RESEARCH ARTICLE

Effect of light intensity on flight control and temporal properties of photoreceptors in bumblebees

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ABSTRACT

To control flight, insects rely on the pattern of visual motion generated on the retina as they move through the environment. When light levels fall, vision becomes less reliable and flight control thus becomes more challenging. Here, we investigated the effect of light intensity on flight control by filming the trajectories of free-flying bumblebees (*Bombus terrestris*, Linnaeus 1758) in an experimental tunnel at different light levels. As light levels fell, flight speed decreased and the flight trajectories became more tortuous but the bees were still remarkably good at centring their flight about the tunnel's midline. To investigate whether this robust flight performance can be explained by visual adaptations in the bumblebee retina, we also examined the response speed of the green-sensitive photoreceptors at the same light intensities. We found that the response speed of the photoreceptors significantly decreased as light levels fell. This indicates that bumblebees have both behavioural (reduction in flight speed) and retinal (reduction in response speed of the photoreceptors) adaptations to allow them to fly in dim light. However, the more tortuous flight paths recorded in dim light suggest that these adaptations do not support flight with the same precision during the twilight hours of the day.

KEY WORDS: Insects, Vision, *Bombus terrestris*, Retina, Behavioural adaptation, Speed control, Position control

INTRODUCTION

In bright light, many insects can be observed to fly rapidly through cluttered natural settings. Information about flight speed and the proximity to nearby surfaces in the environment is extracted from the pattern of visual motion generated on the retina during flight (Baird et al., 2005, 2010; Srinivasan et al., 1996; David, 1982; Fry et al., 2009; Dyhr and Higgins, 2010). However, as light levels fall, vision becomes less reliable. A partial explanation for this is the random and unpredictable nature of photon arrivals: the number of photons absorbed by a photoreceptor from a constant light source per unit time will vary slightly over time. This variation in photon count is referred to as 'shot noise'. If a photoreceptor absorbs N photons, this shot noise will be $\sim\sqrt{N}$ (as a result of the Poisson statistics governing random photon arrivals) (Warrant and McIntyre, 1993). As light levels fall, the shot noise relative to the signal thus gradually increases, which makes it more difficult for the visual system to extract reliable information (Warrant, 2008). In addition to this external source of noise, other internal sources of noise (intrinsic within the photoreceptors themselves, like ion channel noise) also reduce the reliability of vision in dim light (Warrant,

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2008; Barlow, 1956). However, there are ways to improve photon catch and make the eyes more sensitive. Nocturnal and crepuscular insects, like bumblebees, that have apposition compound eyes (Land and Nilsson, 2012) can increase their reliability in dim light by developing wider facets or rhabdoms, and a longer photoreceptor response time (Greiner et al., 2004; Warrant et al., 2004; Frederiksen and Warrant, 2008). It is also likely that adaptations for dim light vision in these insects occur at higher levels in the visual system, particularly via the summation of retinal signals over space and time (Snyder, 1977; Snyder et al., 1977a,b; Warrant, 1999; Pick and Buchner, 1979; van Hateren, 1993). Together, these adaptations would serve to greatly improve the visual system's performance in dim light, but at a cost; spatial and temporal resolution would be reduced (Warrant, 2008, 1999). In other words, signal coding in the visual system tends to become coarser and slower as light levels fall. Despite these limitations, nocturnal and crepuscular insects with apposition eyes fly and see very well at extremely low light intensities (Frederiksen and Warrant, 2008; Somanathan et al., 2008; Baird et al., 2011; Theobald et al., 2007).

One way to compensate for decreased spatial and temporal resolution in dim light is to reduce flight speed. This would enable the insect to gather more visual information per unit distance travelled. Indeed, tethered hornets reduce their flight speed in dim light (Spiewok and Schmolz, 2006) and free-flying honeybees have been observed to do the same (Rose and Menzel, 1981), but this observation has never been quantified. The nocturnal sweat bee *Megalopta genalis*, in contrast, approaches its nest at a constant flight speed over a large range of light intensities (Theobald et al., 2007). However, approaches to the nest became increasingly error prone and erratic in dim light, suggesting that *M. genalis* uses spatial summation, rather than temporal summation, in order to collect enough visual information to control flight under dim light conditions.

Here, we characterised the effect of falling light intensity (from 600 to 3.4 lx) on the flight performance of freely flying bumblebees (*B. terrestris*). We also investigated whether the flight performance can be explained by visual adaptations in the photoreceptors. We found that both behavioural (decreased flight speed) and retinal (increased photoreceptor integration time) adaptations probably evolved to allow bumblebees to fly in dim light. However, as light intensity decreased, the flight trajectories became more tortuous and eventually the bees stopped flying altogether. This suggests that the behavioural and retinal adaptations adopted by the bee's visual system in dim light do not support flight during the twilight hours of the day with the same precision as in bright daylight.

