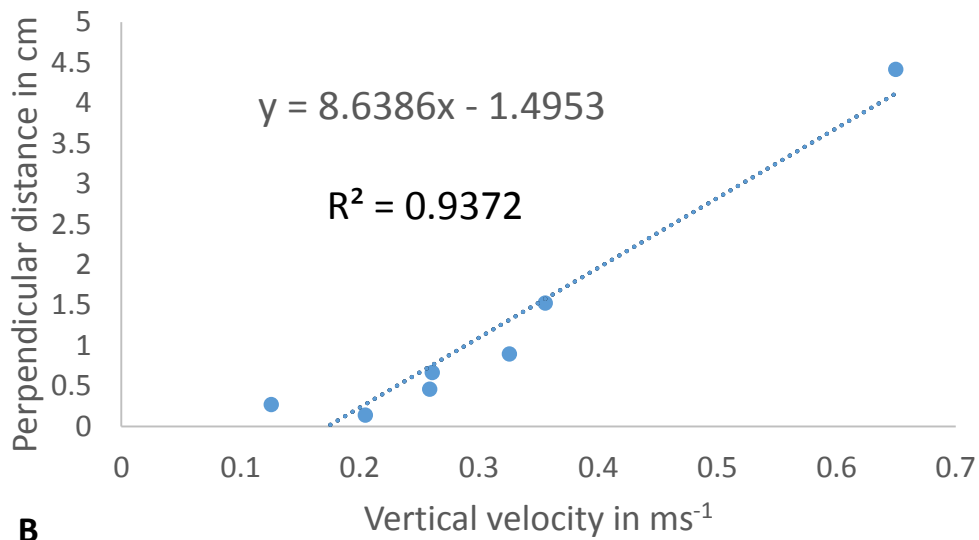
a total of 17 trials, with 14 decelerating before touchdown. In 8 out of 17 trials, the head of the fly bumped into the ceiling (in spite of leg extension before touchdown, summarized in table 1). We classified such landings as “bumps” (See fig 8). In the 14 cases where the flies decelerated before touchdown, 7 bumps were observed. Do “bumps” represent landings that are imperfect, or a misestimation of the time to collision? The flies which performed a smooth landing began decelerating further away from the ceiling at higher vertical velocities (Fig 7 B). There exists a strong correlation between  $d_{\perp}$  and  $V_{\perp}$  at the frame of onset of deceleration ( $R \approx 0.97$ ). A linear equation fits the data accurately ( $R^2 \approx 0.94$ ). However, the flies which bump into the ceiling do not follow this trend (Fig 7 C). The distribution of the time to contact was compared between the smooth landing trials and the trials where the flies bump into the ceiling (Fig 9). The median time to contact was lower for the flies which bump into the ceiling, suggesting that such flies initiate the landing response later than required for a smooth landing. However, the distributions were not significantly different from each other (Mann Whitney U test,  $p \approx 0.21$ ).

Trial number	Decelerate?	Landing?
1	No	Land
2	Yes	Bump
3	Yes	Bump
4	Yes	Land
5	Yes	Land
6	Yes	Land
7	Yes	Bump
8	No	Land
9	Yes	Bump
10	Yes	Land
11	No	Bump
12	Yes	Land
13	Yes	Land
14	Yes	Bump
15	Yes	Bump
16	Yes	Land
17	Yes	Bump

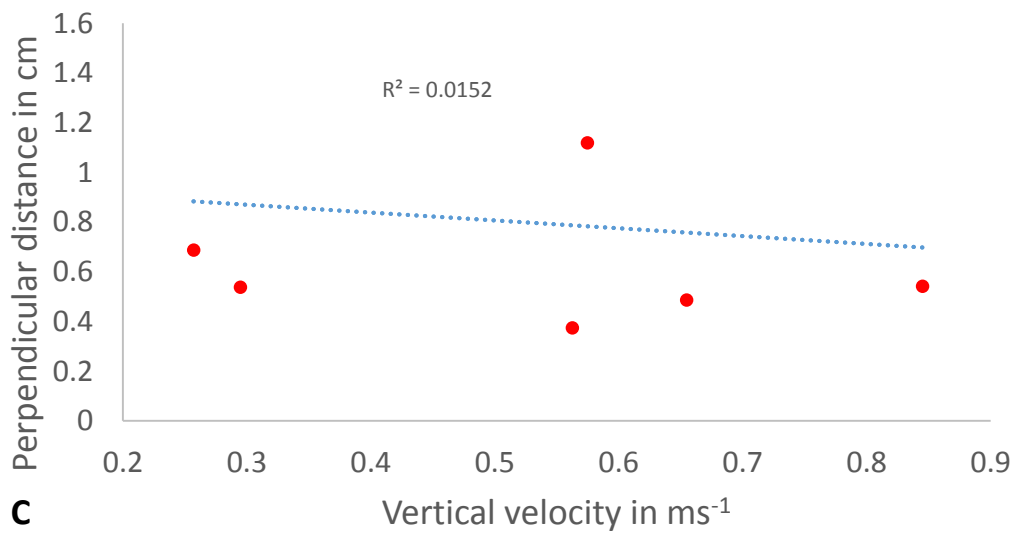
**Table 1. Distribution of smooth landings and bumps in the inverted landing trials.** 3 out of 17 flies do not decelerate before touchdown. Flies bump into the ceiling in 8 trials.



**A**



**B**

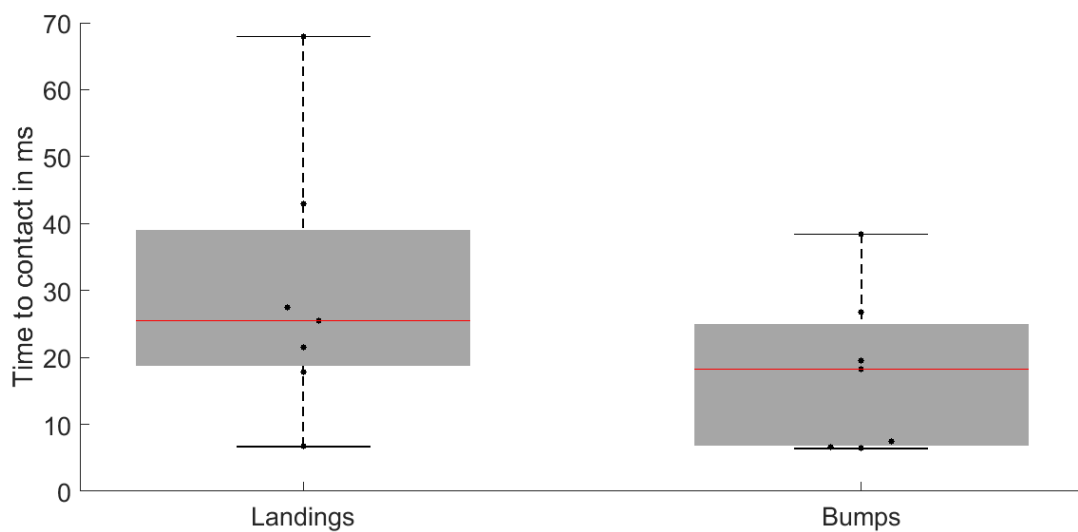


**C**

**Fig 7. Onsets of deceleration for inverted landings. (A)** There is no correlation between  $d_{\perp}$  and  $V_{\perp}$  at the frame of onset of deceleration when all 14 trials are plotted together. Trials in which the flies bump into the ceiling are indicated by red dots. The blue dots represent trials where the fly performs a smooth landing. **(B)**  $d_{\perp}$  is positively correlated  $V_{\perp}$  at the frame of onset of deceleration, for the flies landing on the ceiling. A linear equation fits the data well. **(C)**  $d_{\perp}$  at the frame of onset of deceleration is independent of  $V_{\perp}$  at that frame, for the trials in which the fly which bumps into the ceiling.



**Fig 8. A “bump”.** Note that the head makes contact with the ceiling.



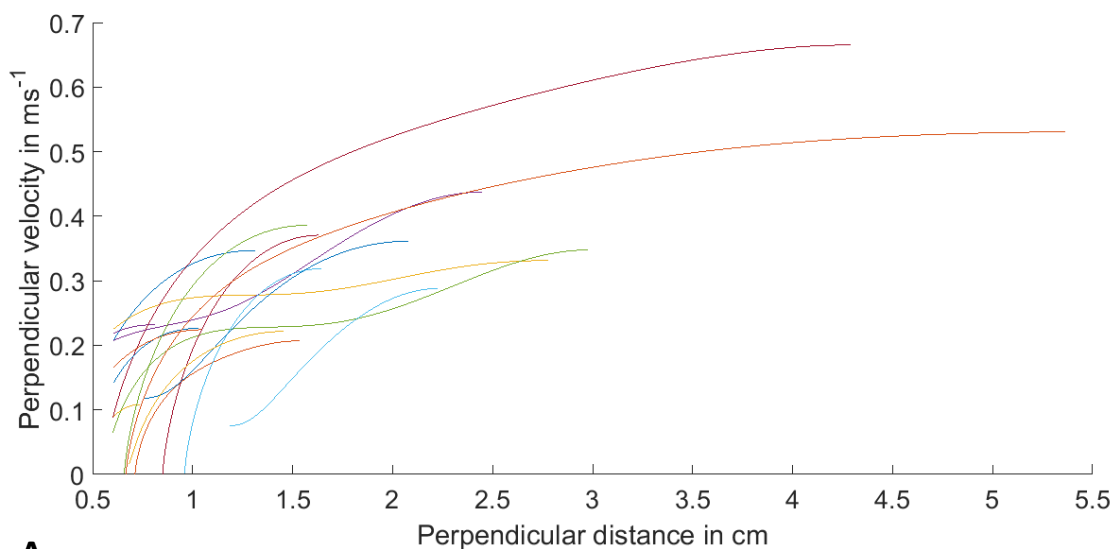
**Fig 9. Time to contact of landings and bumps.** The median time to contact is lower for the flies that bump into the ceiling. However, the distributions are not significantly different from each other (Mann Whitney U test,  $p=0.2$ )

*b) Control of deceleration:*

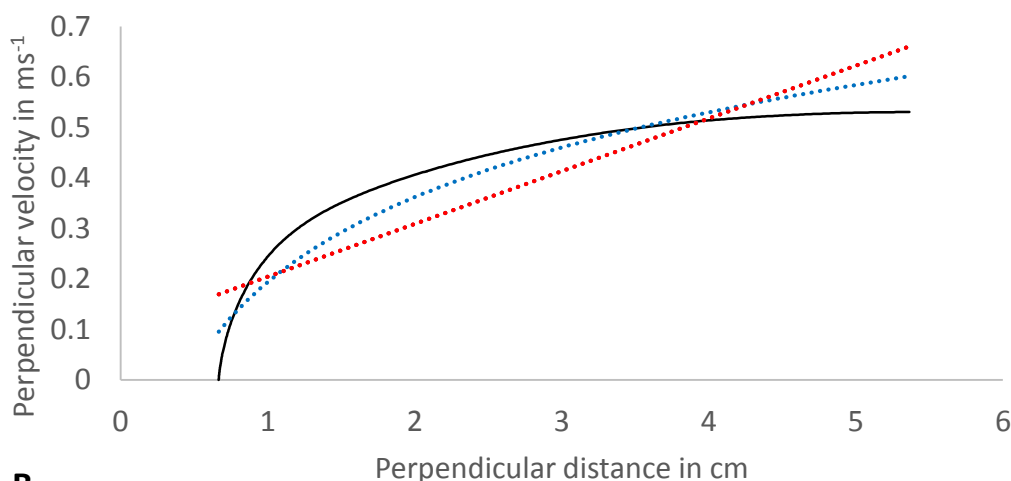
A majority of flies landing on either vertical or inverted surfaces decelerate before touchdown. How is the deceleration controlled? The component of velocity perpendicular to the plane of landing ( $V_{\perp}$ ) is plotted against the displacement from the

landing plane ( $d_{\perp}$ ), for the decelerating portion of the flight trajectories of vertical landings in fig 10 A. Visually,  $V_{\perp}$  seems to decrease as a function of the logarithm of  $d_{\perp}$ . For each trial, we performed both linear and logarithmic regressions (Fig 10 B). The coefficient of determination ( $R^2$ ) was extracted from each fit. The distribution of  $R^2$  for the fits of both the curves are shown in fig 11.  $R^2$  values are significantly higher for the logarithmic fits to the data (Wilcoxon signed rank test,  $p=0.0065$ ).

$V_{\perp}$  is plotted against  $d_{\perp}$  for the decelerating portions of the flight trajectories of inverted landings in fig 12 A. Logarithmic and linear equations were fit to each trial and the  $R^2$  values of the best fit values were computed (Fig 12 B). The distributions of the  $R^2$  values for both logarithmic and linear fits to the data are represented by boxplots in fig 13. The  $R^2$  for logarithmic fits are significantly higher (Wilcoxon signed rank test,  $p=0.0166$ ).

