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By a whisker: the sensory role of vibrissae in hovering flight in nectarivorous bats

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Whiskers are important tactile structures widely used across mammals for a variety of sensory functions, but it is not known how bats—representing about a fifth of all extant mammal species—use them. Nectar-eating bats typically have long vibrissae (long, stiff hairs) arranged in a forward-facing brush-like formation that is not present in most non-nectarivorous bats. They also commonly use a unique flight strategy to access their food—hovering flight. Here we investigated whether these species use their vibrissae to optimize their feeding by assisting fine flight control. We used behavioural experiments to test if bats' flight trajectory into the flower changed after vibrissa removal, and phylogenetic comparative methods to test whether vibrissa length is related to nectarivory. We found that bat flight trajectory was altered after vibrissae removal and that nectarivorous bats possess longer vibrissae than non-nectivorous species, providing evidence of an additional source of information in bats' diverse sensory toolkit.

1. Introduction

Hovering flight is among the most complex and energetically costly forms of locomotion in nature [1–6]. In vertebrates, it is performed by several species of birds and bats [5,7,8]. Similar to hummingbirds and Old World sunbirds, nectarivorous bat species have evolved specialized forms of hovering flight to access nectar in deep-bodied flowers that have co-evolved with their nectarivorous pollinators [9–11]. Many neotropical nectarivorous bat species possess a brush-like growth of vibrissae (long, stiff hairs with a distal taper) around their rostrum (muzzle) that point forward, extending in front of the rostrum and ending in a plane perpendicular to the rostrum (figure 1*b*). This arrangement is noticeably different from most other bat species, which have fewer, shorter vibrissae with no clear directional orientation. Some specialized hairs have a sensory role in bats: the short hairs on the wings of big brown bats (*Eptesicus fuscus*) provide information about air-flow over the wing, assisting with fine flight control [12,13], and the tough hairs growing at the tip of the tail of mouse-tailed bats (*Rhinopoma microphyllum)* are tactile sensors that provide information about roost structure [14]. While bats possess a wide array of sensory modalities [15], no studies have investigated the function of vibrissae in bats. In general, mammalian vibrissae are primarily sensory organs [16], used for a variety of ecological and behavioural functions: in pinnipeds, vibrissae are used for water flow detection and self-movement information [17]; in rodents, vibrissae perform numerous sensory tasks, from obstacle detection to underground passage width estimation [18]; and in felids, vibrissae provide bite-guidance [19].

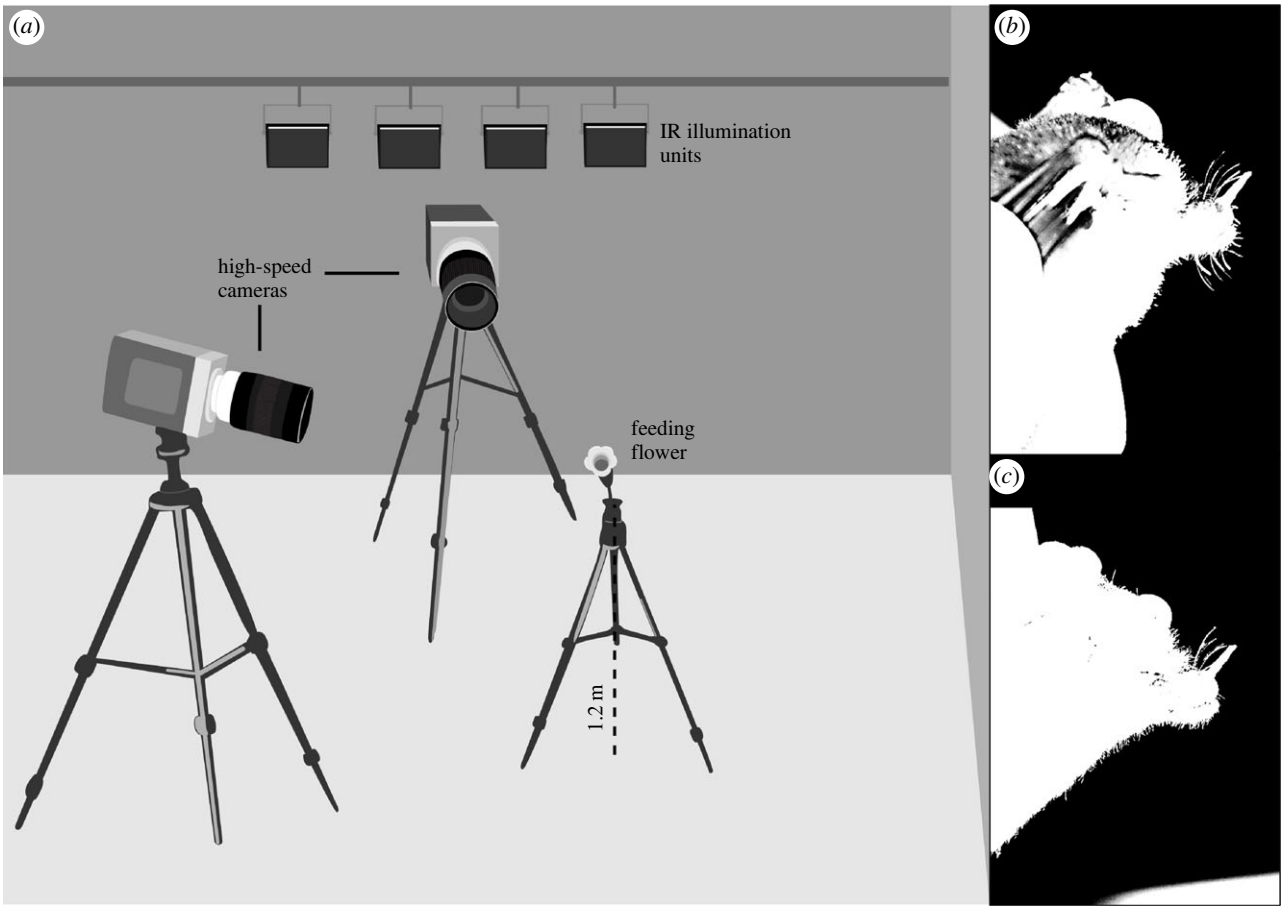

Figure 1. Experimental setup. (*a*) A glass flower containing nectar (honeyed water) was positioned on a tripod in an outdoor flight chamber at a height of 1.2 m. Two high-speed (450 frames s^{-1}, exposure 2200 µs) video cameras were positioned to maximize different angles to the flower while maintaining an overlapping field-of-view of the relevant area (flower and 10 cm in front of it) to enable three-dimensional coordinate extraction. The chamber was illuminated by four infrared (IR) lights with no additional lighting in the visible spectrum. The cameras were operated via a remote trigger by an observer in a separate room divided by a glass wall (thus undetected by echolocation). (*b*) *Glossophaga soricina* with intact vibrissae (control). (*c*) *Glossophaga soricina* with ventral and lateral vibrissae clipped (removed) (treatment). In (*b,c*) pictures were modified (colour, brightness and contrast) to enhance vibrissae visibility.

