

Echolocation and flight behavior of the bat *Hipposideros armiger terasensis* in a structured corridor

Michaela Warnecke, Benjamin Falk, and Cynthia F. Moss

Citation: [The Journal of the Acoustical Society of America](#) **144**, 806 (2018); doi: 10.1121/1.5050525

View online: <https://doi.org/10.1121/1.5050525>

View Table of Contents: <https://asa.scitation.org/toc/jas/144/2>

Published by the [Acoustical Society of America](#)

ARTICLES YOU MAY BE INTERESTED IN

[Dolphins maintain high echolocation vigilance for eight hours without primary \(food\) reinforcement](#)
The Journal of the Acoustical Society of America **144**, 660 (2018); <https://doi.org/10.1121/1.5049585>

[The communication space of humpback whale social sounds in wind-dominated noise](#)
The Journal of the Acoustical Society of America **144**, 540 (2018); <https://doi.org/10.1121/1.5047744>

[On the reliability of acoustic annotations and automatic detections of Antarctic blue whale calls under different acoustic conditions](#)
The Journal of the Acoustical Society of America **144**, 740 (2018); <https://doi.org/10.1121/1.5049803>

[A model and experimental approach to the middle ear transfer function related to hearing in the humpback whale \(*Megaptera novaeangliae*\)](#)
The Journal of the Acoustical Society of America **144**, 525 (2018); <https://doi.org/10.1121/1.5048421>

[Auditory sensitivity, spatial dynamics, and amplitude of courtship song in *Drosophila melanogaster*](#)
The Journal of the Acoustical Society of America **144**, 734 (2018); <https://doi.org/10.1121/1.5049791>

[Understanding sources of variance and correlation among features of Bornean gibbon \(*Hylobates muelleri*\) female calls](#)
The Journal of the Acoustical Society of America **144**, 698 (2018); <https://doi.org/10.1121/1.5049578>



Echolocation and flight behavior of the bat *Hipposideros armiger terasensis* in a structured corridor

Michaela Warnecke,^{a)} Benjamin Falk, and Cynthia F. Moss

Department of Psychological and Brain Sciences, Johns Hopkins University, 3400 North Charles Street, Baltimore, Maryland 21218, USA

(Received 19 April 2018; revised 24 July 2018; accepted 27 July 2018; published online 17 August 2018)

In this study, the echolocation and flight behaviors of the Taiwanese leaf-nosed bat (*Hipposideros armiger terasensis*), which uses constant-frequency (CF) biosonar signals combined with a frequency-modulated (FM) sweep, are compared with those of the big brown bat (*Eptesicus fuscus*), which uses FM signals alone. The CF-FM bat flew through a corridor bounded by vertical poles on either side, and the inter-pole spacing of the walls was manipulated to create different echo flow conditions. The bat's flight trajectories and echolocation behaviors across corridor conditions were analyzed. Like the big brown bat, the Taiwanese leaf-nosed bat centered its flight trajectory within the corridor when the pole spacing was the same on the two walls. However, the two species showed different flight behaviors when the pole spacing differed on the two walls. While the big brown bat deviated from the corridor center towards the wall with sparse pole spacing, the Taiwanese leaf-nosed bat did not. Further, in comparison to *E. fuscus*, *H. a. terasensis* utilized different echolocation patterns showing a prevalence of grouping sounds into clusters of three. These findings indicate that the two species' distinct sonar signal designs contribute to their differences in flight trajectories in a structured corridor. © 2018 Acoustical Society of America.

<https://doi.org/10.1121/1.5050525>

[AMS]

Pages: 806–813

I. INTRODUCTION

Most diurnal animals, including humans, rely on vision to navigate. In contrast, echolocating bats as nocturnal mammals, have developed a specialized active listening system that allows them to represent their environment with sound (Griffin, 1958). Echolocating bats produce high frequency acoustic signals and process information contained in returning echoes to detect, localize and discriminate objects in their surroundings (Griffin *et al.*, 1965; Moss and Schnitzler, 1995; Simmons, 1973). Bats occupy many ecological niches around the world, and forage in environments as diverse as the desert and the tropics (Kunz and Fenton, 2005; Neuweiler, 2000; Schnitzler *et al.*, 2001), solving real-world scene analysis problems using different signal designs. Echolocation signals can be broadly categorized as constant-frequency (CF) and frequency-modulated (FM) sounds. In general, CF signals are typically long (6–100 ms), narrowband signals with a bandwidth of 1–3 kHz, and have a high duty-cycle [Fig. 1(A), top; Moss and Schnitzler, 1995; Schnitzler and Kalko, 2001; Simmons and Stein, 1980]. By contrast, FM signals are relatively short (0.5–15 ms), mostly downward-sweeping sounds with a bandwidth of about 30–100 kHz, and have a low-duty cycle [Fig. 1(A), bottom].

Bats that use broadband FM signals show high localization accuracy, particularly along the range axis, because each frequency in the signal provides a time marker for estimating echo arrival time (Simmons and Stein, 1980). By contrast, bats that use CF signals compensate for velocity-

dependent Doppler shifts in returning echoes by lowering the frequency of their narrowband sonar emissions as they fly (Schnitzler, 1973). This Doppler shift compensation ensures that echoes return to the bat's sonar receiver at the frequency of maximum hearing sensitivity and frequency selectivity (Neuweiler, 1980; Neuweiler *et al.*, 1980), and allow it to detect and discriminate fluttering insect prey in acoustically cluttered environments (von der Emde and Menne, 1989; von der Emde and Schnitzler, 1986, 1990; Kober and Schnitzler, 1990; Neuweiler, 1980; Schnitzler and Flieger, 1983; Trappe and Schnitzler, 1982).