RESULTS

All the flight paths recorded at 600, 60, 6 and 3.4 lx are shown in Fig. 1. Four bees were observed to fly in all of these conditions (highlighted in Fig. 1). Even though the two darkest conditions (6 and 3.4 lx) were presented to the bees more than twice as often as

List of symbols and abbreviations

D	diameter
f_c	corner frequency
FFT	fast Fourier transform
I_{light}	light intensity
T	temperature
t_p	time to peak
V_{flight}	flight speed
VRM	voltage response modulation
Δt	response half-width
$\Delta\rho$	acceptance angle
σ	response skewness

the brighter conditions (see Table 1), we still obtained fewer flights in these conditions. This indicates that the bees are less motivated to fly at light levels below 6 lx. No bees were observed to fly at light levels below 3.4 lx.

The effect of light intensity on flight speed and temporal resolution

We found that the average flight speed (V_{flight}) decreased significantly with falling light intensities (I_{light}) (Kruskal–Wallis test; $P<0.001$, Mann–Whitney U-test; 600–60 lx: $P<0.001$, 60–6 lx: $P<0.001$). Flight speed could also be predicted from log light intensity by the following formula: $V_{flight}=45.09-16.34\times[-\log(I_{light})]$, $R^2=0.94$ (linear regression, see Fig. 2). One possible explanation for this result is that the data contain 'slow' and 'fast' bees that are adapted for flight at different light intensities. The fast

bees would be able to fly as long as the temporal resolution of their photoreceptors allows them to keep their preferred speed, whereas the slow bees would also be able to fly in dim light. To investigate this possibility, we analysed the variation in flight speed for the 12 individual bees that flew in at least four light conditions each (see Fig. 3). We found that in each of these cases there was a positive relationship between log light intensity and flight speed (average slope=15.5; the probability of obtaining 12/12 positive slopes is 0.0002, binomial test). Together, these results strongly suggest that bumblebees fly slower as light levels fall.

To test whether the decrease in flight speed is a behavioural compensation for retinal visual adaptations to dim light conditions, we characterised the temporal resolution of the photoreceptors under the same light conditions. We found that response speed, characterised by f_c (−3 dB corner frequency of the frequency response), dropped by 24% at room temperature (23°C) (Table 2) between the brightest and the lowest light conditions. This drop in f_c was statistically significant when intensity was reduced from 477 to 6 lx and from 6 to 3.4 lx (Kruskal–Wallis test; $P<0.001$, Mann–Whitney U-test; 477–6 lx: $P=0.004$, 6–3.4 lx: $P=0.041$). To match the results of the electrophysiological recordings to the behaviour of the free-flying bumblebees, we calculated the Q_{10} values for the time to peak (t_p) and the skewness of the response (σ): 0.65±0.07 and 0.86±0.08, respectively. These values are in line with previously published values from the fly (Tatler et al., 2000). The temperature of the head of a flying bumblebee was found to be 34.9°C. By using Q_{10}-corrected values for t_p and σ, we estimated that f_c at the flight temperature increases approximately twofold compared with room temperature values (Table 2). This is also well in line with
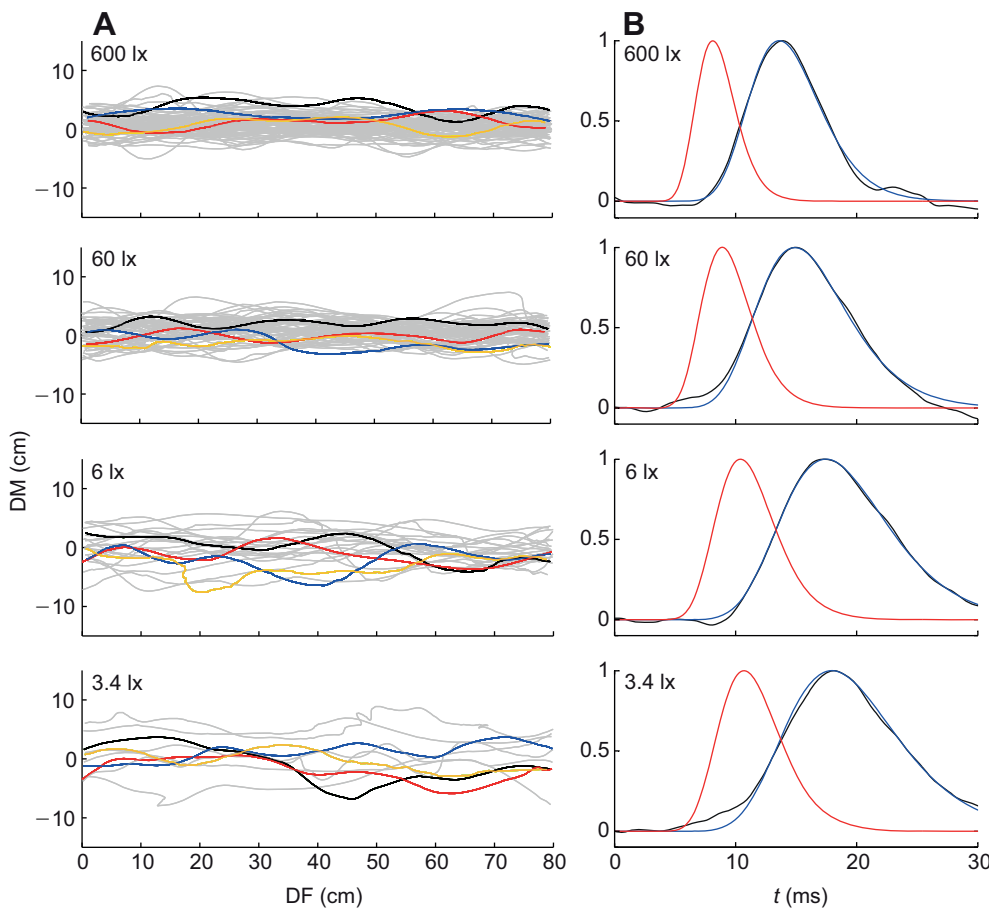