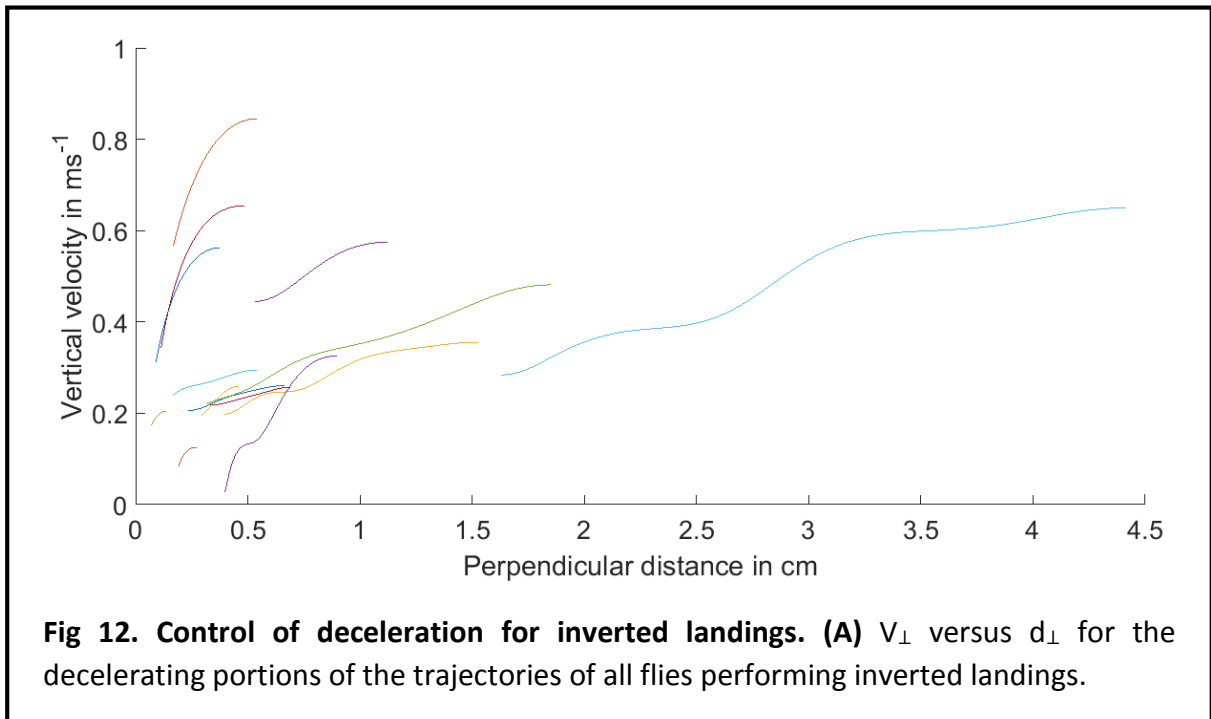
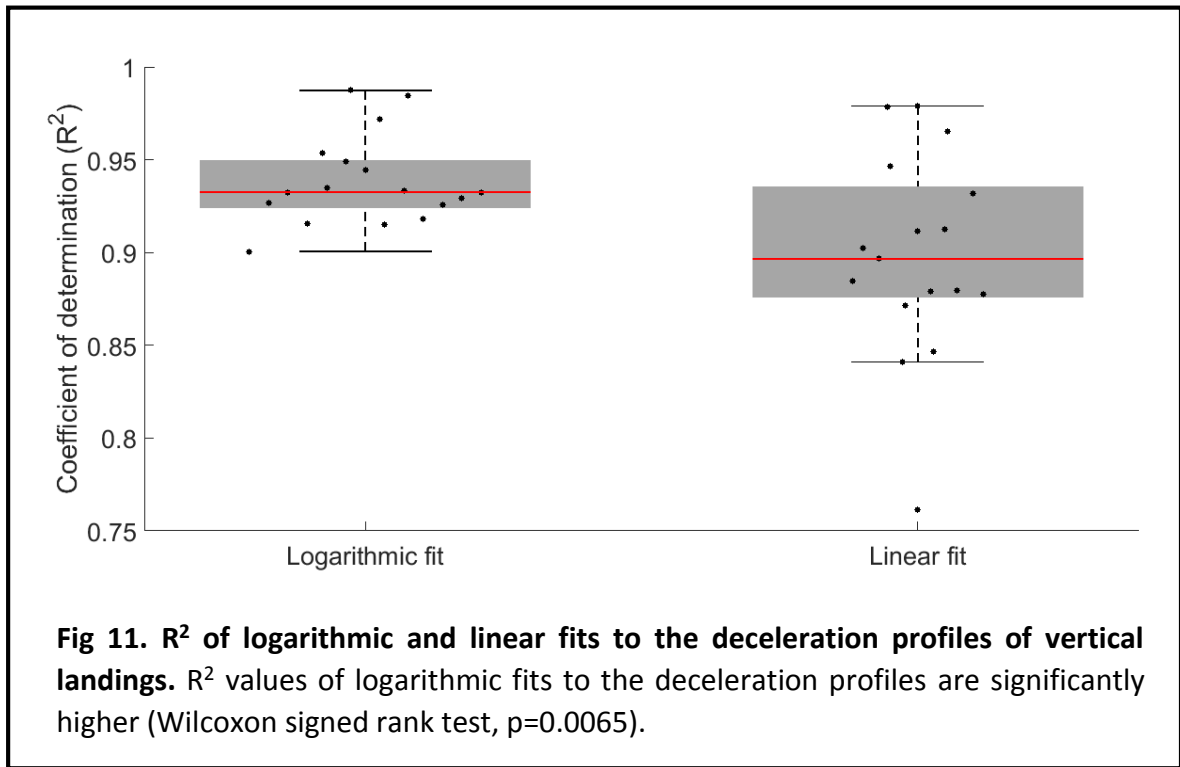


**A**

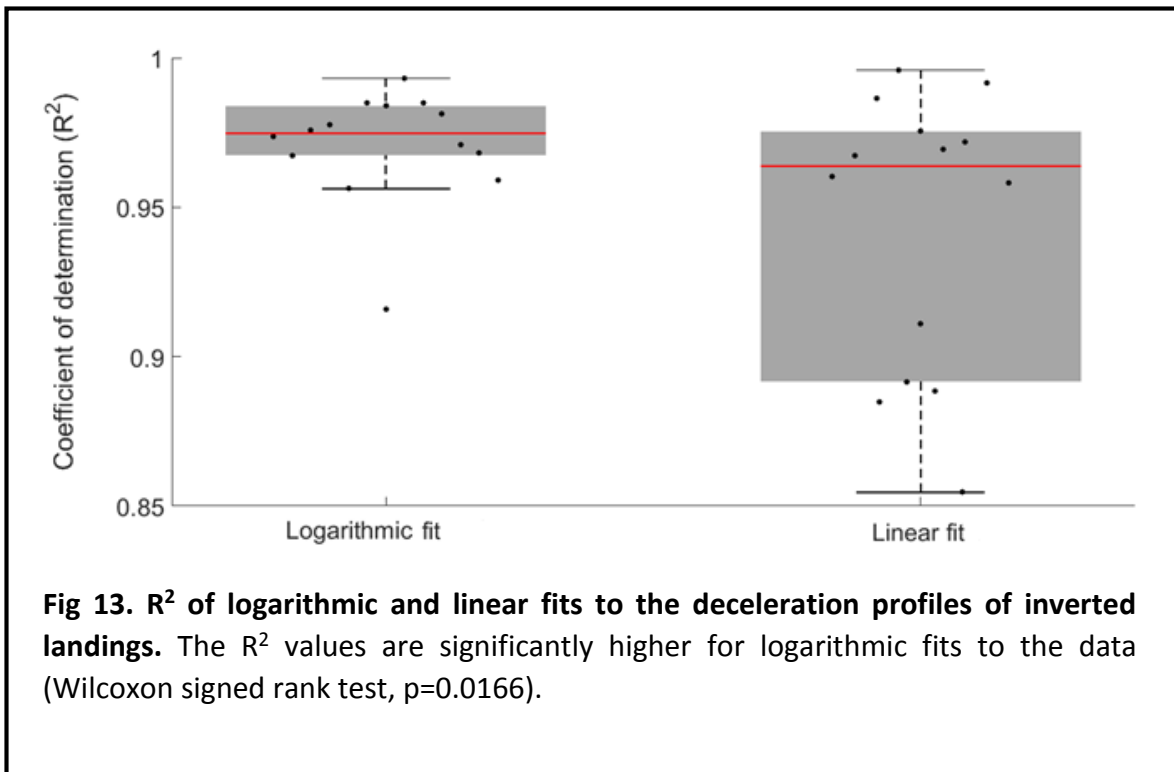
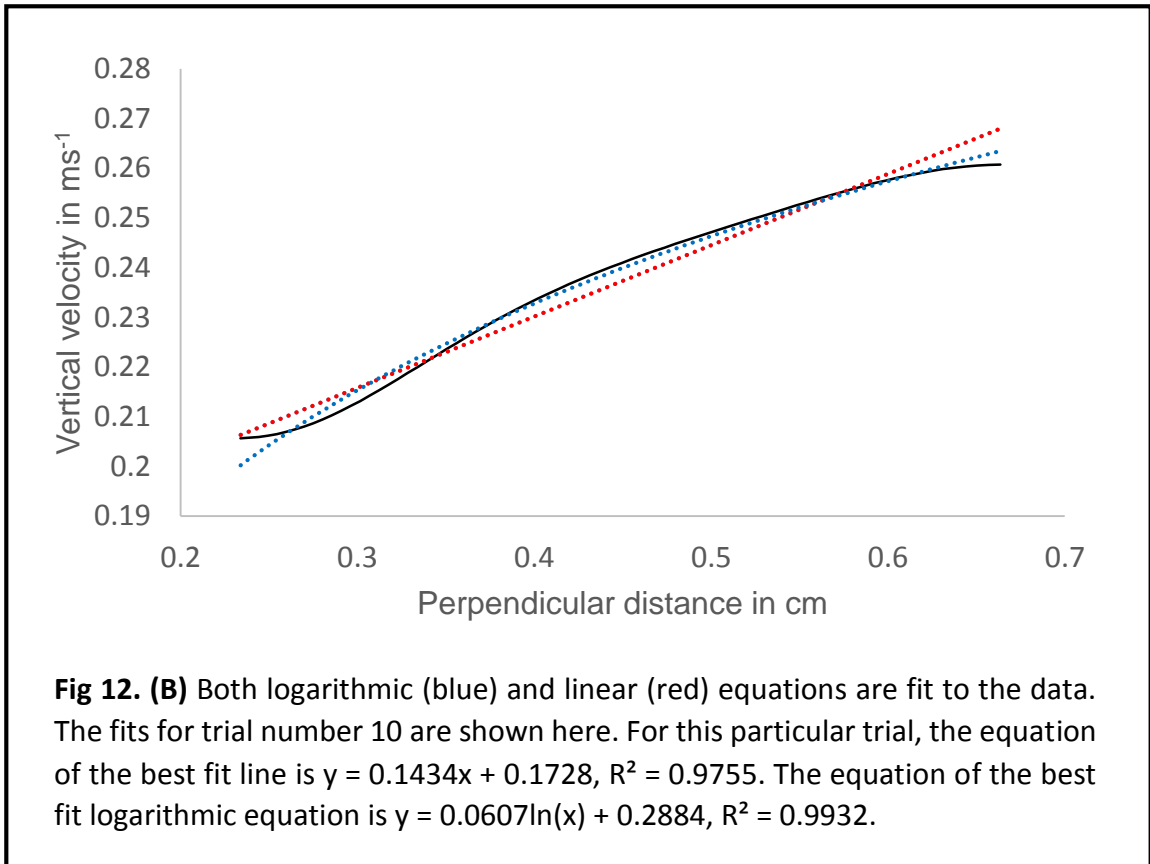


**B**

**Fig 10. Control of deceleration for vertical landings. (A)**  $V_{\perp}$  versus  $d_{\perp}$  for the decelerating portions of the vertical landing trials. **(B)** Both logarithmic (blue) and linear (red) equations are fit to the decelerating portions of the plots for each trial. The fit for trial number 17 is shown here. For trial 17, the equation of the best fit line is  $y = 0.1045x + 0.0999$ ,  $R^2 = 0.7615$ . The equation for the best fit logarithmic equation is  $y = 0.2426 \cdot \ln(x) + 0.1938$ ,  $R^2 = 0.9291$ .