A variety of methods have been employed to investigate echolocation behavior and performance in bats that use FM and CF-FM signals. For example, psychophysical studies have demonstrated the limits of distance, direction, size, shape, movement, and texture discrimination in a variety of bat species (reviewed in Moss and Schnitzler, 1995; Wohlgemuth *et al.*, 2016). Behavioral studies have also considered the contribution of the active control over the spectrum, timing and directional aim of sonar signals to the process of echolocation in different species of bats (von der Emde and Schnitzler, 1986; Gustafson and Schnitzler, 1979; Hiryu *et al.*, 2007; Neuweiler *et al.*, 1980; Schnitzler, 1973; Schnitzler and Flieger, 1983; Schoeppler *et al.*, 2018; Surlykke *et al.*, 2009; Surlykke and Moss, 2000; Trappe and Schnitzler, 1982). Additionally, recent studies show that bats using FM and CF-FM signals adapt the timing between their sonar pulses (pulse interval, PI) to form sonar sound groups, in which the interval between sounds within a group is short, and is flanked by calls at longer intervals [Fujioka *et al.*, 2014; Moss *et al.*, 2006; e.g., Fig. 1(B), top]. The use of

^{a)}Electronic mail: warnecke@jhu.edu

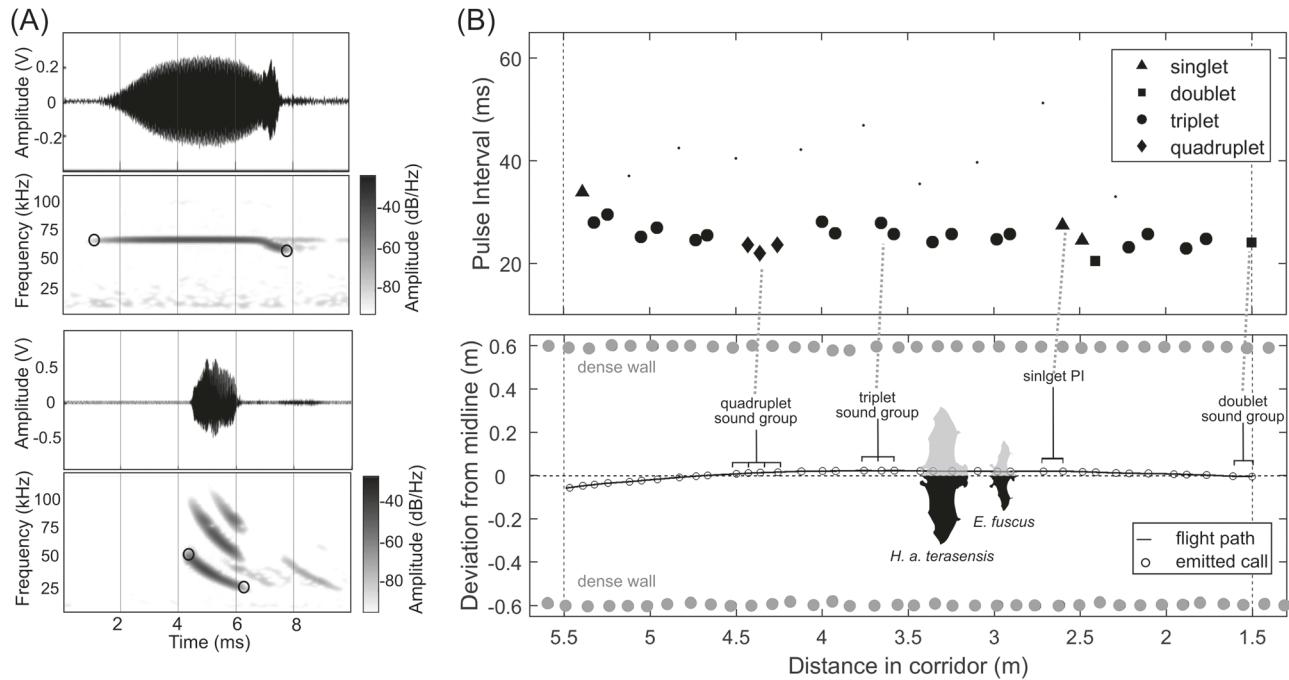


FIG. 1. Call comparison between *Hipposideros armiger terasensis* and *Eptesicus fuscus*, and experimental setup. (A) Top: waveform and spectrogram of typical CF call emitted by *H. a. terasensis*. Bottom: waveform and spectrogram of typical CF call emitted by *E. fuscus*. In both spectrograms, black circles indicate start- and end times typically extracted for audio analysis. (B) Top: Pulse interval (y-axis) plotted across distance (x-axis) bat traverses corridor (bottom). Pulse interval values are plotted in markers that identify their sonar sound group (legend). Small dots represent non-sound group PIs. Bottom: Exemplary plot of flight deviation from the midline (y-axis) across corridor length (x-axis), for the same trial as in the top panel. Black solid line indicates x-y coordinates of bat flight path; white circles indicate locations at which the bat emitted a sonar call. Circle sizes are scaled to call duration. Indicated are singlet, doublet, triplet, and quadruplet sound group classifications, which relate to the data plotted in the top panel (grey dashed arrows). Grey filled circles represent x-y coordinates of poles at dense spacing for this trial. Black dashed vertical lines indicate corridor limitations for analysis (see Sec. II). The sizes of *H. a. terasensis* and *E. fuscus* are sketched to scale.

sonar sound groups is especially prominent in situations where bats would benefit from increased spatial resolution, such as foraging (Kothari *et al.*, 2014) or navigating highly cluttered environments (Hiryu *et al.*, 2008; Petrites *et al.*, 2009; Säding *et al.*, 2014; Warnecke *et al.*, 2016; Wheeler *et al.*, 2016).

Although past work has informed our understanding of adaptive echolocation and flight behavior in diverse bat species, the acoustic basis of sonar-guided navigation in complex environments, where each single echolocation signal results in cascades of echoes, remains an open problem. Previously, Warnecke *et al.* (2016) investigated adaptations in flight and echolocation behaviors in the FM bat, *Eptesicus fuscus* flying in structured corridors, which returned cascades of echoes to the bat. Echo cascades vary with the bat's velocity, head aim, and distance to objects in the environment. In this previous study, FM bats flew through a corridor whose walls were built from individually moveable poles [e.g., Figure 1(B), bottom], and manipulations of the spacing between poles created different echo flow patterns for each emitted biosonar broadcast. The paper reported that bats adapted their flight trajectories and echolocation behavior in response to different spacing between poles on either corridor wall. For example, *E. fuscus* emitted shorter calls in the acoustically most cluttered condition, and typically arranged its calls into pairs of sonar sound groups when navigating the corridor (Warnecke *et al.*, 2016). Kugler *et al.* (2016) further showed similar flight and echolocation behaviors

when *Phyllostomus discolor*, an FM bat that commonly navigates cluttered environments, flew through a corridor of horizontal and vertically arranged wooden slats (Kugler *et al.*, 2016).

Taiwanese leaf-nosed bats use CF-FM signals, and they commonly forage along forest edges and search for prey in confined environments like “corridors under the canopy” (Lee *et al.*, 2012), a natural environment that mimics the experimental setup of the echo flow corridor introduced by Warnecke *et al.* (2016). In the present study, we studied this CF-FM bat species’ adaptations in flight and echolocation behaviors and compared data with that previously obtained from the big brown bat.

