Active Listening in a Bat Cocktail Party: Adaptive Echolocation and Flight Behaviors of Big Brown Bats, *Eptesicus fuscus*, Foraging in a Cluttered Acoustic Environment

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Abstract

In their natural environment, big brown bats forage for small insects in open spaces, as well as in vegetation and in the presence of acoustic clutter. While searching and hunting for prey, bats experience sonar interference, not only from densely cluttered environments, but also from calls of conspecifics foraging in close proximity. Previous work has shown that when two bats compete for a single prey item in a relatively open environment, one of the bats may go silent for extended periods of time, which can serve to minimize sonar interference between conspecifics. Additionally, pairs of big brown bats have been shown to adjust frequency characteristics of their vocalizations to avoid acoustic interference in echo processing. In this study, we extended previous work by examining how the presence of conspecifics *and* environmental clutter influence the bat's echolocation behavior. By recording multichannel audio and video data of bats engaged in insect capture in open and cluttered spaces, we quantified the bats' vocal and flight behaviors. Big brown bats flew individually and in pairs in an open and cluttered room, and the results of this study shed light on the different strategies that this species employs to negotiate a complex and dynamic environment.

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Introduction

Many mammals rely on their visual systems to orient, navigate and forage. However, some species, like bats, dolphins and whales, have evolved specializations to actively probe their environments with sound: echolocating animals send out sonar broadcasts and listen to returning echoes to perceive objects in their immediate surroundings [Griffin, 1958]. They navigate and forage within a 'bat cocktail party', so to speak, as many animals echolocate in the presence of other bats, each producing calls and listening to echoes, which they must sort from the signals of nearby conspecifics.

Eptesicus fuscus, the big brown bat, uses ultrasonic vocalizations to navigate and forage insects in its environment [Griffin, 1958; Surlykke and Moss, 2000]. This bat's sound emissions are frequency-modulated (FM), downward-sweeping echolocation calls containing several har-

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monics in the range of approximately 25–130 kHz, which last between ~0.5 and 15 ms [Simmons, 1979; Surlykke and Moss, 2000]. Call duration and the time between each emission (pulse interval, PI) shorten in length as the bat searches for targets, approaches and finally captures prey (fig. 1a) [Simmons, 1979]. Calls emitted during prey capture are referred to as the 'terminal buzz' which ends in 'buzz 2', where the big brown bat PI is ~6 ms and call duration is ~0.5 ms [Surlykke and Moss, 2000]. Each broadcast echolocation call contains two prominent harmonics: FM1 sweeps from about 65 to 25 kHz, and FM2 sweeps from 130 to 50 kHz (fig. 1b) [Simmons, 1979; Moss and Schnitzler, 1989; Surlykke et al., 2009; Moss and Surlykke, 2010]. The broadband echolocation signals used by *E. fuscus* support accurate sonar localization in azimuth [Simmons et al., 1983], elevation [Lawrence and Simmons, 1982] and range [Simmons, 1973]. To estimate the distance to a given object, the bat relies on the time delay between the emission of a call and the reception of an echo [Simmons, 1973, 1989].

The horizontal sonar beam emitted by *E. fuscus* is spatially broad and can potentially ensonify not just targets of interest in its path, but also surrounding acoustic clutter, such as trees, branches and leaves, as it spans about ±70° at frequencies contained in FM1, and about ±40° at frequencies in FM2 [Hartley and Suthers, 1989; Simmons et al., 1995; Aytekin et al., 2004].

While navigating in its environment, the echolocating bat not only needs to localize and identify different targets [Simmons and Vernon, 1971; Moss and Schnitzler, 1995], but also to discriminate them from background clutter [Simmons, 1973; Bates et al., 2011], such as trees, branches, conspecifics or water surfaces [Simmons, 1973; Chiu et al., 2009; Surlykke et al., 2009; Greif and Siemers, 2010; Bates et al., 2011]. For the bat, echo analysis of its surrounding scene involves the segregation and integration of echo cascades arriving from objects at different locations [Moss and Surlykke, 2001, 2010]. Auditory scene analysis for an echolocating bat is further complicated by the sonar signals of nearby conspecifics.

When approaching a target, *E. fuscus* steers its beam toward a selected prey item and eventually aims its broadcast at the target with an accuracy of 3° during the final capture phase [Ghose and Moss, 2003]. While the bat closely monitors the target's position, successful foraging also depends on minimizing sonar interference from clutter echoes and calls from other nearby bats. It has been suggested that bats attempt to reduce acoustic interference, i.e. background noise, and calls or echoes from nearby conspecifics by either ceasing to echolocate and

potentially 'eavesdrop' on another bat's calls [Barclay, 1982; Balcombe and Fenton, 1988; Gillam, 2007; Chiu et al., 2008] or changing characteristics of emissions such that calls and echoes can be distinguished from those produced by nearby conspecifics [Chiu et al., 2009].

Chiu et al. [2009] reported that the big brown bat shows adaptive spectral changes in its vocalizations when paired with a conspecific in a competitive prey capture situation. The researchers found that (1) as the spatial position of two bats decreased, the difference in their spectral call features increased, and (2) paired bats with similar baseline spectral call characteristics showed larger frequency adjustments compared to those with distinct baseline call designs.

Adjusting sonar call characteristics in the presence of conspecifics is an adaptive strategy for situations in which the bat needs to avoid interference from competing acoustic signals, and must also accurately search for and capture prey by processing target echo information. By changing spectral parameters of its echolocation signals, the bat continues to receive updates about the environment and its location in relation to a selected target, and at the same time it is able to match emission-echo pairs to distinguish its signals from those of another bat that may be broadcasting its sonar signals in close proximity.

