Independent control of slow and fast flight manoeuvres in insects: the roles of vision and antennal mechanoreception

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Flying animals need constant sensory feedback about their body position and orientation for flight control. The visual system provides valuable but slow feedback. In contrast, mechanosensory channels can provide feedback at much shorter response times. How the contributions from the two senses are integrated is still an open question in most insect groups. In Diptera, fast mechanosensory feedback is provided by organs called halteres, and is crucial for the control of rapid flight manoeuvres, while vision controls manoeuvres in lower temporal frequency bands. Here we have investigated the visual-mechanosensory integration in an insect which lacks halteres: the hawkmoth *Macroglossum stellatarum.* It is representative for a large group of insects that use mechanoreceptive Johnston's organs in their antennae to provide gyroscopic feedback on perturbations in body position. High-speed videos of freely-flying hawkmoths hovering at stationary or oscillating artificial flowers show that positional fidelity during flight was reduced in antennectomised animals, but was rescued after antennal re-attachment. Our experiments show that antennal mechanosensory feedback specifically supports fast flight manoeuvres (flower oscillations between 2-6 Hz), but not slow ones. Differences in the reliability of visual feedback (in different light intensities) affected all antennal conditions equally, suggesting there was no compensatory interaction between antennal and visual feedback under the conditions tested. These results establish the importance of antennal mechanosensors in providing rapid mechanosensory feedback for finer control of flight manoeuvres, acting in parallel to visual feedback.

Introduction

The impressive aerobatic manoeuvres of insects provide an insightful model for the neural control of flight and for the development of flying robots [Fuller et al. 2014]. Insect flight requires constant sensory feedback, both on body position relative to the environment, as well as on perturbations to body position. Visual feedback provides key information about flight parameters including ground speed, distance to obstacles and targets, and aerial displacements (for a review see [Srinivasan et al. 1999]). However, visual estimation of self-motion [Fuller et al. 2014, Hung et al. 2013] is limited by its temporal resolution and substantial latency to flight muscle activation [Sherman & Dickinson 2004, Suver et al. 2016]. This is too slow to control very fast aerial manoeuvres, which require rapid sensory feedback before perturbations become too large and thus energetically costly to the animals [Bender & Dickinson 2006].

Avoiding the temporal limitations set by the visual system, insects use mechanosensors to sense their own motion, as these can transduce perturbations on much faster time scales [Yarger & Fox 2016]. The halteres of Dipteran insects are a classic example of gyroscopic function in active flight. Halteres are club-shaped structures evolutionarily derived from the hind-wings that vibrate at the wing beat frequency and sense rotations in any axis [Fraenkel & Pringle 1938, Nalbach 1994, Pringle 1948]. Halteres, however, are a special feature of only Dipteran (and Strepsipteran) insects [Pix et al. 1993]. How do all other flying insects, which require fast feedback for stable flight, control flight manoeuvres without halteres? This question is especially interesting in insects active in dim light, as the visual systems of many insects trade off temporal acuity for sensitivity [Warrant 1999, Warrant 2017], thus rendering visually-based flight control even less reliable than and increasing the need for mechanosensory feedback control.

Sphingids are a group of flying insects that are able to fly in a wide range of light intensities, due to their superposition compound eyes and additional neural adaptations [O'Carroll et al. 1996, O'Carroll et al. 1997, Stöckl et al. 2017, Theobald et al. 2010]. The effect of light intensity on their visual flight control has been quantified [Sponberg et al. 2015, Stöckl et al. 2017]. Moreover, recent studies show that the crepuscular hawkmoth *Manduca sexta* uses information provided by antennal mechanosensors, which may function similar to Dipteran halteres [Sane et al. 2007]. The mechanosensory Johnston's organs, present at the scape-pedicellar joint of the antennae, are stimulated by deflections of the antennal flagellum, is sensitive to a wide range of frequencies [Dieudonné et al. 2014], far exceeding the temporal response range of the visual system [Stöckl et al. 2017, Theobald et al. 2010]. After ablation of their flagella, this organ no longer receives relevant information. Thus *M. sexta* increasingly crash or fly backwards as a result of flight instability, while re-attachment of the flagella significantly improves their flight performance [Sane et al., 2007]. Impaired flight performance after flagellum ablation has also been observed in other Lepidopteran species, such as the tortoise-shell butterfly *Aglais urticae* [Gewecke & Niehaus 1981, Niehaus 1981] and the diurnal swallowtail moth *Urania fulgens* [Sane et al. 2010]. Although these studies underscored the importance of antennal mechanosensors for natural flight, the severe behavioural impairment caused by flagella ablation impeded quantifying the contributions of antennal mechanosensors to flight control, and how they are integrated with the visual sense.

To address these questions, we therefore chose another model, the diurnal hawkmoth *Macroglossum stellatarum*, which retains both the motivation and ability to fly after flagella ablation. *M. stellatarum* feed from flowers while hovering in front of them, and previous studies have underscored the importance of visual feedback on their flower tracking behaviour [Farina et al. 1995, Farina et al. 1994, Kern 1998, Stöckl et al. 2017]. Using this model, we were able to test the role of antennal

mechanosensors for the control of stationary flight, as well as for flight manoeuvres of controlled temporal frequencies, focussing on the integration of visual and mechanosensory information. Here, we show that in *M. stellatarum*, antennal mechanosensors play a key role in the control of hovering flight, specifically in the control of fast flight manoeuvres (rapid turns). Furthermore, we show that visual and antennal mechanosensory feedback operate independently of each other in different frequency bands, with no sign of compensatory interaction.

Results

Antennectomised hawkmoths performed less stable hovering at a stationary flower

To quantify the role of antennal mechanosensory feedback in free flight, we trained hawkmoths (*Macroglossum stellatarum*) in a flight cage to approach and hover in front of an artificial flower with sugar solution provided in the nectary at its centre (see Methods). Individual moths were tested in three antennal conditions: with intact antennae (*control*, blue in Figs 1 and S1.1), with ablated antennae, from here onwards called antennectomised (*antennect*, red) and with re-attached antennae (*reattach*, green). Antennectomized moths were less prone to hover in front of the artificial flower than moths with intact and re-attached antennae (Table 1). Yet, a substantial proportion of antennectomised individuals still approached and fed from the flower (60% in bright and 36% in dim light), which made it possible to study the combined roles of vision and antennal mechanosensory feedback on flight control in more detail.

When approaching the flower, antennectomised moths had distinctly longer and more tortuous flight paths than moths in the control and flagella re-attached conditions (Fig. S2.1). For further quantify flight performance, we focused on the hovering flight of the hawkmoth in front of the flower, where its body position could be closely monitored. Their target position was clearly defined by the position of the flower, from which they fed. For the stationary flower experiment, we analysed the hovering flight of 6 animals, which performed in all three antennal conditions and both light intensities.

When hovering in front of the stationary flower, antennectomised moths moved around their target position to a greater degree than moths in the other two antennal conditions, as evident from their thorax position over time (Fig. 2A). We quantified the amplitude of these thoracic movements across a range of frequencies from 0.5 to 50 Hz. In all three antennal conditions, the magnitude of thoracic movements decreased with increasing frequencies (i.e. the animals performed smaller movements of higher frequencies) (Fig. 2B; Fig. S2.2). At 3000 lux, the thoracic movements of antennectomised moths were significantly larger than those of the other two antennal conditions between 0.7 and 5 Hz, as well as between 8 and 16 Hz (Fig. 2B, Table S2.3), while there was no significant difference between control and re-attached condition. Thus, re-attaching the flagellum restored flight performance close to the control state.

Because hawkmoths use abdominal movements for aerial stabilization during flight [Dyhr et al. 2013, Hinterwirth et al. 2012], we also quantified the movement of the abdomen over the same frequency range. Across all three antennal conditions, the abdominal and thoracic movements revealed similar trends; in antennectomised moths, their magnitude was significantly larger than in control moths or flagella re-attached moths, over the entire range of frequencies tested (Fig. 2C, Table S2.4). Abdominal movements, unlike thoracic movements, differed significantly between control moths and moths with re-attached antennae only at one frequency (1.66 Hz).

Hovering flight is inherently unstable [Liang & Sun 2013, Wu & Sun 2012], and hovering animals need constant sensory feedback to remain at a fixed position [Cowan et al. 2014]. Both the visual system and the antennal mechanosensory system could provide such feedback about deviations from the

target position, which can then be corrected. Because antennectomised moths showed greater corrective movements in our experiment, especially at higher frequencies, we conclude that antennae are required for the control of hovering flight. Without antennal input, the feedback about deviations from the target position (likely supplied by the visual system and possibly by mechanosreceptors on the proboscis) is slower, and causes moths to drift further away from their target position before a corrective manoeuvre can be initiated. This in turn results in greater thoracic and abdominal movements.

