

Flight behaviour of the hawkmoth *Manduca sexta* towards unimodal and multimodal targets

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SUMMARY

Here, we analyse the flight behaviour of the hawkmoth *Manduca sexta* while it approaches three different artificial flower stimuli: a clearly visible blue flower, an invisible scented flower and a flower that is both visible and scented. By tracking the moths in fine temporal detail, we find that flight towards an artificial flower differs depending on whether the stimulus is unimodal (either visual or olfactory) or multimodal (both visual and olfactory). In all three cases, the moth reduces its speed as it nears the target but the speed is higher overall when the visual stimulus is not present. Visual feedback, as well as the concentration gradient of the odour, is used to guide the moths towards the stimulus. The main difference in flight behaviour between an approach towards a visual and a multimodal stimulus is that the olfactory information makes the moths turn more rapidly towards the multimodal stimulus. We also find that moths extend their proboscises in front of a clearly visible feeder independent of whether an odour is present. In contrast, a scented transparent artificial flower only occasionally triggers this response.

Key words: feeding behaviour, hawkmoth, high-speed tracking, olfaction, vision.

INTRODUCTION

The advantages of multisensory processing are well established, and its use is ubiquitous in the natural ecology of animals. For many insects, the two most salient sensory modalities during foraging are vision and olfaction. Depending on whether the insect is active at day or night, one of these modalities is often more prominent than the other. During the day, when light is readily available, vision tends to be the more reliable source of information, whereas olfaction becomes increasingly important as light levels fall (Andersson and Dobson, 2003; Balkenius et al., 2006).

Several hypotheses have been proposed concerning the role of multimodal signals (Hebets and Papaj, 2005). One hypothesis is that a combination of signals facilitates the detection of a target at different distances, where the detection of one sensory modality alerts the insect to the presence of the other. Another hypothesis is that one sensory modality gives the quality of the sender and the other identifies the location of the source. Alternatively, the sender could direct different signals to different receivers (Hebets and Papaj, 2005). Multimodal signals could further act to improve target detection in a noisy environment by adding contributions from different sensory modalities (Hebets and Papaj, 2005). Although there are obvious advantages of using several modalities, as it is a more robust detection method, there are also disadvantages for the sender, such as the energetic cost involved in producing and controlling the output of multiple stimuli rather than only one (Johnstone, 1996; Partan and Marler, 2005). The sender may also become an easier target for unintended receivers, such as predators and parasites (Magnhagen, 1991; Roberts et al., 2007). A possible disadvantage for the receiver is that, with an increased decision speed, there is sometimes a negative correlation with the accuracy of pinpointing the target (Chittka et al., 2003; Passino and Seeley, 2006; Uchida and Mainen, 2003). However, it can sometimes be more advantageous to make a faster decision than an optimal one (Burns, 2005).

During the last decade, the interaction between visual and olfactory input in hawkmoths has been investigated from a behavioural, as well as from a neurobiological, perspective (Balkenius and Kelber, 2006; Balkenius et al., 2009; Goyret et al., 2007; Raguso and Willis, 2002; Rojas and Wyatt, 1999). In some cases, a multimodal output from the flowers enhances the signal to the foraging hawkmoth, whereas in other situations one modality overshadows the other (Balkenius and Kelber, 2006; Partan and Marler, 2005). The localisation of an artificial flower by the hawkmoth *Manduca sexta* is possible even if the flower does not carry any scent (Raguso and Willis, 2002). In contrast, the extension of the proboscis to initiate feeding seems to be controlled by the presence of an odour (Goyret et al., 2007; Raguso and Willis, 2002; Raguso and Willis, 2005). *Manduca sexta* is active at night, when a flower can be detected visually at a shorter distance than under daylight conditions and when odour is very likely to be an important cue used to guide and initiate the approach flight over a longer distance. However, the influence of multimodal signalling on the speed and initiation of the approach flight of the foraging hawkmoth has never been characterised.

The choice made by a foraging hawkmoth between artificial flowers with different odour characteristics (scented and unscented) has previously been studied in wind tunnels with laminar airflow (Goyret et al., 2007), in greenhouses with natural airflow (Raguso and Willis, 2002) and in flight cages with no airflow (Balkenius and Kelber, 2006). Despite the drastically different airflow conditions in these three set-ups, the hawkmoths had no difficulty in locating either a scented or an unscented visual stimulus in any of the studies. It is possible that the localisation of a unimodal olfactory stimulus – which is effectively invisible to the moth – is more dependent on the character of the airflow, but this type of stimulus was not presented to the hawkmoth in any of these studies.

Here we analysed the approach behaviour of *M. sexta* to scented and unscented visual stimuli as well as to a purely olfactory stimulus

in an arena with no airflow. The approach towards the artificial flower, as well as the initiation of feeding, was filmed and analysed in fine temporal detail to behaviourally investigate how multimodal signalling (vision and olfaction) affects the flight behaviour and location of a feeding target by free-flying hawkmoths.

MATERIALS AND METHODS

Animals and environment

Larvae of the hawkmoth *Manduca sexta* L. (Lepidoptera: Sphingidae) were reared on an artificial diet (Bell and Joachim, 1976) with 200 mg β -carotene l^{-1} added (Raguso et al., 2007). The animals were kept under a 16h:8h light:dark cycle at 23–25°C, 40–50% relative humidity. Both male and female moths were used in the experiment, 3–5 days after eclosure. The experiments were conducted in a circular arena with a diameter of 1.5 m. The arena was surrounded by a wall with a height of 0.5 m and was covered with a transparent net (0.1 mm mesh size). A similar set-up has been used in the study of odour localisation and visual feedback in *Drosophila melanogaster* (Frye et al., 2003). The moths were released into the arena one at a time, always from different directions. The light intensity in the visible range was 0.95 $cd\ m^{-2}$. For the moth, this represents an ecologically valid light intensity, while still allowing the camera system to operate.

