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Behavioral and Ecological Influences on the Echolocation of Brazilian Free-Tailed Bats, *Tadarida brasiliensis*

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To the Graduate Council:

I am submitting herewith a dissertation written by Erin H. Gillam entitled "Behavioral and Ecological Influences on the Echolocation of Brazilian Free-Tailed Bats, *Tadarida brasiliensis*." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Ecology and Evolutionary Biology.

Gary Frederick McCracken, Major Professor

We have read this dissertation and recommend its acceptance:

Thomas Hallam, Christine R.B. Boake, Gordon Burghardt, Jim Hall

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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BEHAVIORAL AND ECOLOGICAL INFLUENCES ON THE ECHOLOCATION OF BRAZILIAN FREE-TAILED BATS, *TADARIDA BRASILIENSIS*

A Dissertation Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Erin H. Gillam
May 2007

DEDICATION

This dissertation is dedicated to my parents, Gail and Andrew Gillam.

Though I know I'll never lose affection
For people and things that went before
I know I'll often stop and think about them
In my life I love you more

- John Lennon -

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ABSTRACT

This dissertation investigates variability in the echolocation calls of Brazilian free-tailed bats, *Tadarida brasiliensis* (Chiroptera: Molossidae), and explores how bats adjust echolocation call structure in response to different behavioral and ecological conditions. Substantial geographic variation exists in the echolocation call structure of *T. brasiliensis* throughout the species range in the US, but this variation does not correlate with geographic or climatic patterns. Most variation in call structure is due to differences between and within the calls of individuals. When exposed to broadcasts of high frequency insect sounds, free-flying bats consistently responded by shifting call frequencies away from the broadcast frequencies. This response suggests that bats are sensitive to local acoustic interference that decreases the efficiency of echo reception. In another investigation of reactions to interfering sounds, bats responded to echolocation playbacks by rapidly shifting their call frequencies away from playback frequencies, indicating that a jamming avoidance response was occurring. Bats more frequently shifted their calls upwards to higher frequencies, which may be due to maximal jamming power of the lower frequency portion of echolocation calls. Flexibility in their echolocation calls also was evident in calls produced by *T. brasiliensis* while emerging from roosts in a tight column formation. Bats emitted two distinct call types during emergence, sweep and hook calls, which were substantially different from foraging calls. Call structure differed between roosts, which may be related to differences in the spacing of bats within emergence columns. In a final experiment, it was found that bat activity was substantially greater in response to echolocation playbacks that contained feeding buzz calls compared to broadcasts that did not contain these signals, indicating that bats eavesdrop on the echolocation calls of conspecifics. Overall, this study documents the highly flexible nature of echolocation in Brazilian free-tailed bats and demonstrates that bats respond acoustically to behavioral and ecological influences.

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CHAPTER I

Introduction

Echolocation is a form of biological sonar in which calls emitted by an animal are reflected off objects in the environment and return to the sender as echoes. By comparing the echo to the original signal, the calling animal obtains information about the surrounding environment (Griffin 1958). Echolocation involving simple signals, such as tongue clicks, has been reported in some birds (Konishi and Knudsen 1979), shrews (Tomasi 1979), and megachiropteran bats (Roberts 1975). Substantially more complex forms of echolocation involving the use of highly structured signals and sophisticated neural processing to allow extraction of detailed information about target range and identity occur in cetaceans and microchiropteran bats (Thomas et al. 2004).

Although all microchiropteran bats use some form of echolocation, considerable differences exist between species in the temporal and spectral structure of calls. Bats exhibit substantial interspecific variability in fundamental frequency, call shape, energy distribution (Fenton and Bell 1981), use of harmonics (Simmons and Stein 1980), duty cycle (Jones and Rayner 1989), and tolerance of pulse-echo overlap (Fenton et al. 1995). Intraspecific variability in echolocation of bats also has been documented. In some species, echolocation structure is associated with characteristics of an individual, such as age, sex, or body size (Jones et al. 1992, Barclay et al. 1999). Bats have also been shown to adjust their call structure in reference to conditions at a foraging site, including the proximity of insect prey (Griffin 1958), the amount of vegetative clutter (Kalko and Schnitzler 1993, Obrist 1995), and the presence of nearby conspecifics (Habersetzer 1981, Ulanovsky et al. 2004). Because many bats use echolocation for both orientation and prey capture (Griffin 1958), such adaptability of call structure is critical to maximizing the efficiency of echolocation under different conditions.