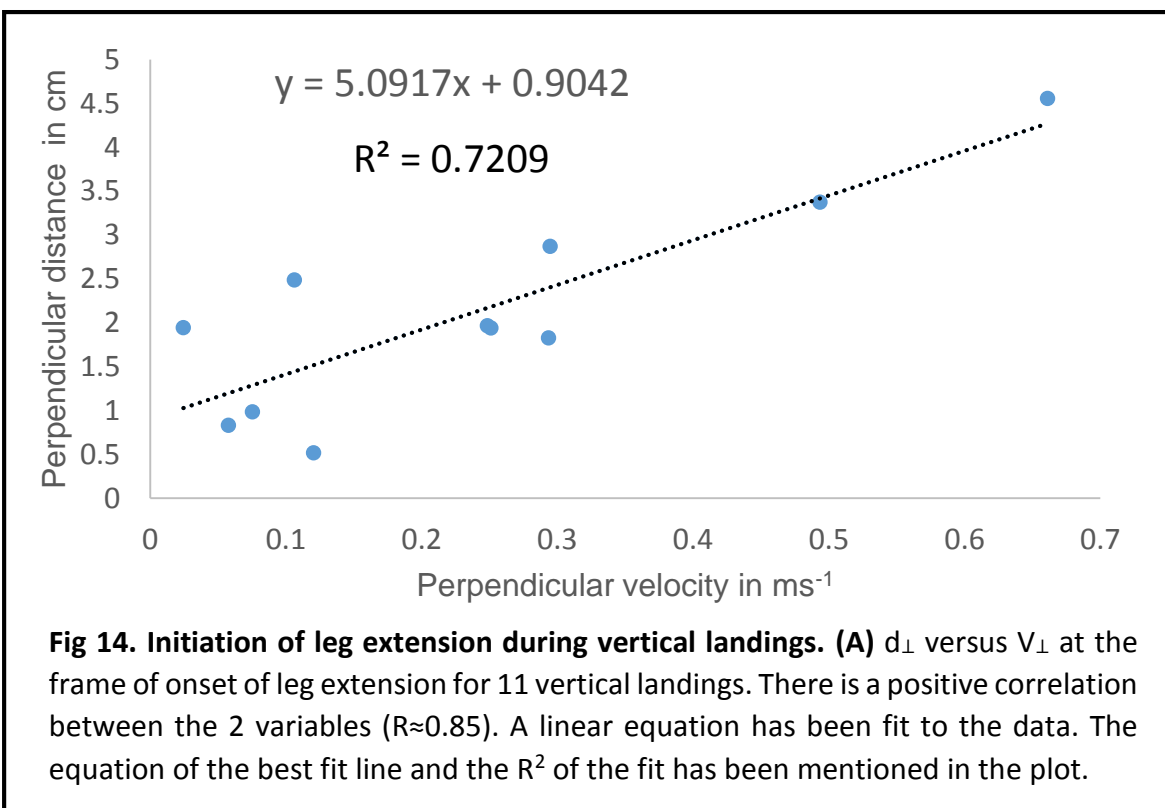




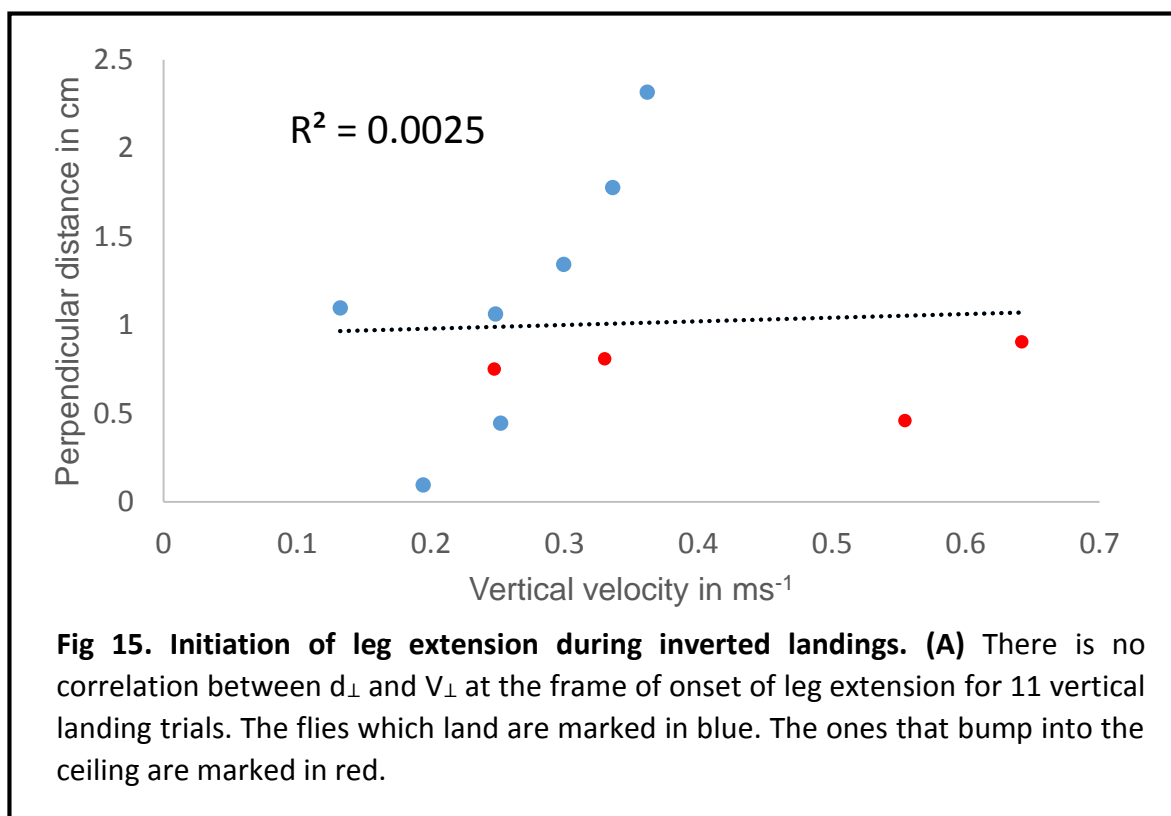
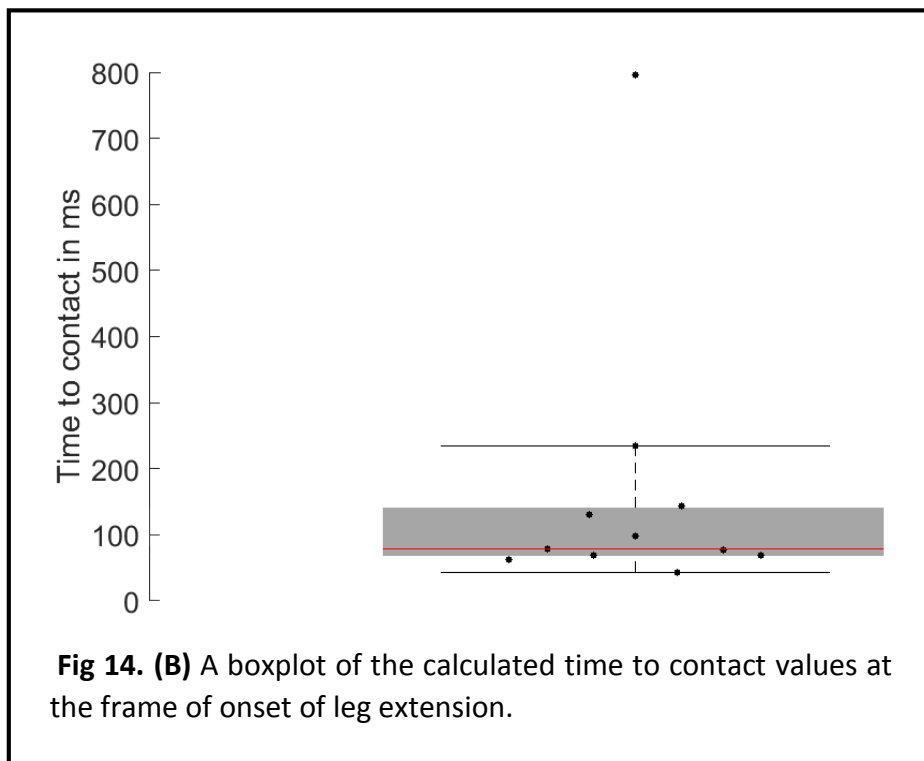
## 2) Initiation of leg extension behaviour as the flies approach the landing target

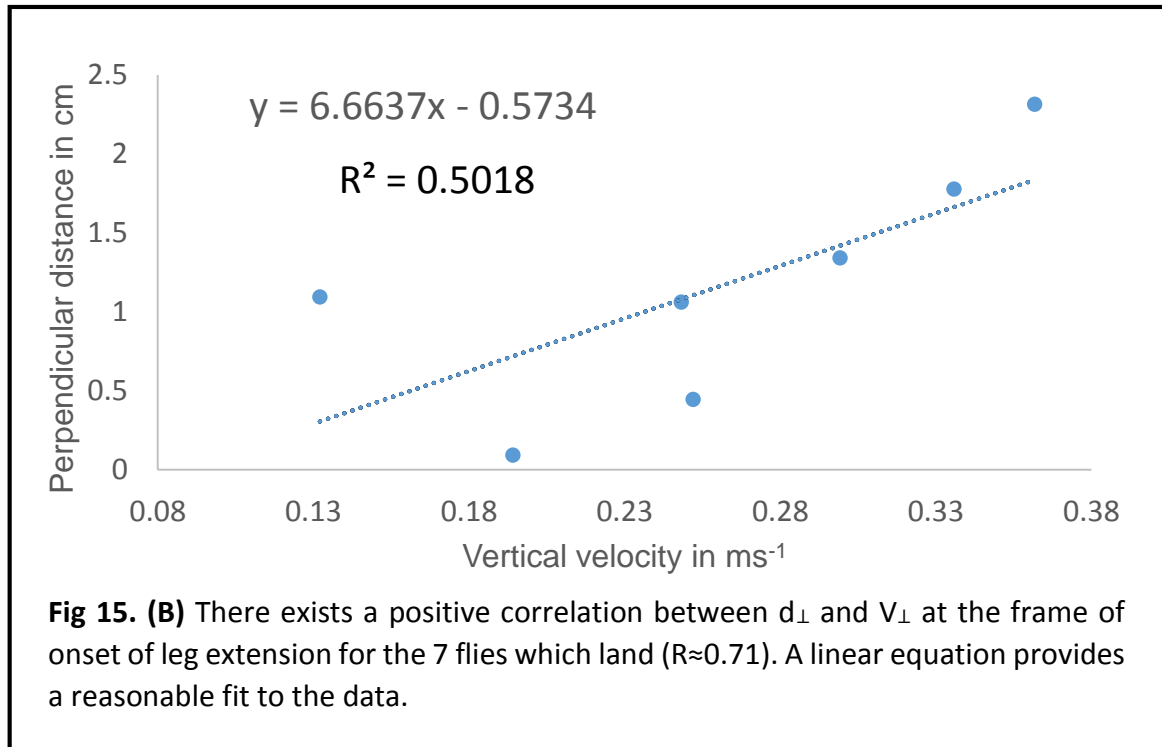
All flies extended their legs before touchdown, regardless of the inclination of the landing surface. Does initiation of leg extension depend on velocity and distance from the landing surface? For the vertical landing trials, the displacement of the frame of onset of leg extension from the landing plane ( $d_{\perp}$ ) is plotted against the component of velocity perpendicular to the landing plane ( $V_{\perp}$ ) at that frame in fig 14 A. In 6 trials, leg extension had already been initiated by the time it was captured by the cameras. The frame of onset of leg extension is therefore, not known for these trials. In one case, the fly extended its legs when it was moving in a direction opposite to the landing surface. These 7 trials have been excluded from fig 14 A. There is a strong positive correlation between  $d_{\perp}$  and  $V_{\perp}$  at the frame of onset of leg extension ( $R \approx 0.85$ ). A linear equation was used to fit the data ( $R^2 \approx 0.72$ ). Do flies extend their legs at a constant value of time to collision to the landing surface? If a linear equation had fit almost perfectly ( $R^2$  close to 1), then all flies would have extended their legs at a constant time to contact to the landing surface. The distribution of the computed time to contacts ( $d_{\perp}/V_{\perp}$ ) is represented by a boxplot in fig 14 B. The coefficient of variation of the time to contact distribution is  $\approx 132\%$  which indicates that the flies do not extend their legs at a constant time to contact to the landing surface.

For the inverted landing trials,  $d_{\perp}$  at the frame of onset of leg extension is plotted against  $V_{\perp}$  in fig 15 A. In 6 trials out of a total of 17, the flies extend their legs even before they took off from the lateral surface of the glass box (see materials and methods). These flies were excluded from fig 15 A. The flies which bump into the ceiling have been marked red and the ones which land are marked in blue. There is no correlation between  $d_{\perp}$  and  $V_{\perp}$  at the frame of onset of leg extension ( $R \approx 0.05$ ). However, if the flies which land smoothly are considered separately, there exists a positive correlation between  $d_{\perp}$  and  $V_{\perp}$  ( $R \approx 0.71$ , fig 15 B).



**Fig 14. Initiation of leg extension during vertical landings. (A)**  $d_{\perp}$  versus  $V_{\perp}$  at the frame of onset of leg extension for 11 vertical landings. There is a positive correlation between the 2 variables ( $R \approx 0.85$ ). A linear equation has been fit to the data. The equation of the best fit line and the  $R^2$  of the fit has been mentioned in the plot.