For nectarivorous bats, vibrissae may play a role during a crucial feeding behaviour—hovering flight. Hovering flight is an extremely energetically costly form of locomotion. The use of hovering flight by nectarivorous animals should require that the energetic value of the nectar meal is greater than the energetic expenditure of obtaining it, and that flight power during hovering is fine-tuned to reduce these costs. To achieve the second condition, an animal hovering at a flower should be able to closely modulate its flight mechanics to track the nectar source in three dimensions, to maintain a stable relative position between the energy source and the mouth, which in turn may require changes in sensory processing [20] and the integration of information from multiple sensory modalities [21]. Although echolocation, vision, olfaction or a combination of these modalities may enable the bat to find an appropriate flower [22,23], they are not suitable for the bat to obtain the necessary information for optimizing flight kinematics while positioning its head in the flower to reach the nectar. At this proximity, scent is completely saturated and cannot provide fine spatial information. In darkness and when the eyes are already in the flower, vision provides little or no information. And at such short distance between the bat and the target, echolocation is also ineffective because the echo overlaps with the pulse that produced it, creating a 'blind zone' (a phenomenon known as 'forward masking zone' [24]). One source of information useful for flight kinematics is the vestibular system, which provides bats with idiothetic (self-generated) cues about the orientation and movement of its own body [25,26]. However, while this can inform the bat about its own movement, the bat still requires information about its location relative to the flower.

In addition to energetic costs, hovering in front of a flower can be costly in terms of predation risk. Arboreal ambush predators such as pit vipers (e.g. *Bothriechis* sp. and *Bothrops* sp.) prey upon nectarivorous and frugivorous bats [27] and have high strike speeds [28], a risk that has arguably led to short, quick feeding events by nectarivorous bats [29].

The unusual arrangement of the facial vibrissae (figure 1*b*) of nectarivorous bats leads to the hypothesis that they provide positional information used by the bats as they perform their specialized feeding strategy. Specifically, we tested the hypothesis that tactile stimuli gained by the vibrissae provide the hovering bat with information regarding its position relative to the flower from which it is feeding, as well as information about its depth within the flower. This information can be used for fine flight control while hovering, to optimize within-flower positioning and to reduce feeding event duration, which will reduce overall energy expenditure and predation risk.

We used two approaches to test this hypothesis. First, we manipulated vibrissa length of nectarivorous Pallas's long-tongued bats (*Glossophaga soricina*) to test whether altered

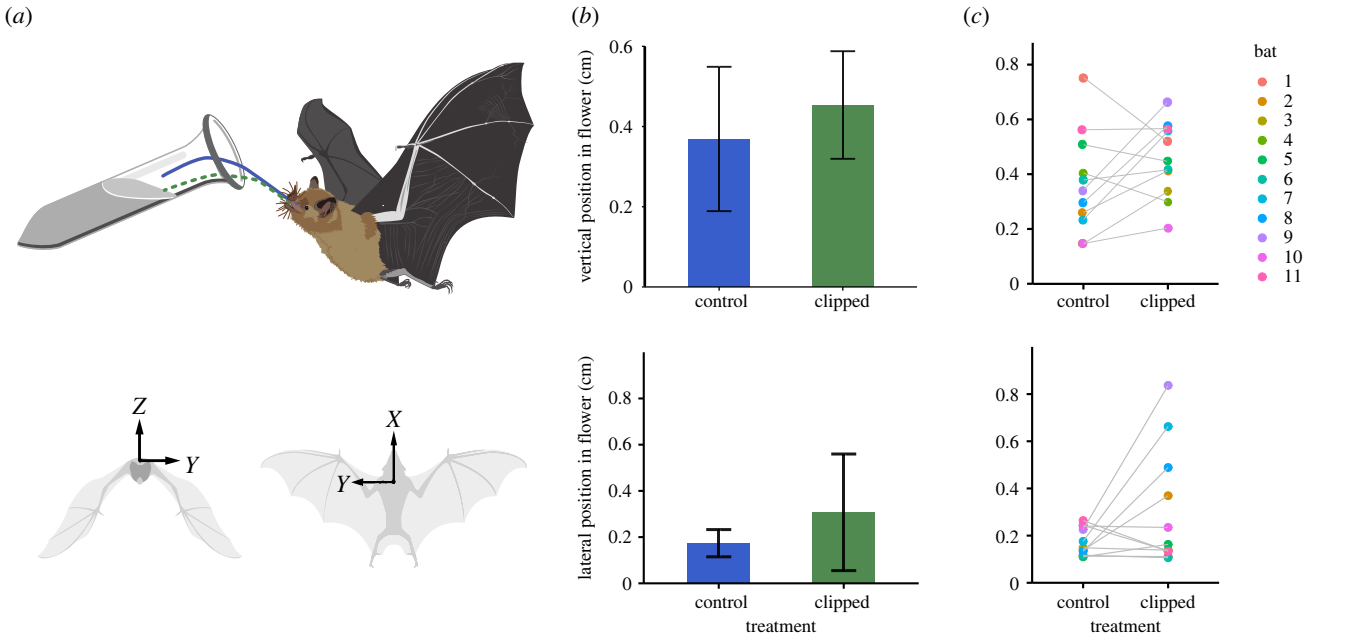

Figure 2. Effect of vibrissa clipping on position within the flower. (*a*) Typical bat approach to the flower: blue solid line depicts a typical approach during control trials; green dashed line depicts a typical approach during treatment trials; axes for position coordinates are indicated below the schematic (see also Material and methods and electronic supplementary material, figure S1). (*b*) Mean relative positions in the flower by treatment for all 11 subject bats. 'Control' shows values for bats with intact facial vibrissae and 'clipped' shows values for bats after the vibrissae were clipped. Error bars indicate s.d. (*c*) Mean relative positions in the flower by treatment for each individual bat.

vibrissae change the bats' approach to and positioning within flowers. Second, we measured vibrissa lengths of preserved museum specimens from 10 nectarivorous and non-nectarivorous species of known phylogenetic relationship to test whether vibrissa length is related to diet. We predicted that if facial vibrissae are important sensory structures for feeding from flowers, then (1) reduced vibrissae length will result in altered approach trajectory and positioning within flower, and (2) vibrissae will be longer in obligate nectarivores than bats with other diets. We found support for both hypotheses: vibrissae play a sensory role in the bats' entry into the flower and vibrissa length is related to diet across bat species.