Antennectomy reduces flower tracking performance at high frequencies in hawkmoths

After comparing the stationary hovering performance between antennal conditions, we examined the differences in performance at specific temporal frequencies. While the hawkmoths were feeding from the nectary, we laterally moved the artificial flower to elicit flight manoeuvres of controlled temporal frequencies and amplitudes while the moths were tracking the flower (Fig. 1C). To probe the moth's manoeuvrability at different temporal frequencies, we used two movement patterns as stimuli: one pattern generated from a sum of sine waves ranging from 0.5 to 8.9 Hz, which decreased in amplitude with increasing frequency, thus retaining the same velocity ("sum-of-sines", Fig. 3A); and a second movement pattern with constant amplitude and an increase of temporal frequency from 0 to 7.3 Hz over time ("chirp", Fig.1D & 3C). We analysed the flight performance of 12 moths, which tracked both stimuli in all antennal conditions and two light intensities. Flight performance was evaluated as tracking error: a metric combining both their accuracy in tracking the amplitude (Fig. S3.1D-F), and the phase relationship (Fig. S3.1FG-I) of the flower movement [Roth et al. 2011, Sponberg et al. 2015].

Tracking performance of hawkmoths in the control conditions agreed with the conclusions of previous investigations of intact individuals of this species [Farina et al. 1995, Farina et al. 1994, Stöckl et al. 2017]. In all antennal conditions, the moths tracked the sum-of-sines stimulus very accurately at low frequencies (Fig.3A): at 3000 lux, their tracking errors were close to 0 for flower movements up to 1 Hz, indicating perfect tracking (Fig.3B). With increasing frequency, tracking errors increased, but did not significantly differ between antennal conditions for frequencies below 2 Hz. At higher frequencies, however, antennectomised moths overshot the flower movements, resulting in a greater lag between the position of the moth and the flower and thus larger tracking errors (Fig.3B): in the frequency range of 2 to 5 Hz, tracking errors were significantly higher for the antennectomised moths than both the control moths and moths with re-attached flagella. In this frequency range, hawkmoths with re-attached flagella also had significantly higher tracking errors than control moths (Table S3.1). Thus, the loss of antennal mechano-sensation impaired flight control specifically at the higher temporal frequencies of flower movement, which compelled the moths to perform fast turns. The lack of effects with frequencies below 2 Hz suggests that vision (and possibly other sensory modalities) provide fast enough feedback to control manoeuvres at these low frequencies.

To ensure that the differences in flight performance between the three antennal conditions did not depend on the specific type of flower movement, we presented the moths with a second stimulus that was quite different in its characteristics. The so-called chirp stimulus retained an amplitude of 20 mm, while the temporal frequency increased continuously, resulting in faster flower movements at higher temporal frequencies (Fig. 1D). At low frequencies, hawkmoths of all antennal conditions tracked this stimulus neatly (Fig. 3C). However, as the temporal frequency increased, antennectomised moths tended to overshoot the position of the flower at the end of each sideways movement, thereby increasing the phase lag on each cycle. The accumulated phase lag and overshoot were eventually large enough to cause the moths to lose contact with the nectary, and abort tracking the flower (Fig. 3C). Only 1 out of 12 antennectomised moths succeeded in following the flower movement during the entire stimulus at 3000 lux. In contrast, almost all control and re-attached moths tracked the flower

successfully up to the maximum frequency that we presented (7.3 Hz). As a measure of flight performance, we quantified the temporal frequency at which hawkmoths in the different antennal conditions aborted flower tracking (Fig.3D). At 3000 lx, there was no significant difference between the control and re-attached flagella moths (Table S3.2). Antennectomised hawkmoths aborted flower tracking at a median frequency of only 4.4 Hz, significantly lower than under the other two conditions.

Despite the differences between the movement patterns and their demands on flower tracking, we observed similar trends in hawkmoth flight performance for both stimuli. Moths in all antennal conditions tracked flower movements well at low temporal frequencies, while antennectomised hawkmoths were significantly impaired at higher temporal frequencies compared to the other two antennal conditions. The average frequency at which antennectomised moths failed to track the chirp stimulus fell within the frequency range for which the tracking error with the sum-of-sines-stimulus was greatest, despite the difference in flower velocity between stimuli. Thus, rather than the velocity of the flower movement, the speed at which the flower changed direction from left to right (which was slow at low, and fast at high temporal frequencies) determined whether the antennectomised hawkmoths could successfully track the flower. These data show that antennal feedback is crucial for fast turns - or directional changes - which are associated with changes in body posture.

Slow visual feedback impaired hovering and tracking performance of all antennal conditions.

In the experiments described so far, hawkmoths of all antennal conditions did not differ in their flight performance at low temporal frequencies (below 0.7 Hz for stationary hovering and below 2 Hz for following a moving flower). At these frequencies, feedback from other sensory modalities likely mitigates the problems in flight control caused by antennal ablation. In particular, visual feedback is known to provide information about changes in insect body (head) position in flight (for a review see [Srinivasan et al. 1999]), albeit with longer latencies and a lower temporal frequency range than mechanosensory feedback [Sane et al. 2007]. We therefore tested how differences in the reliability of visual feedback would affect the hawkmoth's performance, in combination with the antennal conditions. We therefore tested the same group of hawkmoths at an illumination of 30 lux, close to the light intensity limit, at which these diurnal hawkmoths reliably approach and feed from the artificial flowers [Stöckl et al. 2017]. In this low light intensity, the visual system of *M. stellatarum* slows down distinctly [Stöckl et al. 2017]. Thus, if antennectomised hawkmoths relied mainly on visual feedback for flight control when their antennal mechanosensory feedback was reduced, their flight performance should be poorer at lower temporal frequencies, as compared to their performance in bright light. However, we did not expect any difference in the performance of hawkmoths in the control and re-attached conditions, because these moths still receive fast feedback from their antennal mechanosensors, completely independent of light intensity.

As expected, at lower frequencies the tracking error of antennectomised hawkmoths subjected to the sum-of-sines stimulus was greater in dim light than in bright light (Fig S3.1B). However, we observed the same difference in the control and flagella re-attached conditions (Fig S3.1A, C). Experiments using the chirp stimulus further confirmed that the low light intensity affected tracking performance in all antennal conditions (compare Fig.3D and Fig S3.2).

To quantify the differences in the performance of moths in dim and bright light, we compared the difference in the frequency, at which each moth reached a tracking error of 1 with the sum-of-sines stimulus (Fig 4A), as well as the difference in the frequency at which moths aborted tracking with the chirp stimulus (Fig 4B). For both stimuli, we found no significant differences between antennal conditions (Table S4.1 & S4.2), indicating that the visual system does not provide compensation for

the loss of mechanosensory feedback in antennectomised moths. Instead, the slower visual processing impairs flight control equally in all antennal conditions.

This hypothesis was further supported when we compared the moths that were stationary hovering in bright (Fig. 2) and dim light (Fig. S2.2). To quantify the differences in flight performance between the two light intensities, we calculated the difference between the magnitude of thoracic and abdominal movements across a range of frequencies in dim and bright light for individual moths (Fig. 4 C, D). We found no significant effect of antennal condition on the average difference in thorax movements between dim and bright light (Table S4.3), and neither for abdomen movements (Table S4.4). In conclusion, as in the experiment with the moving flower, the stationary hovering experiment did not reveal any effects of visual reliability on flight performance in antennectomised hawkmoths that we did not observe in control moths as well.

Discussion

Insect flight is controlled by both vision and mechanosensation. How their contributions are integrated is in large parts still an open question. In Dipteran flies, which use halteres as gyroscopic sensors, vision and mechanosensation operate in complementary frequency ranges [Mureli & Fox 2015, Yarger & Fox 2016]. We investigated for the first time how vision and mechanosensation contribute to flight control in an insect using antennal Johnston's organs as mechanosensors. We found a similar integration strategy by quantifying both stationary hovering and flight manoeuvres during flower tracking in the hawkmoth *Macroglossum stellatarum*: antennal mechanosensors are crucial to control fast flight manoeuvres, while the visual system is sufficient to control slower ones.

Flagella re-attachment restores flight performance

We have shown that flight control in the diurnal hawkmoth *Macroglossum stellatarum* requires feedback from antennal mechanosensors. As observed in previous experiments [Sane et al. 2007], re-attaching the flagellum restored flight performance by returning load to the Johnston's organs, both for stationary hovering and for tracking moving flowers. This confirms the crucial role of antennal mechanosensation in hawkmoth flight control, and adds to a growing body of evidence [Dieudonné et al. 2014, Gewecke & Niehaus 1981, Niehaus 1981, Sane et al. 2010] that Lepidoptera use Johnston's organs in their antennae as gyroscopic mechanosensor for flight control. Because moths behaved similarly after re-attachment of antennae as in the control condition, the difference in the behaviour of antennectomised moths was unlikely to be caused by the effects of surgery (as the individuals with re-attached antennae had undergone surgery twice, see Methods).

As demonstrated previously [Sane et al. 2007], while flagellum re-attachment improved flight performance significantly compared to the antennectomised condition, it did not restore it to the level of intact animals. One reason for this observation might be that some properties of the re-attached flagella differ from those of intact animals. Re-attached flagella are not connected to the haemolymph system of the hawkmoth and thus dry out, which reduced their weight by more than 50% (see Methods). Since the flagella are thought to provide a mass that inertial forces act on [Sane et al. 2007], changes in weight might alter the sensory input considerably. Changes in the flexibility of the flagella due to moisture loss might also contribute to this effect. Future experiments will be able to shed light onto the exact properties of the flagellum that are required for an unimpaired sensory percept.