### 3) Modulation of body orientation as the flies approaches the landing target

The body orientations have been estimated only for the cases in which the flies land on the vertical surface. For all the trials, the body orientation ( $\theta$ ) has been plotted against time in fig 16 A. The black circles mark the onset of a decrease in  $\theta$ .  $\theta$  decreases as the flies approach the landing surface (except for 2 trials, marked in red). What cues are utilized to trigger this decrease in body orientation? The values of  $d_{\perp}$  at the frame of onset of a decrease in body orientation are plotted against  $V_{\perp}$  at that frame in fig 16 B. The flies began to decrease their body orientation ( $\theta$ ) further away from the landing plane at higher values of  $V_{\perp}$  ( $R \approx 0.77$ , see fig 16 B). A linear equation was fit to the data ( $R^2 \approx 0.6$ ). The distribution of the calculated time to contacts at the frame of onset of decrease in  $\theta$  is represented via a boxplot in fig 17. The coefficient of variation of the time to contact distribution is  $\approx 76\%$ . Therefore, the flies do not initiate the behaviour at a constant time to contact.

### 4) Velocities at touchdown

The component of velocity normal to the landing surface ( $V_{\perp}$ ) at touchdown is significantly higher for inverted landings (Mann Whitney U test,  $p \approx 3 \times 10^{-4}$ , see Fig 18 A).  $V_{\perp}$  at contact was computed for 12 out of the 18 vertical landing trials, as the frame of contact could not be ascertained in 6 trials. Among the inverted landing trials,  $V_{\perp}$  at touchdown is higher for the group of flies which bump into the ceiling (Mann Whitney U test,  $p = 0.0206$ , fig 18 B).  $V_{\perp}$  at touchdown for individuals performing smooth inverted landings is still significantly higher than that of flies performing vertical landings (Mann Whitney U test,  $p = 0.0116$ , fig 18 C).

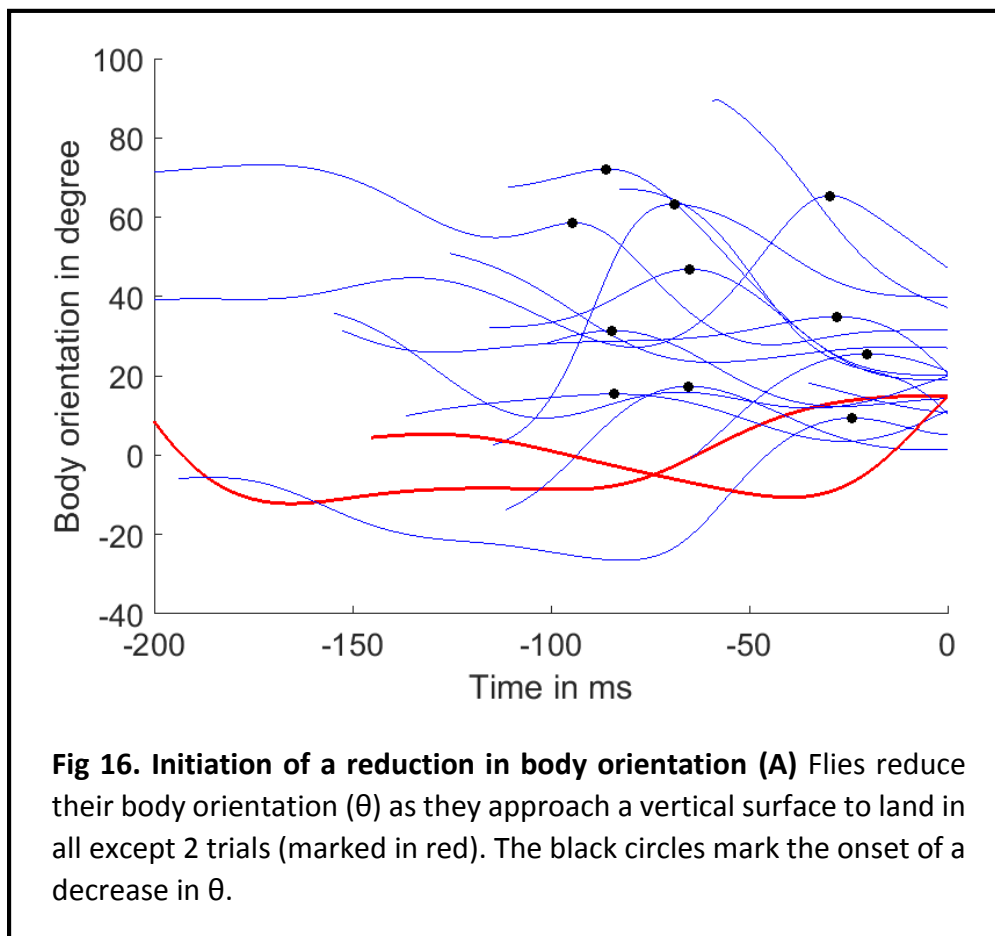
### 5) Time taken for the wings to come to rest after contact

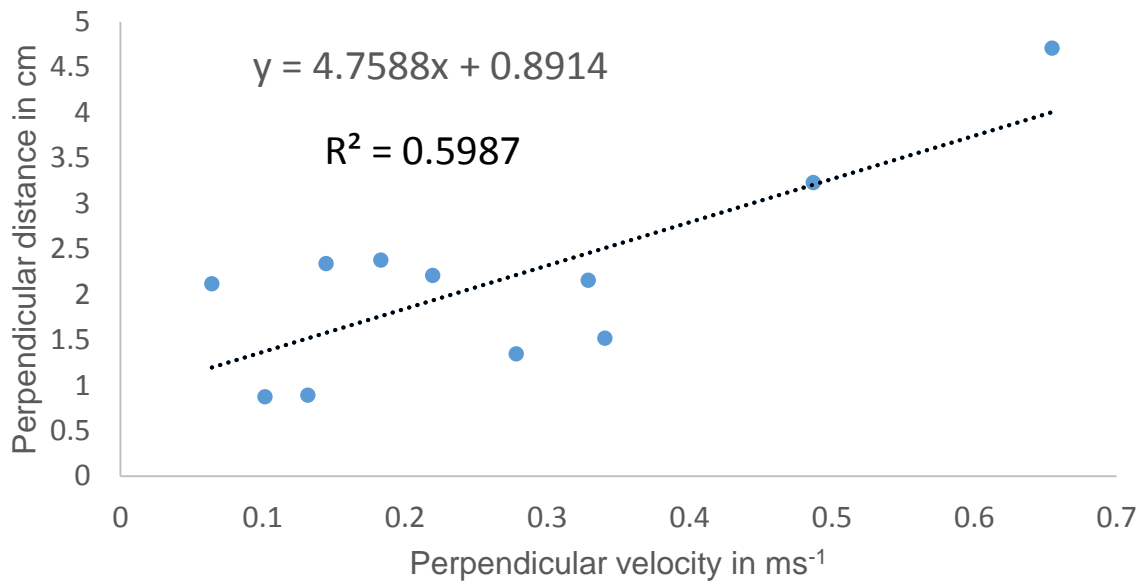
The time taken for the wings to stop beating (stop time) is independent of  $V_{\perp}$  at touchdown for the vertical landing trials ( $R \approx 0.26$ , fig 19 A). However, there is a weak

positive correlation between the stop time and the  $V_{\perp}$  at touchdown, when flies land on inverted surfaces ( $R \approx 0.54$ , fig 19 B). The stop time for the inverted landing trials is significantly longer than that of the vertical landing trials (Mann Whitney U test,  $p \approx 0.0215$ , fig 20).

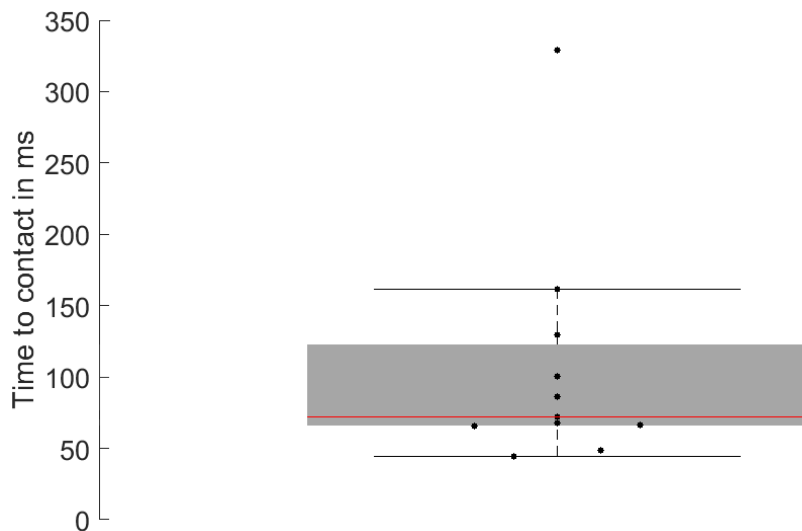
### 6) Order of initiation of various components of the landing behaviour

Are the components of landing behaviour, namely deceleration, leg extension and body orientation towards the landing target, initiated in the same order in all flies? The component of velocity perpendicular to the landing plane ( $V_{\perp}$ ) is plotted against time for all the vertical landing trials in fig 21 A. The onsets of deceleration, leg extension and a decrease in body orientation are marked on the plot. The order of initiation of these components of the landing behaviour, for vertical landings, is listed in table 2. The order is highly inconsistent and changes from trial to trial. Body orientation was not computed for the inverted landing behaviour.  $V_{\perp}$  is plotted against time for all the inverted landing trials in fig 21 B. The onsets of deceleration and leg extension are marked on the trajectories. The order of initiation of these 2 components are listed in table 3. Leg extension occurred first in 8 out of the 10 trials where the points of onset of both leg extension and deceleration are known.

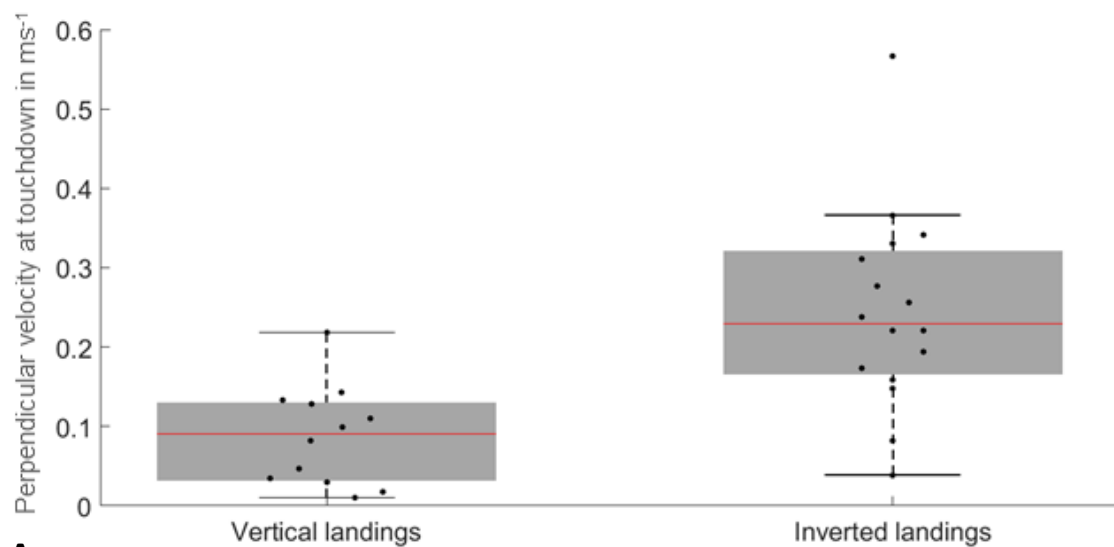




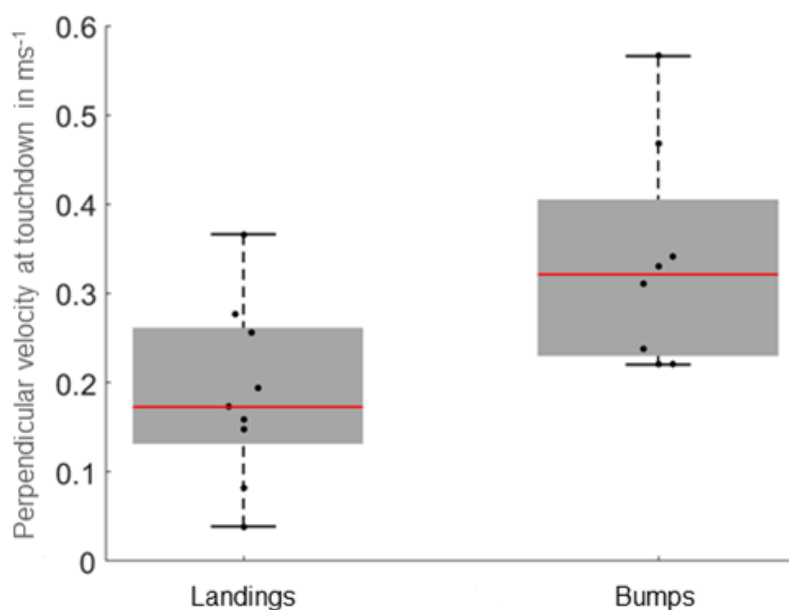
**Fig 16. (B)** There is a positive correlation between  $d_{\perp}$  and  $V_{\perp}$  at the frame of onset of decrease in  $\theta$  ( $R \approx 0.77$ ).