Fig. 1. The effect of light intensity on flight trajectory and photoreceptor impulse responses. (A) Flight trajectory. Flight paths recorded at 600, 60, 6 and 3.4 lx are shown in grey. Four bees flew in all of these conditions (highlighted in black, blue, red and orange). As light levels decreased, the flight trajectories became more tortuous. For the total number of flights in each condition, see Table 1. (B) Photoreceptor impulse responses. Black lines indicate the mean of recorded impulse responses at room temperature. Blue lines indicate the log-normal fit to the mean response. Red lines indicate the log-normal fit with parameter values temperature corrected to a flight temperature of 34.9°C. DM, distance from midline; DF, distance flown.

Table 1. Details of data collection

Condition	Light intensity (lx)	Behavioural experiments			Electrophysiology	
		No. of test sessions	No. of flights	No. of individuals	No. of cells	No. of individuals
1	600*	13	75	41	13	11
2	190	12	68	38	12	10
3	60	16	57	35	13	10
4	19	19	31	23	14	10
5	6	31	23	15	15	10
6	3.4	31	10	6	18	12

As light levels decreased, fewer individuals were motivated to fly to the feeder.
*In electrophysiology, 477 lx. See Materials and methods.

previous results in the fly (Tatler et al., 2000). The drop in f_c between the extreme light conditions was 25% at the flight temperature. Temperature-adjusted f_c could be predicted from log light intensity by the following formula: $f_c = 87.98 - 14.09 \times [-\log (I_{light})]$, $R^2 = 0.84$ (linear regression, see Fig. 4). Thus, the response speed of bumblebee photoreceptors decreases as light levels fall.

Photoreceptor voltage response modulation
We estimated the amount of voltage response modulation (VRM) that the chequerboard patterns on the flight tunnel walls produced on the bumblebee photoreceptors during flight by using the model described by Land (1999). At the brightest light level, VRM of the photoreceptors of a flying bumblebee was ∼0.43 (Fig. 5). As light intensity fell to 3.4 lx, VRM dropped only 3% to ∼0.42. However, the VRM could be predicted from log light intensity by the following formula: $VRM = 0.42 - 0.006 \times [-\log (I_{light})]$, $R^2 = 0.58$ (linear regression, see Fig. 5). This suggests that VRM of bumblebee photoreceptors during flight drops with falling light levels.

The effect of light intensity on position control
A straight track down the centre of the experimental tunnel represents the safest path through the tunnel. In the two brighter conditions, the flight trajectories were relatively straight and smooth. However, as light levels were reduced to 6 and 3.4 lx, the paths became more tortuous and uneven (Fig. 1). This indicates that

it gets more difficult for the bees to control their flight position in the tunnel in dim light. To investigate the effect of light intensity on the straightness of the flight trajectory, we measured the length of the flight paths (Table 3). A longer path length indicates a more tortuous flight trajectory. It may also result from a flight where a bee would pause and turn, fly backwards, and then turn again to continue its flight to the feeder. However, in the few cases where this occurred, the data were excluded from the analysis. In the brightest condition, the average path length over an 80 cm section of the tunnel was 81.9±1.9 cm. Because a perfectly straight path would be 80 cm long, this indicates that in bright light conditions bees fly relatively straight. In the darkest condition, the length of the path increased to 84.0±2.4 cm. Although this small increase in path length may seem negligible, there was a significant difference between path length under the different light conditions (Kruskal–Wallis test; $P < 0.001$). Moreover, path length increased significantly when light intensity was reduced by an order of magnitude from 600 to 60 lx, and then from 60 to 6 lx (Mann–Whitney U-test; 600–60 lx: $P = 0.017$, 60–6 lx: $P = 0.042$). This indicates that the flight paths of bumblebees gradually become longer and more tortuous as light intensity decreases.

To investigate the effect of light intensity on centring behaviour, we also measured the average distance of the bee from the midline of the tunnel under the different light intensities (Table 3). In all six conditions, the flight paths were centred within 0.9 cm (condition 2, see Table 3) to the left or to the right of the midline of the tunnel.
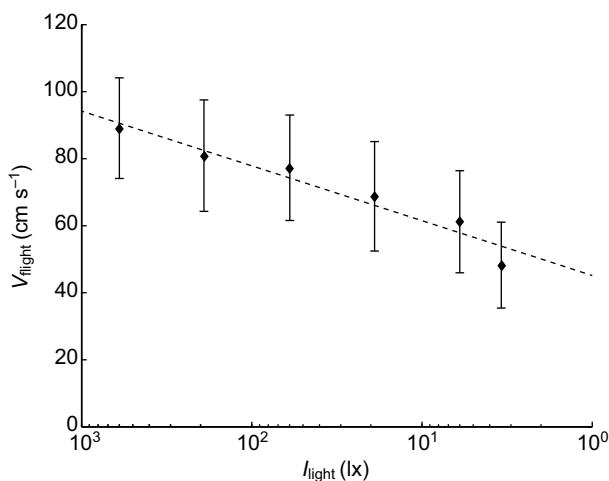