Chiu et al. [2008] reported that pairs of flying big brown bats also exhibit silent behavior when competing for a single prey item. Remarkably, the researchers found that 40% of the time when the inter-bat separation was 1 m or less, one bat would stop echolocating for a minimum of 200 ms. This 'silent behavior' persisted for flight trajectories covering between about 60 cm (200 ms) out to 8 m (2.55 s), and in one bat pair, silent behavior was observed for almost 70% of the analyzed trial time [Chiu et al., 2008]. Bats showed the greatest silent behavior when paired with a conspecific whose signals were naturally similar in spectral characteristics, as measured in baseline recordings, from animals flying alone in an open room. In fact, the more similar the individual bat's echolocation calls were to another bat's signals in baseline recordings, the more likely it was that one bat would go silent when the two were paired [see fig. 4B of Chiu et al., 2008].

The studies by Chiu et al. [2008, 2009] show adaptive sonar behaviors of bats, which serve to minimize jamming avoidance; however, the findings are restricted to situations in which echolocating bats are foraging in open spaces. While big brown bats are known to navigate and forage in open spaces [Simmons et al., 2001], they have
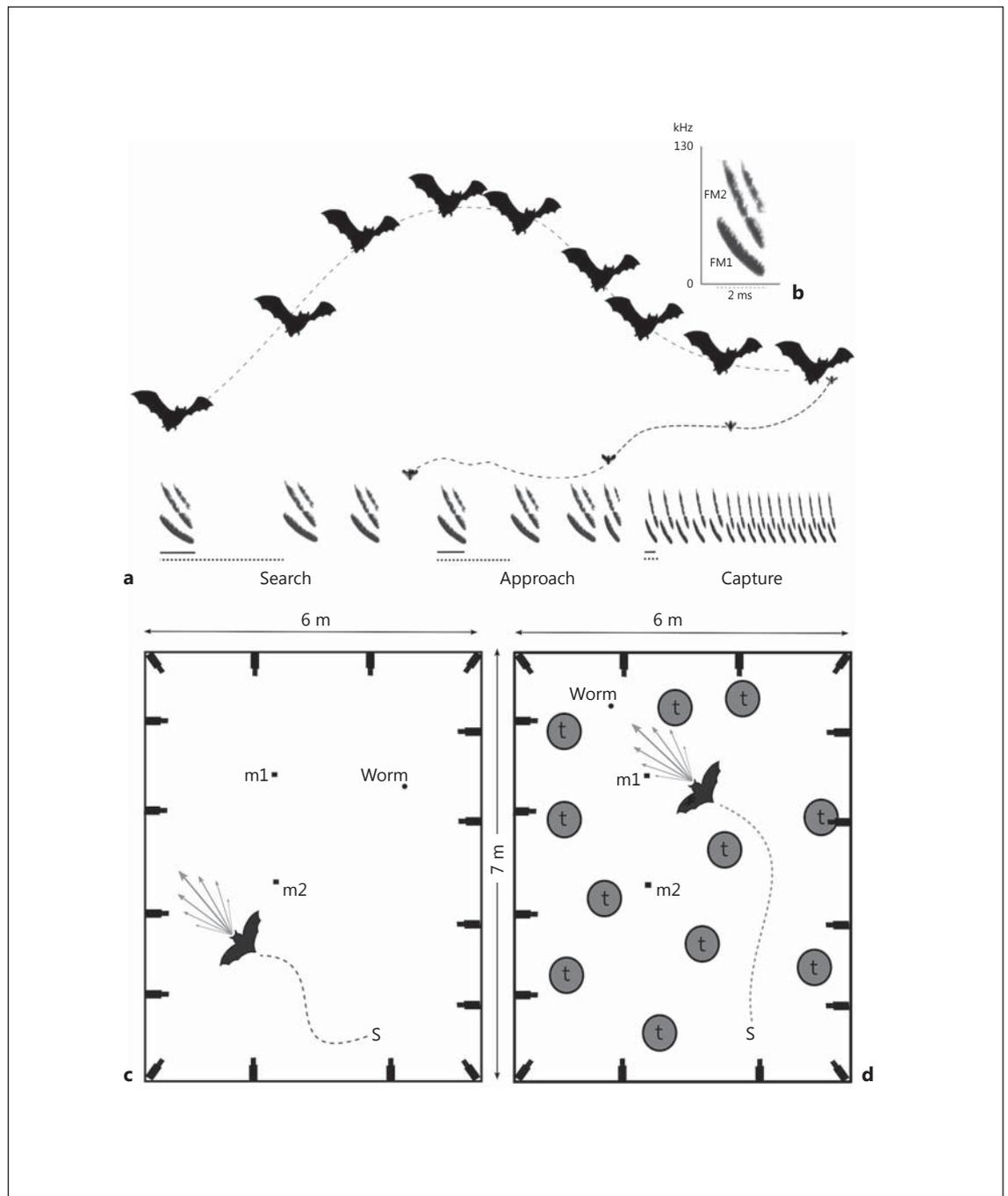
Fig. 1. Vocal adaptations during prey pursuit and flight room arrangement. **a** Schematic of successive positions of an insectivorous echolocating bat searching for approaching and capturing an insect. The foraging process is subdivided into 3 parts (search, approach and capture) during which the call rate increases (see spectral depictions at the bottom), and the duration of each call (solid black line) and PI (black dots) decrease with decreasing distance of the bat to the insect (dashed lines). **b** Typical *E. fuscus* echolocation call. Example of a short, 2-ms-long broadcast sweeping down from ~130 to ~25 kHz in two prominent harmonics, FM1 and FM2. **c** Flight room configuration for open conditions. The walls and ceiling of the flight room ($7 \times 6 \times 2.5$ m, L × W × H) were covered with acoustic foam to minimize reverberation and echoes from walls. Two ultrasonic microphones (m1, m2) were positioned on the floor. Sixteen motion capture cameras were mounted around the perimeter of the room to capture the bat's flight and recreate a 3D flight path (grey dotted line) as the bat searches for the worm (black dot labeled worm). The bat's broadcast is depicted in grey arrows. S = Start of bat flight path. **d** Flight room setup during forest conditions. Black circles represent artificially introduced trees (t). The setup is the same for baseline (depicted here) and experimental conditions. Illustration is not to scale.

also been observed to successfully capture prey in heavily cluttered environments, such as forests [Simmons, 1973; Simmons et al., 2001; Surlykke et al., 2009].