Antennal mechanosensation and vision operate in different frequency bands.

Our experiments quantified the frequency range in which antennal mechanosensation is required for the control of flight and body position in *M. stellatarum* moths. We demonstrate that hawkmoths

without antennae track flowers moving at frequencies below 2 Hz as accurately as hawkmoths with intact antennae (Fig.2 & 3). This suggests that antennal mechanosensory feedback is not required to control slow manoeuvres. Our findings are mirrored in the study of Dipteran flight control: fruit flies rely more strongly on visual feedback than on feedback from the mechanosensory halteres during slow rotations [Sherman & Dickinson 2003]. On the other hand, antennectomised hawkmoths performed significantly worse than moths with intact and re-attached antennae at flower movement frequencies above 2 Hz, where more rapid turns are required to follow the lateral trajectory of the moving flower.

Thus we conclude that mechanosensory feedback from the antennae is required to control fast flight manoeuvres, in which the body position of the hawkmoth cannot be corrected using the slow visual feedback. This again is analogous to the finding that the control of fast saccadic rotations in Dipterans, mainly uses mechanosensory feedback from the halteres, while vision only plays a marginal role [Bender & Dickinson 2006, Sherman & Dickinson 2003].

Vision does not compensate for the loss of antennal mechanosensation in hawkmoth flight control

Both vision and mechanosensation contribute to insect flight control, and how they are integrated is subject of ongoing investigations. In Dipteran flies, vision and haltere mechanosensation operate in complementary frequency ranges, and while both inputs are required for stable flight under most circumstances [Yarger & Fox 2016], each of them seems to be able to independently control some flight behaviours [Mureli & Fox 2015]. In honeybees, airflow on the antennae and optic flow influence antennal positioning in tethered as well as free flight [Khurana & Sane 2016]. In the Oleander hawkmoth *Daphnis nerii*, visual feedback modulates antennal positioning [Krishnan & Sane 2014] in a similar way.

Here we tested whether vision and antennal mechanosensation have a combined effect on flight control and flower tracking performance, or whether they act independently. Using a bright and a low light intensity, we manipulated the temporal resolution of visual responses [Stöckl et al. 2017, Stöckl et al. 2016]. In dim light, the low speed and reduced reliability of the visual input to flight control causes larger tracking errors at higher flower movement frequencies in all antennal conditions (Fig. 4). This effect is explained by the fact that the moths need visual input to identify the movement of the flower relative to the own position and track the flower – this information cannot be provided by the antennal mechanosensors (Fig. 1A). In dim light, because visual processing is slow, and incapable to resolve fast flower movements, the moth fails to track them, as previously demonstrated [Sponberg et al. 2015, Stöckl et al. 2017]. Thus, in dim light, the lower temporal resolution affected the visual monitoring of flower position (and not that of the moth's body position), and therefore affected all antennal conditions. [Roth et al. 2016] have demonstrated that mechanoreceptors on the proboscis also play a role in flower tracking in the crepuscular, long-tongued hawkmoth *M. sexta*. In our experiments we did not test this possibility, however, flight ability was similarly impaired during flower approach (Fig. S2.1), during which the approach phase the proboscis cannot provide no positional cues. Moreover, in the short-tongued *M. stellatarum,* proboscis mechanoreception has been shown to be of minor importance for controlling hovering flight [Goyret & Kelber 2011, Zhou 1991]).

We did not observe a specific effect of light intensity on flight control in the antennectomised moths. This suggests that higher resolution of visual feedback cannot mitigate the instability caused by the loss of mechanosensory feedback. Two main hypotheses could explain this finding: either the contributions of vision and mechanosensation descend to the motor outputs in separate parallel pathways whose functions do not overlap- which is unlikely, as recent recordings of descending neurons in Oleander hawkmoth show that they respond to both visual and mechanosensory

stimulation [Umesh et al. 2017]. Alternatively, vision and mechanosensation might share descending pathways but operate in different frequency ranges, and the visual input is too slow to compensate for the lack of mechanosensation. The latter hypothesis is consistent with physiological studies showing that mechanosensors in Johnston's organ respond to antennal displacements at frequencies of up to 100 Hz in the hawkmoth *M. sexta* [Sane et al. 2007], while the wide-field motion-sensitive neurons of the same hawkmoth species cease to respond to temporal frequencies above 20 Hz [Stöckl et al. 2017], to which most mechanosensors of the Johnston's organ only show a weak response. Eventually, an assessment of the physiological responses of descending neurons that activate the flight muscles will be necessary to reveal how visual and mechanosensory information are combined to control flight in hawkmoths.

Conclusion

Antennal mechanosensation represents one strategy for flying insects to obtain rapid sensory feedback about changes in self-motion, which is crucial for flight control. We showed here that hawkmoths rely on feedback from antennal mechanosensors specifically to control fast manoeuvres and rapid deviations from their hovering position, while they use their visual system to control slower flight manoeuvres. These findings detail a striking similarity to the interaction between mechanosensory halteres and vision in the Dipteran flight control model, and for the first time characterise in detail the antennal system for mechanosensory flight control in a non-Dipteran insect.

Methods

Animals

Wild adult *Macroglossum stellatarum* L. (Sphingidae), were caught in Sorède, France. Eggs were collected and the caterpillars raised on their native host plant *Gallium sp.* The eclosed adults were allowed to fly and feed from artificial flowers similar to the experimental flowers, in flight cages (70 cm length, 60 cm width, 50 cm height) in a 14:10 h light:dark cycle for at least one day before experiments.

All animals were tested with intact antennae first (*control*), then antennectomised (*antennect*), and finally with re-attached antennae (*reattach*) as described below (Figs. 1, S.1.1). Only data from animals that were tested under all three antennal conditions were included in the final data analysis.

Surgery: Antennae ablation and reattachment

For antennectomy, moths were held, by their thorax under a dissection microscope and their flagella were clipped with a pair of surgical scissors, retaining between 5 and 10 annuli (Fig S1.1B). This ensured that Johnston's organs, located at the base of the antennae, were left intact but unloaded. Ablated flagella were preserved in a plastic petri dish with wet tissue to prevent them from drying and losing shape until they were re-attached to the same individual. Moths were left to recover from the antennectomy and tested the following day.

To re-attach the flagella, moths were immobilized by cooling at 3° C for 8 minutes, followed by 2 min at -20° C. Flagella were quickly attached to the flagellar stump with a small amount of superglue (Loctite Super Glue Gel, Henkel, Fig. S1.1C). After ensuring that the flagella were properly attached, moths were placed inside a plastic box (10cm x 10cm x 8cm) on a wet tissue paper for 10 min to keep them quiescent and ensure proper reattachment. In case an animal broke the re-attached antenna, a spare antenna of similar size was used to repeat the re-attachment procedure. Moths were then allowed to recover for a day, before being used in experiments.

We noticed that the flagella lost moisture once re-attached. To quantify the reduction in weight due to moisture loss, we weighed a set of flagella directly after antennectomy and a few days later when they had dried. Dry flagella had significantly lower weights than freshly ablated flagella (moist: 1.2 ± 0.2 mg, dry: 0.4 ± 0.3 mg; median and inter-quartile range, Wilcoxon rank sum test, z-value = -5.915, $p<0.001$). We did not determine the weight of the glue used for reattachment, but expect it to not exceed the difference between the dried and moist flagella, considering the tiny amount of glue used.

Experimental setup

As experimental setup we used the robotic flower pioneered by [Farina et al. 1994, Sponberg et al. 2015], also used in [Stöckl et al. 2017]. A flight cage (same size as the holding cage) was lined with soft muslin cloth and covered with black cloth on the outside, on three sides, while the front and top were sealed with Perspex windows to allow filming. An artificial flower (48 mm in diameter, on a 140 mm stalk) at the centre of the flight cage, with a nectary (opening of 8.3 mm diameter) filled with 10% sucrose solution, could be moved sideways (in arcs around the central pole), controlled by a stepper motor (0.9 degree/step resolution, 1/16 microstepping, Phidgets, Inc.) and a custom-written Matlab program. The cage was illuminated from above with an adjustable white LED panel and diffuser (CN-126 LED video light, Neewer). Intensity was set to 3000 lux for the bright light condition and 30 lux for the dim light condition (measured with a Hagner ScreenMaster, B. Hagner AB, Solna, Sweden, at the position of the artificial flower). In addition, two 850 nm IR LED lights (LEDLB-16-IR-F, Larson

Electronics) provided illumination for the infrared-sensitive high-speed video cameras (MotionBLITZ EoSens mini, Mikrotron) used to film the flower and moths. Videos were recorded at 100 fps, allowing us to record sequences of up to 28 seconds, which were required for our analysis of flower tracking. One camera was placed on top of the cage to film the flower and moth from above during all tests. For experiments with the stationary flower, a second camera providing a rear view was placed on a tripod outside the experimental cage, in approximately 30 cm distance to the artificial flower.

Behavioural experiments

Eclosed moths were taken from their holding cage and placed in small individually marked cardboard boxes, in which they would be kept between trials. For the duration of the experiment, moths were only given access to sucrose from the artificial flower during trials in the experimental cage. A single hawkmoth at a time was introduced into the experimental cage.