Fig. 2. The effect of light intensity on flight speed. The symbols indicate mean values of flight speed (V_{flight}); error bars indicate ±2 s.d., i.e. 95% of the data points fall within this range. A linear correlation {$V_{flight} = 45.09 - 16.34 \times [-\log(I_{light})]$, $R^2 = 0.94$} was found between flight speed and log light intensity I_{light}.
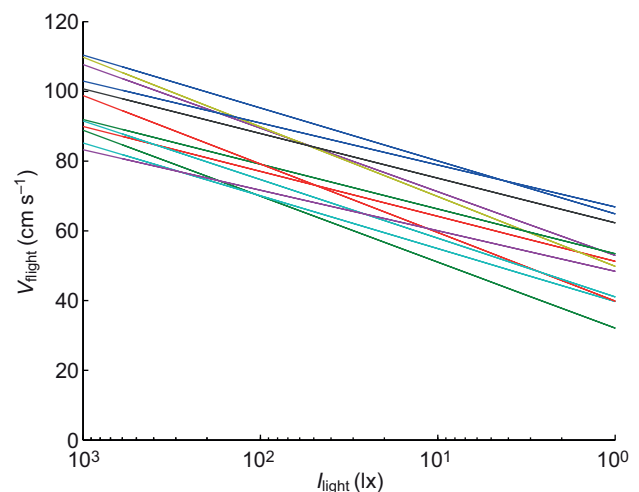

Fig. 3. The effect of light intensity on flight speed in 12 individual bees. Each line indicates the linear relationship between flight speed and log light intensity of each individual bee that flew in at least four light conditions. In all 12 bees, there was a positive relationship between flight speed and log light intensity.

Table 2. Photoreceptor properties under different light conditions

Condition	Room temperature (23°C)				Flight temperature (34.9°C)			
	t_p (ms)	σ	Δt (ms)	f_c (Hz)	t_p (ms)	σ	Δt (ms)	f_c (Hz)
1	13.7±0.8	0.23±0.01	7.3±0.6	61.3±5.0	8.2±0.5	0.19±0.01	3.6±0.3	120±10.0
2	13.9±1.1	0.22±0.02	7.1±1.0	63.0±8.4	8.3±0.6	0.18±0.01	3.6±0.5	123±16.6
3	14.6±1.3	0.22±0.02	7.6±1.2	59.0±9.5	8.7±0.8	0.19±0.01	3.8±0.6	116±18.2
4	15.6±1.7	0.22±0.02	8.2±1.5	55.8±9.0	9.3±1.0	0.19±0.01	4.1±0.7	108±17.9
5	16.1±1.6	0.23±0.01	8.7±1.3	52.7±7.3	9.6±0.9	0.19±0.01	4.3±0.6	101±15.2
6	17.1±1.7	0.25±0.02	10.0±1.7	46.7±7.3	10.2±1.0	0.21±0.02	5.0±0.9	89.6±15.1

On the left are the recorded values at room temperature (23°C). Values are means±s.d. As light levels fell, the speed of the impulse responses increased (response half-width Δt and corner frequency f_c decreased). On the right are Q_{10}-corrected values of time to peak t_p and response skewness σ at flight temperature (34.9°C), which were used to calculate temperature corrected Δt and f_c values.

This indicates that the bees centre remarkably well, even in dim light. However, the s.d. increased with falling light levels, which implies that the bees fly further away from the midline in dim light.

DISCUSSION

Insect flight control behaviour has been studied extensively, but very little is known about the mechanisms supporting visual flight control in dim light (Warrant, 2008; Baird et al., 2011). In this study, we investigated the effect of light intensity on flight control in freely flying bumblebees, *B. terrestris*. We also explored the adaptations of the retina for vision at declining light levels by studying the temporal properties of the green-sensitive photoreceptors, which are known to produce the input for achromatic motion vision in insects (Yamaguchi et al., 2008; Wardill et al., 2012; Kaiser, 1974; Srinivasan and Lehrer, 1984).

The effect of light intensity on flight speed and temporal resolution

The effect of light intensity on flight speed was so obvious that it could easily be observed with the naked eye (see Figs 2 and 3, and Table 3). In the dimmest condition (3.4 lx), the bees flew twice as slow (48.1±12.8 cm s^{-1}) as in the brightest condition (600 lx, 88.9± 15.0 cm s^{-1}). Flight speed decreased more or less linearly in response to a logarithmic decrease in light intensity. This result is consistent with the findings of a previous study, which showed that

tethered hornets fly slower in dim light (Spiewok and Schmolz, 2006). In that study, the flight speed of hornets was measured at three different light intensities, similar to the ones used in our study (850, 5 and 0.5 lx). The authors found a significant difference in flight speed in hornet workers between the highest and the lowest light intensity. However, the authors did not further analyse the relationship between light intensity and speed. Our results can also be directly compared with foraging nocturnal ants that show a gradual decrease in walking speed when foraging around twilight (Narendra et al., 2013).