Here, we extended Chiu et al.'s [2008, 2009] work by investigating vocal and flight adaptations in paired big brown bats that competed for a single prey item in a cluttered space. Specifically, we investigated whether two bats competing for a single prey item (1) adjusted their sonar call structure to avoid sonar interference from a conspecific and (2) exhibited silent behavior when they foraged and navigated obstacles in the presence of a conspecific. We hypothesized that bats would adjust parameters of their sonar calls in the cluttered environment to negotiate obstacles when flying alone and in pairs, similar to findings reported by Chiu et al. [2009]. Additionally, we hypothesized that paired bats would exhibit little or no silent behavior in the presence of obstacles, as each bat must continuously monitor its surroundings. The aim of this study was to shed light on behavioral and vocal adaptations of echolocating bats as they transition from open space to cluttered environments, and specifically to address how bats minimize interference from the calls of conspecifics, while also monitoring the location of obstacles.

Materials and Methods

Animals

Six wild-caught female big brown bats *(E. fuscus)* were individually trained to catch a tethered mealworm during flight. Bats' weights were maintained between 14 and 16 g, and they received mealworms *(Tenebrio molitor)* daily to maintain this weight for the period of training and testing. Bats were housed in individual cages under reversed 12-hour light/dark cycle in a colony room kept at 24–28°C at 30–50% relative humidity. The experimental procedures were approved by the University of Maryland Animal Care and Use Committee.

Behavioral Experiments

This study investigated the vocal and flight behavior of bats, alone and in pairs, in an empty room and in an artificial forest. Experiments were run between July and September 2013 in a large flight room (7 × 6 × 2.5 m) equipped with two ultrasound microphones placed on the floor, and a sixteen-camera motion tracking system (fig. 1c, d). Only dim long-wavelength lighting (Reed plastic filters, >650 nm, red filter) was used during experimental and baseline flights to restrict bats from using visual cues [Hope and Bhatnagar, 1979].

Each of the six bats was first individually trained to catch a tethered mealworm in an empty flight room. Once each bat had reached at least 80% success, baseline experiments began. All six bats took part in two baseline [single bat in an empty room (S-ER) and single bat in a forest condition (S-F)] and two experimental conditions [paired bats in an empty room (P-ER) and paired bats in a forest condition (P-F)]. Two experimenters were present for each trial of the experiment. In paired trials, bats were individually marked with reflective markers to identify and distinguish between them in video recordings. After the tethered insect was set up and the recording equipment was ready for a trial, the experimenter released the single bat or two bats simultaneously into the flight room. Bats were free to fly until one of them caught the mealworm. Once the mealworm was caught, a second experimenter end-triggered the synchronized data acquisition system, which saved 8 s of the preceding audio and video recordings. After the audio and video data were saved, a new trial was started.

To replicate the study by Chiu et al. [2008], we ran the first baseline condition with each single bat flying in an empty room (S-ER; fig. 1c). Subsequently, we conducted a second set of baseline trials, flying individual bats in a 'forest', with artificial tree trunks constructed from nets hanging from circular 20-cm-diameter rings (S-F; fig. 1d). After each bat completed the baseline conditions in the open room and in the forest, individual bats competed for a tethered insect in the presence of another bat. All possible combinations of bat pairs were tested (15 pairs) in the empty room (P-ER) and forest (P-F) conditions. The bat release points varied throughout both baseline and experimental conditions. For the analysis, we used all trials available with good quality audio and video recordings (S-ER: n = 75, S-F: n = 88). Data for paired condition analysis were also selected according to the quality of audio recordings and reliable 3D flight path reconstruction (P-ER: n = 121, P-F: n = 110). We analyzed data from 13 of 15 bat pairs, as the signal:noise ratio of the audio data for two bat pairs was not high enough to extract echolocation call parameters.

Data Recording

For each trial, we recorded audio and video data. Audio data were recorded using two ultrasonic microphones (UltraSound Advice, London, UK) filtered (10–100 kHz) and digitized at a sample rate of 250 kHz (National Instruments). Flight data were recorded using a sixteen-camera infrared motion tracking system (Vicon Motion Systems Ltd., Oxford, UK), which was mounted along the perimeter of the room (fig. 1c, d) and tracked infrared reflective markers attached to each bat. The Vicon motion tracking system also acquired data on the position of the floor microphones, the tethered mealworm and the obstacles mimicking the artificial forest in the flight room. Synchronized Vicon cameras ran at 300 frames/s. Data from the tracking system were used to reconstruct 3D bat flight paths.

Audio and video data were analyzed off-line using custom-written MATLAB programs (MathWorks, Natick, Mass., USA).