We performed two sets of experiments: one, in which we filmed the moth's approach to and hovering at a stationary flower with both the top and the rear camera, and one in which we filmed the moth tracking a moving flower using only the top camera. In this second set of experiments, we started moving the artificial flower once the moth began to feed from it. We used two different types of movements, the "sum-of-sines" stimulus and the "chirp" stimulus, in the same flight bout. The first 16 s of the sequence was thus comprised of the pseudo-random sum-of-sine stimulus composed of the following 14 frequencies, which were prime multiples of each other to avoid harmonic overlap: 0.5, 0.7, 1.1, 1.3, 1.7, 1.9, 2.3, 2.9, 3.7, 4.3, 5.3, 6.1, 7.9, 8.9 Hz. High frequencies had lower amplitudes and vice versa, to assure equal velocities at all frequencies (Fig.1D). The sum-of-sines stimulus was followed by a brief stationary phase of 0.5 s, and then the 10 s lasting chirp stimulus with fixed movement amplitude of ≈20 mm and frequencies increasing over time from 0 up to 7.3 Hz (Fig.1D) with the relation: frequency = 1.15 ^ (time -1).

The protocols were similar for both sets of experiments. Each individual was tested six times: in three antennal conditions (intact *control*, *antennectomized* and with *reattached* antennae), and in two light intensities (3000 and 30 lux). Because *M. stellatarum* were less motivated to fly in dim light, we tested the moths in dim light first, when they were hungriest and had the highest motivation to forage, and in bright light (3000 lux) later the same day. If a moth did not track both the sum-of-sines and the chirp stimulus (or the stationary flower for at least 6 s), we repeated the test the next day, until a full set of data was collected and the experiment moved on to the next condition. This experimental strategy gave antennectomised (and re-attached) moths a chance to adjust to their altered mechanosensory feedback, and indeed practice flying and tracking the flower on several days before succeeding and entering our data-analysis. Indeed, our observations suggest that the hawkmoths learned to adjust their flight to the lack or change of mechanosensory feedback, as the initial flight attempts of many antennectomised (and to a lesser degree re-attached) moths showed much more severe impairments than consecutive attempts. Our final dataset includes only individuals that tracked the flower in all three antennal conditions in both light intensities. We used 6 individuals for the experiment with the stationary flower, and 12 different individuals for the experiment with the moving flower.

Data Analysis

The positions of the flower and the hawkmoth were digitised from the videos using the DLTdv5 software for Matlab [Dyhr et al. 2013]. In experiments with stationary flowers, both the approach, and the stationary hovering period were digitised, while during experiments with the moving flower, only sequences during which the proboscis of a moth was in contact with the nectary were rated as 'tracking' and digitized (as in [Sponberg et al. 2015, Stöckl et al. 2017]). In the top view, a point on the

flower, and a reliably identifiable point on the pronotum of the moth were used for reference. From the rear view videos, we used the centre of the nectary, the centre of the pronotum and the centre tip of the abdomen (Fig. 3).

General behaviour: We characterised the general behaviour of all hawkmoths tested in the moving flower experiments, including those that did not complete all antennal conditions and light intensities, and thus were not included in any further analysis. We classified their behaviour into three different categories: non-flying (animals which would not take off after 5 minutes in the experimental cage), flying (animals which flew but would not feed from the flower) and tracking (animals feeding from and tracking the flower, at least partially), see Table 1. We compared the proportion of hawkmoths of the different antennal conditions performing these three behaviours using a logistic regression model and a generalized linear hypothesis test ("glth" function) with Tukey multiple comparisons correction for post-hoc testing.

Stationary flower experiments: to compare the stability of hovering flight between the different antennal conditions and light intensities, we analysed the position of the thorax and abdomen for a 6 s interval of hovering at the flower nectary during feeding (given perfect hovering, the thorax should retain a stable position, since the flower did not move). We quantified the amplitude of thorax and abdomen movements across different frequencies by Fourier transforming their position over time (Fig. 2B,C). In order to assess the effect of antennal condition across frequencies, we applied a linear mixed-effects model with antennal condition, temporal frequency and their interaction as fixed effects and individual identity as a random effect on the log-transformed magnitudes of body movement. We confirmed that the full model did explain the variance better than reduced versions of the model (deviance test) before performing post-hoc comparisons using the "difflsmeans" package in R.

To compare these measures across light intensities, we calculated the difference between the log-transformed magnitude spectra of thorax and abdomen position in bright and dim light for each antennal condition (Fig. 3C,D). We then compared these using general linear models of the same form as above.

Sum-of-sines movement: we used system identification analysis [Cowan et al. 2014] to characterise hawkmoth flower tracking performance. This analysis is possible because the sum-of-sines stimulus fulfils the requirement of linearity, i.e. it does create the same flower tracking performance at different amplitudes and phase relationships (see Supplement, [Stöckl et al. 2017]. Hawkmoth flower tracking can be described by two components: the gain and phase [Farina et al. 1994, Sponberg et al. 2015, Stöckl et al. 2017]. The gain gives the relationship between the amplitude of flower movement and hawkmoth movement (1 in case of perfect tracking), while the phase describes the lead or lag of the hawkmoth with respect to the flower's movement (0 in the case of ideal tracking). We used a metric, which incorporates both the effects of gain and phase to quantify the tracking performance of the hawkmoths: the tracking error [Roth et al. 2011] (Fig.3B). A tracking error of 0 means perfect tracking (comprising a gain of 1 and a phase lag of 0), while the tracking error is 1 if the hawkmoth and flower movement had no correlation (for example, when either the hawkmoth remains stationary and the flower moves or vice versa). We calculated average tracking errors within antennal conditions, as well as confidence intervals, by averaging data in the complex plane, to avoid artefacts resulting from separating gain and phase components when transforming them and averaging in the non-complex plane (see [Stöckl et al. 2017] for discussion).

Because the tracking error is an inherently complex value, and was only transformed into the non-complex plane after averaging across individuals, it is not straightforward to find appropriate statistical

tests to compare tracking error (as well as gain and phase) across antennal conditions and light intensities. Linear mixed effects models would be well suited, but complex data might not fulfil all of the assumptions these models are based on. Lacking an alternative, we had to rely on these tests, as did previous studies with the same approach [Roth et al. 2016, Sponberg et al. 2015] to compare the effect of antennal conditions across frequencies as fixed effects, including individual identity as a random effect. We are confident that overall trends identified as significant by these models are indicative of biologically relevant effects, but advise caution when interpreting differences in significance at individual movement frequencies isolated from the overall trend.

To compare tracking performance across light intensities, we calculated the difference in the frequency at which tracking error reached 1 for both dim and bright light intensity within antennal conditions (similar to [Stöckl et al. 2017]), and compared these across antennal conditions (Fig.3A). A Friedman test was used for statistical comparisons, as a non-parametric test that accounts for repeated measures.

Chirp movement: since the chirp stimulus does not fulfil the linearity criterion (does not generate the same flower tracking performance at different amplitudes and phase relationships, but rather contained a saturation non-linearity), the system identification analysis we used for the sum-of-sines stimulus could not be applied [Roth et al. 2011]. We therefore determined the temporal frequency, at which each individual lost proboscis contact with the flower (i.e. failed at tracking the flower) as a measure of flower tracking performance across frequencies. This measure gave an absolute cut-off frequency at which moths could no longer track the oscillating flower. Since this data was non-parametric and included repeated measures, we used a Friedman test to compare the paired data.

To compare the tracking performance across light intensities, we calculated the difference in termination frequency between dim and bright light for each antennal condition (taking the difference for each individual). These differences between light conditions were then compared across antennal conditions using a Friedman test to retain information about the paired data (Fig.3B).

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Author contributions

AS, SS and AK conceived the study, AD performed the experiments, AD digitized the data, JF conceived the statistical analysis, AS analysed the data with input from AD, AK and JF, AS wrote the initial draft of the manuscript, and all authors discussed and commented on the manuscript.