II. MATERIALS AND METHODS

A. Animals

Three Taiwanese leaf-nosed bats, *H. a. terasensis*, served as subjects in this experiment after having been collected from the field in Taiwan and transported to Johns Hopkins University under CDC import permit 2015-03-146. The bats were fed with mealworms (*Tenebrio molitor*) daily to maintain their individual weights between 52 and 63 g. All animals were housed in a single colony room on a reversed 12-h light/dark cycle, which was kept at 24°C–28°C at 40% to 50% relative humidity. The experimental procedures were approved by the Johns Hopkins University Institutional Animal Care and Use Committee.

B. Experimental paradigm

The experimental setup and paradigms were the same as previously described in [Warnecke et al. \(2016\)](#). Briefly, in a large carpeted flight room ($6 \times 7 \times 2.5$ m) a 6.2 m long and 1.2 m wide corridor was built from individually moveable wooden poles (2.5 cm diameter). The spacing between poles on the left and right corridor sides could be experimentally manipulated and several conditions with different pole spacing were tested in the experiment. The opening to the corridor was lined with a black felt curtain that had an elliptic opening (31 \times 38 cm) to prevent the bat from gaining information about the different wall configurations before it began its flight through the corridor on each trial. *H. a. terrensis* entered the experiment completely novice to the environment and had not undergone any prior training. We ran two baseline conditions in which the opposite walls of the corridor were symmetric: left dense spacing, right dense spacing [LD-RD; Fig. 1(B), bottom], or left sparse spacing, right sparse spacing (LS-RS). We also collected data in two test conditions in which the opposite walls of the corridor were asymmetric: left sparse spacing, right dense spacing (LS-RD), or left dense spacing, right sparse spacing (LD-RS). For all conditions, dense spacing refers to a 12 cm gap between two poles, and sparse spacing refers to a 36 cm gap between two poles. These gap measurements were chosen to be able to directly compare the data to a previously collected data set ([Warnecke et al., 2016](#)). To test if the effect of deviation changes for different corridor widths, we collected data for the LS-RD condition at corridor widths of 1.0, 1.2, and 1.5 m.

Prior to each experiment, the test bat was removed from its cage and water-soluble glue (Grimas Mastix Water Soluble, Heemstede, Holland) was used to attach a custom-built marker to the bat's head. The marker was shaped like a triangle and had three small (5 mm diameter) reflective spheres glued to each corner. At a total weight of less than 0.1 g, the marker was positioned between the bat's ears to track the animal's position during flight. The experiment started when the marker was securely attached to the bat's head, and all recording systems were ready to collect data. Bats were released at ca. 20 cm distance from the curtain, and they entered the corridor by flying through the elliptical opening. After collecting data over at least nine trials, the head and body markers were carefully removed, and the animal was returned to its cage.

Each day the bats were tested in the same order and at approximately the same time of day. Due to the time required to prepare each corridor setup, a single condition was tested on each day. For each trial, three experimenters were present: one experimenter released the bat from behind the curtain and remained in that location. A second experimenter was responsible for catching the bat after a trial had ended and safely return it to the curtain-enclosed space. The third experimenter recorded notes on every trial and triggered the audio-video recording system for trial capture (see below).

All data collection was carried out in a room that was solely illuminated with dim infrared light for motion-tracking

detection of the reflective markers on the bat ([Hope and Bhatnagar, 1979](#)). Measurements of the light levels in the flight room at different locations within the corridor each revealed a light intensity of $<10^{-2}$ lux. Measurements were made with a spectrophotometer (GS-1500, Gamma Scientific, San Diego, CA) under experimental conditions.

C. Data recording

For each trial, synchronized audio and motion-tracking data of the flying bat were captured. Audio data were recorded using seven ultrasonic microphones (six D500X external microphones, Pettersson Elektronik Uppsala, Sweden, and one UltraSound Advice microphone, Costa Mesa, London, UK) bandpassed between 10 and 100 kHz (Alligator Technologies, CA). Four microphones were mounted at the end of the corridor, and three were mounted in the beginning of the corridor. Microphones at the end of the corridor were used to collect the bats' calls, and to be able to extract temporal and spectral features of each call in combination with three dimensional (3D) flight track information, while microphones mounted at the beginning of the corridor more effectively sampled the echoes returning for each emitted call. All audio data were sampled at 250 kHz (NI PXI board 6143). The bat's flight trajectory was recorded using 13 high-speed IR motion-capture cameras (Nexus, Vicon, Centennial, CO, USA) mounted on the ceiling within the corridor. The motion-tracking system tracked the reflective spheres attached to each bat at 100 frames per second and allows for millimeter precision. After all trials for the day were collected, the motion-tracking system also captured the position of the microphones, the location of the curtain entrance hole, and the poles that comprised the corridor walls. Every trial was manually triggered by an experimenter after the bat had traversed the full length of the corridor. Data acquired within 4 s prior to the trigger were stored for analysis.

D. Data processing and analysis

Motion-capture data were processed with custom-written MATLAB code to reconstruct 3D tracks of each bat's flight trajectories on a given day. In subsequent processing, we computed the bat's 3D deviation from the midline of the corridor. Data points are calculated with reference to the distance from the end of the corridor, which is defined as the plane created by the last poles on the left and right sides.

Echolocation calls produced by bats flying in the corridor were manually processed using custom-written MATLAB programs. For each trial, we extracted the call start and end times of each call that was emitted during the portion of the flight path (Fig. 1, black circles). With these parameters, we then calculated the call duration, duty cycle, call rate, and pulse interval of each call.

To exclude positions in which the bat first entered the corridor or may have been planning its exit, data points 1.5 m from the entrance and 1 m from the exit were excluded. Only audio and track data collected from the middle portion (a total of 4 m) of the corridor were analyzed. Trials in which the bats did not fly along at least [3] [4]

of the length of the entire corridor were excluded. One bat's data was excluded from analysis in the LS-RS condition, because only three flights through the corridor could be collected. As such, only data from two animals will be considered for the LS-RS condition, while data from all three animals contribute to all other conditions (LD-RD, LD-RS, LS-RD).

We used the statistical data analysis software JMP (SAS) to run statistical analyses on the data set. To evaluate whether the flight deviation (deviation from midline between two corridor sides) differed across conditions, we averaged flight deviation across conditions and bats, and subsequently used a mixed effect model analysis with condition ($N = 4$) as a fixed factor, and bat ($N = 3$) as a random factor. To evaluate whether the flight behavior of *Eptesicus fuscus* differed from that of *Hipposideros armiger terasensis*, we calculated the difference between the mean deviation of each species per condition [see Fig. 2(A), dashed black lines], and tested whether that value differed significantly from zero. If both species showed similar flight path selections in a given condition, the difference between their mean deviations would be small. By contrast, if the species selected different flight paths in a given condition, the difference between their mean deviations would be large. We used a z-test to measure if the difference between mean deviations was different from zero.