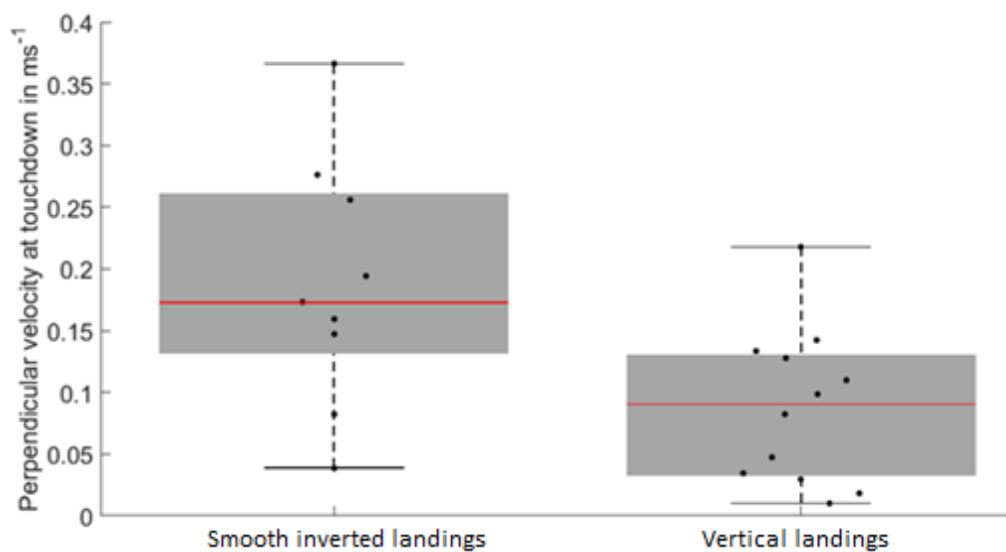
**Fig 17.** Time to contacts of the points of onset of a decrease in body orientation.



**A**

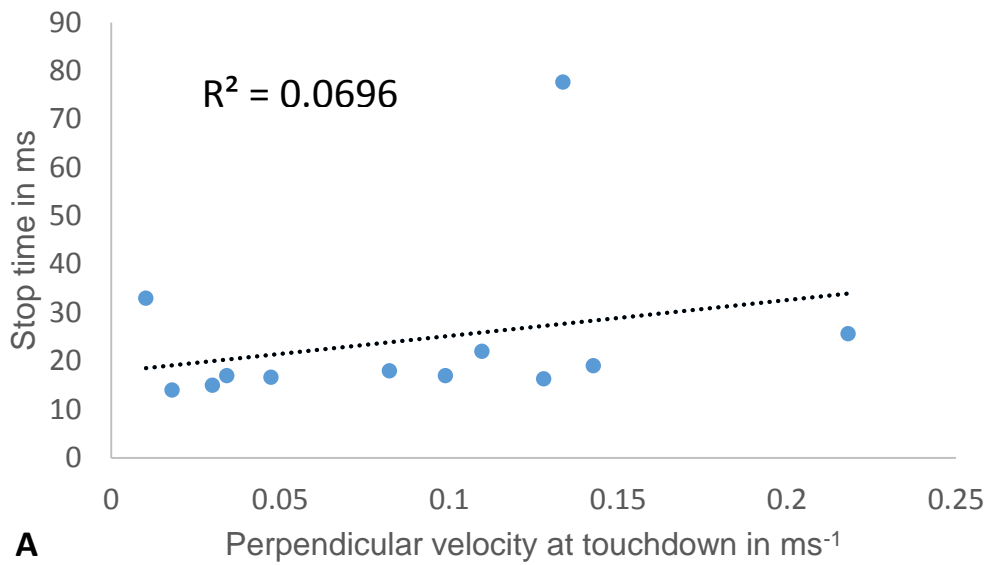


**B**

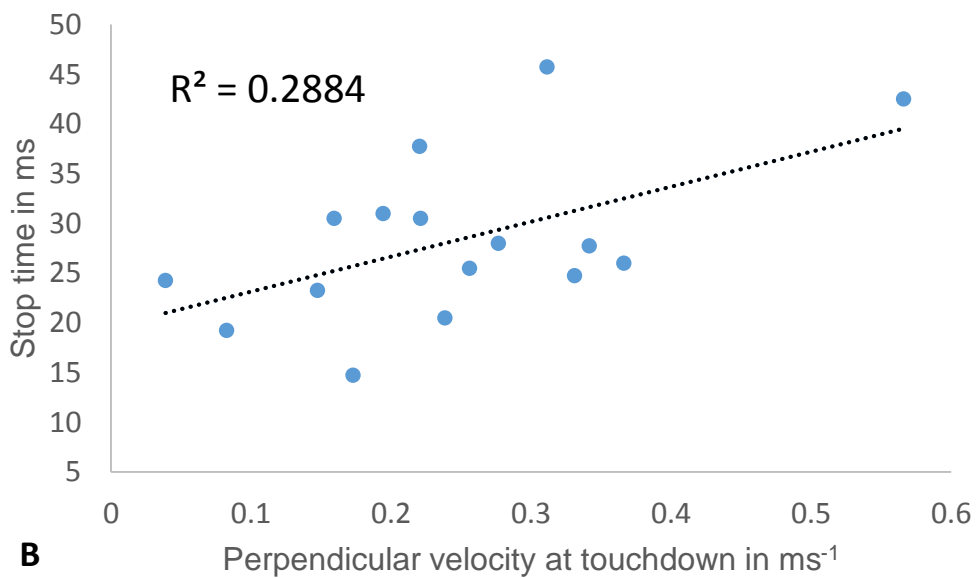


**C**

**Fig 18. Perpendicular velocities at touchdown. (A)**  $V_{\perp}$  at touchdown is significantly higher for landings on the inverted surface (Mann Whitney U test,  $p \approx 3 \times 10^{-4}$ ) **(B)** Among the inverted landing trials,  $V_{\perp}$  at touchdown is significantly higher for flies that bump into the ceiling (Mann Whitney U test,  $p = 0.0206$ ). **(C)**  $V_{\perp}$  at touchdown of the flies that land smoothly on the ceiling is still significantly higher than the  $V_{\perp}$  at touchdown for flies performing vertical landings (Mann Whitney U test,  $p = 0.0116$ ).



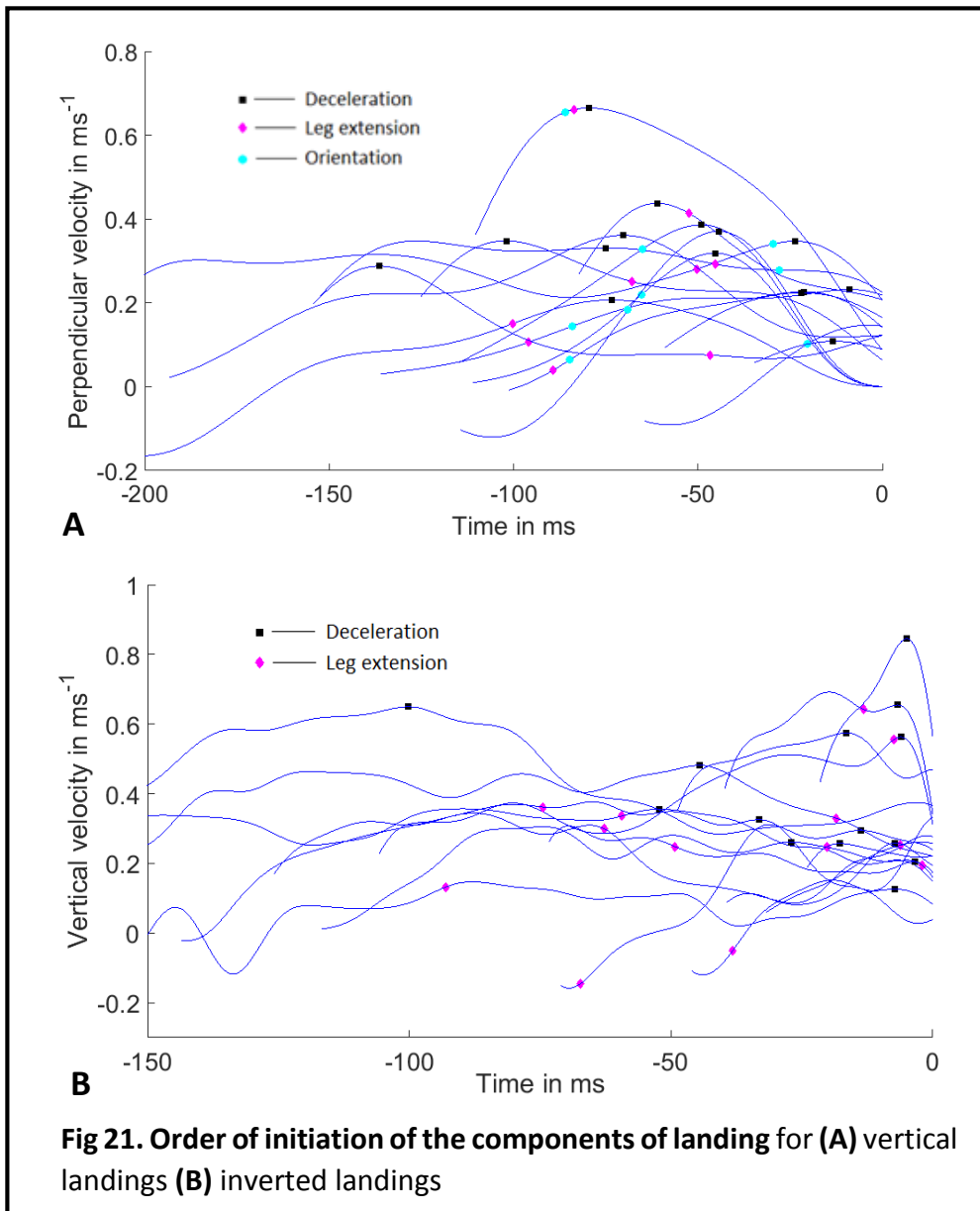
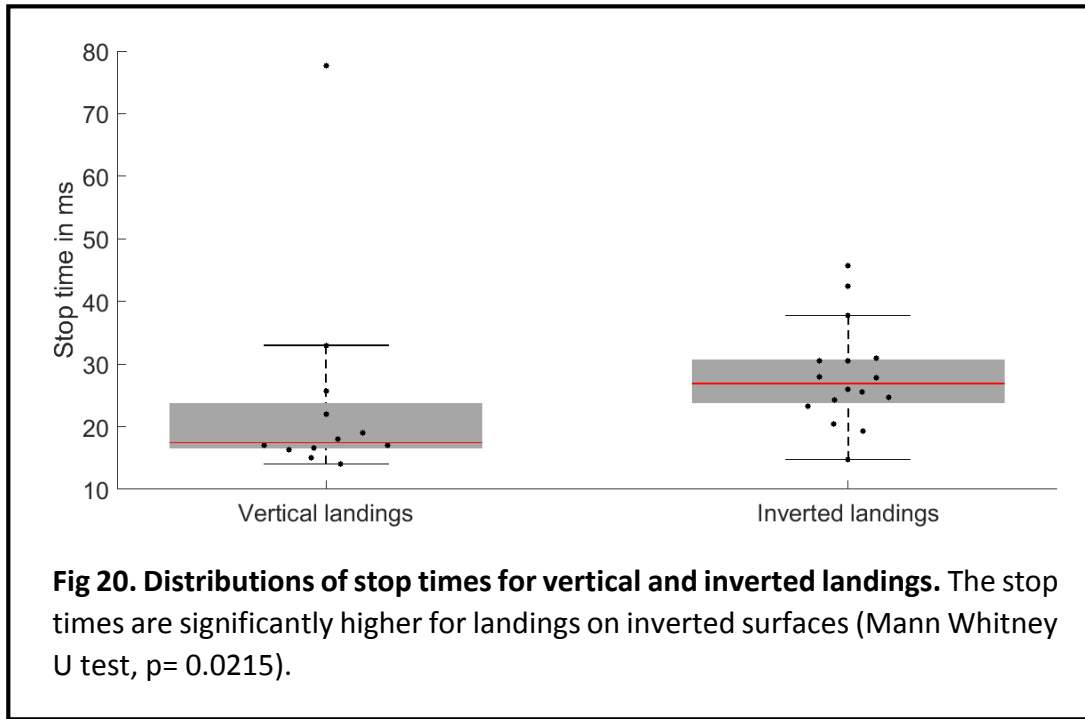
**A**



**B**

**Fig 19. Time taken for the wings to come to rest after touchdown is (A) almost independent of the perpendicular velocities ( $V_{\perp}$ ) at touchdown for vertical landings ( $R \approx 0.26$ ) (B) weakly correlated to  $V_{\perp}$  for landings on the inverted surface ( $R \approx 0.54$ ).**





Trial no.	Deceleration	Leg extension
1	-	First
2	Second	First
3	First	-
4	Second	First
5	Second	First
6	Second	First
7	Second	First
8	-	First
9	Second	First
10	First	Second
11	-	First
12	Second	First
13	First	-
14	First	-
15	First	Second
16	First	-
17	Second	First

**Table 2. Order of initiation of the components of the landing behaviour during inverted landings.** For a given trial, if a particular component of the landing behaviour does not occur, or its frame of onset is unknown, the box pertaining to that component is marked with a dash ‘-’.