We further found that the response speed of the green-sensitive photoreceptors decreased in relation to the decrease in light intensity. The −3 dB corner frequency dropped 24% from 61.3± 5.0 Hz to 46.7±7.3 Hz between the brightest and dimmest condition at room temperature (see Table 2). Even though visual reliability in dim light is improved with a longer integration time, the drawback is that fast-moving objects become blurred (Warrant, 2008; van Hateren, 1993). The reduction in flight speed that we observed compensates for this by making the world move more slowly across the retina of the bee.

The effect of light intensity on position control

To test whether retinal adaptations (decreased photoreceptor response speed) and behavioural adaptations (decreased flight speed) allowed bumblebees to fly with the same precision over a
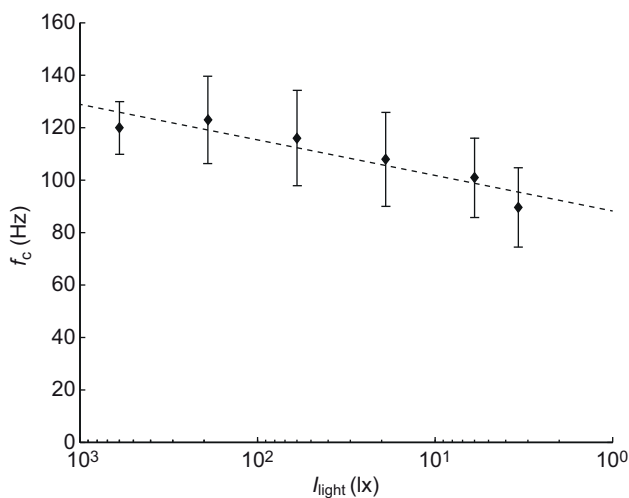

Fig. 4. The effect of light intensity on photoreceptor response speed characterised by the −3 dB corner frequency of the response. The symbols indicate means of −3 dB corner frequency (f_c); error bars indicate ±2 s.d. A linear correlation {f_c=87.98−14.09×[−log (I_{light})], R^2=0.84} was found between −3 dB corner frequency and log light intensity.
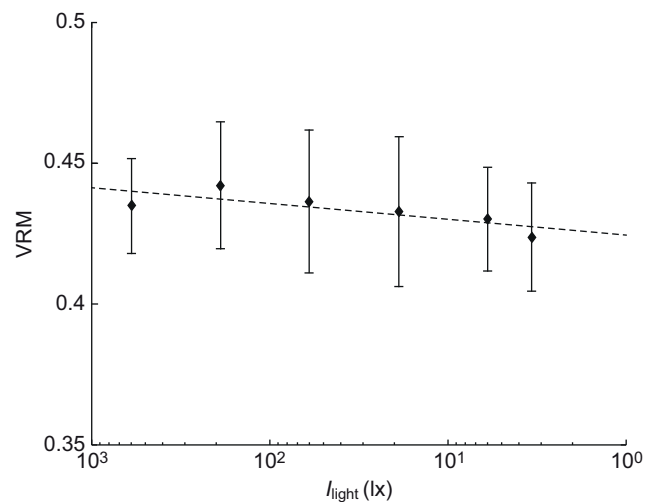

Fig. 5. The effect of light intensity on the estimate of the voltage response modulation experienced by the photoreceptors during free flight. The symbols indicate mean voltage response modulation (VRM); error bars indicate ±2 s.d. A linear correlation {VRM=0.42−0.006×[−log (I_{light})], R^2=0.58} was found between VRM and log light intensity.

Table 3. Flight data recorded at different light conditions

Condition	Flight speed (cm s^{-1})	Path length (cm)	Centring (cm)
1	88.9±15.0	81.9±1.9	0.7±1.2
2	80.7±16.6	82.4±2.1	0.9±1.4
3	77.1±15.7	82.4±2.2	0.6±1.3
4	68.6±16.3	82.7±2.0	0.4±1.2
5	61.2±15.2	83.0±1.9	−0.3±1.9
6	48.1±12.8	84.0±2.4	0.4±2.8

As light levels fell, flight speed decreased, whereas path length increased. The bees stayed remarkably well centred in all conditions. Values are means±s.d.

range of light levels, we performed a detailed analysis of the flight path over 80 cm of the tunnel. This revealed that the length of the flight path increased significantly as light levels fell, an indication of a more tortuous flight. This observation is consistent with recent studies on nocturnal ants (Narendra et al., 2013) and tropical bees (Theobald et al., 2007) that both move along less straight paths as light levels fall. Bumblebees have also been found to search for flowers for a longer time in dimmer light conditions (Chittka and Spaethe, 2007), but as the bees were not tracked in this study, it is not possible to determine whether longer foraging times were the result of a decrease in flight speed or an increase in the length of the flight path, or both. However, in our experiments the mean path length of the bumblebees only increased from 81.9±1.9 cm at our brightest light level to 84.0±2.4 cm at our dimmest (after which the bees stopped flying altogether; see Table 3). In addition, the average distance of the bees from the midline never deviated by more than 0.9 cm even in the dimmest light condition. This indicates that, for as long as the bees are able to fly, their flight control system is still reliable enough to support a relatively straight flight down the safe midline of the tunnel, implying that in the earlier study by Chittka and Spaethe (2007), longer foraging times in dimmer light are probably due mostly to declining flight speeds.