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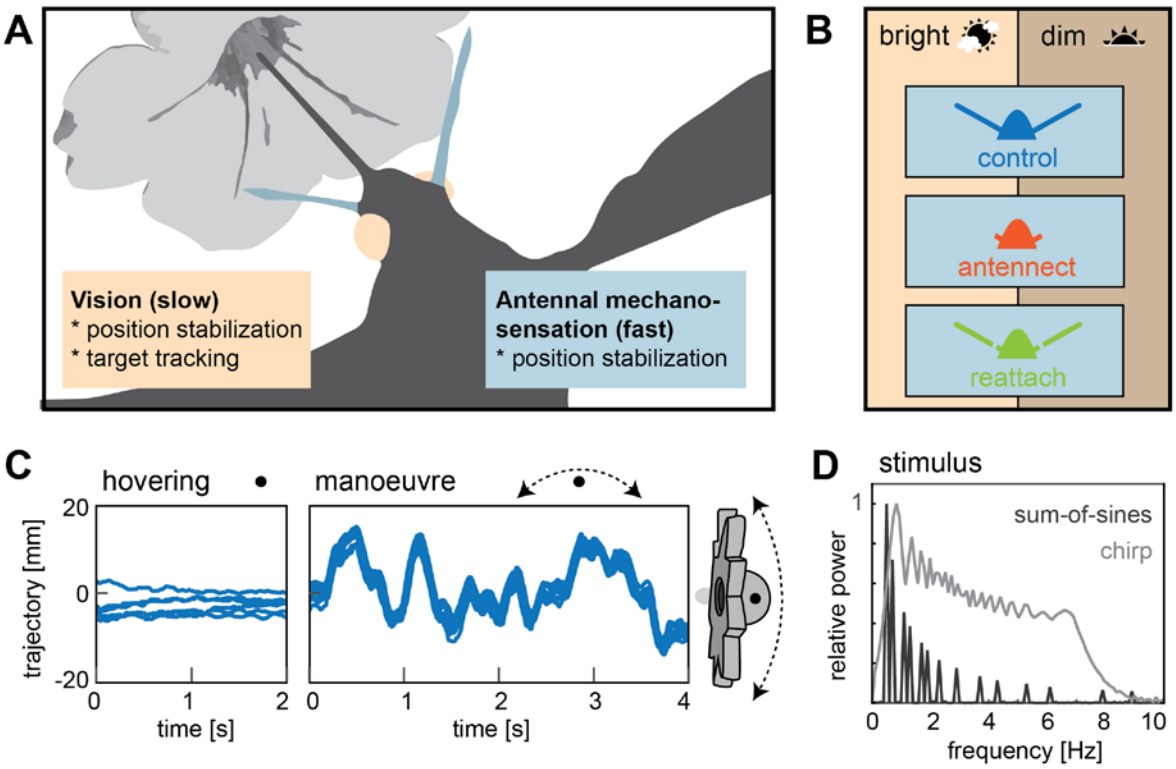

Fig. 1 Flight control in hawkmoths requires vision and mechanosensation

A. Flight control in insects requires sensory feedback about perturbations in body position. The visual system supplies such feedback, but with rather long response latencies. In addition, insects use mechanosensory systems to control their position in the air, which provide rapid feedback and thus are crucial for fast flight manoeuvres. Here, we investigated the role of antennal mechanosensation and vision on flight control in the hummingbird hawkmoth.

B. In order to quantify the effects of antennal mechanosensation in free flight, we subjected each hawkmoth to three treatments: intact antennae (*control*, blue), ablated antennae (*antennect*, red) and re-attached antennae (*reattach*, green). To quantify the role of vision, we tested these three antennal treatments in two different light intensities (*bright*: 3000lux and *dim*: 30 lux).

C. All conditions were tested in free hovering flight at artificial flowers, which could be kept stationary (*hovering*), or moved at different temporal frequencies (*manœuvre*).

D. We used a stimulus composed of a *sum-of-sines* to sample distinct frequencies with similar velocities (amplitude adjusted accordingly), as well as a stimulus ramping up in frequency, while retaining similar amplitude (*chirp*).

See also Fig. S1.1

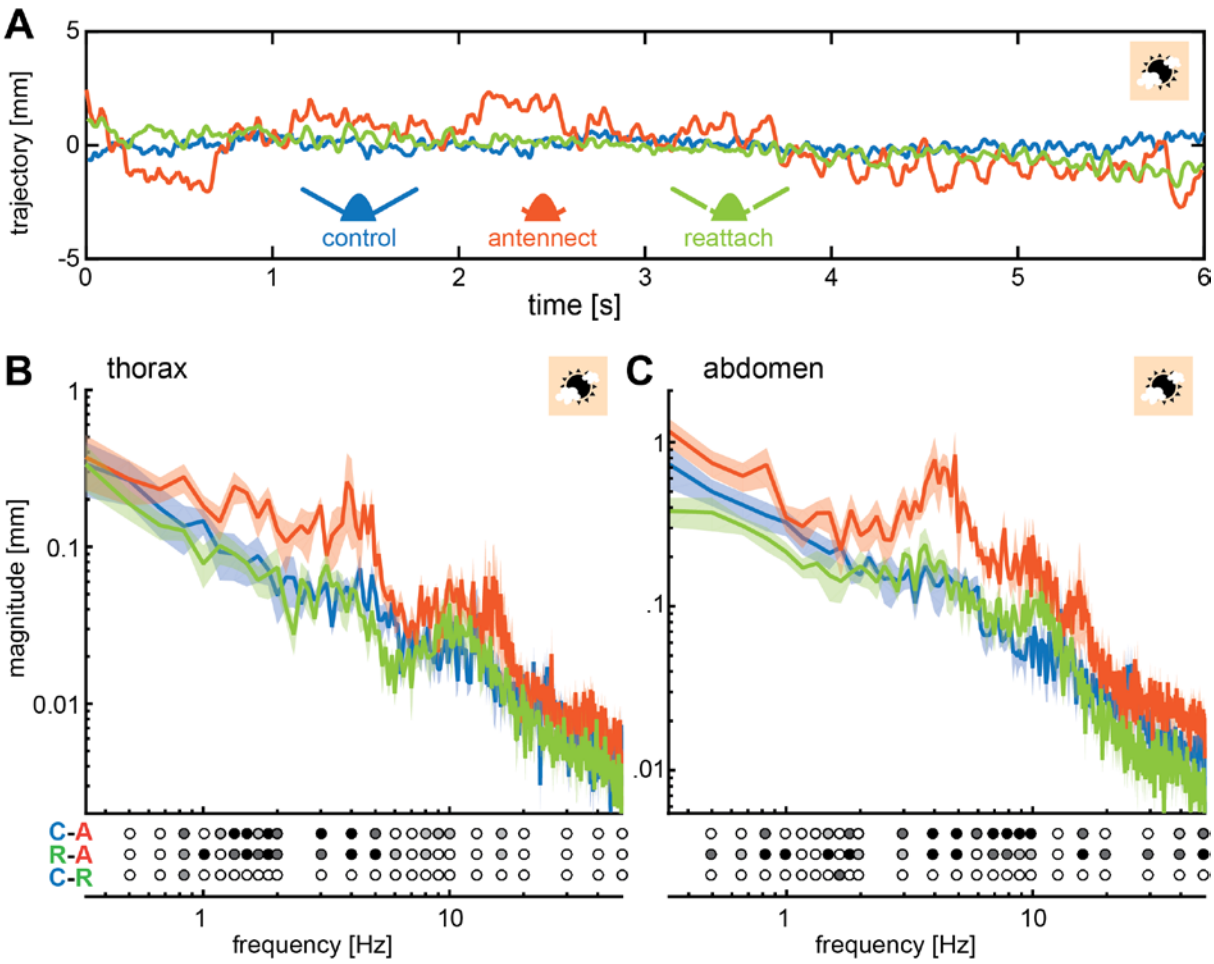

Fig. 2 Antennectomised hawkmoths showed greater thorax and abdomen movement during hovering flight at a stationary flower.

A. When moths hovered in front of a stationary flower at 3000 lux, it was notable that antennectomised moths moved around their target position with larger amplitudes than moths in the other two antennal conditions, as quantified by their thorax position. The nectary is centered at 0 mm in this graph.

B. The thorax of moths with ablated antennae moved with distinctly higher magnitude at frequencies between 1 and 8 Hz than the other two antennal conditions. There were significant differences in thorax movement between the antennectomised treatment and the other two treatments in the range between 0.7-4 Hz. There was no significant difference between control and re-attached moths. Post-hoc tests were performed as part of a general linear model including antennal treatment and frequency (binned to the logarithmic scale) as factors. Statistical significance is indicated below the plot as: black $p<0.001$, dark grey: $p<0.01$, grey $p<0.05$, white $p>0.05$, see Table S2.1.

C. Because hawkmoths are known to use abdomen movements to correct their position in the air, we also quantified these movements in the three antennal treatments, showing a similar trend to that for thorax movements: the antennectomised moths exhibited significantly larger abdomen movements in the frequency range between 0.5 and 10 Hz than the other two treatments (Statistical significance is indicated as in **B**, see Table S3.2).