The approaches of a hawkmoth to a feeding target were analysed under three experimental conditions: in the presence of a visual stimulus (V), a multimodal stimulus (M) or a transparent odour stimulus (O). All three conditions were presented an equal number of times during each experimental session. This was to control for possible effects of external factors, such as temperature and air pressure on the foraging behaviour of the moth. Each session was at least 1 week apart from the previous session, and the visual stimulus was always presented first to avoid the possibility of any odour from earlier trials still being present in the cage. The arena and the experimental room were thoroughly ventilated to extract the air from the arena between each session. The total number of animals tracked was 31 ($N_V=8$, $N_M=10$, $N_O=13$).

During the experiments, one of the three different feeding targets was placed at the centre of the arena. The visual target was composed of a flower-like blue feeder (Pfaff and Kelber, 2003), the multimodal target was composed of a scented flower-like blue feeder and the odour target consisted of a transparent, scented thin glass tube (1.5 mm diameter) (Fig. 1A). The blue target reflects preferentially at a wavelength of 450 nm (Fig. 1B).

Bergamot oil (5 μ l) (aroma, essential oil *Citrus aurantium bergamia*) was used to scent the targets. This oil contains linalool and monoterpenoid odours, which are released by many night-blooming flowers (Raguso and Pichersky, 1999), has previously been used to stimulate feeding in *M. sexta* (Goyret et al., 2007; Goyret et al., 2009). In a separate experiment to control for the visibility of the odour stimulus, the arena was fitted with three unscented glass tubes placed 20 to 120 cm away from each other. Of the eight moths that were flown in the arena one at a time, none made any apparent attempts to avoid the transparent tubes, and three moths collided with the transparent tubes in mid-air.

The shape and position of the odour plume from the multimodal and odour stimuli were simulated by applying dry ice at the same location as the odour source in the arena. This established that there were no air currents in the arena and that the concentration of the applied odour was influenced only by passive diffusion and the turbulence from the flying moth.

In all experiments, the moths were gently released at random locations on the floor and allowed to warm up. Moths were then

allowed to fly until they had approached the target or had flown for a maximum of 5 min. The moths were then removed from the arena and were not used again. Moths that did not fly, or landed before they had approached the target, were discarded from any further participation in the experiments. After take-off, the moths typically flew upwards and stabilised their flights ~10 cm below the net ceiling of the arena.

Data collection

Flights of moths in the arena were recorded at 100 frames s^{-1} using a high-speed camera (MotionBLITZ; QZEO, San Diego, CA, USA) with a 12.5 mm lens. The arena was illuminated with four infrared (IR) lamps. IR light is invisible to the moths but increases the signal-to-noise ratio of the captured images. Recording of the state variables (see below) started the first time the moth came within 400 mm of the centrally placed target. The location of the target stimulus (x_t , y_t) was manually indicated in one of the images under each experimental condition. From the captured images, it was also possible to determine whether the moths extended their proboscises at any time during the approach to the target stimulus. This was detected for 81 animals ($N_V=29$, $N_M=30$, $N_O=22$).

Data analysis

The recorded images were converted into QuickTime movies (Apple, Cupertino, CA, USA) without compression, and the analysis of the recorded image sequences was performed in four stages, as detailed below.

Foreground detection

The moths were localised using an adaptive foreground-detection method (Stauffer and Grimson, 1999). This method robustly detects parts of an image that are different from the learned distribution of grey levels in each pixel. To increase localisation accuracy, we removed detected foreground pixels that could be classified as shadows. A foreground pixel was classified as a shadow if the local neighbourhood of the pixel was sufficiently similar to an intensity-scaled version of the average background around that pixel.

Preliminary tracking

Next, the detected foreground pixels were clustered to find the centroid, which indicates the preliminary position of the moth (Duda et al., 2000). Finally, a number of target positions before and after the current time were averaged using a Gaussian window ($\sigma=10$) to produce a smooth trajectory. The resulting tracking was able to accurately localise the animal, but the exact position drifted slightly along the body of the moth as the wings moved relative to the body.

Active shape matching

The result of the preliminary tracking was used to initiate the position of an active shape model (Cootes et al., 1995), which was used to track the moth more exactly. The shape model consisted of a number of contour points around the moth together with contour normals estimated from a standard moth image. The initial orientation of the model was set to the direction of the second moment of the foreground pixels. In addition, the last estimated position and orientation of the moth was used to construct an alternative match of the shape model. The best match of the shape model was iteratively calculated and the relative matches of the different hypotheses were compared to obtain the estimated location (x , y) and orientation (θ) of the moth (Fig. 2). The resulting position estimation was accurate to approximately a single pixel resolution, which corresponds to 1.7 mm in the recorded scene.