Trial no.	Deceleration	Leg extension	Body orientation
1	Third	First	Second
2	Second	First	-
3	First	Second	Third
4	Third	First	Second
5	First	Second	-
6	First	Second	-
7	Third	Second	First
8	Third	First	Second
9	-	-	First
10	First	-	-
11	First	-	-
12	First	Second	-
13	Second	-	First
14	Second	-	First
15	Second	-	First
16	First	Third	Second
17	First	Second	Third
18	First	Second	-

**Table 3. Order of initiation of the components of landing behaviour during vertical landings.** For a given trial, if a particular component of the landing behaviour does not occur, or its frame of onset is unknown, the box pertaining to that component is marked with a dash ‘-’.

### Discussion:

In our study we have examined the landing behaviour of houseflies on 1. The underside of a horizontally orientated surface (inverted landings), and 2. A vertically oriented surface (vertical landings). Flies landing on both surfaces, decelerate and extend their legs as they approach the landing surface. Flies performing vertical landings orient their body vectors in the direction of the landing plane. We have not queried this behaviour for inverted landings. When flies approach the vertical surface, the points of onset of deceleration, leg extension and a reduction in the body orientation, are functions of both the component of flight velocity normal to the landing surface ( $V_{\perp}$ ) and the displacement from the landing plane ( $d_{\perp}$ ). However, the order of initiation of the components of the landing behaviour differed across individuals. Close to half the flies bump into the ceiling while performing inverted landings, even though they extend their legs before touchdown. The point of onset of deceleration and leg extension is a function of both  $V_{\perp}$  and  $d_{\perp}$  for the flies which perform smooth landings on the ceiling. Leg extension is initiated first in most of the flies. Flies reduce their

velocity in a logarithmic manner when approaching both kinds of surfaces to land. Even though most flies decelerate as they approach the underside of horizontal surface to land, they contact this surface with greater velocities as compared to their velocity at touchdown on vertical surfaces. Among the flies which perform inverted landings, the flies that bump into the ceiling contact it with higher velocities. Additionally, they take a longer to time stop beating their wings after touchdown.

**What are the cues used to trigger the initiation of the components of the landing behaviour?**

The relative retinal expansion velocity model states that the landing response is triggered when the ratio of image expansion to image size, called the relative retinal expansion velocity (RREV), reaches a threshold value (Wagner, 1982). Houseflies landing on spherical objects (Wagner, 1982) and *Drosophila* landing on cylindrical objects (van Breugel and Dickinson, 2012) decelerate at a threshold value of RREV. Can the RREV model explain the initiation of deceleration, leg extension and a reduction in body orientation in our experiments? The RREV for the landing surface can be calculated by estimating at each frame, the ratio of the rate of change of solid angle of the landing surface to the solid angle of the landing surface. It is however, non-trivial to calculate the solid angle subtended by a square surface at any arbitrary point. Therefore, we have modelled the simplest case of a fly approaching a planar square surface along an axis perpendicular to the surface and passing through the centre of the surface.

Consider a fly flying towards a square planar surface with a side  $s$ , at a perpendicular distance  $z$  from the landing surface, with a velocity  $v$  in the direction perpendicular to the surface (Fig 22).

The solid angle ( $\Omega$ ) is computed by modifying the formula to calculate the solid angle of a triangle (Van Oosterom and Strackee, 1983).

$$\Omega = \tan^{-1} \left( \frac{s^2}{2z(4z^2 + 2s^2)^{\frac{1}{2}}} \right) \tag{6}$$

The RREV can be obtained by differentiating the value of  $\Omega$  by time  $t$  and multiplying it with  $1/\Omega$ . The derived equation of RREV is as follows,

$$\text{RREV} = \frac{s^2 \cdot v \cdot f(z) \cdot g(z) \cdot \gamma}{\tan^{-1} \beta}$$

$$\text{Where, } \beta = \frac{s^2}{2z(4z^2 + 2s^2)^{\frac{1}{2}}}$$

$$f(z) = (4z^2 + 2s^2)^{-\frac{1}{2}}$$

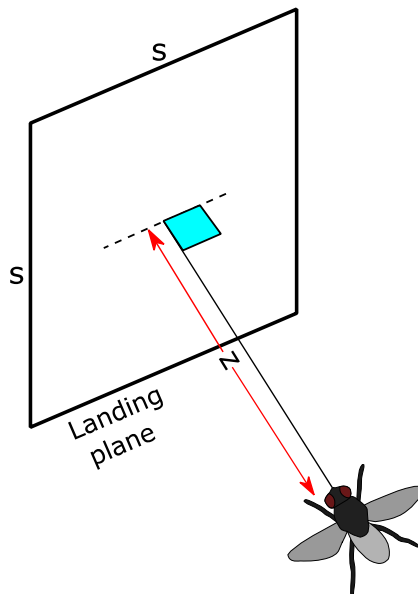
$$g(z) = \frac{2}{(4z^2 + 2s^2)} + z^{-2}$$

$$\gamma = \frac{1}{1 + \frac{s^4}{4z^2(4z^2 + 2s^2)}}$$

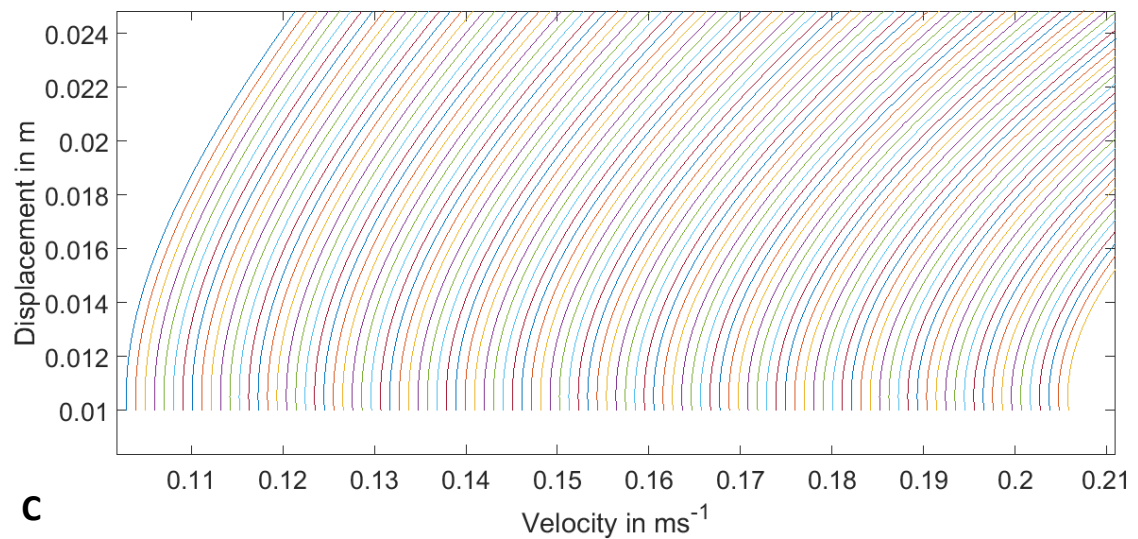
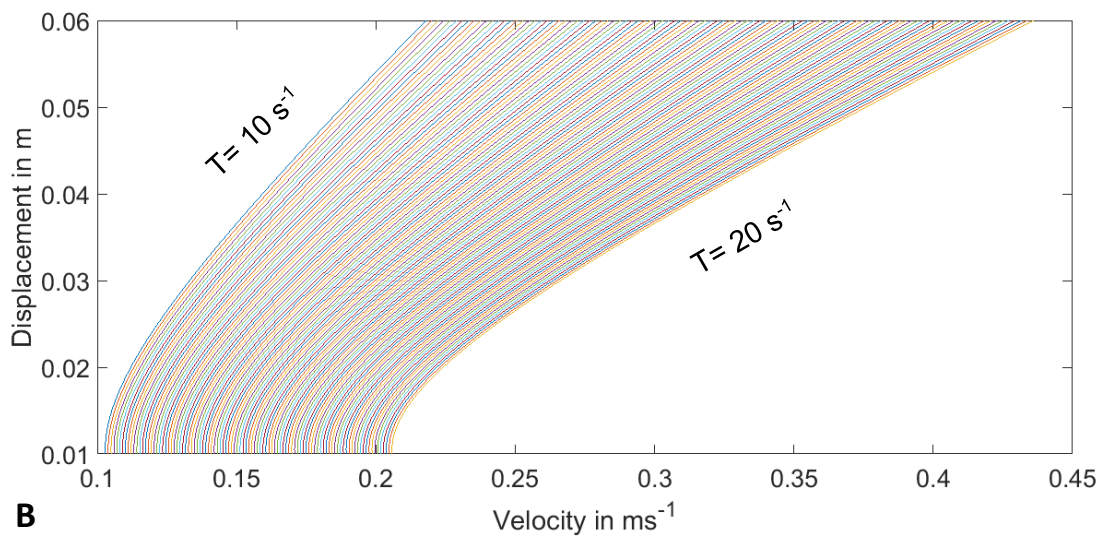
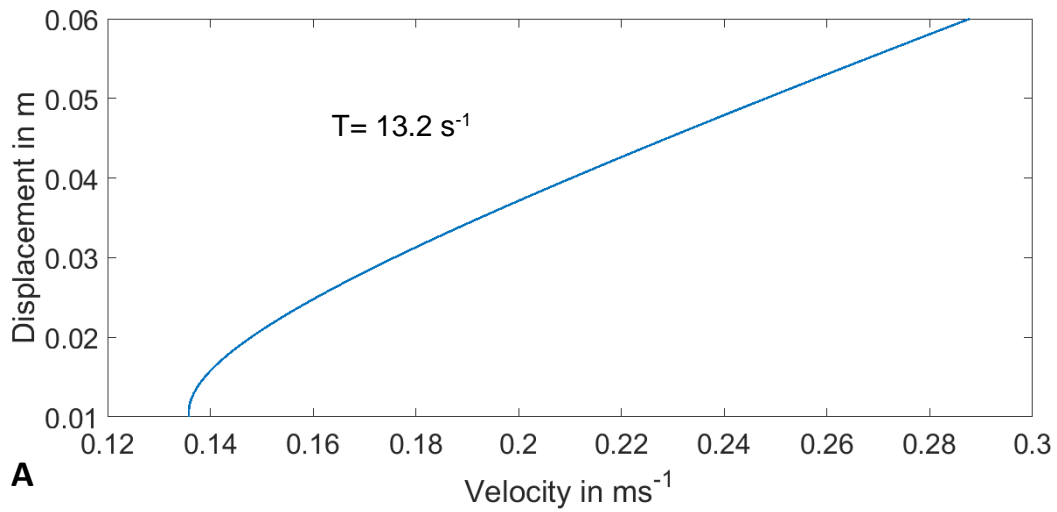
Let the threshold value of RREV for the initiation of deceleration be  $T$ . Then, the value of flight velocity at the threshold value is,

$$v = \frac{T \cdot \tan^{-1}(\beta)}{s^2 \cdot f(z) \cdot g(z) \cdot \gamma} \quad (7)$$

The side of the square  $s$  is chosen to be 0.045 m, same as the dimensions of the object. The range of  $z$  is chosen to be between 0.06 and 0.01 m, roughly the range of the  $d_{\perp}$  versus  $V_{\perp}$  plots at the frame of onset of deceleration in fig 6 A. Flies initiate deceleration at an RREV value of  $13.2 \text{ s}^{-1}$ , while landing on spherical surfaces (Wagner, 1982).  $z$  has been plotted against  $v$  for this value of threshold in fig 23 A. Deceleration is triggered at a greater distance  $z$ , at higher values of velocity  $v$ . The rate of decrease of  $z$  with  $v$  is almost linear at higher velocities, indicating that the flies decelerate at a constant time to contact in this region. There is a sharp increase in the rate of reduction of  $z$  with  $v$  at lower values of  $v$ .  $z$  is plotted against  $v$  for values of threshold RREV ranging between 10 and  $20 \text{ s}^{-1}$ , with a step size of 0.1 in fig 23 B. The slope of the linear portions of the plot decrease with an increase in the threshold. The curves for the various thresholds do not intersect, at the range of  $z$  used in the simulations (Fig 23 C). Hence, if multiple components of the landing behaviour like leg extension, deceleration and body orientation are triggered at different thresholds, the order of initiation of the components would not change, provided the thresholds are inside the range used in the simulation.