Data Analysis

For baseline conditions (S-ER and S-F), data analysis required tracking the bat's position in space at points sampled every 3.3 ms and analyzing the bat's corresponding acoustic emissions. A custom-written MATLAB program was used to track the bat's flight position and manually work through the audio recording of each trial and mark a single bat's start and end frequency, as well as start and end time of each call emitted in a given trial. These markings allowed us to later analyze six signal parameters of interest: start and end frequency, bandwidth, sweep rate, PI and duration. We analyzed data starting 3 s before the final buzz in each trial, and only used data points during confirmed bat flight. Buzz calls were excluded from analysis.

Central to the analysis of this data set was assigning each call to the vocalizing bat in both paired conditions (P-ER and P-F). After reconstruction of each bat's flight path, these position data were loaded into a custom-written MATLAB program to combine it with the recorded audio signals from the two floor microphones. Loading the position data into the audio analysis program allowed us to use the time difference of arrival of the signals at each of the microphones and assign calls to one bat or another by referencing to their marked location in the room at the time of call emission. For a more detailed description of this process refer to Chiu et al. [2009]. Only time segments with reliable audio recording and 3D flight path reconstruction were used for analyses, and, as noted above, buzz calls were excluded.

All echolocation call measurements (start and end frequency, bandwidth, sweep rate, PI and duration) were taken from the FM1 component for all bats and all conditions. After preprocessing, raw data were analyzed by discriminant function analysis (DFA) using the measured raw call variables to assess the similarity of call designs between bats [Chiu et al., 2008]. Differences in raw call features as the bat transitioned from single to paired, and empty to forest conditions were assessed by running full factorial (2-level) ANOVAs with bat as a random factor in JMP. A total of 546 calls were identified as outliers (>3 SDs, 1.79%) and excluded from the analysis. *Adjustments* of call parameters in the single conditions were calculated by taking the difference between each successive call feature for the duration of the trial for each individual bat when it flew alone in S-ER and S-F. Call adjustments for paired conditions were calculated the same way for each individual bat. This was possible because video position data, combined with measurement of signal onset times at each microphone, allowed us to correctly assign each call to the vocalizing bat. Differences between *call adjustments* at the level of single/paired and empty room/forest conditions were calculated using full factorial ANOVAs with bat as a random factor in JMP; 369 call adjustment measurements were identified as outliers (>3 SDs, 1.23%) and excluded from the analysis.

Results

The present study aimed to identify behavioral and vocal changes in big brown bats as they foraged alone in an open space or a cluttered environment, as well as paired individuals in the same environments, where the two bats competed for a single prey item.

Our results show that bats adapt the temporal and spectral structure of their vocalizations, as well as their flight behavior, when they transition from single to paired flight, and from empty to cluttered environments. Further, we report that bats make a larger adjustment of call parameters when navigating cluttered environments compared to open spaces.

The mean call features (black square) taken across individual bats (grey circles) and all four conditions (x-axis) are plotted in figure 2a. After Bonferroni correction (p = 0.0083), all variables show a main effect of condition (sin-

gle/paired) and environment (forest/empty). Specifically, as bats transition from flying alone to navigating in pairs, bat calls show an increase in start frequency ($F_{1, 593}$ = 178.24, p < 0.0001, single: 64.79 kHz, SE = 0.96, paired: 69.82 kHz, SE = 0.95), end frequency ($F_{1, 593}$ = 17.49, p < 0.0001, single: M = 21.80 kHz, SE = 0.45, paired: M = 22.41 kHz, SE = 0.40), bandwidth ($F_{1, 593}$ = 151.37, p < 0.0001, single: M = 42.98 kHz, SE = 0.759, paired: M = 47.42 kHz, SE = 0.78), PI ($F_{1, 593}$ = 26.07, p < 0.0001, single: M = 61.22 ms, SE = 2.21, paired: M = 66.98 ms, SE = 2.13) and duration ($F_{1, 593}$ = 159.3, p < 0.0001, single: M = 3.26 ms, SE = 0.09, paired: M = 3.75 ms, SE = 0.11), as well as a decrease in sweep rate ($F_{1, 593}$ = 23.58, p < 0.0001, single: M = 14.13 kHz/ms, SE = 0.31, paired M = 13.41 kHz/ms, SE = 0.3). This suggests that the presence of another echolocating bat evokes dynamic changes in sonar vocalizations to navigate the environment successfully. There was also a main effect of environment in all measured call parameters showing changes in spectral parameters as bats transitioned from empty room to forest conditions in start frequency ($F_{1, 593}$ = 137.35, p < 0.0001, empty: M = 69.54 kHz, SE = 1.00, forest: M = 65.06 kHz, SE = 1.04), end frequency ($F_{1, 593}$ = 121.94, p < 0.0001, empty: M = 22.92 kHz, SE = 0.36, forest: M = 21.29 kHz, SE = 0.37), bandwidth ($F_{1, 593}$ = 61.88, p < 0.0001, empty: M = 46.62 kHz, SE = 0.92, forest: M = 43.78 kHz, SE = 0.79) and sweep rate ($F_{1, 593}$ = 113.7, p < 0.0001, empty: M = 12.99 kHz/ms, SE = 0.23, forest: M = 14.55 kHz/ms, SE = 0.23). Temporal call parameters show significant changes in PI and duration when bats flew in the forest compared to the open room, as indicated by a lower PI during flights in the forest ($F_{1, 593}$ = 92.73, p < 0.0001, empty: M = 69.63 ms, SE = 1.83, forest: 58.58 ms, SE = 1.42) and shorter call durations in the forest ($F_{1, 593}$ = 233.33, p < 0.0001, empty: M = 3.8 ms, SE = 0.09, forest: M = 3.21 ms, SE = 0.08). Analyses revealed no significant interactions of condition by environment.