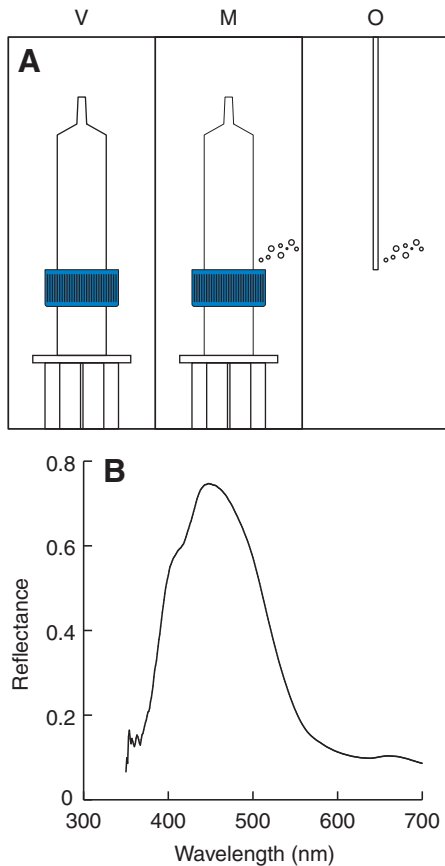


Fig. 1. (A) The artificial flowers used as feeding targets consisted of a visual blue feeding target (V), a multimodal feeding target with both odour and colour (M) or an odour target, consisting of a thin glass tube scented with bergamot oil (O). (B) Reflection curve for the visual stimulus that reflects preferentially in the blue region of light at a wavelength of 450 nm.

Calculation of state variables

In the last stage, a number of values were calculated. For the moth, the velocity was mapped onto forward, lateral and rotational components (v_x, v_y, v_r) (Fig. 2). The moth can control its flight independently along all axes depending on the situation. Forward flight (v_x) moves the moth forwards along the central axis of its body, whereas lateral flight (v_y) moves the body perpendicular to the central axis. Moths are able to fly sideways to adjust the position of their body even when they are hovering without any forward speed. They can also rotate around their own body (v_r) while remaining at the same location. Most often, a moth's movement through air is a combination of these three components, which allows it to move along an arbitrary trajectory.

The distance and direction to the target stimulus (d_t, r_t) relative to the moth were also calculated (Fig. 2). From these variables, the distribution of angles towards the target and the distribution of the position of the moth during the entire approach can be obtained. The distribution of forward, lateral and turning speeds of the moth was also calculated.

Statistical analysis

To determine the functional relationship between the stimulus position and the flight behaviour of the moth, we used the recorded data to estimate four functions: (1) forward velocity as a function of target distance, (2) forward velocity, (3) turning velocity and (4)

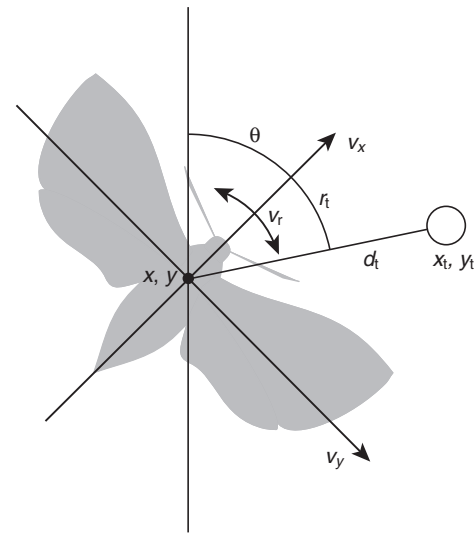


Fig. 2. Measured flight parameters. From the position and orientation of the moth (x, y, θ) in successive frames, the velocity of the moth can be mapped onto forward (v_x), lateral (v_y) and rotational (v_r) components. The distance (d_t) and angle (r_t) to the target stimulus (x_t, y_t) relative to the moth was also calculated. From these variables the distribution of angles towards the target and the distribution of the position of the moth during the entire approach were obtained.

lateral velocity as a function of angle to target. Each function was estimated for each of the three stimulus conditions using locally weighted regression (Cleveland, 1979) with the function 'lowess' in the statistics package R (R Development Core Team, 2009). The symmetry of the control functions for turning and lateral velocity was exploited to merge mirrored data for target angles to the left and right of the moth. The correlations between the different control functions for lateral movement and rotation were calculated by resampling the data at regular intervals (0.005 rad) for each of the three conditions to allow the standard correlation computation to be performed.

RESULTS

Proboscis extension

A moth needs to extend its proboscis to initiate feeding. This extension is controlled by the proboscis extension reflex. When flying towards the multimodal target, 47% of the moths extended their proboscis. When approaching the visual target, the fraction of proboscis extensions increased to 58%. The difference between these two conditions (V and M) was, however, not significant (Fisher's exact test, $P=0.62$). When the visual stimulus was removed – and an odour was presented as the only target cue – the fraction of proboscis extensions decreased drastically to 4% (Fig. 3). This is significantly lower than that observed under both the multimodal and the visual feeding conditions (Fisher's exact test; V and O, $P<0.001$; M and O, $P<0.001$). The proboscis extension reflex thus seems to be initiated by the presence of a visual target alone, decoupled from the olfactory modality of the stimulus. The fraction of proboscis extensions did not differ between males ($N=48$) and females ($N=33$) for any of the feeding targets (Fisher's exact test, all $P=1.00$).

Approaching the target

The approach of the moth towards the feeding targets was analysed from a distance of 0.4 m. Fig. 4 shows three examples of approaches

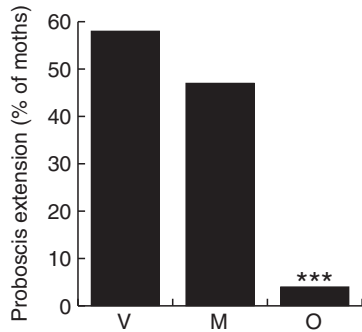


Fig. 3. The number of proboscis extensions in each of the three conditions: visual target (V), multimodal target (M) and odour target (O). The number of proboscis extensions was significantly larger ($***P<0.001$) when the visual stimulus was present (V and M) compared with the condition with only odour (O).

under each of the three types of feeding condition: visual, multimodal and olfactory. When approaching the visual or multimodal target, the moths typically stopped before reaching the target. The flight paths also showed a clear forward and backward component, suggesting that the moths actively adjusted their distance from the visual object. This forward and backward motion was absent from approaches towards the odour target when, instead, the moths rapidly turned back once they had passed the invisible target. At no time did we observe a zigzag-shaped track towards the odour source. Instead, the moths flew either along a straight line or in a curved path towards the target.