See also Fig. S2.1, S2.2

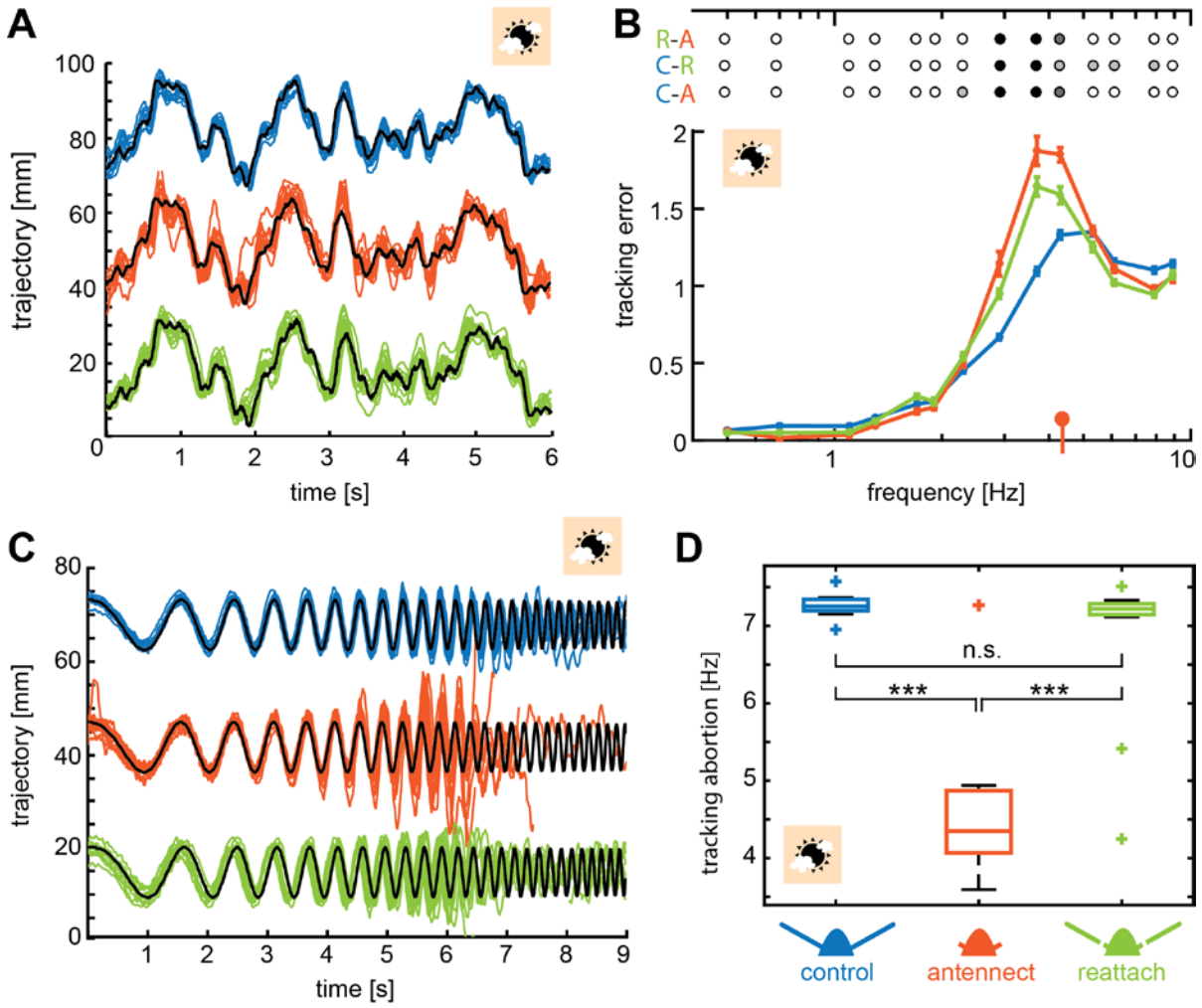

Fig. 3 Antennectomised hawkmoths showed reduced tracking performance of flowers moving at high temporal frequencies.

A, C. When tracking a moving robotic flower at 3000 lux, hawkmoths with ablated antennae often overshot the movements of the flower both with the sum-of-sines (**A**) and chirp (**C**) stimulus, specifically at higher frequencies. With increasing temporal frequencies, moths also lagged behind the phase of flower movements more strongly. While the amplitude in the sum-of-sines stimulus was adjusted such that moths of all conditions could track the entirety of the stimulus, the chirp stimulus forced moths that overshot too much and whose phase-lags were too large to loose contact with the flower, and abort tracking (see red tracks in **C**).

B. Together, overshooting and phase-lags resulted in an increased tracking error of antennectomised moths following the sum-of-sines stimulus at frequencies between 2 and 6 Hz, compared to both the control and re-attached condition. Linear mixed-effects models were used to compare the tracking error of the different antennal treatments with respect to frequency, colours indicate significance (black $p<0.001$, dark grey: $p<0.01$, grey $p<0.05$, white $p>0.05$, Table S3.1). The red indicator on the x-axis gives the median frequency at which antennectomised moths aborted tracking the chirp-stimulus (**D**). Curves show the mean and 95% confidence intervals of the mean, calculated in the complex plane.

D. For the chirp stimulus, we compared the movement frequency of the flower, at which the moths aborted tracking, across antennal treatments, showing that antennectomised moths lost contact with the flower at significantly lower frequencies than the control and re-attached condition. A Friedman test was used to compare between the treatments (*** $p<0.001$, **: $p<0.01$, * $p<0.05$, Table S3.2).

See also Fig. S3.1, S3.2

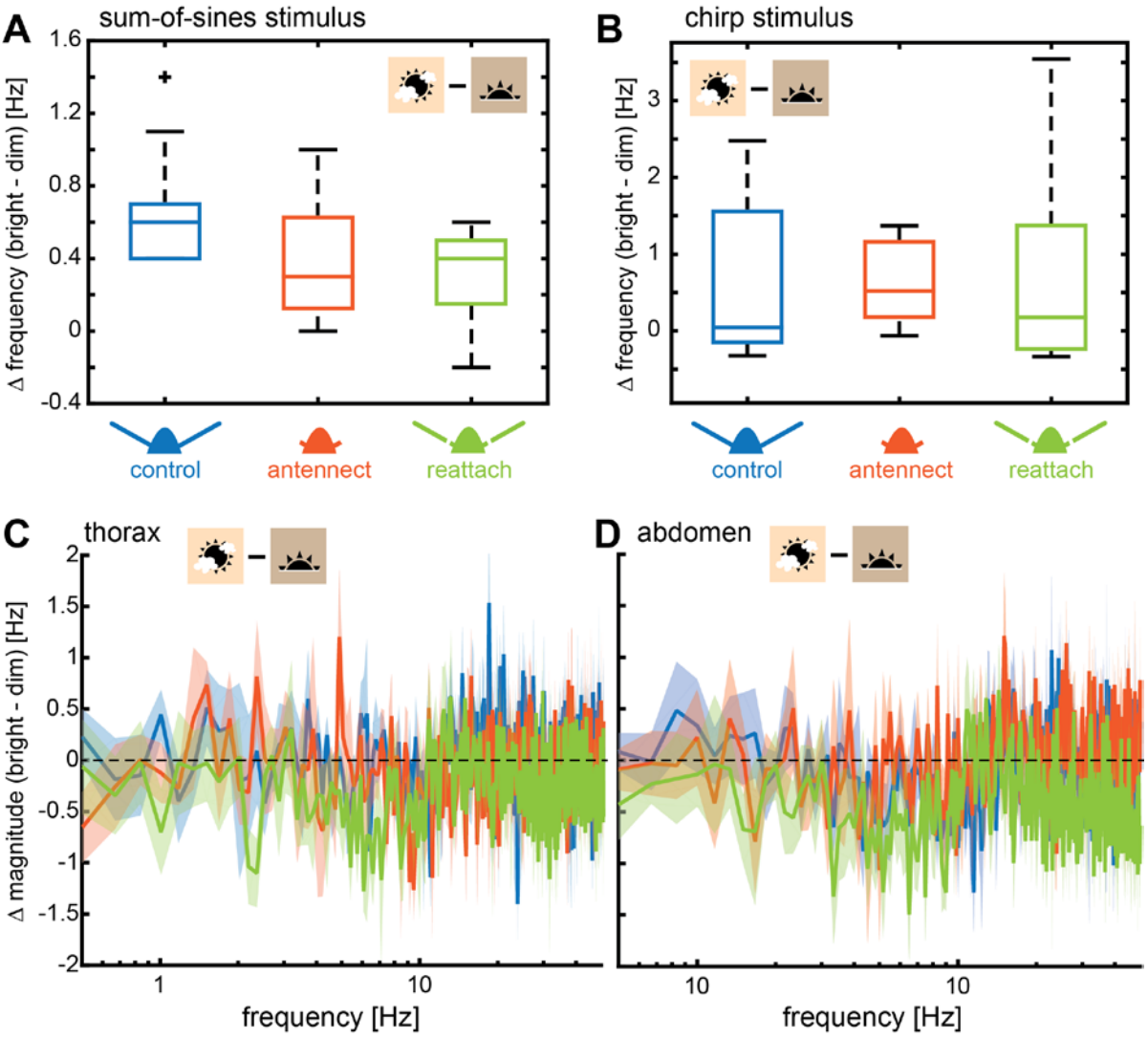

Fig. 4 Light intensity had the same effect on all antennal treatments.
To test the effect of visual feedback, and its possible interaction with antennal mechanosensory feedback, on flower tracking, we performed all experiments both a bright (3000 lux) and dim (30 lux) light intensities. Hawkmoths showed reduced tracking performance of artificial flowers moving at higher frequencies in dim light, due to the slowing of the visual system (Fig. S2.2, S3.1., S3.2). Here we compare tracking performance between bright and dim light across antennal treatments.
A. We quantified the difference in frequency between light intensities at which moths reached a tracking error of 1 with the sum-of-sines stimulus. There was no significant difference (Table S4.1) between antennal conditions, suggesting that vision reduced tracking performance in dim light irrespective of the presence or absence of mechano-sensory feedback.
B. Similarly, there was no significant difference between the tracking performance in dim and bright light for the chirp stimulus (quantified as the difference of tracking abortion frequency at the two light conditions) (Table S4.2).
C-D. We determined the difference between the log-transformed magnitude spectra for thorax (**C**) and abdomen (**D**) movement in bright and dim light. No significant effect of antennal condition was found using Friedman comparisons of the average difference in thorax or abdomen movements (Tables S4.3 & S4.4).