Photoreceptor voltage response modulation

The observed gradual increase in the tortuosity of the flight paths with falling light levels could be a consequence of bees no longer being able to resolve the optic flow cues from the centre of the tunnel as a result of the increase in visual blur. To estimate the visual blurring experienced by the flying bumblebee, we estimated the VRM encoded by the photoreceptors. With falling light levels, the increasing shot noise and photoreceptor integration time decreased photoreceptor VRM (i.e. increased blurring). The drop in flight speed counteracts this drop in VRM, but only partially. We found that the estimated photoreceptor VRM dropped 3% between the brightest and dimmest light level (see Fig. 5). Our behavioural results suggest that even this small drop in VRM significantly degrades the bumblebee's perception of the optic flow as the flight speed decreases and the tortuosity of the flight path increases with dimming light conditions. This contradicts Land's conclusion that blur does not hinder resolution in bees at angular velocities less than 100 deg s^{-1} (Land, 1999). However, in addition to angular velocity, the number of photons arriving at the retina affects the amount of blur as a result of the stochasticity of the process. Thus, at low light intensities, the increased level of shot noise could affect bee vision so that they have to slow down in order to prevent resolution loss.

It is also possible that other neural adaptations, such as spatial and temporal summation in higher brain areas, would provide an additional increase in visual reliability in dim light (Warrant, 1999). Earlier experiments on vertebrates (Barlow, 1958), flies (Pick and Buchner, 1979), honeybees (Rose and Menzel, 1981; Chittka and

Spaethe, 2007), hornets (Spiewok and Schmolz, 2006) and the nocturnal bee *M. genalis* (Theobald et al., 2007) have pointed towards higher order summation of visual signals as an adaptation for vision at low light levels. Such summation, along with the behavioural and retinal adaptations examined in this study, would enable foraging flights earlier in the morning and later in the evening, albeit with reduced efficiency – a cost that is probably worth paying in the competition for nectar and pollen. To see whether we could infer the existence of such neural adaptation mechanisms, we compared the drop in photoreceptor response speed with the effects of falling light intensity on the motion-detecting neurons of other insects. In dipterans, the spiking rate of the motion-detecting neuron H1 does not change when light intensity falls from daylight levels to dusk levels (Egelhaaf et al., 2001) while the latency of the response increases by about 40 ms (more than twofold) with an intensity drop of three orders of magnitude from dim daylight levels (Spavieri et al., 2010). This study shows that in bumblebee photoreceptors, as light levels fall by over two orders of magnitude, the latency (described by the time-to-peak) increases by 24% and the response speed (described by corner frequency) decreases by 25% (see Table 2). Assuming that the effects of light intensity on the motion-detecting neurons of the bumblebee were comparable to those in dipterans, our results suggest that the post-photoreceptor temporal summation does not change motion resolution appreciably within the intensity range studied although the timing of signals in the brain is likely to be delayed.

MATERIALS AND METHODS
Animals and experimental setup

Experiments were performed on bumblebees (*B. terrestris*) from a commercial breeder (Koppert, Berkel en Rodenrijs, The Netherlands). In the behavioural experiment, bumblebee hives were placed in an indoor flight cage made from aluminium netting (2.3 m long, 2.0 m high and 2.0 m wide). The temperature in the room was held constant (~21°C). Dimmable fluorescent lamps (BIOLUX®, OSRAM GmbH, Munich, Germany) were mounted inside the cage and could be further dimmed with a neutral density filter (210 0.6 ND, LEE Filters, Bellalite AB, Växjö, Sweden). Experiments were conducted under six different light intensities: 600, 190, 60, 19, 6 and 3.4 lx measured in the middle of the experimental tunnel (see below) at a 15 cm elevation using a portable light meter (Hagner ScreenMaster, B. Hagner, Solna, Sweden). Compared with natural lighting conditions, the experimental light intensities ranged from the light level of sunrise or sunset on a clear day (600 lx) down to the dark limit of civil twilight under a clear sky (3.4 lx) (Johnsen et al., 2006). An experimental tunnel (2.0 m long, 0.3 m high and 0.3 m wide) was placed inside the cage. The walls and floor of the tunnel were lined with a random chequerboard pattern (2×2 cm black and white squares with Michelson contrast 0.92) to provide the bees with optic flow cues. The top of the tunnel was covered with insect netting to allow video recording from above. The bees were individually marked with plastic number plates on their thorax and trained to fly along the full length of the tunnel to a feeder containing sugar solution and pollen grains. Bees flying through the tunnel were recorded at 60 frames s^{-1} using a camera (MotionBLITZ Eo*Sens*® mini, Mikrotron GmbH, Unterschleissheim, Germany) mounted above the centre of the tunnel. For better discrimination of the bees in the videos, infrared illuminators (IR Illuminator silver, TV6700, Abus, Elfa Distrelec AB, Järfälla, Sweden) were used in the darker conditions (6 and 3.4 lx).