For the analysis of audio data and flight speed we merged the conditions of different pole spacings, LD-RS and LS-RD, into a single variable "S/D," as we did not expect nor observe differences in echolocation behavior or flight speed depending on whether the densely spaced poles were on the left or right side of the corridor. We used a mixed effect model analysis to evaluate differences across conditions, with conditions ($N = 3$) as a fixed effect and bats ($N = 3$) as a random effect. After the initial analysis, if necessary, we performed a multiple comparison Tukey's HSD to evaluate (1) whether the bat's flight behavior differed between baseline (LD-RD and LS-RS) and test conditions (S/D), (2) whether echolocation parameters (duration, duty

cycle, call rate) differed between conditions, and (3) whether pulse timings (PI, and number of sonar sound groups) differed between different sonar sound groups. A total of 124 trials (LD-RD: $N = 35$, LD-RS: $N = 38$, LS-RD: $N = 29$, LS-RS: $N = 22$) were analyzed.

III. RESULTS

In this study, we tested whether different configurations of pole spacing along the sides of a corridor influenced the flight and echolocation patterns of the CF-FM bat, *Hipposideros armiger terasensis*. Figure 2 plots the flight data and represents the bat's deviation from the midline of the corridor. Below, we first outline the flight behavior, and then describe the changes of echolocation parameters across conditions (Fig. 3).

A. Flight behavior

The average deviation from the midline of the corridor for each of the four different conditions is shown in Fig. 2. Figure 2(A) plots the mean deviation of each bat (open circles), as well as the overall mean and standard error of deviation (black) for all bats across conditions (x-axis). Deviation from the midline is plotted along the y-axis: deviations toward the right are shown on a positive scale, whereas deviations toward the left are shown on a negative scale. Grey plots indicate the mean deviation of *E. fuscus* for the same condition (see Sec. IV; Warnecke *et al.*, 2016). Figure 2(B) shows the raw distribution of deviations (x-axis) for each condition (y-axis), with the mean of each distribution indicated by a black line for *H. a. terasensis*, and a grey dashed line for *E. fuscus* (Warnecke *et al.*, 2016). Baseline (symmetric) conditions, LD-RD and LS-RS, presented the bat with similar spacing of poles on the left and right corridor sides. When both sides were constructed from densely spaced poles (LD-RD), *H. a. terasensis* centered its flight path around the midline and on average deviated about 0.28 cm (SE = 0.6 cm) away from the midline toward the right side of the corridor. When the bat flew through the corridor constructed from two sides of sparsely spaced poles

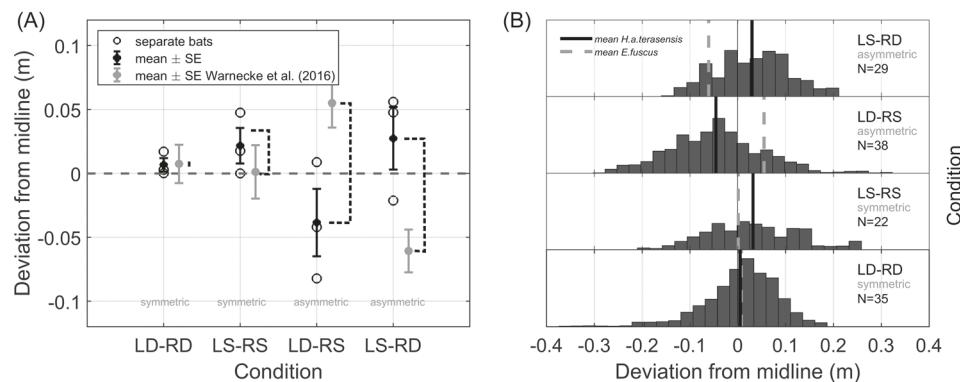


FIG. 2. Comparison of flight behavior across conditions. (A) Scatter plots of mean deviation per bat (open circles) across conditions (x-axis) are shown alongside plots of mean \pm standard error per condition (black). Added in grey are the data points for the mean deviation of the same conditions collected by Warnecke *et al.* (2016) for the big brown bat. Dashed black lines indicate the magnitude of the difference value between means of each species per condition. (B) Histograms representing the deviation from the midline (x-axis) for all bats across four conditions (y-axis). Black line indicates the mean deviation for *H. a. terasensis*. Grey dashed line indicates the mean deviation for the *E. fuscus* in the same conditions (Warnecke *et al.*, 2016).

(LS-RS), the flight paths showed a broader distribution around the midline [Fig. 2(B), LS-RS], with a slight shift to the corridor's right (mean = 3.2 cm, SE = 1.3 cm). When the bat flew through the corridor with different pole spacings (asymmetric) on the left and right corridor walls (LD-RS, LS-RD) *H. a. terasensis* deviated slightly toward the wall that was built from densely spaced poles (LD-RS: mean = -4.1 cm, SE = 2.7 cm; LS-RD: mean = 2.8 cm, SE = 2.5 cm), however, our statistical analyses do not show a difference in the flight path distributions of deviations of *H. a. terasensis* from the midline across conditions ($F_{3,4} = 4.9, p = 0.075$).

To evaluate whether the flight behavior of *H. a. terasensis* differed from that of *E. fuscus*, we calculated the difference between the mean deviation of the two species for each corridor condition [Fig. 2(A), black dashed lines], and tested whether that difference was reliably larger than zero. If the two species selected similar flight paths, the difference between their mean deviations would be close to zero [Fig. 2(A), LD-RD, LS-RS], and if the two species selected different flight paths, the mean deviations would be greater than zero [Fig. 2(A), LS-RD, LD-RS]. Statistical analyses show that the difference between mean deviations from center in symmetric conditions do not differ from zero (LD-RD: difference = 0.36 cm, $z = 0.13, p = 0.44$; LS-RS: difference = 2.87 cm, $z = 0.76, p = 0.22$). However, in asymmetric

conditions, the difference of mean deviations between *E. fuscus* and *H. a. terasensis* flight trajectories is significantly larger than zero (LS-RD: difference = 8.8 cm, $z = 2.02, p = 0.021$; LD-RS: difference = 9.59 cm, $z = 1.92, p = 0.027$). Thus, there is a statistical difference between flight trajectories of the two species in the asymmetric corridor conditions.