Table 1. **Proportion of moths performing specific behaviours across antennal conditions and light intensities**. Proportion of trials, in which animals performed the following behaviours: *no flight*, *flight*

(but no tracking of the flower), *tracking*. This dataset is based on the animals participating in the moving flower experiments. Of the total numbers of moths, 27 control, 22 antenna-ablated, and 14 re-attached moths were tested in both light intensities. Some moths were tested multiple times to collect the necessary tracking data, and thus have contributed multiple trials to this dataset. Statistical comparisons within light intensity performed using logistic regression. Shown are z-values of post-hoc tests, background colour indicates significance, white: p>0.05, light grey: p<0.05, black: p<0.001

		control	antennect	reattach	C-A	C-R	A-R
bright	No flight	0.03	0.11	0.05	1.4	0.5	-0.8
	Flight, no tracking	0.15	0.29	0.1	1.3	-0.6	-1.5
	Flight & tracking	0.82	0.60	0.85	-2.1	0.3	1.9
	Total	**38**	**42**	**20**			
dim	No flight	0.03	0.34	0.08	2.7	0.8	-2.2
	Flight, no tracking	0.09	0.30	0.08	2.3	-0.1	-2.1
	Flight & tracking	0.88	0.36	0.84	-4.4	-0.5	3.7
	Total	**35**	**66**	**25**			

Supplementary Figures

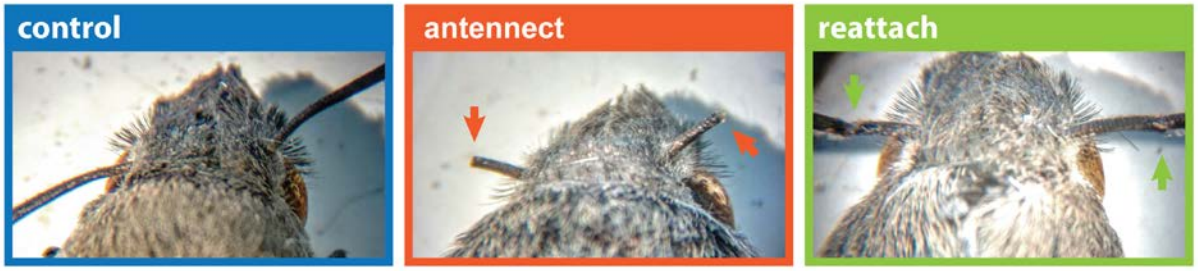

Fig. S1.1: **Antennectomy and re-attachment.** Antennae were cut between the 5th and 10th annulus (red arrows in middle panel). Flagella were re-attached to the same animal with a small amount of super glue (red arrows, right panel).

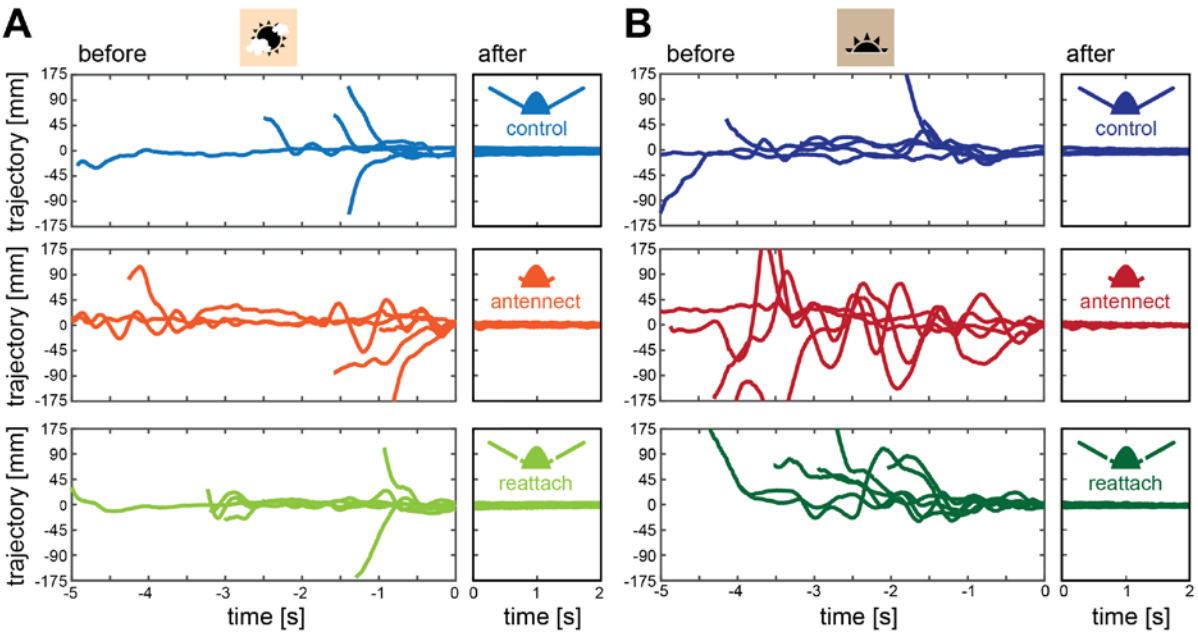

Fig. S2.1: **Hawkmoths approaching the moving flower** in bright (3000 lux) and dim (30 lux) light. *Before* refers to time intervals before their proboscis made contact with the nectary of the flower, *after* to the period after contact was initiated.

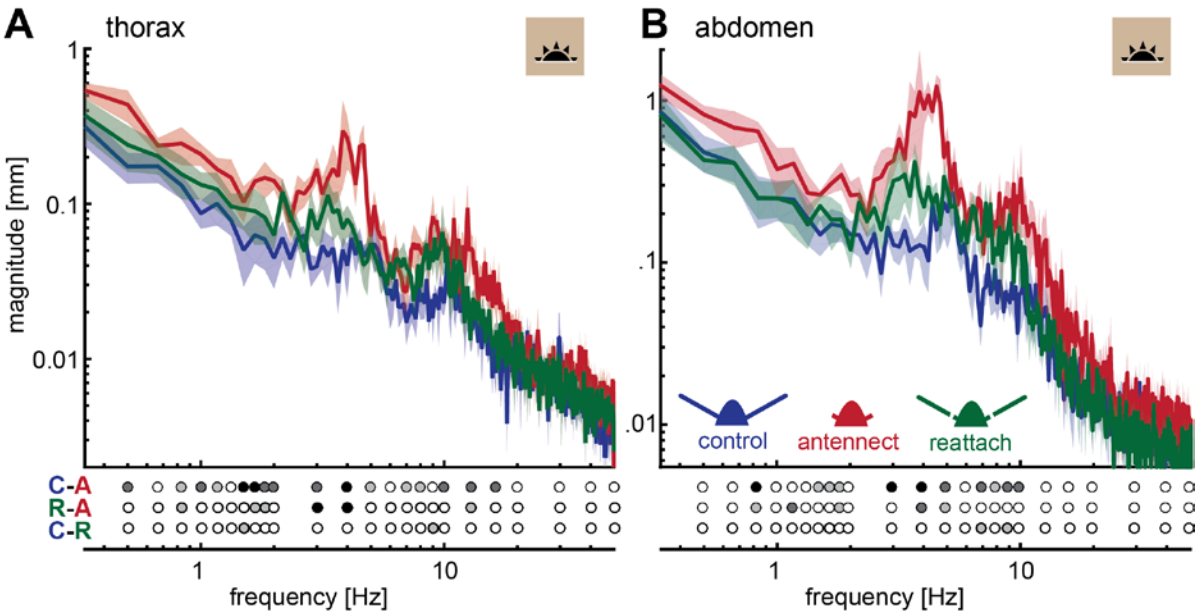

Fig. S2.2 **Antennectomised hawkmoths perform less stable hovering at a stationary flower at 30 lux.**

Thorax (**A**) and abdomen (**B**) position of moths hovering in front of a stationary flower at 30 lux. Post-hoc tests were performed as part of a general linear model including antennal treatment and frequency (binned to the logarithmic scale) as factors. Statistical significance is indicated below the plots as: black p<0.001, dark grey: p<0.01, grey p<0.05, white p>0.05, see Tables S2.3 and S2.4.