2. Results

We trained 11 wild-caught *G. soricina* bats to fly to and feed from an elevated glass flower positioned in an outdoor flight chamber. Experiments were carried out at night with infrared (IR) illumination and recorded using two synchronized high-speed cameras (figure 1). We defined a successful feeding event as a flight to and from the flower that included hovering in front of and drinking from the flower. For each individual, we compared the following trajectory parameters between control flights (vibrissa intact) and treatment flights (ventral and lateral vibrissae clipped): (*A*) *vertical position within flower*, (*B*) *lateral position within flower*, (*C*) *depth in flower when tongue first extended*, (*D*) *latency of tongue extension*, (*E*) *total feeding event duration*, (*F*) *dorsoventral head tilt angle*, and (*G*) *smoothness of entry*. See electronic supplementary material, figure S1 for graphical representation of parameters *A–D* and *F*. See Material and methods for detailed description of experimental setup and procedures. We analysed a total of 158 feeding events: on average 7.4 control and 7.0 treatment events per individual (see electronic supplementary material, table S1; and Material and methods).

(a) Effect of vibrissa clipping on entry into flower

Success rates (the proportions of the approaches bats made to a flower for which they succeeded in drinking from it) were very high during both control and treatment (clipped vibrissae) events (0.98 for control and 0.92 for treatment) and the difference was not significant (two-sample *t*-test, $t_{10} = 1.22$, $p = 0.24$). We subsequently used only successful feeding events for three-dimensional trajectory analysis. For each of the variables listed below (*A–G*), we tested for the effect of vibrissa clipping during the bats' entry into the flower using a linear mixed model with treatment as a categorical fixed effect (intact or clipped vibrissae) and bat individual and flight number as random effects.

A—Vertical position within flower. We predicted that if bats use tactile information from their ventral vibrissae to assess their distance to the internal surface of the flower, then the vertical position of the bat when entering the flower would differ with clipped compared with intact vibrissae. Vibrissa removal affected the bats' vertical position during flower entry (figure 2; $t_{130.6} = 2.46$, $p = 0.015$). Interestingly, bat responses to vibrissa clipping differed between some individuals. The bats usually approached the flower from below. When the ventral (and to a lesser degree the lateral) vibrissae touched the rim of the flower, the bats entered the flower maintaining approximately the same distance from the flower bottom wall during entry by maintaining contact between the flower and tips of the vibrissae (electronic supplementary material, video S1). Most individuals entered the flower *lower* after vibrissa clipping, guided by their chin instead of the then-missing vibrissae (electronic supplementary material, video S2). Individuals 1 and 5 were positioned *higher* in the flower during entry after their vibrissae were clipped. Bat 1, after not contacting the rim of the flower with its missing vibrissae, continued its ascent and

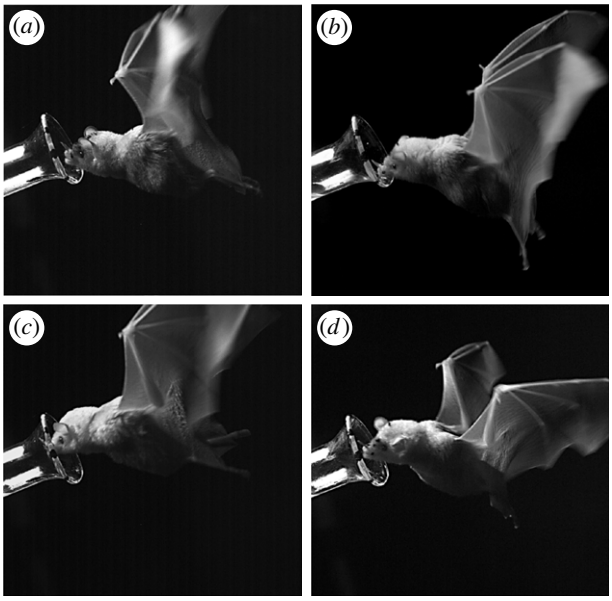

Figure 3. Entry strategies. Typical entry strategies into the flower during feeding. (*a*) Typical entry with vibrissae guiding along the bottom of the flower. (*b*) Typical entry along the bottom of the flower with clipped vibrissae, guided by the chin. (*c*) Entry along the top of the flower, guided by the nose leaf (e.g. bat 5). (*d*) Entry along the midline of the flower, presumably guided mostly by echolocation (e.g. bat 1).

entered from the middle of the flower rather than following the bottom. Bat 5 switched from entry guided by vibrissae to entry guided by the tip of the nose leaf (electronic supplementary material, video S3), thus following the top of the flower during treatment flights, resulting in a much higher position within the flower. Figure 3 shows the different entry strategies.

B—Lateral position within flower. We predicted bats would enter the flower further left or right from the centre of the flower following vibrissa clipping if bats use tactile information from their lateral vibrissae to assess their distance to the sides of the flower. The lateral direction of approach was not consistent across trials; hence clipping the lateral vibrissae had no clear directional effect (left versus right). However, the absolute lateral distance between the centre of the bats' muzzle (marker 3: tip of nose leaf) and the central line of the flower (a virtual line passed through the geometrical centre of the flower from the centre of the opening to the back of the flower) was greater after clipping the lateral vibrissae compared with the control condition ($t_{137.7} = 2.73$, $p = 0.007$, figure 2*b*,*c*).

C—Depth in flower when tongue first extended. We predicted that bats with clipped vibrissae would fly further into the flower before extending their tongues following vibrissa clipping than when vibrissae are intact if treatment delays or weakens effective transmission of information about the relative position of the bat to the flower. Contrary to our prediction, the depth of the bat in the flower when it started to extend its tongue was not affected by vibrissa clipping ($t_{133.4} = 0.54$, $p = 0.592$).