H. a. terasensis' wingspan is almost thirty percent greater than that of *E. fuscus* [Fig. 1(B)], and their flight path selection might have been impacted by the corridor width. To test whether the deviation from the midline was influenced by the width of the corridor, we also tested each *H. a. terasensis* in the LS-RD condition at three different corridor widths (1.0, 1.2, 1.5 m). Comparing mean flight deviation for this condition across the three corridor widths showed no significant difference of deviation ($F_{2,4} = 4.3, p = 0.1$; 1.0 m width: mean = 1.31 cm, SE = 2.95 cm; 1.2 m width: mean = 2.75 cm, SE = 2.45 cm; 1.5 m width: mean = 3.65 cm, SE = 3.07 cm).

We investigated whether flight speed differed across pole density conditions, and did not find a difference in flight speed across conditions [Fig. 3(A), grey; $F_{2,5,1} = 0.6, p = 0.64$]. On average, bats flew at about 3.5 m/s, with slightly slower speeds in LD-RD (mean = 3.4 m/s, SE = 0.2 m/s) compared to LS-RS (mean = 3.53 m/s, SE = 0.08 m/s) or either of the test conditions (S/D: mean = 3.52 m/s, SE = 0.09 m/s).

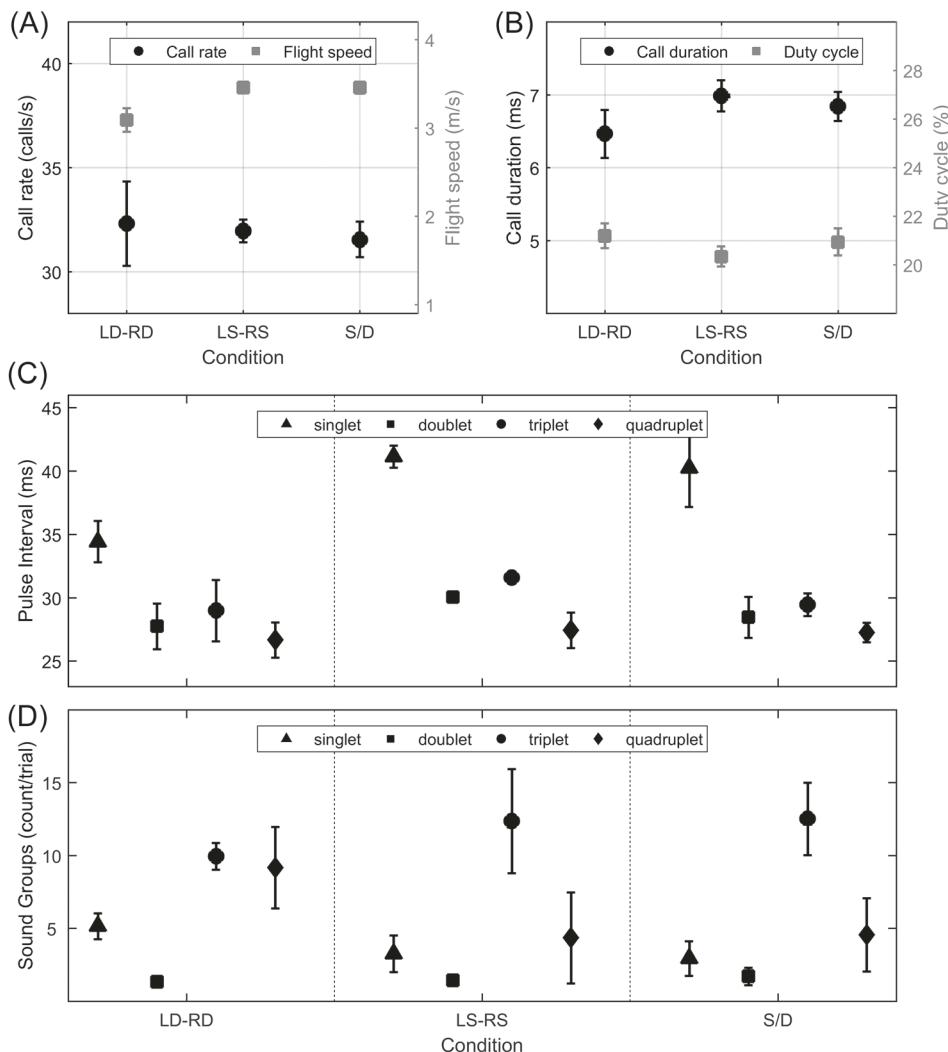


FIG. 3. Echolocation behavior of *H. a. terasensis*. (A) Scatter plots indicating the call rate (left y-axis, black) and flight speed (right y-axis, grey) across conditions (x-axis) as mean \pm standard error. (B) Scatter plot of call duration (left y-axis, black) and duty cycle (right y-axis, grey) across conditions (x-axis). (C) Scatter plots of pulse interval (y-axis) across conditions (x-axis) per sonar sound group type (legend). (D) Scatter plots of number of sonar sound group per trial (y-axis) across conditions (x-axis) for each sonar sound group type (legend).

B. Echolocation behavior

Our results indicate that the bats did not alter their call rates across corridor conditions [Fig. 3(A), black; $F_{2,6} = 0.005$, $p = 0.99$], and consistently emitted on average 31 calls per second (LD-RD: mean = 31.8 call/s, SE = 2.7; LS-RS: mean = 31.6 calls/s, SE = 0.76; S/D: 31.8 call/s, SE = 0.73).

Overall, *H. a. terasensis* emitted calls that were about 6.8 ms long [Fig. 3(B), black]. We observed a tendency for this species to produce shorter calls in LD-RD compared to the other conditions, but the difference was not statistically reliable [LS-RS, S/D; Fig. 3(B); $F_{2,6} = 5.07$, $p = 0.054$; LD-RD: mean = 6.4 ms, SE = 3.2; LS-RS: mean = 6.9 ms, SE = 0.26; S/D: mean = 6.8 ms, SE = 0.13].

Across all conditions, the bats oftentimes grouped echolocation calls together into sonar sound groups. The bats' echolocation behavior fell into one of four categories: grouping calls into sets of two (doublets), three (triplets), or four (quadruplets) calls, or emitting sounds that were not grouped (singlets). Standards previously introduced to categorize sonar sound groups (e.g., Kothari *et al.*, 2014; Warnecke *et al.*, 2016) were slightly modified to match the patterning of pulse intervals used by *H. a. terasensis* into similar categories [see Fig. 1(B), decreased island criterion = 1.1, increased tolerance = 0.08, see Kothari *et al.*, 2014]. The prevalence of each of these four sound group categories produced by *H. a. terasensis* was statistically different [Fig. 3(D); $F_{3,36} = 19.1$, $p < 0.0001$]. Specifically, *H. a. terasensis* emitted triplet sonar sound groups significantly more often [Fig. 3(D), diamond] than any other group (singlet-triplet: $t = -5.7$, $p < 0.0001$; doublet-triplet: $t = -7.18$, $p < 0.0001$; quadruplet-triplet: $t = -3.96$, $p = 0.0018$).