During their approach to the target, the long body axes of the moths were directed mainly towards the target location (Fig. 5). As the moth homed in on the target to initiate feeding, the distribution of long body axes directions decreased with distance to the target for both the multimodal and the visual targets. With the olfactory target, the distribution of directions during the approach was much wider overall and was not biased towards the target, even at close range.

The mean velocities of the forward, lateral and rotational components (v_x , v_y , v_r) for each flight under the three different stimulus conditions are summarised in Fig. 6. An ANOVA, followed by a *post hoc* test (Tukey's HSD), showed that the mean forward speed for the olfactory stimulus differed significantly from the mean forward speed of both the visual and the multimodal stimulus ($P<0.001$). In contrast, the calculated means of the velocity of the lateral and rotational components of flight did not differ between the three different stimulus conditions (v_y , $P=0.24$; v_r , $P=0.1$). Nor did the mean velocities of the forward, lateral and rotational components for each flight differ between males and females (v_x , $P=0.22$; v_y , $P=0.59$; v_r , $P=0.69$).

A more detailed analysis of the functional relationship between the different flight parameters clearly shows that the location of the target relative to the moth controls the flight in different ways for the different stimulus conditions (Fig. 7). Forward velocity, v_x , decreased differently as the moth approached the visual, multimodal or olfactory target (ANOVA, V, $P<0.001$; M, $P<0.01$; O, $P<0.001$) (Fig. 7A). The forward velocity did not decrease to zero at the target location during an approach towards the invisible odour stimulus.

With a visible target in sight (V and M), the forward velocity is influenced by the angle to the target (Fig. 7B). Moths slightly decreased their forward velocity when the visual or multimodal

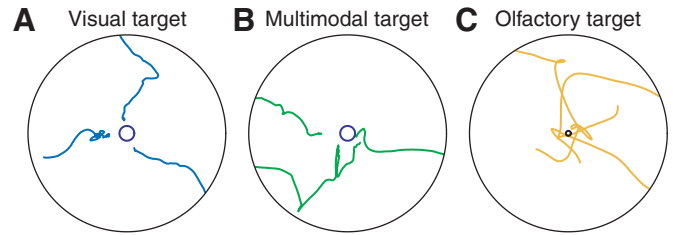


Fig. 4. Example of flight paths with (A) a blue visual target, (B) a multimodal target (scented blue visual target) and (C) an invisible olfactory target. Note that the moth stops before it reaches the target in A and B, and passes by the target and turns back in C.

stimulus was straight ahead, and increased it with larger angles to the target (ANOVA, V, $P<0.0001$; M, $P<0.01$). Some effect of the angle to the target on forward velocity was also identified during approaches towards the transparent odour stimulus, but the effect was significantly smaller than for the visual and multimodal targets (ANOVA, O, $P<0.05$).

The speed of rotation also depends on the angle to the target (Fig. 7C). For the visual stimulus, the speed of rotation increased with larger angles to the target up to ~ 23 deg (0.4 rad). For larger angles, the turning speed decreased again. This differs from the reaction to the olfactory and the multimodal stimulus, where the turning speed continued to increase with the angle to the target. A mixed-model ANOVA revealed that the location of the target influenced the turning speed differently depending on the stimulus condition ($P<0.001$). As long as the reorientation of the flying moth is in the direction of the flower, the positive correlation between rotational speed and the angle to the target (up to 23 deg for the visual target) will serve to quickly realign the long axis of the moth with the flower. The observation that the body of the moth is highly directed towards the visual target along the recorded flight paths (Fig. 5, V and M) indicates that the moths do indeed turn in a valid direction if they are not already oriented towards the flower. This alignment with the flower was not obvious for the flights towards the odour target (Fig. 5, O). This is partly because the moths will also look away from this invisible target as they fly past it.

The lateral and rotational velocities during the approach towards the multimodal stimulus were strongly correlated ($R=0.91$), which means that the moths both turned and translated their bodies sideways in a curved path towards the stimulus. This correlation weakened for flights towards the visual target ($R=0.46$), and was negligible during approaches towards the invisible odour stimulus ($R=0.02$). This again suggests that the approaches towards the multimodal target are more accurately controlled than the approaches towards a unimodal target.

The interaction between angle to the target and turning velocity for the different conditions was further analysed by dividing the area around the moth into two zones (Fig. 7C): a frontal zone with the target within a 45 deg ($\pi/4$ rad) angle to the moth and a lateral zone with larger angles to the target. A *post hoc* test of the turning velocities in the frontal and lateral zones under the visual, multimodal and odour conditions showed that there were no significant differences between the turning velocities for the different conditions when the target was in front of the animal, whereas there were significant differences between the turning velocities for an approach towards the visual stimulus and the two other conditions when the feeding target was in the lateral zone (Tukey's HSD,