D—Latency of tongue extension. We predicted that the latency for extending the tongue would be greater for bats with clipped compared with intact vibrissae for the same reasoning as for *C*. As in that case, clipping the vibrissae had no significant effect; the amount of time between entry of the bat's head into the flower and the start of tongue extension did not change with clipping treatment ($t_{139.9} = 1.24$, $p = 0.217$).

E—Total feeding event duration. If vibrissae aid bats in identifying optimal hovering locations within a flower, then we predict that it would take longer to find the ideal location in the flower with clipped vibrissae, and the total time that the bat's head is in the flower for a feeding event will be greater with clipped compared with intact vibrissae. Contrary to this prediction, there was a significant decrease in flower visit duration after the vibrissae were clipped ($t_{132.5} = 2.35$, $p = 0.020$). This result is likely driven by a large decrease in duration for two bats following treatment; the other nine bats displayed small and inconsistent differences between control and treatment durations. See electronic supplementary material, figure S2 for graphical representation of parameters *C*–*E* values.

F—Dorsoventral head tilt angle. If the vibrissae guide the entrance to the flower, head angle may be influenced by vibrissal sensing, leading to our prediction of different or more variable head orientation for bats with clipped compared with intact vibrissae. However, there was no significant difference in head angle between treatments ($t_{144.3} = 0.63$, $p = 0.531$), and the coefficients of variation (CV) calculated for each individual based on multiple feeding events were not significantly different between treatments ($t_{10} = 0.13$, $p = 0.901$).

G—Smoothness of entry. We predicted that there would be more variability in the positions of the bat's head as it enters the flower with clipped compared with intact vibrissae if the vibrissae guide the entrance to the flower. This parameter was quantified as the CV of the change in horizontal and vertical position (calculated separately) of marker 1 from one frame to the next for five frames before to five frames after entering the flower. There was no significant difference between treatments for CVs of horizontal movement ($t_{136.3} = 1.85$, $p = 0.067$) or vertical movement ($t_{137.5} = 0.72$, $p = 0.472$).

(b) Relationship between diet and vibrissae length

To test whether long facial vibrissae could be an adaptation to nectarivory across bat species, we measured vibrissa lengths of preserved specimens from nine species from family Phyllostomidae divided into three diet groups: obligate nectarivory (the species is reported to always use nectar as a regular food source); complementary nectarivory (the species' diet may include nectar-feeding but nectar is not a primary dietary element or is employed in a facultative manner); no nectarivory (the species has not been reported to drink nectar while hovering at flowers), as well as one outgroup species from the family Mormoopidae (a sister taxon to Phyllostomidae). The nine phyllostomid species were selected such that diet categories were spread across different clades. This was to ensure that any relationship between diet and vibrissa length was not confounded by close phylogenetic relationships. We measured two relative vibrissa length parameters: ventral vibrissa ratio (ratio between ventral vibrissa length and height of rostrum at the base of the nasal opening), and lateral vibrissa ratio

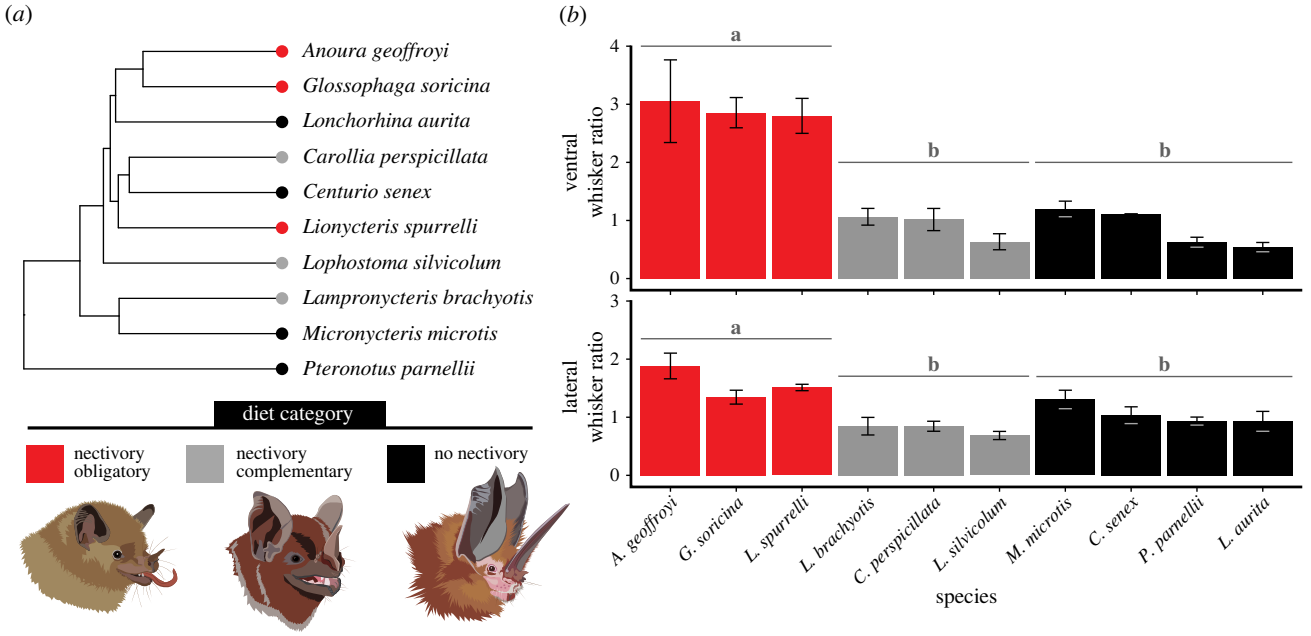

Figure 4. Relative vibrissa lengths for 10 bat species. (*a*) Phylogeny for the species used to compare vibrissae length, with nine species from the family Phyllostomidae and one outgroup species (*Pteronotus parnellii*, Mormoopidae). (*b*) Ratio of the ventral vibrissa length to skull rostrum height (top), and ratio of the lateral vibrissa length to skull rostrum width (bottom). Bars show means, error bars show s.d., colour indicates diet category, and different letters above bars show statistically significant differences by diet category.

(ratio between lateral vibrissa length and width of rostrum at the base of the nasal opening). See Material and methods for a detailed description and list of specimens.

Using a phylogenetic ANOVA to control for phylogenetic relationships, we found that vibrissa length differed by diet group (ventral vibrissae: $F = 62.3$, $p = 0.001$; lateral vibrissae: $F = 13.0$, $p = 0.007$; figure 4). Specifically, obligate nectarivorous species had significantly longer ventral and lateral vibrissae than facultative nectarivores and non-nectarivores, and there was no significant difference between the latter two categories.