In the electrophysiological experiments, bees were extracted from the hive and prepared for electrophysiological recordings as described in Vähäkainu et al. (2013). Briefly, each bee was fixed in a holder using a beeswax–resin mixture. A small hole was cut in the dorsal cornea of the left eye and sealed with paraffin-based grease. Recording electrodes were pulled using 1 mm diameter borosilicate or quartz glass capillaries with a laser puller (Model P-2000, Sutter Instruments, Novato, CA, USA) and filled with 2 mol l^{-1} (borosilicate) or 3 mol l^{-1} (quartz) KCl solution.

Electrode resistances were mostly in the range 100–130 MΩ (borosilicate) or 180–220 MΩ (quartz) when inserted into the retina. The reference electrode was inserted into the right eye. In the electrophysiological setup, two light sources were used. The main light source was identical to the one used to illuminate the behavioural experiments (BIOLUX bulb with neutral density filtering), directed via a dual-branch optical fibre to a position approximately 5 cm from the eye. This allowed the accurate targeting of the optic axes of the photoreceptors. A spectrometer (USB4000, Ocean Optics, Dunedin, FL, USA) was used to calibrate the intensity of the light stimulation at the eye to match the values of the five lowest light levels used in the behavioural experiments. The highest level of light intensity could not be attained in the electrophysiological setup and the maximum value of 477 lx was used instead. A secondary LED light stimulus was used to stimulate the photoreceptors via the other branch of the dual-branch light guide. Voltage responses to bright flashes from three LEDs with peak wavelengths of 405, 466 and 525 nm were used to distinguish the green photoreceptors from other spectral classes. Following identification, only the 525 nm LED was used for light stimulation in addition to the fluorescent bulb.

The effect of light intensity on flight control

Before recording commenced, the bees were given 30 min to adapt to the test light intensity. Each experimental trial lasted for 30 min and the presentation of the six light conditions was randomised over the day (08:00 h–17:00 h) to exclude circadian influence on the flight behaviour. The darker conditions, under which the bees were more reluctant to fly, were presented more frequently than the brighter conditions in an attempt to obtain a comparable number of flights in each light condition (see Table 1). At light intensities below 3.4 lx, the bees stopped flying altogether. Different parameters of flight control – flight speed, path length and 'centring performance' (a measure of how well the insect flies along the midline of the tunnel; Kirchner and Srinivasan, 1989) – were analysed over a distance of 80 cm in the middle section of the tunnel. The position of the bee was determined in each frame using an automated tracking program (Lindemann, 2005). The bee position data were converted to centimetres using a known size reference pattern placed 15 cm above the floor of the tunnel. In other words, the bees were estimated to fly at the average height of 15 cm. The flight height of the bees did not seem to change as light levels fell and the distance error associated with bees flying at different heights in the tunnel was therefore considered to be minimal. The average flight speed was calculated by dividing the two-dimensional distance each bee travelled between two successive frames (including the forward and lateral components) by the time between two frames (1/60 s). The path length of each flight was calculated by summing the two-dimensional distance travelled between successive frames. The centring performance of the bee was analysed by finding the distance from the midline in each frame. For flight speed and centring, the average value of each flight was calculated and used in the analyses. A minimum of six individuals in each condition, with a maximum of three flights each, were included in the analysis. Flights from the same individual were treated as independent data points (Dyhr and Higgins, 2010).

The temporal properties of the photoreceptors

Electrophysiological recordings were performed on green class photoreceptors. The *in vivo* intracellular recordings were made using a SEC-05L amplifier (npi electronic GmbH, Tamm, Germany) in discontinuous single-electrode current-clamp mode (Brennecke, 1974; Finkel and Redman, 1984) with a 10 kHz switching frequency (Weckström et al., 1992; Juusola et al., 1994; Heimonen et al., 2006). Photoreceptor responses were filtered at 700 Hz, digitised at 5 kHz, and saved to the computer for analysis. A comparable electrophysiology setup was used for the determination of temperature coefficients (Q_{10} values). Impulse response recordings were performed by adapting the cell to one of the six light levels with the fluorescent bulb for 100 s and then recording, at that light level, the photoreceptor responses to 400×2 ms long light flashes of low amplitude, produced with the 525 nm LED. The amplitude of the LED stimulus was set so that the amplitude of the voltage response was between 0.5 and 1.5 mV above the steady-state level. At this range, linearity

could be assumed and thus the response was well fitted with a log-normal function (Howard et al., 1984):

$$v(t) = \exp-\left(\frac{(\ln(t/t_p))^2}{2\sigma^2}\right), \tag{1}$$

where t_p is the time to peak and σ is the skewness of the response. These two parameters were used to derive the response half-width (Δt), a measure of response speed (sometimes called the 'integration time'), to an accuracy of 1% (Howard et al., 1984) as follows:

$$\Delta t = 2.35 \times t_p\sigma . \tag{2}$$

A minimum of 10 individuals in each condition, with recordings from a maximum of three cells from each individual, were included in the analysis. Recordings from cells of the same individual were treated as independent data points.