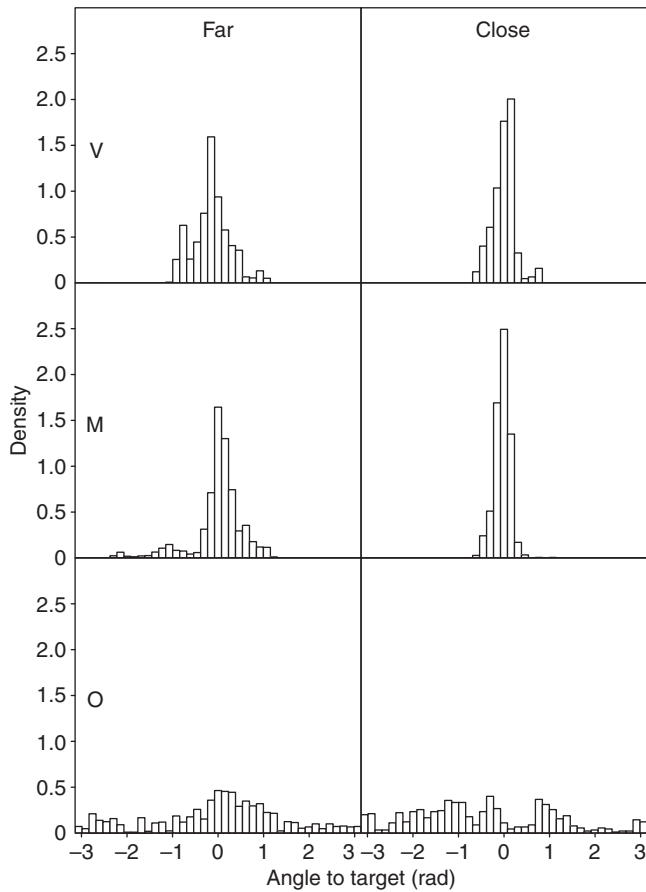


Fig. 5. Distribution of the orientation of the moth in relation to the target when the moth is far from (left) and close to the target (right). The area of each bar represents the probability that the moth has that particular orientation. The width of each bin is $\pi/4$ rad. The angle to the target decreased when the moth was close to the visual stimulus compared with when it was further away (V and M). When no visual cue was present (O), there was a tendency for the moth to be directed towards the target at a longer distance, but not when the moth was close to it.

$P < 0.05$). This strongly indicates that vision is primarily used to identify the feeding target when it is within a limited visual angle in front of the moth. In contrast, olfactory information controls turning velocity, even when the target is far off to the side, where it causes a maximal turning velocity for both the olfactory and the multimodal stimulus (Fig. 7C). This suggests that the moths rely on the concentration gradient of the odour stimulus to locate the target even in the absence of airflow. Studies with *Drosophila* in a still-air arena have also shown that they can localise the odour source without airflow (Frye et al., 2003; Stewart et al., 2010).

When a visual target (V or M) was within a limited angle in front of the moth, the moth moved sideways towards the target location under both visual conditions (Fig. 7D). However, when the angle to the unimodal visual target was larger than $\sim 35^\circ$ (0.6 rad), the moth moved away from it. A multimodal stimulus at the same location generated a lateral movement component towards it at all angles to the moth. An odour stimulus alone did not cause any lateral movement, regardless of its location relative to the moth. A mixed-model ANOVA showed an interaction between the stimulus condition and the relation between lateral movement and direction to target ($P < 0.001$).

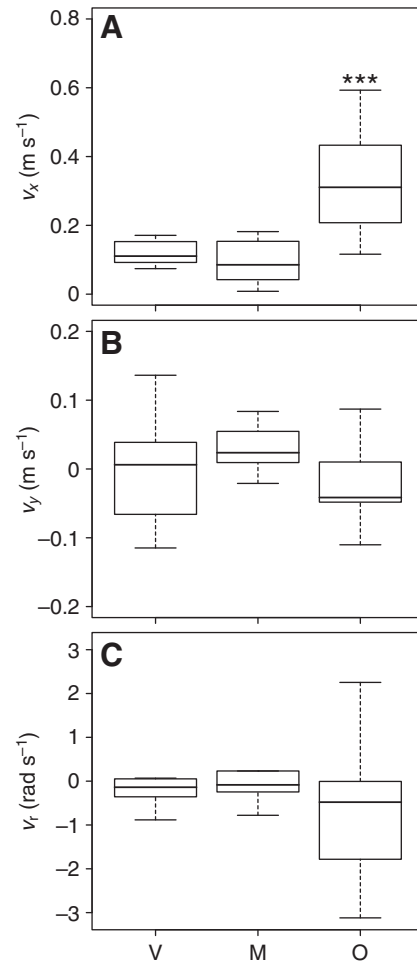


Fig. 6. Boxplots of (A) forward (v_x), (B) lateral (v_y) and (C) turning velocity (v_r) for visual (V), multimodal (M) and olfactory (O) stimuli. Boxes indicate the distance between the lower and upper quartile values, black lines indicate the median values and whiskers indicate the maximum and minimum. The mean forward speed for the olfactory stimulus was significantly higher ($***P < 0.001$) than when a visual stimulus was present.

DISCUSSION

In this analysis of the flight behaviour of hawkmoths under dim light conditions, we found that the flight towards a target stimulus was different depending on whether the stimulus was unimodal (either visual or olfactory) or multimodal (both visual and olfactory). With odour as the single cue to define the position of the feeder, the moths approached it at high speed and did not slow down appreciably, nor did they extend their proboscises, even when close to the target. This shows that odour is used for goal-directed behaviour towards a flower, but not necessarily for the feeding behaviour of the moth. In contrast, the moths slowed down in front of a feeder and extended their proboscises as soon as a visual stimulus is present, either on its own or in combination with an odour. A multimodal stimulus further generated a lateral movement component in the flight towards it at all angles to the moth, whereas moths moved away from the unimodal visual target when the angle to it was greater than $\sim 35^\circ$. This suggests that odour guides the moth towards the flower from any position in space, whereas visual cues facilitate an approach towards the flower when the moth is heading more directly towards it.

Multimodal signalling (olfactory and visual) by a flower increases foraging accuracy by combining the merits of the two modalities.