We also investigated the PI of each sonar sound group category and found a reliable difference in pulse interval [Fig. 3(C); $F_{3,36} = 25.25$, $p > 0.0001$]. Specifically, the PI of singlets was significantly longer [Fig. 3(C), triangle] than that of any of the other sonar sound groups (singlet-doublet: $t = -6.7$, $p < 0.0001$; singlet-triplet: $t = 5.4$, $p < 0.0001$; singlet-quadruplet: $t = -8.9$, $p < 0.0001$).

We did not explicitly measure Doppler shift compensation for *H. a. terasensis*, because recent work suggests that the resting frequency of this species varies considerably between individuals and over time (Schoeppler *et al.*, 2018).

IV. DISCUSSION

Echolocating bats navigate in dark, acoustically complex surroundings with great agility and apparent ease (e.g., Griffin, 1958; Moss and Surlykke, 2001; Neuweiler, 1990; Simmons *et al.*, 2001). In this study, we investigated flight and sonar adaptations of the echolocating Taiwanese leaf-nosed bat, *Hipposideros armiger terasensis*, in a confined, and reverberant corridor. We compare these adaptations to those observed in the big brown bat, *Eptesicus fuscus*, which navigated the same corridor (Warnecke *et al.*, 2016), and propose that species differences in flight and echolocation behavior may stem from adaptations that *H. a. terasensis* and *E. fuscus* have evolved to their natural soundscape.

A. Flight path selection

We predicted that *H. a. terasensis* would select different flight trajectories in corridors with asymmetric pole spacing on opposite walls, compared to the FM bat, *Eptesicus fuscus*, because the former commonly navigates in dense vegetation and might prefer to follow the wall with dense pole spacing. However, *H. a. terasensis* does not show statistically reliable differences across conditions in their flight path selection. When the bats flew through the corridor with the same approximate pole spacing on opposite walls (symmetric), they centered their flight paths along the midline [Fig. 2(B)]. When flying through the corridor in test conditions (asymmetric), the bats' mean deviation was toward the side with densely spaced poles [Fig. 2(B); black lines]. Figure 2(A) illustrates that in asymmetric conditions two out of three bats consistently veered toward the densely spaced corridor wall, while one bat did not. This behavior is intriguing when one considers the flight paths of the big brown bat, *E. fuscus*, in the same corridor conditions, which show a distinctly different pattern [Fig. 2(B); grey dashed lines]. Specifically, statistical analyses show that flight path selection in the asymmetric conditions between *E. fuscus* and *H. a. terasensis* show significant differences in their mean deviations.

H. a. terasensis operates in cluttered environments, flying along forest edges, within the canopy, and foraging within dense vegetation (Lee *et al.*, 2012). In these environments, each sonar call the bat produces results in a cascade of echoes returning to it from trees and shrubs at different distances. Thus, the CF-FM bat *H. a. terasensis* may commonly experience and use cascades of echoes for flight guidance in its natural habitat. The big brown bat, on the other hand, commonly forages in comparatively open spaces and over ponds, where echo cascades from vegetation may be rather sparse (Moss and Surlykke, 2010; Simmons *et al.*, 2001; Surlykke *et al.*, 2009). We propose that each species' common foraging grounds could be a factor in their bias to deviate toward one side of the corridor in the asymmetric wall condition.

B. Echolocation behavior

Echolocating bats adjust the timing of their sonar calls with respect to their environment and the task at hand (Hiryu *et al.*, 2005; Kothari *et al.*, 2014; Moss *et al.*, 2006; Petrites *et al.*, 2009; Schnitzler and Kalko, 2001a; Warnecke *et al.*, 2016). In all conditions tested in this study, we observed that *H. a. terasensis* grouped its calls into clusters of sonar sound groups, as previously described in other bat studies (Kothari *et al.*, 2014; Moss *et al.*, 2006; Wheeler *et al.*, 2016). Schoeppler *et al.* (2018) recorded *H. armiger* calls at rest and during flight using wall-mounted microphones, while Hiryu *et al.* (2005) and Hiryu *et al.* (2008) recorded echolocation calls of the same species while carrying a custom-made telemetry microphone. Both studies reported sonar sound group production in bats engaged in the search and approach phases and preparing to land (Hiryu *et al.*, 2005; Hiryu *et al.*, 2008; Schoeppler *et al.*, 2018). This observation is consistent with previous reports of sonar sound group production patterns by the big brown bat in situations which

demand high special localization accuracy (Kothari *et al.*, 2014; Moss *et al.*, 2006; Säding *et al.*, 2014; Warnecke *et al.*, 2016 and Wheeler *et al.*, 2016).

Audio analysis of echolocation behavior of bats in the present study revealed that *H. a. terasensis* more commonly produced sonar call triplets [Fig. 3(D), circles], compared to other sound groups. Interestingly, in the acoustically most cluttered condition (LD-RD), animals also produced an increased number of quadruplets (diamonds). This differed from the other conditions (LS-RS and S/D), in which bats produced triplets more often than all other sound group categories, and suggests that the bat's production of triplets and quadruplets in the most cluttered corridor condition might be helpful in navigating through this complex soundscape.

In this context, it is noteworthy that the overall call rate of *H. a. terasensis* does not differ across corridor conditions [Fig. 3(A), black], showing that the bats re-arranged the temporal patterning of calls to create triplets and quadruplets, rather than just “adding” or “deleting” calls to create a different sound group. Further, this implies that the use of higher order sound groups, i.e., triplets or quadruplets, may yield a sharper representation of the soundscape, and therefore support navigation through the acoustically most complex condition.

The prevalent use of sonar call triplets by *H. a. terasensis* contrasts with the big brown bat's prevalent use of doublets, followed by singlets. In fact, *E. fuscus* rarely made use of triplets or quadruplets (Warnecke *et al.*, 2016). Overall, while the Taiwanese leaf-nosed bat most often used triplets, followed by quadruplets to navigate any echo flow condition, and as shown in Fig. 1(A), the bat does not have a strict order for when a specific sound group is used. By contrast, the big brown bat used those two sound groups the least, and relied on doublets and singlets. By emitting higher order sound groups, the Taiwanese leaf-nosed bat receives multiple “snapshots” of the environment as it flies by the poles. Both bat species pattern higher order sound groups at pulse intervals of around 30 ms [Fig. 3(C); Warnecke *et al.*, 2016], but the prevalence of triplets used by the Taiwanese leaf-nosed bat may mean that it receives more “snapshots” in the same stretch of time compared to the big brown bat. This hypothesis is further supported by the fact that *H. a. terasensis* has a higher call rate (~31 calls/s) compared to *E. fuscus* (~23 calls/s; Warnecke *et al.*, 2016).