3. Discussion

The diversity of bat sensory adaptations, an extensively studied aspect of bat biology, is a key factor in their extraordinary evolutionary success and diversity [15]. In this study we used two complementary approaches—behavioural experiments and phylogenetic comparative methods—to investigate bats' use of facial vibrissae, sensory structures that are widely used across mammals but have hitherto not been studied in bats.

(a) Behavioural experiments

We clipped the ventral and lateral vibrissae of the nectarivorous bat *G. soricina* and quantified the effect on the bats' trajectory when entering and feeding inside a flower. We found vibrissa clipping significantly affected the vertical (Z-axis) and lateral positioning (Y-axis) of the bat's head in the flower. In both dimensions, the bats entered the flower farther from the centre of the flower (i.e. closer to the flower wall) during clipped vibrissa compared with control trials (figure 2), thus supporting the hypothesis that vibrissae provide positional information to the bat.

We found no significant difference between control and treatment trials in the other measured parameters (depth in flower when tongue first extended, latency of tongue extension, total feeding event duration, head vertical angle, smoothness of entry). We considered feeding event duration to be an important variable, not as a proxy for nectar intake, but because hovering flight is energetically expensive [1–3], bats are vulnerable to predators while feeding [27–29], and bats are competing with conspecifics for a finite resource [30,31]. Bats may have learned quickly that the flower was stable, its nectar was not depleted, and its surroundings could not conceal predators, and therefore were not strongly motivated to minimize feeding time.

Feeding success rate (the proportion of the approaches bats made to a flower for which they succeeded in drinking from it) was not significantly influenced by treatment, although it was lower after vibrissa clipping. While we could not record all feeding events owing to technical limitations of the system (see Material and methods), we always recorded the first feeding event of each individual bat in both control and treatment trials, and the events that were missed after the first event had no systematic bias. The extremely quick nature of the feeding event (674.6 ± 19.3 ms (mean ± s.e.), range: 188.9–1437.8 ms) meant that the experimenter did not know in real time whether a particular event was successful or not, thus removing potential bias towards successful events. The overall high success rate recorded in our analysis, therefore, reflects the true success rate at the artificial flowers. It is possible that in natural conditions the bats would abort an attempt if the sensory input were less than ideal and that would have resulted in lower success rate after vibrissa clipping.

It is worth noting that tactile input from vibrissae can be used in much more nuanced ways than we tested here for flight control. For example, we know that hawkmoths alter wingbeat amplitude and frequency when hovering to recover from perturbations [32]. Based on studies of bats recovering from perturbations [33] and on comparisons of hovering dynamics in bats, birds and insects [11], nectar-feeding bats would likely alter wing extension/flexion and wing protraction/retraction to adjust their body orientation (e.g. roll and

pitch) to keep their heads as steady as possible when hovering in the flower. These should be investigated in future studies.

(b) Individual behavioural differences

Individual variation provides an interesting, if complicating, aspect to this study. Individuals had different but consistent baseline behaviours (figure 3*a–c*). For example, bat 3 consistently did not rely on vibrissa contact to determine entry point. Some bats also had different but consistent reactions to treatment (figure 3*d*). For example, bat 5 switched to following the top of the flower using its nose-leaf after vibrissae were removed. While the observation period here was short (2–3 days), if these individual differences are consistent over longer time periods they may represent behavioural reaction norms—BRNs [34]. BRNs are consistent ways in which individuals differ from each other while engaged in the same behavioural tasks and are an important and useful way to quantify aspects of animal personality. BRNs have now been documented in bats both in a sensory context [35,36] and in a behavioural foraging context [37], and they promise to be an important aspect in future research of bat behavioural ecology. Although it is possible that these differences arise from slight differences in vibrissa trimming procedures, we think that it is unlikely because we consistently trimmed vibrissae to the length of the surrounding fur and checked that no visible vibrissa tips were left protruding from the chin and sides. Whether the differences described here are consistent enough to be considered BRNs remains to be tested.

Not only do individual bats differ from one another behaviourally, but each individual can vary its behavioural strategies for accessing the flower across feeding events. For example, bat 11 used the range of strategies (low entry guided by vibrissae, high entry guided by nose leaf, entry through middle; figure 3*a*,*c*,*d*). This behavioural flexibility allows bats to switch between or integrate sensory modalities [15]. In addition, bats' wings are highly articulated and controllable [38], enabling bats to use multiple solutions for each kinematic challenge.

(c) Relationship between diet and vibrissa length

Numerous morphological and behavioural traits have been linked to dietary differences in bats (e.g. tooth structure [39], bite force [40], nose-leaf shape [41]). These relationships have been particularly well studied in the family Phyllostomidae, which has the greatest dietary diversity of all bat families [42]. Our behavioural results show that the nectarivorous bat *G. soricina* uses long facial vibrissae to gain tactile information when feeding from flowers, and our comparative analyses complement these results by showing that these long facial vibrissae are morphological features specific to bats that are obligate nectarivores. By selecting distantly related species of obligate nectarivores, we show that this adaptation evolved independently at least twice. The difference was greatest for the ventral vibrissae, which were three times longer in the nectarivorous species than in those that feed primarily on fruit or insects. This suggests that using the long ventral vibrissae to gain tactile feedback from the bottom of the flower is a common sensory mechanism in nectar-feeding specialists—specifically those that hover while feeding. All the obligate nectarivores in our study hover as they access nectar, while the facultative ones do not. In this view, it is not the diet *per se* that selects for long vibrissae, but the specialized way of accessing it. This hypothesis remains to be tested behaviourally in other nectarivorous bat species and would be interesting to investigate in nectarivorous pteropodid bats which are phylogenetically far from phyllostomids—should they exhibit longer vibrissae than their frugivorous relatives this may represent diet-related parallel evolution.

One insectivorous species in our sample, *Micronycteris microtis*, has relatively long lateral vibrissae compared with other non-nectarivorous species. This species uses a specialized gleaning hunting strategy in which it approaches stationary prey on leaf surfaces from oblique angles to detect the insect using echolocation [43]. It is possible that its vibrissae allow it to detect the leaf surface when attacking prey and may explain why this species evolved long vibrissae.