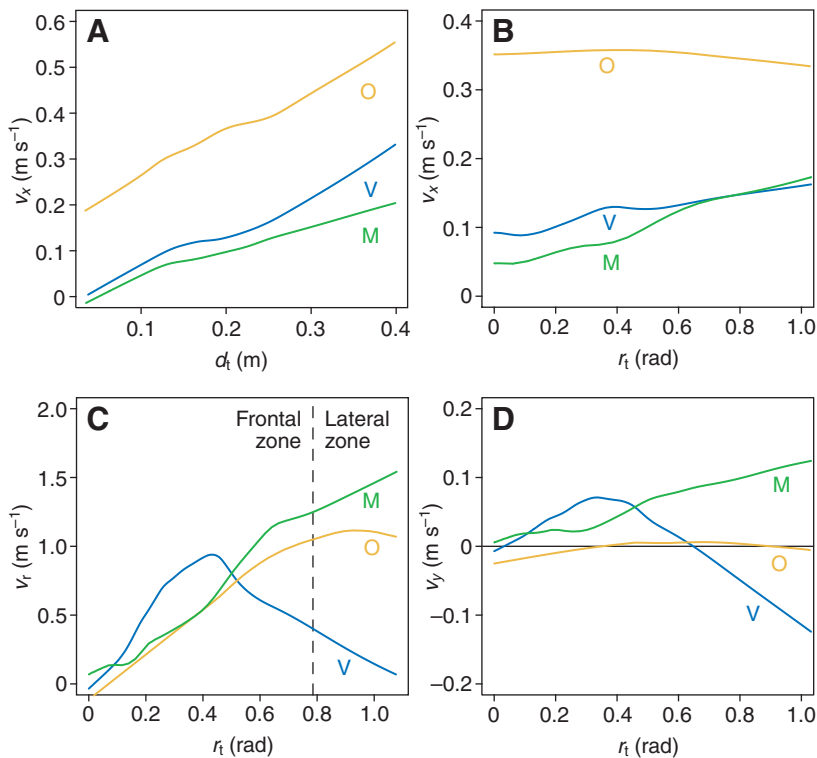


Fig. 7. Flight control for the approach to the target stimulus [blue, vision only (V); green, multimodal (M); yellow, olfaction only (O)]. The curves were generated using local estimation of the underlying function. (A) Forward velocity (v_x) as a function of distance to the target (d_t). Forward velocity increased with increased distance to the target in all conditions. When the visual stimulus was present, the velocity decreased to zero before the moth reached the target. (B) Forward velocity as a function of angle to the target (r_t). The forward velocity was lower when the target was in front of the moth. (C) In all conditions, the turning velocity (v_r) of the moth depended on the angle to the target. With a visual target, the velocity of the rotation increased with larger angle to the target up to an angle of ~ 0.4 rad, where the turning velocity decreased again. The vertical line indicates the border between the frontal and lateral zones. (D) Lateral velocity (v_y) as a function of angle to the target.

The odour makes the moth react to the flower in situations, whereas the visual information plays a minor role, for example when the flower is to one side of the moth (Fig. 7C). The visual information is necessary for the moth to accurately slow down at the flower (Fig. 7A), and for lateral flight control (Fig. 7D). This ability requires fast visual feedback, as has been measured in wide-field motion-sensitive neurons in hawkmoths (Theobald et al., 2010). Visual information also contributes to the stabilisation of the moth in front of a flower while foraging (Kern and Varjú, 1998; Sprayberry and Daniel, 2007). It is conceivable that the decreased velocity that was observed when the moths approached the visual stimulus also aids in the estimation of the distance to the target.

Our present results show interactions between vision and olfaction when guiding the approach flight in the multimodal condition. From the tracks in Fig. 4C, it is clear that the moths are able to locate the odour source, but without a visual cue they pass by it. At this point, their velocity rapidly decreases and they instantly turn back to search for a visual cue. The moths could clearly smell the food source, but appeared confused when a visual signal indicating the position of the flower was absent.

In the present study, the moths did not attempt to feed without a visual stimulus. This is well in line with earlier observations by Raguso and Willis (Raguso and Willis, 2002), where the moths were reluctant to extend their proboscises toward flowers without strong visual contrast. The consistent attempts of the hawkmoths in the present study to feed from a clearly visual but unscented feeder do, however, differ from earlier experiments in the wild and in the laboratory, where *M. sexta* also required odour for foraging (Raguso and Willis, 2002; Raguso and Willis, 2005). It is possible that these previous experiments in the wild could have included females searching for an oviposition site, and we do not expect the moths to extend their proboscises while in this mode. Another explanation for the differences in willingness to extend the proboscis in front of the scentless flowers is that the moths used in the present study

were naïve and had never seen or smelled a real flower, or even tried to fly, before the experiments. That different types and numbers of artificial flowers were used in the present and previous studies can also have influenced the proboscis extension reflex. A possible problem with the present study is that scented and unscented feeders were presented to the moth at different times in the same arena but, because the arena was thoroughly ventilated for at least 1 week between each experimental session, we do not consider residual scent to be a confounding factor for our results. That odour is not critical to initiate a proboscis extension reflex in *M. sexta* has been further confirmed in a recent study on feeding behaviour in this moth (Balkenius and Balkenius, 2010).