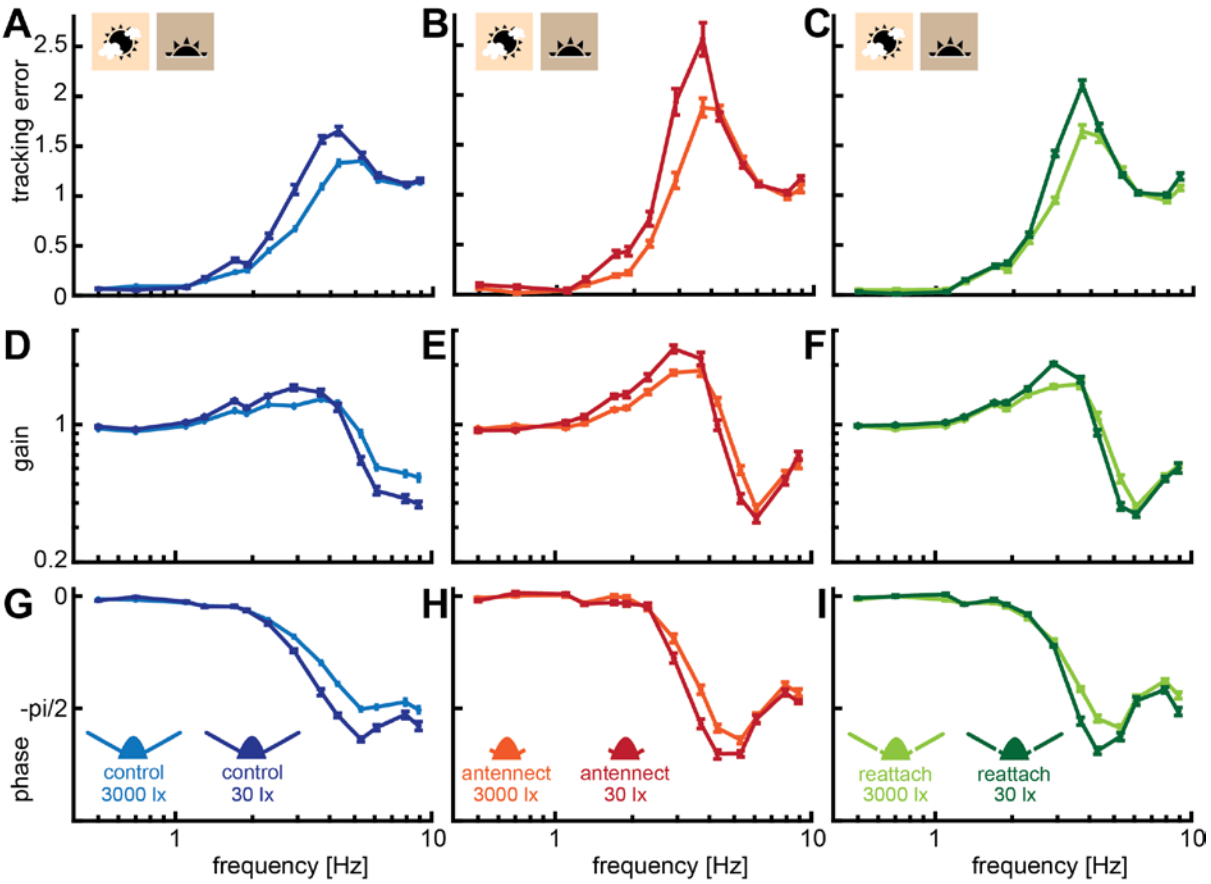

Fig. S3.1 **Hawkmoth tracking performance with the sum-of-sines stimulus in bright and dim light.**

Tracking error (**A-C**), gain (**D-F**) and phase (**G-I**) of hawkmoths tracking a moving robotic flower which moved as a sum-of-sines (see Methods) at 3000 and 30 lux. Curves show the mean and 95% confidence intervals of the mean, calculated in the complex plane.

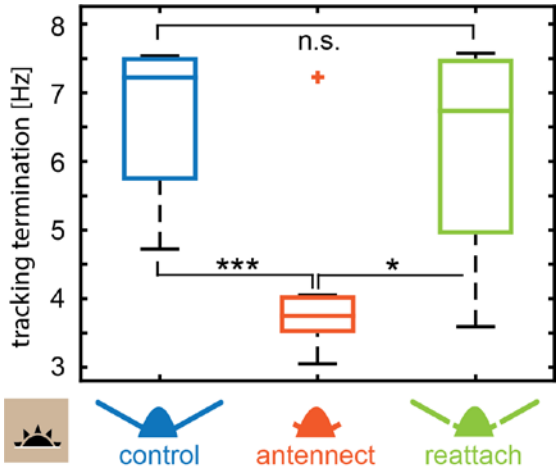

Fig. S3.2 **Flower tracking performance of chirp stimuli in dim light.**

Comparison of the temporal frequency of the flower at which the moths aborted tracking across antennal treatments at 30 lux. A Friedman test was used to compare between the treatments (*** p<0.001, **: p<0.01, * p<0.05, Table S3.2).

Supplementary Tables

Table S2 Results of the statistical models assessing the effect of antennal treatment in the stationary experiment in bright light. A general linear model was constructed with antennal treatment and frequency (binned to the logarithmic scale) as factors: log(response) ~ antenna * freq + 1|individual

Table S2.1 **Results of the statistical model assessing the effect of antennal treatment on thorax movements** in bright light (Fig. 1B).

Treatment	Estimate	t-value	DF	p-value
control - antenn	-0.664	-10.92	385	<0.001
control - reatt	-0.002	-0.04	385	0.97
antenn - reatt	0.662	10.88	385	<0.001

Table S2.2 **Results of the statistical model assessing the effect of antennal treatment on abdomen movements** in bright light (Fig. 1B).

Treatment	Estimate	t-value	DF	p-value
control - antenn	-0.658	-12.07	385	<0.001
control - reatt	0.149	2.74	385	0.007
antenn - reatt	0.807	14.81	385	<0.001

Table S2.3 **Results of the statistical model assessing the effect of antennal treatment on thorax movements** in dim light (Fig. S2.2A).

Treatment	Estimate	t-value	DF	p-value
control - antenn	-0.742	-11.63	385	<0.001
control - reatt	-0.257	-4.03	385	0.0001
antenn - reatt	0.485	7.60	385	<0.001

Table S2.4 **Results of the statistical model assessing the effect of antennal treatment on abdomen movements** in dim light (Fig. S2.2B).

Treatment	Estimate	t-value	DF	p-value
control - antenn	-0.656	-10.37	385	<0.001
control - reatt	-0.234	-3.71	385	0.0002
antenn - reatt	0.421	6.67	385	<0.001

Table S3 Results of the statistical model assessing the effect of antennal treatment on flower tracking performance in moving flower experiments.

Table S3.1 **Results of the statistical model assessing the effect of antennal treatment on flower tracking performance with the sum-of-sines stimulus** in bright light (Fig. 2B): a general linear model was constructed with antennal treatment and frequency (binned to the logarithmic scale) as factors: log(response) ~ antenna * freq + 1|individual

Treatment	Estimate	t-value	DF	p-value
control - antenn	-0.160	-9.04	913	<0.001
control - reatt	-0.043	-2.42	913	0.016
antenn - reatt	0.117	6.62	913	<0.001

Table S3.2 **Results of the statistical model assessing the effect of antennal treatment on flower tracking performance with the chirp stimulus** in bright light (Fig. 2B): a Friedman test was performed, with a Tukey-Kramer post-hoc comparison correction for multiple comparisons.

Treatment	X^2	p-value
overall	19.48	<0.001
control - antenn		<0.001
control - reatt		0.68
antenn - reatt		0.002

Table S3.3 **Results of the statistical model assessing the effect of antennal treatment on flower tracking performance with the sum-of-sines stimulus** in dim light (Fig. S3.1): a general linear model was constructed with antennal treatment and frequency (binned to the logarithmic scale) as factors: log(response) ~ antenna * freq + 1|individual

Treatment	Estimate	t-value	DF	p-value
control - antenn	0.184	-6.36	462	<0.001
control - reatt	0.028	0.98	462	0.328
antenn - reatt	0.156	5.38	462	<0.001

Table S3.4 **Results of the statistical model assessing the effect of antennal treatment on flower tracking performance with the chirp stimulus** in dim light (Fig. S3.2): a Friedman test was performed, with a Tukey-Kramer post-hoc comparison correction for multiple comparisons.

Treatment	X^2	p-value
overall	16.7	<0.001
control - antenn		<0.001
control - reatt		0.56
antenn - reatt		0.012

Table S4 Results of the statistical model comparing the effect of antennal condition between light conditions for all experiments. A Friedman test was performed, with a Tukey-Kramer post-hoc comparison correction for multiple comparisons.

Table S4.1 **Results of the statistical model assessing the effect of antennal condition on the difference in flower tracking error between light conditions** with the sum-of-sines stimulus (Fig. 4A).

Treatment	X^2	p-value
overall	2.71	0.258
control - antenn		0.303
control - reatt		0.357
antenn - reatt		0.994

Table S4.2 **Results of the statistical model assessing the effect of antennal treatment on the difference in flower tracking performance between light conditions** with the chirp stimulus (Fig. 4B).

Treatment	X^2	p-value
overall	0.67	0.710
control - antenn		0.693
control - reatt		0.912
antenn - reatt		0.912

Table S4.3 **Results of the statistical model assessing the effect of antennal treatment on the difference in thorax stability during hovering between light conditions** (Fig. 4C).

Treatment	X^2	p-value
overall	2.33	0.311
control - antenn		0.480
control - reatt		0.319
antenn - reatt		0.955

Table S4.4 **Results of the statistical model assessing the effect of antennal treatment on the difference in abdomen stability during hovering between light conditions** (Fig. 4D).

Treatment	X^2	p-value
overall	4.33	0.115
control - antenn		0.832
control - reatt		0.319
antenn - reatt		0.107