(d) Biological relevance

The biological importance of our findings is clear in the small but significant changes in head position during nectar-feeding following removal of vibrissae. Event duration was not significantly different between treatments in our experiment, so at this point, we have no evidence of specific time-saving benefits of using sensory input from vibrissae. When nectivorous bats feed in nature, however, flexible flowers and stems can move while the bat is feeding. Our flowers were rigid, fixed in space and lacked internal structures such as stamens, making the positional task easier. Finding the opening of variable natural flowers and keeping three-dimensional position within a *moving* flower while negotiating internal obstacles is arguably much more challenging. We suspect that repeating our experiments with real flowers will result in greater differences between control and treatment for positional data and could possibly affect feeding success rate and event duration as well. In addition, we argue that keeping as close as possible to the central line of the flower is important for another reason—it could minimize the vibrations caused by the bat when it first enters the flower. This has two potential benefits. First, it reduces cues for ambush predators such as snakes. Pit vipers routinely prey upon nectarivorous bats [27] and strike at speeds of 100–120 cm s^{-1} [28], which could be at the lower end of their abilities, as rattlesnakes, a closely related group, have been shown to strike at speeds 3–6 times greater [44]. While this selective pressure has arguably contributed to the short duration of nectar-feeding events [29], reducing the amplitude of vibrational cues when entering a flower could delay the response of snakes to the presence of a bat. Reducing vibrations could also prevent nectar from spilling from the flower as the bat enters, ensuring the net energetic gain of each feeding event remains as high as possible. In our setup, the artificial flower was fixed and angled upwards, so that we could not measure vibrations or nectar spillage. A setup using more natural features of the flowering plant should be used in future research to test this prediction.

Central alignment within the flower might also affect the efficacy of nectar extraction. Nectarivorous bats' tongues are highly specialized tools relying on hair-like papillae for nectar extraction [45]. The amount of nectar extracted depends on how many of the papillae are inserted into the nectar, and the timing of papilla erection by blood engorgement. Unlike our flowers, real flowers have a limited

quantity of nectar and possess internal structures such as stamens. It is likely that these two factors affect nectar extraction, and that correct alignment during entry helps the bat mitigate these factors.

4. Conclusion

We used behavioural experiments and phylogenetic comparative methods to test nectarivorous bats' use of tactile sensory input provided by vibrissae. We found that vibrissae provide the bat with useful information that helps it to maintain central alignment as it enters deep-bodied flowers, a typical flower shape of plants pollinated by bats or birds [10]. Our comparative analyses suggest that vibrissa length evolved as a response to nectarivory, adding sensory adaptation to the already fascinating story of co-evolution of plants and their pollinators. Vibrissae are known to be an important sensory organ across Mammalia [16], but to our knowledge they have never been studied in bats—an order that represents a fifth of all extant mammal species.

This study highlights the importance of field observations and natural history for biological research. This study began with an observation of the stark difference between the vibrissae of neotropical nectarivorous and palaearctic insectivorous bats. Even in a period characterized by advanced molecular methods, sophisticated computational capabilities, and hi-tech research technologies, 'basic' naturalist skills are irreplaceable for identifying interesting and biologically relevant questions.

5. Material and methods

(a) Facilities and study animals

Experiments were conducted at the Smithsonian Tropical Research Institute (STRI) facility in Gamboa, Republic of Panama. Eleven adult male *Glossophaga soricina* (see note on taxonomy below) bats were used in this study. The bats were captured in two separate roosts in the vicinity of Gamboa using mist-nets and hand-held nets. For the duration of the study (4–6 nights per bat), the bats were housed in an ambient outdoor flight cage (henceforth 'aviary', dimensions: 1.7 × 1.5 × 2.3 m) with light and temperature conditions similar to those in the roosts (fully shaded but not completely dark, 26–30°C, following ambient temperatures). In the first night of captivity, we trained the bats to feed from glass flowers mounted on camera tripods in a setup identical to the one used in the experiments. We also gave each bat at least one more night to acclimate before we began experiments. During acclimation and after each experiment, food (30% honeyed water enriched with commercial bee pollen) was available ad libitum overnight in the aviary, and we weighed all bats every morning to ensure no individual experienced significant weight loss while in captivity.

All procedures were approved by the Institutional Animal Care and Use Committees of both Dartmouth College (protocol no. 2045) and STRI (protocol no. 2017-0102-2020). Bats were captured by permission of the Ministry for Environmental Protection of the Republic of Panama (Ministerio de Ambiente permit no. SE/AP-22-19).

Several authors have recently suggested that *G. soricina* may in fact be a species complex [46] and that some subspecies should be elevated to species status based on genetic and morphological data [46,47], designating the Panamanian population as *Glossophaga mutica* (possibly synonymous with *Glossophaga handleyi*). Since this suggestion was published after the completion of our work, and since it will not affect the conclusions drawn by either the experimental or the phylogenetic aspects of this study, we opted to continue using the traditional taxonomy in this manuscript.

(b) Experimental setup

We trained the bats to feed from a hand-blown glass flower (DuGrenier, USA) that contained honey-water enriched with bee pollen powder. The flower shape was a simplified representation of a typical bat-pollinated flower: tubular in shape and with a deep hypanthium (the tube-shaped 'body' of the flower) (figure 2) [9,10]. Our flower's hypanthium was 1.8 cm wide with a slight widening at the top (entrance) and a 5 mm wide lip perpendicular to and surrounding the opening. The flower was positioned at a height of 1.2 m from and an angle of 30° to the ground. Feeding events were captured by a set of two synchronized high-speed video cameras (Phantom Miro 340 cameras (Vision Research, USA)). One camera was positioned 40 cm to the left of the flower when viewed facing the flower opening and 10° above it to provide a side view. The second camera was 40 cm behind the flower, 10° to the left (toward camera 1), and 30° above the flower (figure 1) to provide the front view. The positioning of the cameras allowed sufficient field-of-view overlap for successful calibration and three-dimensional tracking (see 'Video analysis' below). We used four IR illumination units (850 nm; Axton, USA) positioned behind the flower to enable videography. The videos were recorded at 450 frames s^{-1}, 2200 µs exposure and 1920 × 1080 resolution. A typical feeding event lasted approximately 0.5–0.8 s. We used an external hand-held trigger to record videos of 2 s duration (1.5 s pre-trigger, 0.5 s post-trigger video) when bats visited the flower. The setup was near the back wall of a medium-size flight chamber (1.7 × 2.1 × 2.3 m) located in the outskirts of Gamboa and adjacent to rainforest. The experimenter was behind a glass wall so that bats could be observed, but bats could not detect the experimenter by means of echolocation. The camera setup was calibrated each night using a chequerboard composed of 6 × 8 2cm squares and the open-source motion tracking software XMALab and following the protocol described in [48].