The differences in the triggering of proboscis extensions between earlier laboratory experiments (Raguso and Willis, 2002; Raguso and Willis, 2005) and the present study could also depend on a carotenoid deficiency in the moths of these earlier studies (Raguso et al., 2007). Such a deficiency would decrease the amount of light captured by the photoreceptors, and might explain why odour, rather than visual cues, became crucial to initiate a feeding behaviour in these earlier studies (Goyret et al., 2009). The present study also used a slightly higher light intensity compared with earlier studies, and it is well known that the responsiveness to visual stimuli in the crepuscular–nocturnal *M. sexta* increases with increasing light intensity (Goyret et al., 2009). Like the moths in our experiment, diurnal honeybees also extend their proboscises to odourless visual cues (Daumer, 1958; Niggerbrügge et al., 2009).

The overall flight paths of the moths towards the different feeding targets obtained in this study differ from those recorded in many earlier studies in terms of their total absence of a pronounced lateral component (Fig. 4). The behaviour of moths flying towards an odour source has traditionally been studied in a wind tunnel, where the moths can clearly be observed to fly in a zigzag track along the odour plume (Belanger and Arbas, 1998; Vickers and Baker, 1994; Willis and Arbas, 1991). This regular counterturning in contact with

the odour plume is one of the most stereotypical features of moth flight and is modulated preferentially by fluctuations in odour (Baker et al., 1984), but also by the visual cues generated by wind drift (Kennedy, 1983; Preiss and Kramer, 1986). The counterturning appears when the moths lose the odour in the wind plume and respond by turning back across it. The lack of this counterturning in the flight paths recorded in the present study is most likely due to the lack of a uniform airflow in our arena, where, instead, the local concentration gradients and the plume fragments provide information about the location of the odour source (Fraenkel and Gunn, 1961). Straight flight in moths has also been reported previously from flight conditions with homogeneous or turbulent odour plumes (Mafra-Neto and Cardé, 1994), as well as in still air (Brantjes, 1978; Brantjes and Bos, 1980).

When approaching the multimodal target – which was both clearly visible and carried an odour – the moths turned directly towards it (Fig. 5) and were also able to locate it from any visual angle (Fig. 7). These parameters of flight behaviour for target finding are of major importance for feeding efficiency if the flower has to be located in the vast natural habitat of the moth, rather than in our relatively small arena. In bumblebees, it has previously been shown that multimodal cues are not superior to olfactory cues during the learning phase, but flowers with multimodal signals increased the decision speed and accuracy of the foraging behaviour (Kulahci et al., 2008). Our results suggest that multimodal signals are also likely to increase the accuracy of foraging behaviour in moths.