The *adjustments* in call parameters that bats made from call to call as bats foraged in each of the conditions are presented in figure 2b, plotting the average adjustments made by a given bat for each call parameter (grey circles) along with the mean adjustments across conditions (black square). After Bonferroni correction (p = 0.0083), the call adjustments that bats made when tested in single flight compared to paired flight show a main effect of condition (single/paired) only in start frequency ($F_{1, 593}$ = 30.92, p < 0.0001, single: M = 5.32 kHz, SE = 0.08, paired: M = 4.91 kHz, SE = 0.04) and bandwidth ($F_{1, 593}$ = 31.13, p < 0.0001, single: M = 5.51 kHz, SE = 0.08, paired: M = 4.92 kHz, SE = 0.04). There
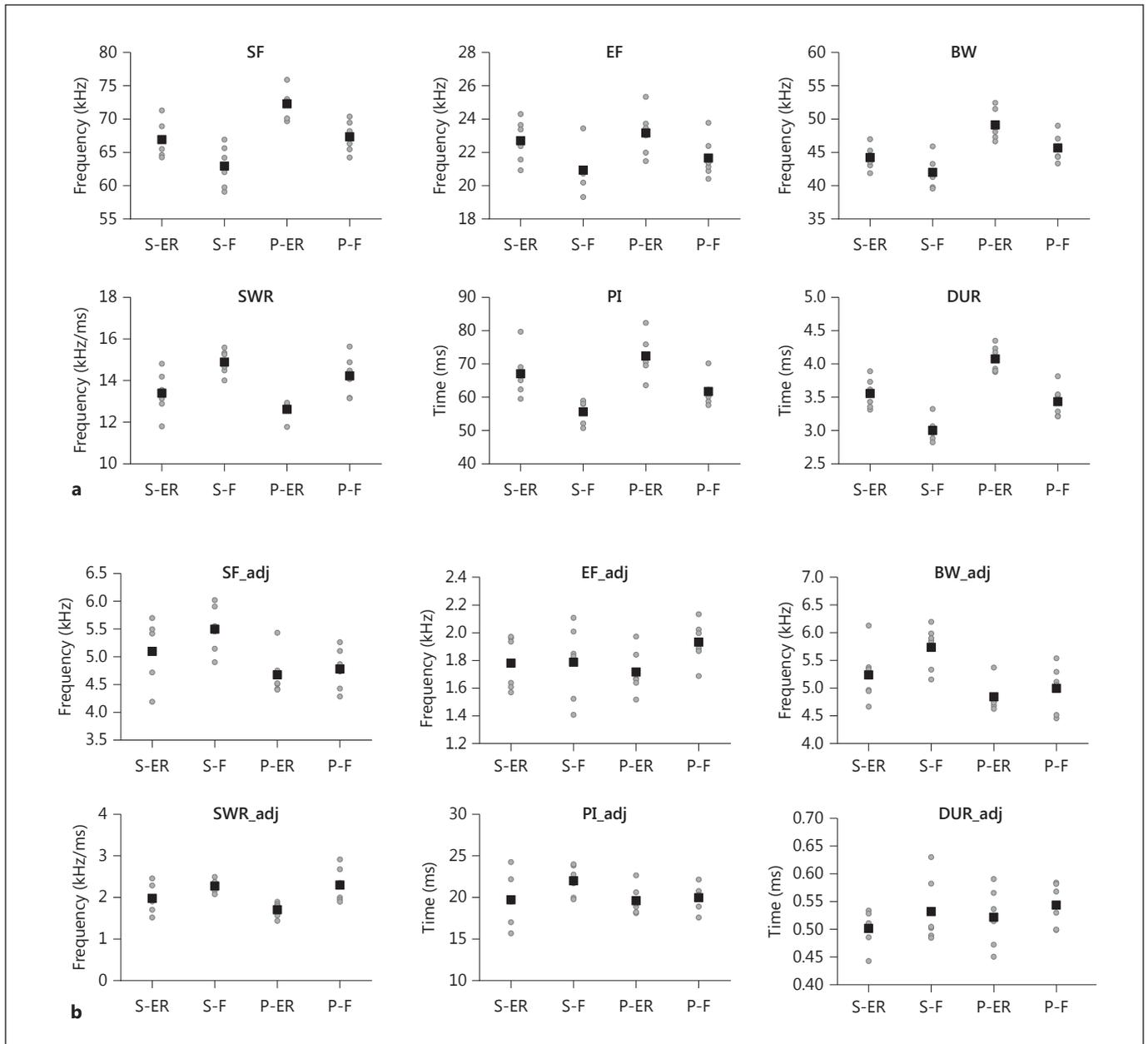
Fig. 2. Changes in call features and call adjustments across conditions. **a** Averages (black squares) across bats (grey circles) of the respective raw call feature variable in each condition (S-ER, S-F, P-ER and P-F). **b** Mean value of call-to-call *adjustment* (_adj) across bats (grey circles) for each condition (S-ER, S-F, P-ER and P-F). Black square plots mean value across bats per condition to illustrate main effects and interactions between main effects (see Results). Signal parameters of interest were start (SF) and end frequency (EF), bandwidth (BW), sweep rate (SWR), PI and duration (DUR).

was a main effect of environment, such that bats consistently made larger adjustments from one call to the next when flying in the forest, compared to the open room (start frequency: $F_{1, 593} = 8.34$, p < 0.0001, empty: M = 4.76 kHz, SE = 0.06, forest: M = 4.99 kHz, SE = 0.06;

end frequency: $F_{1, 593} = 8.55$, p = 0.0036, empty: M = 1.72 kHz, SE = 0.02, forest: M = 1.89 kHz, SE = 0.02; bandwidth: $F_{1, 593} = 14.8$, p = 0.0001, empty: M = 4.91 kHz, SE = 0.05, forest: M = 5.21 kHz, SE = 0.06; sweep rate: $F_{1, 593} = 72.82$, p < 0.0001, empty: M = 1.77 kHz/ms,