Bumblebee flight muscles need to attain a temperature surpassing 30°C before they can fly (Krogh and Zeuthen, 1941) and this also elevates the temperature of their head (Heinrich, 1980). Heating up the retina increases its response speed (Weckström et al., 1985; Roebroek et al., 1990; Tatler et al., 2000). To match the results of the electrophysiological recordings to the behaviour of the free-flying bumblebees, we estimated the head temperature of a flying bumblebee by recording the surface head temperature of bumblebees with a thermal camera (FLIR ThermaCam A325, FLIR Systems AB, Täby, Sweden). To resolve the bees in the videos, the bees were recorded just after landing at a feeder. We recorded 23 landings by six individual bees. We found no significant difference in temperature between individual bees (ANOVA; $P=0.268$); hence, recordings from the same individual were treated as individual data points. The average head temperature for all the landings was calculated. To estimate temperature dependence, Q_{10} values were determined for t_p and σ by recording photoreceptor impulse responses at two different temperatures (23 and 33°C) and fitting them with the log-normal function. Q_{10} is defined as:

$$Q_{10} = \left(\frac{r_2}{r_1}\right)^{\frac{10}{T_2-T_1}}, \tag{3}$$

where T is temperature and r is the variable examined (t_p or σ). Recordings were made at each temperature from 13 cells from six individuals with a maximum of four cells from each individual. Recordings from cells of the same individual were treated as independent data points. The linear frequency response was calculated on the basis of the recorded impulse response with a fast Fourier transform (FFT)-based method.

The quality of the electrophysiological recordings was evaluated by using three parameters: resting potential, maximum light response and input resistance in darkness. If any of these parameters changed significantly during the recordings, the recordings were discontinued. The resting potential and the maximum light response were used as criteria upon starting recording from a cell. The resting potential of the photoreceptor had to be lower than −50 mV and the maximum light response ≥50 mV above the resting potential.

Photoreceptor voltage response modulation

Movement causes image blur on a retina. We used the method reviewed by Land (1999) to estimate the VRM experienced by a photoreceptor of a flying bumblebee to describe how much the image produced is degraded by movement. Briefly, as a black-and-white grating pattern moves relative to a photoreceptor, the light intensity entering the photoreceptor, as a function of the angular position of the grating, is the convolution of a square-wave intensity function and a Gaussian angular acceptance function. By dividing this by the angular speed, a function of time is produced. This is then convolved with the photoreceptor temporal response function and results in a partially modulated sinusoidal function that estimates the voltage response of the photoreceptor. VRM is then the Michelson contrast between the maximum (r_{max}) and minimum (r_{min}) values of this function:

$$\text{VRM} = \frac{r_{max} - r_{min}}{r_{max} + r_{min}}. \tag{4}$$

Thus, a VRM of 1.0 represents maximum modulation and a VRM of 0 represents zero modulation. Ideally, the square-wave intensity function used

by this algorithm switches between values of 0 (i.e. complete darkness) and 1 when normalised with maximum intensity. To account for the actual measured contrast of 0.92, we used a minimum value of 0.04. Moreover, we included the effect of shot noise by estimating the number of photons (N) arriving at a single photoreceptor within one photoreceptor integration time (Δt) with the equation:

$$N = 0.62 \times I_{light}D^2(\Delta\rho)^2, \qquad (5)$$

where I_{light} is the light intensity in photons m^{-2} sr^{-1} s^{-1}, D is the diameter of a compound eye facet and $\Delta\rho$ is the acceptance angle (Land, 1997). For *B. terrestris*, D=28 μm and $\Delta\rho$=3.8 deg (Meyer-Rochow, 1981). Shot noise ($\sqrt{N}$) was calculated for the light stimulation supplied to the eye by both the white (ON) and black (OFF) squares of the tunnel at each light level. To reflect the degrading effect of shot noise on VRM, we further modified the square-wave intensity function for each light level by subtracting the s.d. of shot noise ($\sqrt{N}$) of the ON stimulus from the maximum value of 1 and adding the s.d. of shot noise of the OFF stimulus to the contrast-corrected minimum value of 0.04. This is justified in the sense that the size of the elements in the random chequerboard pattern (2×2 cm) is seen by the photoreceptors at an angle of ~7.5 deg, when the bees are flying near the centre of the tunnel. This angle is much larger than the receptive field of the photoreceptors (Meyer-Rochow, 1981).

Statistics

An assessment of the normality of the behavioural, as well as the electrophysiological data, was made using normal *Q–Q* plots and the Shapiro–Wilk test, using SPSS (IBM SPSS Statistics 20). As not all samples were normally distributed, the data sets were analysed using non-parametric tests. Kruskal–Wallis tests were used for multi-sample comparisons and in the cases where there was a significant difference between at least two groups, Mann–Whitney *U*-tests were used to compare pairs of samples. Linear regression on the mean values was used in the analysis of flight speed, −3 dB corner frequency and VRM. In the analysis of flight speed of the 12 individual bees that flew in at least four light conditions, linear regression on all values for each bee was performed to obtain the average slope. To analyse the probability of obtaining 12/12 positive slopes, a binomial test was used. Values given in the text are means±s.d. (unless otherwise indicated). Significance was set at *P*<0.05.

Competing interests
The authors declare no competing or financial interests.

Author contributions
T.R. performed the flight control experiments and wrote the manuscript. A.V. performed the electrophysiological experiments and the voltage response modulation and wrote the corresponding parts of the manuscript. All authors participated in the design and analysis of the experiments, and in the final version of the manuscript.

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