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REFERENCES

- Andersson, S. and Dobson, H. E. (2003). Behavioral foraging responses by the butterfly *Heliconius melpomene* to *Lantana camara* floral scent. *J. Chem. Ecol.* **29**, 2302–2318.
- Baker, T. C., Willis, M. A. and Phelan, P. L. (1984). Optomotor anemotaxis polarizes self-steered zigzagging in flying moths. *Physiol. Entomol.* **9**, 365–376.
- Balkenius, A. and Balkenius, C. (2010). Behaviour towards an unpreferred colour: can green flowers attract foraging hawkmoths? *J. Exp. Biol.* **213**, 3257–3262.
- Balkenius, A. and Kelber, A. (2006). Colour preferences influence odour learning in the hawkmoth *Macroglossum stellatarum*. *Naturwissenschaften* **93**, 255–258.
- Balkenius, A., Rosén, W. and Kelber, A. (2006). The relative importance of olfaction and vision in a diurnal and a nocturnal hawkmoth. *J. Comp. Physiol.* **192A**, 431–437.
- Balkenius, A., Bisch-Knaden, S. and Hansson, B. (2009). Interaction of visual and odour cues in the mushroom body of the hawkmoth *Manduca sexta*. *J. Exp. Biol.* **212**, 535–541.
- Belanger, J. H. and Arbas, E. A. (1998). Behavioral strategies underlying pheromone-modulated flight in moths: lessons from simulation studies. *J. Comp. Physiol.* **183A**, 345–360.
- Bell, R. A. and Joachim, F. A. (1976). Techniques for rearing laboratory colonies of tobacco hornworms and pink bollworms. *Ann. Entomol. Soc. Am.* **266**, 365–373.
- Brantjes, N. B. M. (1978). Sensory responses to flowers in night-flying moths. In *The Pollination of Flowers by Insects* (ed. A. J. Richards), pp. 13–19. Dorchester: Dorset Press.
- Brantjes, N. B. M. and Bos, J. J. (1980). Hawkmoth behaviour and flower adaptation reducing self pollination in two *Liliflorae*. *New Phytol.* **84**, 139–143.
- Burns, J. G. (2005). Impulsive bees forage better: the advantage of quick, sometimes inaccurate foraging decisions. *Anim. Behav.* **70**, 1–5.
- Chittka, L., Dyer, A. G., Bock, F. and Dornhaus, A. (2003). Bees trade off foraging speed for accuracy. *Nature* **424**, 388.
- Cleveland, W. S. (1979). Robust locally weighted regression and smoothing scatterplots. *J. Am. Stat. Assoc.* **74**, 829–836.
- Cootes, T. F., Taylor, C. J., Cooper, D. H. and Graham, J. (1995). Active shape models: their training and application. *Comput. Vis. Image Underst.* **61**, 38–59.
- Daumer, K. (1958). Blumenfarben, wie sie die Bienen sehen. *Z. Vgl. Physiol.* **41**, 49–110.
- Duda, R. O., Hart, P. E. and Stork, D. G. (2000). *Pattern Classification*. New York: John Wiley & Sons.
- Fraenkel, G. and Gunn, D. (1961). *The Orientation of Animals*. New York: Dover Publications.
- Frye, M. A., Tarsitano, M. and Dickinson, M. H. (2003). Odor localization requires visual feedback during free flight in *Drosophila melanogaster*. *J. Exp. Biol.* **206**, 843–855.
- Goyret, J., Markwell, P. M. and Raguso, R. A. (2007). The effect of decoupling olfactory and visual stimuli of the foraging behaviour of *Manduca sexta*. *J. Exp. Behav.* **210**, 1398–1405.
- Goyret, J., Kelber, A., Pfaff, M. and Raguso, R. A. (2009). Flexible responses to visual olfactory stimuli by foraging *Manduca sexta*: larval nutrition affects adult behavior. *Proc. R. Soc. Lond. B Biol. Sci.* **276**, 2739–2745.
- Hebets, E. A. and Papaj, D. R. (2005). Complex signal function: developing a framework of testable hypotheses. *Behav. Ecol. Sociobiol.* **57**, 197–214.
- Johnstone, R. A. (1996). Multiple displays in animal communication: 'backup signals' and 'multiple messages'. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **351**, 329–338.
- Kennedy, J. S. (1983). Zigzagging and casting as a programmed response to wind-borne odor: a review. *Physiol. Entomol.* **8**, 109–120.
- Kern, R. and Varju, D. (1998). Visual position stabilization in the hummingbird hawk moth *Macroglossum stellatarum* L. I. Behavioral analysis. *J. Comp. Physiol.* **182A**, 225–237.
- Kulahci, I. G., Dornhaus, A. and Papaj, D. (2008). Multimodal signals enhance decision making in foraging bumblebees. *Proc. R. Soc. Lond. B Biol. Sci.* **275**, 797–802.
- Mafra-Neto, A. and Cardé, R. T. (1994). Fine-scale structure of pheromone plumes modulates upwind orientation of flying moths. *Nature* **369**, 142–144.
- Magnhagen, C. (1991). Predation risk as a cost of reproduction. *Trends Ecol. Evol.* **6**, 183–186.
- Niggebrügge, C., Leboulle, G., Menzel, R., Komischke, B. and Hempel de Ibarra, N. (2009). Fast learning but course discrimination of colours in restrained honeybees. *J. Exp. Biol.* **212**, 1344–1350.
- Partan, S. R. and Marler, P. (2005). Issues in the classification of multimodal communication signals. *Am. Nat.* **166**, 231–245.
- Passino, K. M. and Seeley, T. D. (2006). Modeling and analysis of nest-site selection by honeybee swarms: the speed and accuracy trade-off. *Behav. Ecol. Sociobiol.* **59**, 427–442.
- Pfaff, M. and Kelber, A. (2003). Ein vielseitiger Futterspender für anthophile Insekten. *Entomol. Z. Insektenbörse* **113**, 360–361.
- Preiss, R. and Kramer, E. (1986). Mechanism of pheromone orientation in flying moths. *Naturwissenschaften* **73**, 555–557.
- R Development Core Team (2009). *R: A Language and Environment for Statistical Computing*. Technical report, Vienna, Austria: R Foundation for Statistical Computing.
- Raguso, R. A. and Pichersky, E. (1999). A day in the life of a linalool molecule: chemical communication in a plant–pollinator system. *Plant Species Biol.* **14**, 95–120.
- Raguso, R. A. and Willis, M. A. (2002). Synergy between visual and olfactory cues in nectar feeding by wild hawkmoths *Manduca sexta*. *Anim. Behav.* **69**, 407–418.
- Raguso, R. A. and Willis, M. A. (2005). Synergy between visual and olfactory cues in nectar feeding by naive hawkmoths, *Manduca sexta*. *Anim. Behav.* **64**, 685–695.
- Raguso, R. A., Ojeda-Avila, T., Desai, S., Jurkiewicz, M. A. and Woods, H. A. (2007). The influence of larval diet on adult feeding behaviour in the tobacco hornworm moth, *Manduca sexta*. *J. Insect. Physiol.* **53**, 923–932.
- Roberts, J. A., Taylor, P. W. and Uetz, G. W. (2007). Consequences of complex signalling: predator detection of multimodal cues. *Behav. Ecol.* **18**, 236–240.
- Rojas, J. C. and Wyatt, T. D. (1999). Role of visual cues and interaction with host odour during the host-finding behaviour of the cabbage moth. *Entomol. Exp. Appl.* **91**, 59–65.
- Sprayberry, J. D. H. and Daniel, T. L. (2006). Flower tracking in hawkmoths: behavior and energetics. *J. Exp. Biol.* **210**, 37–45.
- Stauffer, C. and Grimson, W. E. L. (1999). Adaptive background mixture models for real-time tracking. In *Proceedings 1999 IEEE Computer Society Conference on Computer Vision and Pattern Recognition*. Washington, DC: IEEE Press.
- Stewart, F. J., Baker, D. A. and Webb, B. (2010). A model of visual-olfactory integration for odour localisation in free-flying fruit flies. *J. Exp. Biol.* **213**, 1886–1900.
- Theobald, J. C., Warrant, E. J. and O'Carroll, D. C. (2010). Wide-field motion tuning in nocturnal hawkmoths. *Proc. R. Soc. Lond. B Biol. Sci.* **1683**, 853–860.
- Uchida, N. and Mainen, Z. F. (2003). Speed and accuracy of olfactory discrimination in the rat. *Nat. Neurosci.* **6**, 1224–1229.
- Vickers, N. J. and Baker, T. C. (1994). Reiterative responses to single strands of odor promote sustained upwind flight and odor source location by moths. *Proc. Natl Acad. Sci. USA* **91**, 5756–5760.
- Willis, M. A. and Arbas, E. A. (1991). Odor-modulated upwind flight of the sphinx moth, *Manduca sexta* L. *J. Comp. Physiol.* **169A**, 427–440.