SE = 0.03, forest: M = 2.29 kHz/ms, SE = 0.03; duration: $F_{1, 593}$ = 9.0, p = 0.0028, empty: M = 0.51 ms, SE = 0.006, forest: M = 0.54 ms, SE = 0.005, and PI: $F_{1, 593}$ = 7.19, p = 0.0075, empty: M = 19.7 ms, SE = 0.34, forest: M = 20.6 ms, SE = 0.29). These results show that bats make greater adjustments in their call structure while navigating cluttered environments, compared with open spaces. Further, sweep rate is the only variable showing a significant interaction of condition by environment ($F_{1, 593}$ = 7.72, p = 0.0056). The cluttered environment revealed greater adjustments in the bat's sweep rate when it flew by itself or in a pair.

A second mechanism to avoid interference from the acoustic signals of conspecifics, reported by Chiu et al., is silent behavior in paired bats [Chiu et al., 2008]. We hypothesized that paired bats would exhibit silent behavior in the paired empty room condition (P-ER), and they would exhibit reduced, if any, silent behavior in all other conditions (S-ER, S-F and P-F). Our data show an absence of silent behavior in single bat conditions, as well as while paired bats fly in a cluttered environment (S-ER: M = 0.44%, SD = 0.69, S-F: M = 0.46%, SD = 0.46, P-F: M = 0.27%, SD = 0.72). However, we also did not find evidence for silent behavior in the empty room, paired bat condition (P-ER: M = 0.73%, SD = 0.99).

Given the report by Chiu et al. [2008] of silent behavior of paired bats in an open room (on average, 40% of analyzed trial time when inter-bat separations were ≤1 m), the absence of silent behavior in this study motivated a closer investigation of the flight behavior of paired bats in the empty and cluttered environments. Chiu et al. [2008] reported that silent behavior was most prevalent when the inter-bat distance was ≤1 m, and, additionally, when the two bats were in a following-flight configuration [Chiu et al., 2008]. Two-tailed paired-sample t tests revealed that bats in the present study did not exhibit significantly different flight behaviors in open or cluttered environments, with the exception of converging flight (fig. 3). Figure 3 illustrates flight configuration data and, importantly, shows that the bats used in this study spent only 8–10% of their time flying at distances of ≤1 m. Even less time was spent at 1 m or closer distance while in following flight.

Although paired bats spent similar amounts of time in diverging flight (P-ER: M = 41.14%, SD = 10.82, P-F: M = 36.59%, SD = 4.81) and following flight (P-ER: M = 43.79%, SD = 13.61, P-F: M = 38.88%, SD = 8.66) in P-ER and P-F conditions, the amount of time spent converging in the cluttered environment (M = 24.72%, SD = 6.48) was significantly higher than in the empty room [M = 15.06%, SD = 6.24, t(12) = −4.005, p = 0.002]. Further,
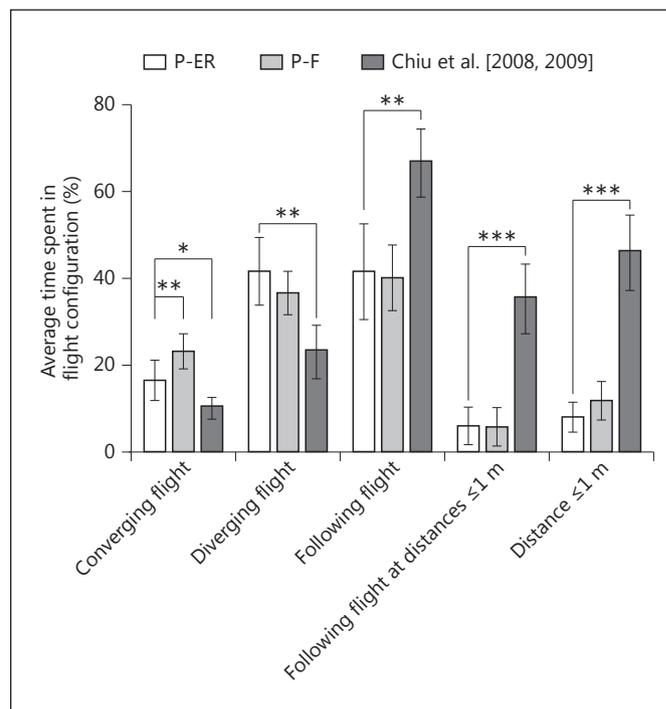

Fig. 3. Flight configurations during P-ER and P-F conditions, and data derived from the studies by Chiu et al. [2008, 2009]. Bar graphs plot the percent of time (y-axis) all bats spent in a different flight configuration (x-axis) of P-ER (white) and P-F (grey) conditions, as well as data by Chiu et al. [2008, 2009] (dark grey). Bats spent little time at distances ≤1 m or in following flight configurations. Flight configurations differ significantly between P-ER and data reported by Chiu et al. [2008, 2009]. Bats in the current study spent less time following one another or at close proximity, an indication of increased call adjustment and silent behavior.

there is no significant difference of inter-bat distance in the two environments, and no significant difference in the amount of time two bats spent following each other at distances of ≤1 m.