How might this difference in temporal patterning of echolocation calls affect the echo flow soundscape in each species? First, at flight speeds of ~3.5 m/s [Fig. 3(A)], the bat will travel approximately 10 cm between calls emitted within a sound group at ~30 ms interval. The spacing between dense poles is 12 cm, so when the bat moves past them it will receive successive echo cascades from different sets of dense poles for each call within a sonar sound group. By contrast, sparse poles, which are at 36 cm spacing, will return echoes from largely the same set of poles for the entirety of a doublet or triplet sonar sound group. Since, however, *H. a. terasensis* more commonly utilizes triplet sonar sound groups, and uses a higher call rate, its updates about the echo scene are more frequent. Thus, it may be possible for this bat to follow or represent echo patterns more

reliably than *E. fuscus*. Second, when *H. a. terasensis* produced singlets, the PI was about 37 ms, which is considerably longer than any other sound group PI (~29 ms). At the entrance of the corridor the bat would receive a 30–40 ms long cascade of echoes of decreasing in amplitude. As such, the singlet PI would allow for echoes of the entire corridor to be received, before a subsequent call was emitted. At shorter intervals, like those in doublet, triplet or quadruplet sound groups, the bat would have been emitting a new call when echoes from the previous sonar emission were still arriving. We propose that the longer PI of singlets may have served as a “break” from rapid processing of echo information that occurred when emitting calls at shorter intervals (i.e., doublet, triplet and quadruplet PIs). In contrast to the Taiwanese leaf-nosed bat, singlet PIs emitted by *Eptesicus fuscus* in this experimental setup are much longer (~60 ms), and thus may give the big brown bat a longer “break” from processing between sonar sound groups. We propose that this difference in patterning might reflect each species' adaptations to their natural environments: *H. a. terasensis* may be more accommodated to rapid echo processing due to the cluttered vegetation in its natural environment. It would be of interest to learn whether *Phyllostomus discolor*, an FM bat that navigates cluttered vegetation, might show temporal patterns that more closely resemble that of the big brown bat or the Taiwanese leaf-nosed bat, to better understand the extent to which signal differences and natural habitat might influence echolocation behavior in such structured environments (see Kugler *et al.*, 2016).

Differences in the interval and duration of sonar signals produced by *E. fuscus* and *H. a. terasensis* yield species differences in sonar call duty cycle. The big brown bats' calls are considerably shorter (~2 ms) than those of the Taiwanese leaf-nosed bat (~6.8 ms). These differences in duration, coupled with the prevalence of call triplets produced by *H. a. terasensis*, yield sonar duty cycles in this species that are several times higher than those of *E. fuscus*. [*H. a. t.*: ~18%–22%, see Fig. 3(B), grey; *E. f.*: 3%–5%]. The differences in duty cycle will dramatically influence the echo patterns used by the two species to represent the corridor.

In summary, we compared the echolocation and flight behavior of the CF-FM bat, *H. a. terasensis*, with that of the FM bat, *E. fuscus*, in a structured corridor and quantified differences in the two species' behaviors. This study revealed that a corridor returning asymmetric echo flow patterns from opposite walls resulted in significantly different flight path selection and adaptive echolocation behavior in the two species. These findings may arise from the distinct echolocation call designs of the two species, and future work may elucidate differences in echo flow processing by FM and CF-FM bats.

ACKNOWLEDGMENTS

We thank Traia Roper, Daniel Schwartzbaum, Alexia Huggins, Sydney Donofrio, Grace Lee, and Eyal Li for help during data collection. We thank Dallas DeFord and Brittney L. Boublil for help with the audio analysis. We also thank three anonymous reviewers for comments on earlier versions

of this manuscript. This research was supported by the ERP Graduate Fellowship of the Deutsche Studienstiftung to MW; the National Science Foundation IOS1460149 and 1734744, the Human Frontiers Science Program RGP0040, the Office of Naval Research N00014-12-1-0339, N00014-17-1-2736, and the Air Force Office of Scientific Research FA9550-14-1-0398. The authors declare no conflict of interest.

Fujioka, E., Aihara, I., Watanabe, S., Sumiya, M., Hiryu, S., Simmons, J. A., Riquimaroux, H., and Watanabe, Y. (2014). "Rapid shifts of sonar attention by *Pipistrellus abramus* during natural hunting for multiple prey," *J. Acoust. Soc. Am.* **136**, 3389–3400.

Griffin, D. R. (1958). *Listening in the Dark: The Acoustic Orientation of Bats and Men* (Yale University Press, New Haven, CT).

Griffin, D. R., Friend, J. H., and Webster, F. A. (1965). "Target discrimination by the echolocation of bats," *J. Exp. Zool.* **158**, 155–168.

Gustafson, Y., and Schnitzler, H.-U. (1979). "Echolocation and obstacle avoidance in the hipposiderid bat *Asellia tridens*," *J. Comp. Physiol.* **131**, 161–167.

Hiryu, S., Hagino, T., Shiori, Y., Riquimaroux, H., and Watanabe, Y. (2007). "Compensation behaviors in echolocating bats measured by a telemetry microphone during flight," in *Symposium on Underwater Technology and Workshop on Scientific Use of Submarine Cables and Related Technologies* (IEEE, New York), pp. 535–539.

Hiryu, S., Katsura, K., Lin, L.-K., Riquimaroux, H., and Watanabe, Y. (2005). "Doppler-shift compensation in the Taiwanese leaf-nosed bat (*Hipposideros terasensis*) recorded with a telemetry microphone system during flight," *J. Acoust. Soc. Am.* **118**, 3927–3933.

Hiryu, S., Shiori, Y., Hosokawa, T., Riquimaroux, H., and Watanabe, Y. (2008). "On-board telemetry of emitted sounds from free-flying bats: Compensation for velocity and distance stabilizes echo frequency and amplitude," *J. Comp. Physiol. A* **194**, 841–851.

Hope, G. M., and Bhatnagar, K. P. (1979). "Electrical response of bat retina to spectral stimulation: Comparison of four microchiropteran species," *Experientia* **35**, 1189–1191.