(c) Experiment design

Experiments began each night an hour after sunset; food was not available to bats before experiments began. We marked each bat with two black dots using a non-toxic marker: one near the distal end of the upper lip and one on the upper side of the muzzle, just behind the base of the nose leaf. Together with three natural points on the bat's head these formed the landmarks that were used for movement tracking (1. Upper lip; 2. Eye; 3. Nose-leaf tip; 4. Tip of tongue. See 'Video analysis' below and electronic supplementary material, figure S3). After marking we released the bat into the flight chamber and let it feed voluntarily. Typically, the bat would explore the flight chamber for approximately 10–15 min before locating the flower and feeding. Most bats performed 5–10 feeding events in quick succession, followed by a period of inactivity or non-feeding flight, and then another series of feeding events. Bats had 1–20 feeding bouts each night, resulting in a potential large number of feeding events for analysis. Unfortunately, the actual number of recorded feeding events was much lower (electronic supplementary material, table S1) because at the end of each recorded event the video had to be downloaded to the computer—a process lasting approximately 40 s, during which some bats performed 2–5 feeding events. A bat that performed 10 or more feeding events (recorded or unrecorded) and was then inactive for 30 min was returned to the aviary and was not used again that night. A bat that performed fewer than 10 feeding events and was then inactive for 45 min was returned to the aviary and was not used again that night.

For each bat, the first night of trials was used as control, with unmanipulated vibrissae. For individuals for which we recorded fewer than 6 feeding events in the first night, we ran a second night of control trials to increase sample size. Otherwise, the second night was used for treatment trials. Prior to treatment trials, we used scissors to trim the vibrissae from the bottom and both sides of the muzzle at their base to the same length as the surrounding fur, leaving only the upper vibrissae intact (figure 1). Vibrissa trimming is not expected to have a lasting negative effect on the bats. Although we are not aware of other studies manipulating bat vibrissae, considerable research has been done with rodent vibrissae, and while clipping vibrissae in infant rats has damaged their tactile discrimination long-term, clipping of adult vibrissae had no effect on the animals' performance [49]. Vibrissae subsequently grow, and while vibrissa growth has not been investigated in bats, in mice the normal vibrissa growth rate is 1–1.5 mm day^{-1} [50], and in pinnipeds the growth rate is 0.024 cm day^{-1} [51]. Since vibrissae can be damaged or shed during the natural life cycle of animals [50–52], we expected the effect to be transient and short-term, until the brain starts to compensate for the altered vibrissae, probably over seconds to minutes. For that reason, we began treatment trials immediately after vibrissa removal and completed them the same night. Consequently, sample sizes for some individuals are smaller than desired. On average we analysed 7.4 control trials (range 4–14) and 7 treatment trials (range 3–14) per individual (see electronic supplementary material, table S1).

(d) Video analysis

Videos were analysed using a double-blind protocol where the person analysing a video did not perform experiments and had no knowledge of the bats' identity or whether the trial was control or treatment. A successful attempt was an event in which a bat approached the flower and interacted with it, and subsequently drank nectar (electronic supplementary material, videos S1–S3). An unsuccessful event was one in which a bat approached the flower and interacted with any part of its opening but aborted mid-try or did not drink (no contact between tongue and nectar; electronic supplementary material, video S4). We used four landmarks on the bat's head, one marked and three natural: 1. Upper lip (marked); 2. Eye; 3. Tip of nose leaf. 4. Tip of tongue (electronic supplementary material, figure S3). We obtained three-dimensional coordinates for each landmark using the open-source motion tracking software XMALab [48]. Each landmark was marked manually frame-by-frame in the videos from both cameras using the tracking feature in XMALab software. We marked landmarks in each frame, starting when the bat was approximately 4 cm away from the flower and until the tongue was retracted for the first time. In the videos from the side-view camera, the wing periodically occluded the facial landmarks, leading to 7–10 frames (15–22 ms) of missing data from this camera. We used the linear interpolation feature of XMALab to complete these missing datapoints for the two-dimensional trajectories. As the bat entered the flower, the bent glass of the rim caused refraction that shifts the landmark position in the video. The refraction effect stops when the bat has moved beyond the bent rim and into the flower (approx. 5 frames or 11 ms). Owing to the different positions of the two cameras and the angle of the flower, the refraction does not occur at the same time in the simultaneously recorded videos, allowing us to use a feature of XMALab—the epipolar line—to correct for the refraction manually and mark the landmark in its true position. Once we had complete two-dimensional coordinates from each camera (i.e. two sets of two-dimensional coordinates for each landmark) we used XMALab to calculate three-dimensional trajectories for each landmark, which were exported to MatLab (Mathworks, USA) for analysis.

We calculated all kinematic measurements relative to a global coordinate system defined using four landmarks along the rim of the flower (12, 10, 9 and 7 o'clock; electronic supplementary material, figure S3), which we refer to in our custom MATLAB scripts as the Flower Coordinates System (FCS), (X_F, Y_F, Z_F) (see electronic supplementary material, figure S1) [53]. The flower coordinate system had its origin at the centre of the opening of the flower, which we computed based on the arc established by the flower rim landmarks using the circlefit3d function in MATLAB [54]. We defined the Z-axis (Z_F) using a vector from the origin to the 12-o'clock landmark (vertical); the Y-axis (Y_F) using a vector from the origin to the 9-o'clock landmark (lateral); and the X-axis (X_F) as the cross-product of Y_F and Z_F (horizontal, into the flower). For a bat approaching the flower from below to assume a position at the flower centre, it would thus move along the X_F from negative to positive values ($X_F = 0$ denotes flower entry), would have a constant Y_F value ($Y_F = 0$ denotes centred laterally within flower), and would move along the Z-axis from negative values toward 0 as it approaches the flower from below ($Z_F = 0$ denotes centred vertically within flower).