Discussion

Echolocating big brown bats forage in open spaces, as well as in heavily cluttered vegetation [Simmons, 1973; Simmons et al., 2001; Surlykke et al., 2009]. The presence of physical obstacles poses challenges to the echolocating bat, as it has to monitor its surroundings continuously to avoid collisions. Recent work has shown that single bats adjust temporal characteristics of their calls in cluttered environments, compared to open spaces [Falk et al., 2014]. Falk et al. [2014] report a decrease in call duration

Table 1. Mean flight velocity and sonar call rate in baseline trials

Bat	Flight velocity, m/s				Mean call rate per trial			
	S-ER	S-F	S-ER	S-F	S-ER	S-F	S-ER	S-F
G82	2.72	2.77	3.34	2.96	27.42	41.51	29.01	26.61
O33	2.81	2.62	3.12	2.91	26.44	38.59	27.32	30.93
O92	2.50	2.34	2.88	2.69	30.51	41.88	24.36	27.46
R14	2.93	2.54	3.30	2.92	27.42	42.39	26.52	29.64
R20	2.62	2.53	2.94	2.70	28.79	48.70	31.02	27.43
R24	3.05	2.63	2.52	2.38	24.29	42.94	33.18	28.79
Mean ± SD	2.77±0.20	2.57±0.14	3.02±0.31	2.76±0.22	27.48±2.11	42.67±3.32	28.57±3.19	28.48±1.62

Significance test	Velocity			Call rate		
	DFDen	F-ratio	p	DFDen	F-ratio	p
S/P	15	7.46	0.016	15	34.16	<0.0001
ER/F	15	8.39	0.011	15	45.34	<0.0001
Interaction	15	0.13	0.728	15	46.48	<0.0001

Summary of baseline flight velocity and call rate. Each cell represents the average flight velocity per bat or call rate of that bat across all trials analyzed in S-ER, S-F, P-ER and P-F, respectively. Means ± SD are added, along with significance between each condition (2-way ANOVA; bat added as random factor; JMP). DFDen = Denominator degrees of freedom.

and flight speed, along with an increase in call rate, when bats fly in an artificial forest compared with an empty room. Our results are consistent with these findings (table 1) and further indicate that bats additionally modulate all parameters that we measure in this study when they fly in a cluttered environment (fig. 2).

Foraging in their natural habitat, however, not only poses the challenge of negotiating physical objects, but also that of acoustic interference from nearby foraging conspecifics. Previously, it was shown that echolocating bats make use of two different strategies to overcome the challenge of acoustic interference from other bat signals.

Chiu et al. [2009] report that one strategy to avoid acoustic interference is dynamic vocal adjustment in the spectral features of calls, namely start and end frequency, and bandwidth of the FM sweep. Furthermore, call adjustments were largest when bats flew at inter-bat separations of ≤1 m.

In the present study, we found reliable vocal changes in paired bats, similar to those reported by Chiu et al. [2009]. Specifically, our data show that when bats adapt from flying alone to being paired with a conspecific, they show significant changes in call features. In the present study, there were main effects of condition (single/paired) and environment (empty/forest) on bat echolocation call design, suggesting that adaptive call behavior is influenced by the presence of another conspecific *and* the surrounding environment. In other words, both the potential acoustic interference of another bat and physical obstacles drove changes in bat call design.

Given changes in bat call design across conditions, we considered how these adjustments differ between single or paired conditions, and in empty or cluttered environments. Our results indicate that bats make larger adjustments of all call parameters when they are navigating in a cluttered environment, as compared to an open space. Further, bats make significant adjustments in start frequency and bandwidth when they fly with a conspecific compared with flying alone. Collectively, these findings suggest that adjustments in call parameters from one vocalization to the next allow the bat to better distinguish its own call and echo from the other bat's signal.

Chiu et al. [2008, 2009] reported that call adjustments and silent behavior were most prevalent at inter-bat distances of ≤ 1 m. Surprisingly, in the present study, silent behavior was not observed in the empty room, paired bat condition (P-ER), which prompted an investigation of flight behavior in both paired conditions (P-ER and P-F). Comparing the amount of time that bats in the present study spent separated by ≤ 1 m (M = 9.1%, SD = 3.9) with that reported by Chiu et al. [2008, 2009; M = 45.89%, SD = 8.6], it becomes evident that the bats in this study
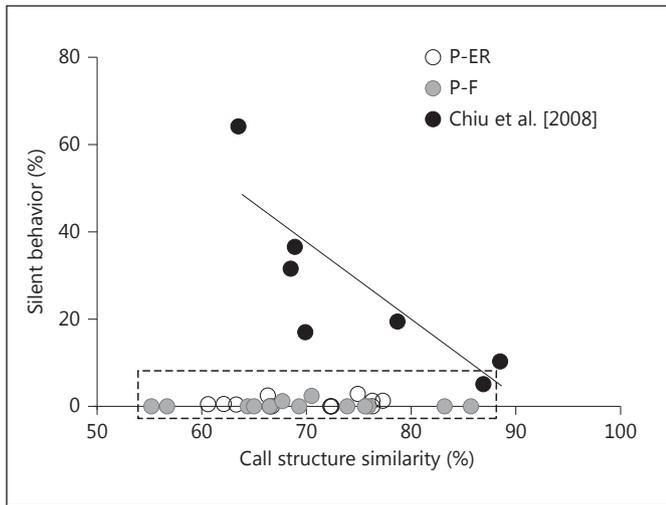
Fig. 4. Comparison of silent behavior of current data and those by Chiu et al. [2008]. Scatter plot shows silent behavior by similarity of baseline call structure. Data illustrate distinct differences between distributions of the silent behavior in the current P-ER (white circles) and P-F (grey circles) conditions, compared to silent behavior reported by Chiu et al. [2008] (black circles). Dotted rectangle includes all female-female bat pairs run in both studies. Pearson's r = –0.83, y = –2.7x + 1.54, R^2 = 0.69.