**Fig 22. Variables used to test the RREV and REV models.** The fly flies to the centre of a square plane with a side  $s$  along an axis which is perpendicular to the plane and passing through its centre. The instantaneous displacements of the fly from the landing plane are denoted by  $z$  and the instantaneous velocities are denoted by  $v$ .



**Fig 23. Predictions of RREV model for different thresholds (A)** Displacement ( $z$ ) versus velocity ( $v$ ) at a threshold value of RREV of  $13.2 \text{ s}^{-1}$ , the threshold value for deceleration observed by Wagner, 1982. **(B)**  $z$  versus  $v$  for threshold values of RREV between 10 and  $20 \text{ s}^{-1}$ . **(C)** The  $z$  versus  $v$  curves for different threshold values do not intersect each other.

In our setups for both vertical and inverted landings, flies approach the landing plane along various directions. Therefore, the results of the simulation cannot be extrapolated to explain the trend observed by us. However, it does demonstrate that a positive correlation can be achieved between the distance from the landing surface at which the landing response is triggered and the flight velocity, if the landing response is triggered at a certain threshold value of RREV. No explicit computation of distances or velocities are required. How would the nature of the  $z$  versus  $v$  plots change if the fly initiated the landing response at a threshold value of image expansion (the retinal expansion velocity, REV)? Then,  $v$  at threshold can be computed by the following equation,

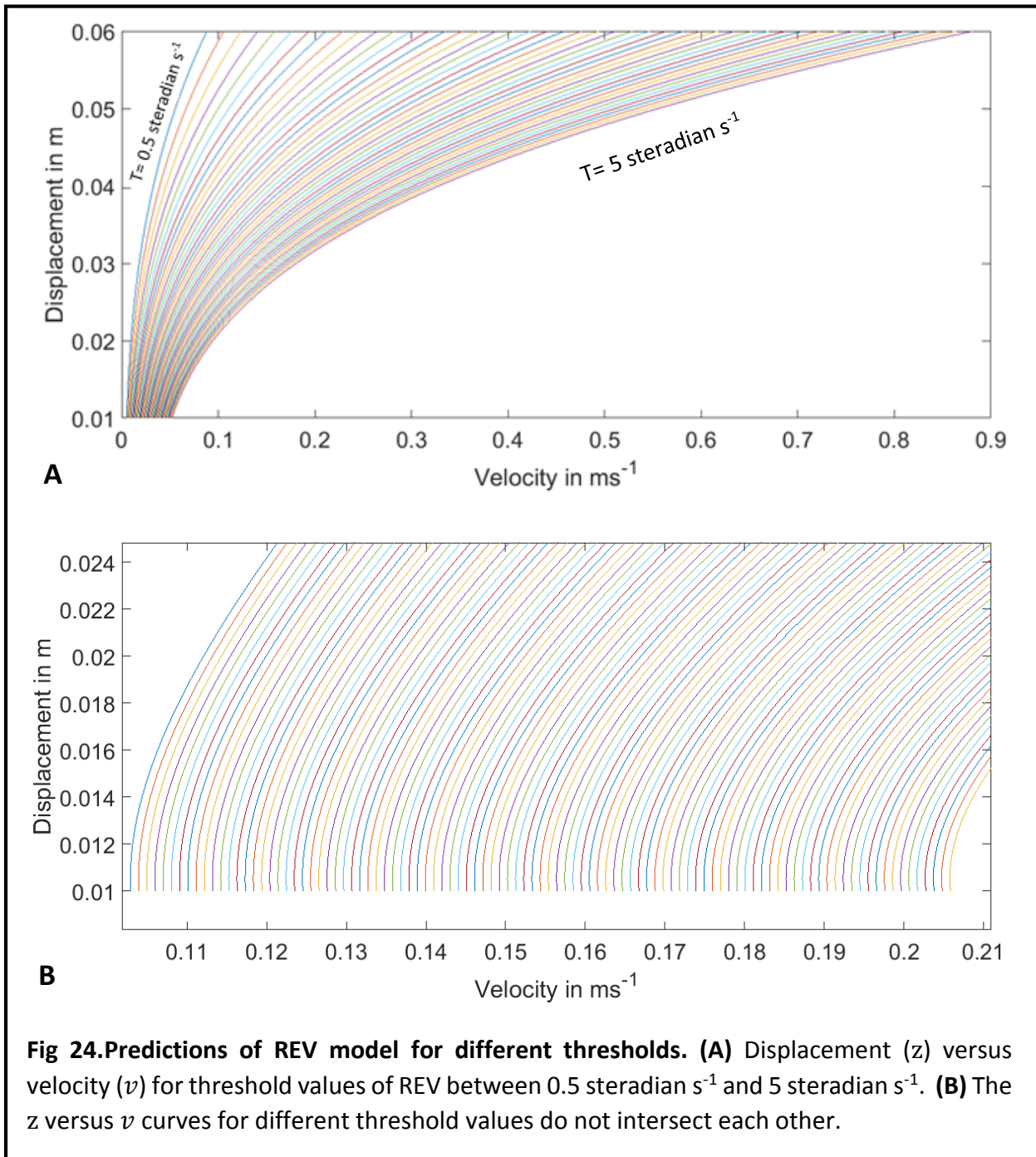
$$v = \frac{T}{s^2 \cdot f(z) \cdot g(z) \cdot \gamma} \quad (8)$$

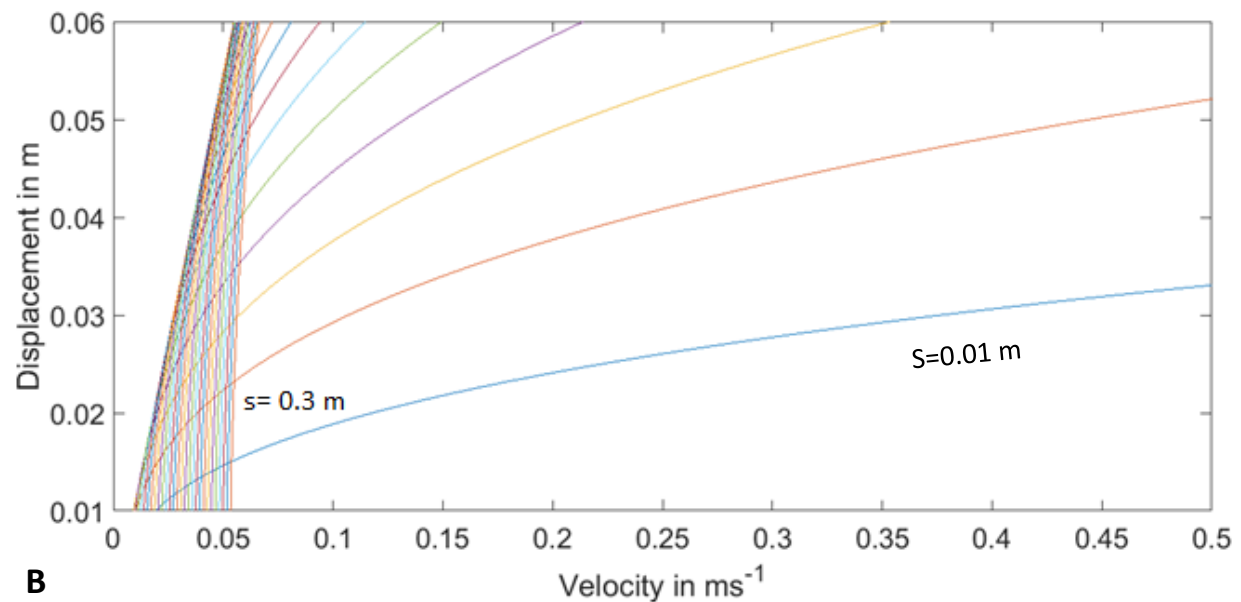
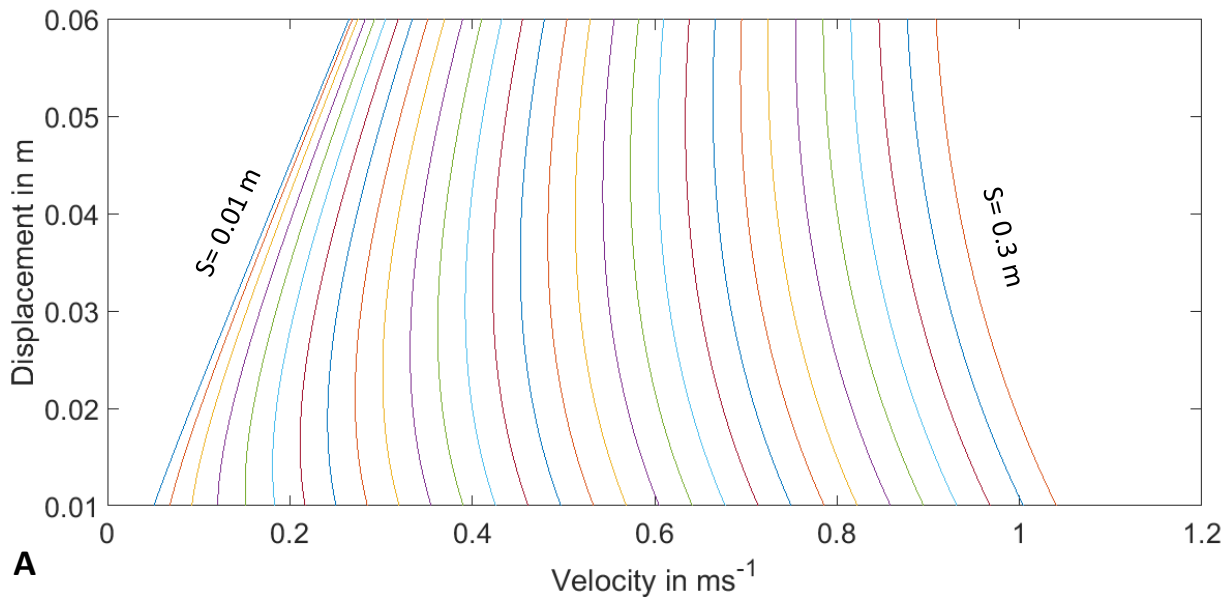
We plotted  $z$  against  $v$ , for a range of threshold values of retinal expansion velocity (REV) between  $0.5 \text{ steradian s}^{-1}$  and  $5 \text{ steradian s}^{-1}$  with a step size of  $0.1$  (Fig 24 A).  $z$  appears to increase with  $v$ , as a logarithmic function of  $v$ . A higher threshold means that the landing response is triggered at a greater velocity for a given distance from the landing surface  $z$ . The steepness of the curves increase with a decrease in threshold. The curves do not intersect (Fig 24 B). If the various components of the landing behaviour are triggered at different thresholds, the components are predicted to increase in a fixed order.

Therefore, the pattern of the landing response being initiated further away from the landing surface at higher velocities is obtained even if the fly triggers the landing response at a threshold value of REV. However, this relationship is non-linear. The simulations for both the models predict a conserved order of initiation of the components of landing. However, we are not sure how various approach angles alter the  $z$  versus  $v$  profiles of the onset of the landing response when it is initiated either at a constant value of RREV or REV. If an oblique trajectory can alter the  $z$  versus  $v$  profiles such that the curves at different thresholds intersect, then a change in the order of the components of landing at different approach velocities would not go against either the RREV or the REV models. In vertical landing experiments, the flies had more freedom to approach the landing object from various angles. In the inverted landing trials, the flies were constrained to approach the landing plane within an area of  $(5 \times 5) \text{ cm}$ . The approach trajectories were closer to the trajectory in the simulations. Maybe this is the reason that the order of the landing components are more preserved for this orientation (see table 3).

We have assumed the flies estimate the rate of expansion of the entire landing object. This would mean that the value of RREV for a given value of flight velocity and distance from the ceiling is dependent on the dimensions of the landing object (at least for a square planar object). We plotted  $z$  versus  $v$  at a threshold value of RREV of  $13.2 \text{ s}^{-1}$ , for different values of the side of landing object  $s$  (Fig 25 A). We varied  $s$  from  $0.01 \text{ m}$  to  $0.3 \text{ m}$ . As  $s$  grows larger,  $z$  begins to decrease with increasing  $v$ , at higher values of  $v$ . This would mean that a fly flying at higher velocities would initiate the landing response closer to the landing object. This strategy would not be feasible, as it would

result in crash landings when landing on large objects. We also tested the effect of object size, when flies initiate the landing response at a critical value of REV. The results of the simulations are illustrated in fig 25 B. The threshold retinal expansion velocity was chosen to be 1 steradian  $s^{-1}$ . At higher values of  $s$ , the slopes of the decrease in  $z$  with  $v$  reach close to  $90^\circ$ . This would mean that at higher velocities, the fly would initiate the landing response at distances tending to infinity. This strategy is highly impractical. Therefore, our simulations suggest that it is unlikely that the flies estimate the expansion rates of the entire object, especially when landing on larger objects.