Kober, R., and Schnitzler, H. (1990). "Information in sonar echoes of fluttering insects available for echolocating bats," *J. Acoust. Soc. Am.* **87**, 882–896.

Kothari, N. B., Wohlgemuth, M. J., Hulgard, K., Surlykke, A., and Moss, C. F. (2014). "Timing matters: Sonar call groups facilitate target localization in bats," *Front. Physiol.* **5**, 1–13.

Kugler, K., Greiter, W., Luksch, H., Firzlaff, U., and Wiegrefe, L. (2016). "Echo-acoustic flow affects flight in bats," *J. Exp. Biol.* **219**, 1793–1797.

Kunz, T. H., and Fenton, M. B. (2005). *Bat Ecology* (University of Chicago Press, Chicago, IL).

Lee, Y. F., Kuo, Y. M., Chu, W. C., Lin, Y. H., Chang, H. Y., and Chen, W. M. (2012). "Ecomorphology, differentiated habitat use, and nocturnal activities of *Rhinolophus* and *Hipposideros* species in East Asian tropical forests," *Zoology* **115**, 22–29.

Moss, C. F., Bohn, K., Gilkenson, H., and Surlykke, A. (2006). "Active listening for spatial orientation in a complex auditory scene," *PLoS Biol.* **4**, 615–626.

Moss, C. F., and Schnitzler, H.-U. (1995). "Behavioral studies of auditory information processing," in *Hearing by Bats, Springer Handbook of Auditory Research*, edited by R. Fay and A. Popper (Springer Verlag, Berlin), Chap. 5, pp. 87–145.

Moss, C. F., and Surlykke, A. (2001). "Auditory scene analysis by echolocation in bats," *J. Acoust. Soc. Am.* **110**, 2207–2226.

Moss, C. F., and Surlykke, A. (2010). "Probing the natural scene by echolocation in bats," *Front. Behav. Neurosci.* **4**, 1–16.

Neuweiler, G. (1980). "How bats detect flying insects," *Phys. Today* **33**(8), 34–40.

Neuweiler, G. (1990). "Auditory adaptations for prey capture in echolocating bats," *Physiol. Rev.* **70**, 615–641.

Neuweiler, G. (2000). *The Biology of Bats* (Oxford University Press, New York).

Neuweiler, G., Bruns, V., and Schuller, G. (1980). "Ears adapted for the detection of motion, or how echolocating bats have exploited the capacities of the mammalian auditory system," *J. Acoust. Soc. Am.* **68**, 741–753.

Petrites, A. E., Eng, O. S., Mowlds, D. S., Simmons, J. A., and Delong, C. M. (2009). "Interpulse interval modulation by echolocating big brown bats (*Eptesicus fuscus*) in different densities of obstacle clutter," *J. Comp. Physiol. A* **195**, 603–617.

Sändig, S., Schnitzler, H.-U., and Denzinger, A. (2014). "Echolocation behaviour of the big brown bat (*Eptesicus fuscus*) in an obstacle avoidance task of increasing difficulty," *J. Exp. Biol.* **217**, 2876–2884.

Schnitzler, H.-U. (1973). "Control of Doppler shift compensation in the greater horseshoe bat, *Rhinolophus ferrumequinum*," *J. Comp. Physiol.* **82**, 79–92.

Schnitzler, H.-U., and Flieger, E. (1983). "Detection of oscillating target movements by echolocation in the Greater Horseshoe bat," *J. Comp. Physiol.* **153**, 385–391.

Schnitzler, H.-U., and Kalko, E. K. V. (2001). "Echolocation by insect-eating bats," *Bioscience* **51**, 557–569.

Schnitzler, H.-U., Kalko, E. K. V., and Denzinger, A. (2001). "Evolution of echolocation and foraging behavior in bats," in *Echolocation in Bats and Dolphins*, edited by J. A. Thomas, C. F. Moss, and M. Vater (University of Chicago Press, Chicago, IL), pp. 331–338.

Schoeppler, D., Schnitzler, H.-U., and Denzinger, A. (2018). "Precise Doppler shift compensation in the hipposiderid bat, *Hipposideros armiger*," *Sci. Rep.* **8**, 4598.

Simmons, J. A. (1973). "The resolution of target range by echolocating bats," *J. Acoust. Soc. Am.* **54**, 157–173.

Simmons, J. A., Eastman, K. M., Horowitz, S. S., O'Farrell, M. J., and Lee, D. N. (2001). "Versatility of biosonar in the big brown bat, *Eptesicus fuscus*," *Acoust. Res. Lett. Online* **2**, 43–48.

Simmons, J. A., and Stein, R. A. (1980). "Acoustic imaging in bat sonar: Echolocation signals and the evolution of echolocation," *J. Comp. Physiol. A* **135**, 61–84.

Surlykke, A., Ghose, K., and Moss, C. F. (2009). "Acoustic scanning of natural scenes by echolocation in the big brown bat, *Eptesicus fuscus*," *J. Exp. Biol.* **212**, 1011–1020.

Surlykke, A., and Moss, C. F. (2000). "Echolocation behavior of big brown bats, *Eptesicus fuscus*, in the field and the laboratory," *J. Acoust. Soc. Am.* **108**, 2419–2429.

Trappe, M., and Schnitzler, H.-U. (1982). "Doppler-shift compensation in insect-catching horseshoe bats," *Naturwissenschaften* **69**, 193–194.

von der Emde, G., and Menne, D. (1989). "Discrimination of insect wingbeat-frequencies by the bat *Rhinolophus ferrumequinum*," *J. Comp. Physiol. A* **164**, 663–671.

von der Emde, G., and Schnitzler, H.-U. (1986). "Fluttering target detection in Hipposiderid bats," *J. Comp. Physiol. A* **159**, 765–772.

von der Emde, G., and Schnitzler, H.-U. (1990). "Classification of insects by echolocating greater horseshoe bats," *J. Comp. Physiol. A* **167**, 423–430.

Warnecke, M., Lee, W.-J., Krishnan, A., and Moss, C. F. (2016). "Dynamic echo information guides flight in the big brown bat," *Front. Behav. Neurosci.* **10**, 1–11.

Wheeler, A. R., Fulton, K. A., Gaudette, J. E., Simmons, R. A., Matsuo, I., and Simmons, J. A. (2016). "Echolocating big brown bats, *Eptesicus fuscus*, modulate pulse intervals to overcome range ambiguity in cluttered surroundings," *Front. Behav. Neurosci.* **10**, 125.

Wohlgemuth, M. J., Luo, J., and Moss, C. F. (2016). "Three-dimensional auditory localization in the echolocating bat," *Curr. Opin. Neurobiol.* **41**, 78–86.