Using the three-dimensional movement reconstructions of the landmarks, we compared the following parameters between control and treatment trials for each individual: *A, vertical position within flower*: the absolute distance (cm) of the bat's upper jaw (landmark 1) above or below the central line of the flower (its Z_F value, a virtual line passing through the geometrical centre of the flower from the centre of the opening to the back of the flower); *B, lateral position within flower*: the absolute distance (cm) from the midline of the bat's head (landmark 3) to either side of the flower's central line (its Y_F value); *C, depth in flower when tongue first extended*: the distance (cm) between the tip of the bat's tongue (landmark 4) and the edge of the flower along the flower's long axis when the bat begins extending its tongue (its X_F value); *D, latency of tongue extension*: the amount of time (ms) between the bat entering the flower (landmark 1 passes rim) until tongue extension (first appearance of landmark 4); *E, total feeding event duration*: the time elapsed (ms) between entry to and exit from flower (landmark 1 passes rim on way in and on way out); *F, dorsoventral head tilt angle*: the angle (°) between the vector connecting landmarks 1 and 2 (upper lip to eye, forming anterior–posterior axis for bat's head) and X_F (central line running the length of the flower) in the X_F–Z_F plane; *G, smoothness of entry*: the coefficients of variation of the displacement (cm) of landmark 1 per frame along the horizontal (X) and vertical (Z) axes for 5 frames before to 5 frames after passing the 0 coordinate (11 measurements for each axis).

We compared these parameters between control and treatment flights for each bat at the critical time of entry into the flower, where echolocation is no longer useful (five frames (−5 to 5) before and after entry: 24 ms, corresponding to approximately 2.5 mm before and after entry; mean of 11 frames for parameters *A* and *B*, frame 0 for parameter *F*, and the relevant frames for the other parameters). All data used for analyses are available in electronic supplementary material, S4. The Matlab script used for generating these data is available in electronic supplementary material, S5.

(e) Vibrissa length measurements

To test whether vibrissa length is an adaptation to feeding strategy, we measured vibrissa length and skull morphometrics of 82 individuals from 10 neotropical bat species. Following the phylogeny and diet categories of [55], we chose three nectarivorous species from predominantly nectarivorous genera from two separate clades (*Anoura geoffroyi, Glossophaga soricina* (Glossophaginae) and *Lionycteris spurrelli* (Lonchophillinae)); three non-nectarivorous species from genera where nectarivory is

complementary, from three separate clades (*Lampronycteris brachyotis* (Micronycterinae), *Lophostoma silvicolum* (Phyllostominae) and *Carollia perspicillata* (Carolliinae)); three non-nectarivorous species from genera where nectarivory is absent, from three separate clades (*Micronycteris microtis* (Micronycterinae)), *Lonchorhina aurita* (Lonchohininae) and *Centurio senex* (Stenodermatinae); and one outgroup species not from family Phyllostomidae (*Pteronotus mexicanus*). This last species belongs to the family Mormoopidae—a sister taxon to Phyllostomidae. Measurements were performed on skull and skin preparations of specimens preserved at the American Museum of Natural History (see electronic supplementary material, S4 for specimen list). To ensure we did not measure broken or damaged vibrissae we only included those with a complete and gradual distal taper in our measurements.

We used ImageJ software [56] to measure skulls. We photographed each skull twice (top and side views) with a scale visible to calibrate ImageJ measurements. All photos were taken using a Canon EOS R6 digital mirrorless camera fitted with a Canon RF 100 mm macro lens (Canon, Japan) with the camera always mounted perpendicular to and above the skull using a copy stand.

We measured vibrissa length directly from the preserved skin specimens using calipers (accuracy to 0.02 mm). We used the length of the longest vibrissa in each vibrissa group. Since preserved skins do not maintain the natural orientation of the vibrissae (slightly bent and pointing forward), we did not measure the distance between the tip of the vibrissa and the rostrum but instead measured the absolute length of a straightened vibrissa.

We calculated relative length of the vibrissae for each individual for both the ventral and the lateral vibrissae. For the ventral vibrissae, we divided the length of the ventral vibrissae (interramal vibrissae, [57]) by the height of the rostrum at the caudal edge of the nasal aperture. For the lateral vibrissae, the length of the main lateral vibrissa (characters 14 and 15 in [57]) was divided by the width of the rostrum at the same point. These relative measurements provide the ratio between vibrissa length and rostrum size, and we named them ventral and lateral vibrissae to rostrum ratio (V_V/R and V_L/R), respectively.

(f) Statistical analyses

Statistical analyses were run in R v. 4.1.3 [58]. To test for the effect of vibrissa clipping on bat feeding behaviour, we ran linear mixed models using the *lmer* function from the *lme4* package (https://github.com/lme4/lme4). For most of the variables described in the video analysis methods, we generated linear mixed models with treatment as a categorical fixed effect (intact or clipped vibrissae) and bat individual and flight number as random effects. Significance was assessed using *p*-values calculated by the *lmerTest* package (https://github.com/runehaubo/lmerTestR) using the Satterthwaite approximation to estimate degrees of freedom. Variables that deviated substantially from normality based on quantile–quantile plots (*lateral position within flower* (*B*) and *latency of tongue extension* (*D*)) were log-transformed to achieve normality before statistical analyses. To test whether long facial vibrissae are associated with a nectarivorous diet, we used phylogenetic ANOVA (*phylANOVA* function, *phytools* package: https://github.com/cran/phytools/blob/master/R/phylANOVA.R). The phylogeny from [55] was imported into R and pruned to the 10 bat study species. The factor 'diet category' had three levels (nectarivory obligatory, nectarivory complementary, no nectarivory) with 3 or 4 species per category. *p*-values were calculated by *phylANOVA* using phylogenetic simulations, and the Holm method was used for *post hoc* tests. The R script used for analyses as well as a summary containing all details of every statistical test performed in the study are available in electronic supplementary material, S5.

Data accessibility All the data used for this study are available in the electronic supplementary material. Electronic supplementary material, S4 contains all raw data extracted from videos, cleaned data used for trajectory analyses, skull and vibrissae measurements and museum specimens list. Electronic supplementary material, S5 includes four subfolders containing the following: MATLAB scripts and input spreadsheets; R scripts and input spreadsheets; Xmalab landmarks tracking files for all flights; skull photo used for measurements. In all data file and event names, we use the following nomenclature: YYYYMMDD_bat#_flight#_treatment. Electronic supplementary material, S5 is available online: https://data.mendeley.com/datasets/yfrwkdpgsc/2.

Authors' contributions E.A.: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, supervision, validation, visualization, writing—original draft, writing—review and editing; D.B.B.: data curation, formal analysis, methodology, software, writing—review and editing; R.A.P.: methodology, project administration, resources, supervision, writing—review and editing; S.M.S.: data curation, methodology, project administration, resources, supervision, writing—review and editing; H.M.t.H.: data curation, formal analysis, funding acquisition, methodology, project administration, resources, software, supervision, visualization, writing—original draft, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed herein.

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