**Fig 25. Dependence of the RREV and REV models on object size. (A)** The  $z$  versus  $v$  curves of the points of onset of deceleration, at a threshold value of retinal expansion velocity of  $13.2 \text{ s}^{-1}$ , for object sizes ranging from a side length of  $0.01 \text{ m}$  to  $0.3 \text{ m}$ . **(B)** The  $z$  versus  $v$  curves of the points of onset of deceleration, at a threshold value of retinal expansion velocity of  $1 \text{ steradian s}^{-1}$ , for object sizes ranging from a side length of  $0.01 \text{ m}$  to  $0.3 \text{ m}$ . The curves become very steep at larger object sizes.

However, flies could be estimating the rate of expansion of the landing object by measuring the rate of expansion of a point on the landing object, as suggested by Baird et al., 2013. If so, do the values of the rate of expansion depend on the point chosen? Consider a fictitious circle drawn around the centre of the square. The radius of the circle is less than  $0.5 s$  (Fig 26). Let  $\alpha$  be the angle subtended by a point at a



distance  $r$  from the centre of the circle, on the retina. The rate of expansion of the point is given by the equation derived in Baird et al., 2013,

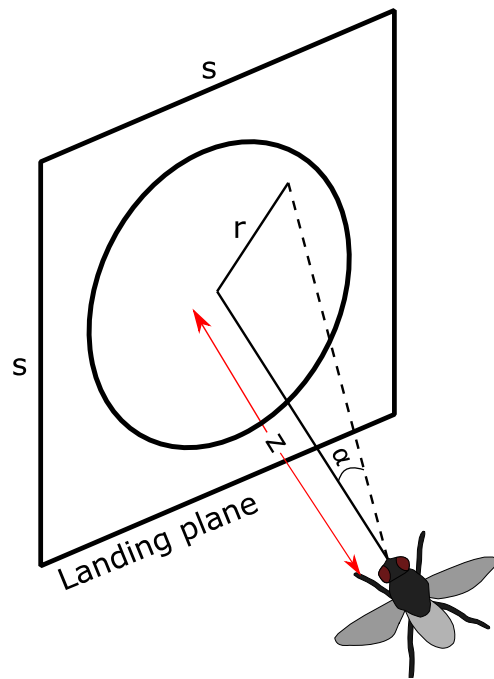
$$\frac{d\alpha}{dt} = \frac{v}{2z} \sin 2\alpha$$

Therefore, the rate of expansion is dependent on the angular position of the point. The relative retinal expansion velocity of the point is,

$$\frac{1}{\alpha} \frac{d\alpha}{dt} = \frac{v}{2z\alpha} \sin 2\alpha$$

The relative retinal expansion velocity is also a function of the angular position of the point. If the flies utilized expansion cues from an arbitrary point on the landing object to initiate landing, then one would not expect a correlation between  $z$  and  $v$  at the point of onset of the landing response like we observe in our results. The onsets of initiation of deceleration while landing on cylindrical and spherical objects occur at a threshold value of RREV (van Breugel and Dickinson, 2012; Wagner, 1982). Wagner, 1982, used spheres of different sizes (0.25 to 1 cm in radii). However, he did not quantify the effect of object size on the initiation of the landing response. Also, he made an approximation in the calculation of RREV, which removed the dependence of the RREV on the radius of the sphere. It would be interesting to study the initiation of the various components of landing on objects having drastically different sizes.

The spatio temporal integration model (STIM) has been proposed to explain the landing response (Borst, 1989, 1990; Borst and Bahde, 1988; see introduction for more details). STIM entails the landing response to be a function of optic flow along the entire visual field. It is almost impossible to test in free flight, as one cannot ensure that each fly landing on an object receives the same set of visual stimuli. In tethered conditions, the landing response has been shown to be dependent on the size and velocity of the looming object, as predicted by STIM (Borst and Bahde, 1988). In our study, despite the variance in the trajectories of the flies we do observe a positive correlation between the velocity and distance of the fly from the landing surface, at the points of onset of a landing component. The results are similar for other studies of landing in free flight (van Breugel and Dickinson, 2012; Wagner, 1982). Such results suggest that expansion of the image of the landing object is the most important stimulus for initiation of the landing behaviour. The same object can be kept in various visual backgrounds and the initiation of the landing response can be compared to see if the visual panorama plays a role in initiation. When *Drosophila* approach a cylindrical object, the point of onset of deceleration was found to be dependent on the velocity and distance from the landing object but not on its contrast (van Breugel and Dickinson, 2012). This result provides goes against the predictions of STIM, as STIM predicts a dependence on the landing response on the contrast of the object.



**Fig 26. Expansion of a point on the landing object.** Flies could be estimating the expansion of the object by measuring the expansion of an arbitrary point on the landing surface.  $r$  is the distance of an arbitrary point from the centre of the landing surface.  $z$  and  $s$  have the same meaning as in fig 22.  $\alpha$  is the angle subtend by the point on the retina of the fly.

The time to contact model of landing states that flies initiate a landing response at a constant time to collision from the landing surface. Our study provides some support to this model as we obtain almost linear relationships between the distance and velocity from the landing surface at the point of initiation of the components of the landing response. However, we obtained distributions of time to contact values, with high values of coefficient of variation. Therefore, it does not seem that the flies are releasing landing behaviours at a constant time to contact (Fig 6 B, 9, 14 B, 17). The sample size of the study should be increased to check if the variance of the time to contact distribution reduces and consequently, the  $R^2$  of the regression between the distance and velocity at the point of onset of deceleration increases. If this happens, then the time to contact hypothesis will gain more ground. The RREV model predicts that the flies would initiate landing behaviours at a constant time to contact at higher flight velocities when approaching plane square surfaces along an axis perpendicular to the plane and passing through its centre (Fig 23 A). When flies approach spherical surfaces, the RREV is approximately inverse of the time to contact, provided that the angular size of the object is small (van Breugel and Dickinson, 2012). It seems highly unlikely that flies can compute the time to contact by measuring absolute distances and velocities (discussed later).

Flies extended their legs at a displacement range of 0.5 cm to 5 cm from the landing surface, when the flight velocities at the onset of deceleration were roughly between 0.1 and 0.7  $\text{ms}^{-1}$ . Tethered houseflies extended their legs much earlier (at a distance between 10 and 20 cm) when discs with varying diameters were brought towards the fly at similar speeds (Borst and Bahde, 1988). This is not surprising because flies in tethered conditions do not receive all the sensory stimuli that freely flying flies would

experience, like for example, the airflow sensors in the head would receive little or no sensory stimulation in tethered individuals. In our experiment, the leg extension behaviour was a function of both distance from the landing target and velocity. The flies also reduce their body angle with respect to the landing surface on approach, similar to honeybees (Evangelista et al., 2010) and bumblebees (Reber et al., 2016). However, unlike these 2 insects, they do not hover before touchdown (Fig 5 A, B). *Drosophila* flying towards a rod shaped landing object extend their legs at a critical distance from the landing surface, with little dependence on flight velocity. They also do not orient towards the landing post before touchdown (van Breugel and Dickinson, 2012). This is interesting because it indicates that these 2 species of dipterans have evolved differences in their landing response. van Breugel and Dickinson, 2012, found that the relationship between the distance from the landing target and flight velocity at the onset of deceleration is well approximated by a linear equation ( $R^2=0.8$ ,  $n=140$ ). Houseflies landing smoothly (excluding bumps) on both vertical and inverted surfaces showed a similar trend, with  $R^2$  values of the regression being  $\approx 0.7$  and  $\approx 0.9$  respectively. High values of  $R^2$  were obtained despite the number of trials being small, 16 for vertical landings and 7 for inverted landings. Does this mean that dipterans begin deceleration at a point that is a function of both velocity and distance from the landing target, but independent of the actual nature of the landing target? More studies need to be performed to confirm this observation.

### ***How is deceleration controlled?***

It has been proposed that after deceleration is initiated, it is controlled by maintaining the angular velocity of an arbitrary point on the landing surface at a constant value. This model predicts a uniform deceleration when landing is carried out on plane surfaces (Baird et al., 2013). However, in our experiments, we observe the curves of velocity versus distance to be better approximated by a logarithmic regression, although lines also fit the data well (Fig 10, 11, 12, 13). The shapes of our deceleration profiles for both inverted and vertical landings are similar to the deceleration profiles of *Drosophila* approaching cylindrical surfaces to land (see fig 8 A in van Breugel and Dickinson, 2012). It indicates that the control of deceleration could be independent of the nature of the landing surface. If you look closely at our velocity versus distance plots, the magnitude of deceleration is initially low, increases and then almost remains constant. Physiological or mechanical constraints might prevent the fly decelerating uniformly right after the onset of deceleration. The fly may be able to decrease velocity uniformly only after a certain time lag. Therefore, we cannot rule out the possibility that the flies control the final stage of deceleration by maintaining the angular velocity of a point on the landing surface at a constant value.

### ***Differences between vertical and inverted landings***

We observed that close to 50% of the flies (8 out of 17) bumped into the underside of the horizontal surface, even though they extended their legs before touchdown. We did not observe any bumps when flies landed on the vertically oriented surface. One obvious explanation for this observation is that the flies contacted the inverted surface at higher velocities (Fig 18 A). The higher momentum could be responsible for the head colliding with the ceiling. This hypothesis is further corroborated by the

observation that in the inverted landing trials, the ones that bumped into the ceiling had higher velocities at touchdown (Fig 18 B). The flies which performed a smooth landing on the inverted surface still had significantly higher velocities at touchdown than the flies performing vertical landings (Fig 18 C). Why do flies land on inverted surfaces with higher velocities? For performing inverted landings, flies have to orient themselves in the upside down direction at the end of the manoeuvre. However, we have not come across any studies mentioning that flies can fly upside down stably for long periods. Therefore, the flies have to rotate their bodies either before or after touchdown. We mentioned in the materials and methods section that the turning frequencies were higher when the flies performed inverted landings. Such a manoeuvre, with a larger number of components is bound to be more error prone. It could also be the case that the higher velocities at touchdown could have an adaptive value, possibly reducing the energetics of the landing manoeuvre. Studies on the biomechanics of landing could help to test this hypothesis. We observed that flies took a longer time to stop beating their wings after contact, while performing inverted landings (Fig 20). This trend cannot be completely explained by the observation of greater velocities at touchdown for inverted landings, as the correlation between the time taken for the wings to rest and the velocity at touchdown is weak (Fig 19). In most cases, the fly inverted itself upside down only after contact with the ceiling. We observed very little body rotation after touchdown on vertical surfaces. Therefore, for inverted landings, it is reasonable to assume that the wings beat a longer time to complete the body rotation. However, this hypothesis has to be explicitly tested.

### ***Can flies use cues other than image expansion to initiate and control the landing response?***

The lenses of compound eyes cannot change their focus. This rules out the possibility of distance estimation by accommodation. The eyes of insects are immobile, therefore they cannot estimate distances by comparing the angles moved by both the eyes to view the object (Srinivasan, 1992). Can flies use stereoscopic vision like us to estimate depth? Insects in general have closer spaced eyes and lower visual acuity compared to mammals. Thus, it is unlikely that the images formed by each eye have sufficient angular separation to estimate distances by stereopsis. In fact, the only known insect where depth estimation by stereopsis is confirmed is a species of praying mantis, which has widely spaced eyes (Rossel, 1983; Srinivasan, 1992). The fact that the landing response can be elicited in one eyed flies by a looming stimulus (Goodman, 1960) indicates that stereopsis is not being used to estimate depth while landing. Some insects can estimate depth by a phenomenon called motion parallax, wherein the insect translates its head to one side and depth is determined by the optic flow rate of the object (Srinivasan, 1992). We do observe the flies oscillating with each wing beat. However, depth perception by motion parallax requires translation. Flies have been shown to extend their legs in a fashion similar to the landing response when the light intensities are suddenly reduced (Borst, 1986; Goodman, 1960). When flies approach close to surfaces, the light intensities would reduce. We cannot rule out this model as a potential cause to initiate the landing response in the vertical landing experiments. However, we attracted flies to land on the inverted surface by shining

U.V light on it. All the flies still showed at least the leg extension response. Controlled experiments with different profiles of light intensities near the landing surface can be used to test the “reduction in light intensity hypothesis”. In one vertical landing trial the fly extended its legs while moving away from the landing surface. 6 flies performing inverted landings extended their legs during takeoff, and continued to keep them extend till touchdown. These results indicate that cues other than image expansion can be used to initiate at least certain components of the landing behaviour.

### **Conclusion and future directions**

Our study provides support to the hypothesis that flies use cues from the expansion of the landing target to initiate and guide landings. However, it is still unclear as to how they measure image expansion, and how the optic flow of the surroundings can impact the initiation and control of the landing response. Intuitively, landing on the ceiling seems more difficult. This idea is supported by the observation that flies make more errors while performing inverted landings. Future experiments could be directed at testing the impact of the size, and contrast of the landing object on the landing response. Figuring out the role of the visual environment surrounding the landing object, as well as that of light intensity profiles around the object is another major question to be addressed. Flies touchdown at relatively higher velocities while performing inverted landings. The mechanics of how they how they avoid injuries despite high contact velocities is an exciting problem for the future.

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