Table 2. Silent behavior in male and female bats [Chiu et al., 2008]

Bat pair		Gender		Silence		Total silence/ pair
bat 1	bat 2	bat 1	bat 2	bat 1	bat 2	
g32	*g48*	f	f	29.77%	70.23%	5.09%
p17	hp54	f	m	17.15%	82.85%	17.01%
p17	y31	f	m	3.42%	96.58%	19.47%
g48	g78	f	m	26.91%	70.93%	31.56%
hp54	y31	m	m	60.24%	39.76%	10.31%
g67	g78	m	m	17.55%	69.37%	64.17%
g41	g78	m	m	18.35%	79.49%	36.57%

Summary of silent behavior separated by gender for each bat used in the study by Chiu et al. [2008]. Each cell represents the percent silent behavior that a given bat exhibited throughout the length of all analyzed trial time. The last column shows the total silent behavior for a given bat pair. Whenever female bats were paired with male bats, males produced more silent behavior than females. The female-female pair showed least silent behavior.

spent significantly less time in close proximity [t(20) = –14.038, p = 0.0001; fig. 3, dark grey bars; Chiu et al., 2008] and also significantly less time engaged in following flight at this separation [t(18) = 20.539, p = 0.0001; fig. 3]. In fact, figure 3 shows that following flight oc-

curred significantly more often in their data set compared to the current study [t(2) = –3.187, p = 0.005], and also that bats in the current study showed significantly more converging [t(18.588) = 2.140, p = 0.046] and diverging flight [t(20) = 3.585, p = 0.002] than reported by Chiu et al. [2008, 2009]. It is noteworthy that Chiu et al. [2008, 2009] did not report increased call adjustments when bats engaged in converging and diverging flight paths. In contrast to the bats in their study, which spent more than half of their time engaged in following flight at close inter-bat separations (≤1 m), bats in the present study were engaged predominantly in converging and diverging flights, an indication of uncoordinated flight patterns between the individuals in the pair. The differing flight configurations of bats in the two studies could possibly account for reported differences in vocal behavior.

A second strategy employed by big brown bats to avoid acoustic interference with conspecifics is silent behavior [Chiu et al., 2008]. Bat silent behavior is correlated with baseline call design similarity of two bats, such that two bats with very similar baseline call design showed more silent behavior than paired bats with less similar baseline call design [Chiu et al., 2008]. In the present study, we hypothesized that bats would show silent behavior in the empty room condition when flying in pairs, similar to reports by Chiu et al. [2008]. To our surprise, however, the bats in the present study exhibited very little silent behavior in the empty room (P-ER) or the forest (P-F) condition. In fact, the small amount of silent behavior that was observed in the current study differs significantly from that reported by Chiu et al. [2008]: t(6.017) = –3.7, p = 0.01. Figure 4 plots the percent silent behavior as a function of baseline call similarity between paired bats (correctly classified by DFA) from Chiu et al. [2008]. Along with the data from Chiu et al. [2008] (black circles), we plotted the data points from the current study for silent behavior in paired empty (white circles) and forest (grey circles) conditions, with their respective correctly classified baseline DFA scores. This plot reveals that the data sets collected in the two studies are distinctly different.

The surprising finding that paired bats in the open room showed little silent behavior in this study led us to investigate the factors that could possibly contribute to differences from the findings reported by Chiu et al. [2008]. Just as the distinct patterns in flight behavior observed in the two studies could account for differences in call adjustments, different flight configurations could possibly explain the absence of silence.

In addition to differences in flight configurations of paired bats in the two studies, we note differences in the gender composition of subjects in the present study, compared to those in Chiu et al. [2008, 2009]. Specifically, animals in the present study were exclusively female, creating 15 female-female pairs, 13 of which were used for analysis. In contrast, Chiu et al. [2008] used five male and three female bats, and tested three male-male, three male-female and only one female-female pair. Interestingly, the one female-female pair used in the study by Chiu et al. [2008] showed only about 5% silent behavior of analyzed trial time in the open room, compared to up to 70% in one male-male pair. For better visualization, we have enclosed all female-female pairs from the current study and the one female-female pair of their data in a dashed black rectangle in figure 4. While all female bats in the current study employed circular flight behavior patterns, Chiu et al. [2008] report a predominance of following flight behavior between paired bats. In fact, their single female-female pair showed the least total amount of following flight behavior (55.82%) and even less following flight behavior at distances of ≤1 m (27.11%), compared to all other pairs tested in their study averaging 68.40% (following) and 36.72% (following ≤1 m), respectively. Additionally, we analyzed data from previous studies by Chiu et al. [2008, 2009] and confirm that in male-female pairs, the male bat consistently exhibited more silent behavior than the female bat (table 2).

Taken together, the data reported here suggest a possible gender difference in the strategies used by bats to avoid acoustic interference while foraging with conspecifics. Results from Chiu et al. [2008, 2009] indicate that male bats engage in chasing-like behavior, following one another at close inter-bat distances in flight, ceasing echolocation and thereby possibly eavesdropping on the other bat. By contrast, the data from the present study, along with a subset of their data, show that female bats, when paired together, engage in more circular flight paths at greater inter-bat distances and avoid being close to one another, thereby reducing acoustic interference from the conspecific, and hence show less silent behavior, as well as smaller adjustments in their echolocation call design. It is important to note, however, that these studies were conducted at different time points and include bats from different colonies.

In summary, we report strategies that bats employ to avoid acoustic interference, not just from nearby echolocating conspecifics, but also while navigating in cluttered spaces. The intriguing differences between the data collected from female bats in this study and the male bats in the studies by Chiu et al. [2008, 2009] raise compelling questions about gender and social hierarchy influences on flight and echolocation behavior in the big brown bat. These questions provide fertile ground for future research.

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