The research presented in this dissertation expands our understanding of intraspecific variation in the echolocation of bats, by investigating behavioral and ecological influences on call structure that have not been thoroughly explored in the past, if at all. This dissertation includes six chapters; the first of which is this introduction and overview. Chapters 2 – 5 are manuscripts that are in press, submitted and under review, or in preparation, that describe my studies investigating variation in the echolocation calls of Brazilian free-tailed bats, *Tadarida brasiliensis*, in different geographical, behavioral and ecological contexts.

Chapter 2 (in press, Animal Behaviour) describes geographic variation in the echolocation calls of Brazilian free-tailed bats throughout their range and in a variety of different habitats in the southern United States. Substantial differences in call structure are documented, but these differences do not correspond to geographic or climatic patterns, and are attributed to substantial flexibility in individual call structure. In chapter 2, I also assess the effects of local acoustic environments on variation in echolocation calls by examining the response of bats to broadcasts of high frequency insect sounds.

Chapter 3 (in press, Proceedings of the Royal Society of London B: Biological Sciences) provides the first experimental evidence that bats shift call frequencies to avoid spectral overlap with the calls of nearby conspecifics, a phenomenon described in the literature as a jamming avoidance response (JAR). Previous research has suggested that bats exhibit jamming avoidance, but these studies relied on behavioral correlations rather than well-controlled experimental playbacks. Bats consistently exhibited a rapid jamming avoidance response to playback stimuli, and showed an asymmetrical response toward shifting their calls upwards to higher frequencies, which I hypothesize is due to the greater jamming power of lower frequency call components

Chapter 4 (for submission to Acta Chiropterologica) describes the calls emitted by Brazilian free-tailed bats during evening emergences from major roosts in South Central Texas. Bats exiting roosts experience a highly cluttered acoustic environment that presents very different challenges for orientation than foraging for insects in open airspace. In this chapter, I describe two very different, high frequency call types that are produced by emerging bats. I examine whether call structure changes in relation to the number of emerging bats, as estimated from thermal imaging video recordings, and speculate on the possible functions of these calls.

Chapter 5 (in review, Canadian Journal of Zoology) demonstrates that free-flying bats eavesdrop on the echolocation calls of conspecifics and are attracted to playbacks of ‘feeding buzz’ calls that indicate successful foraging. While eavesdropping on echolocation calls has been reported in the past, this research expands on previous studies by comparing responses to realistic echolocation playbacks, which allows me to demonstrate that the feeding buzz component of call sequences attracts bats to apparent “hotspots” of successful foraging.

Chapter 6 concludes the dissertation with a summary of the important findings from chapters 2 - 5, and suggestions for potential areas of productive research in the future

CHAPTER II

Variability In the Echolocation of Tadarida brasiliensis: Effects of Geography and Local Acoustic Environment

This chapter is a version of a paper by the same name currently in press in the journal *Animal Behavior* by Erin H Gillam and Gary F McCracken:

Gillam, E.H. and McCracken, G.F. Variability in the echolocation of *Tadarida brasiliensis*: effects of geography and local acoustic environment. *Animal Behaviour* in press.

My consistent use of “we” throughout this chapter is in reference to my co-author, Gary McCracken and myself. I was the primary contributor to this work, which involved the following tasks: (1) development of project design and all data collection, (2) measurement of acoustic signals and statistical analysis of the dataset, (3) gathering and interpretation of the relevant literature, and (4) all of the writing.

ABSTRACT

We examined variation in the echolocation calls of Brazilian free-tailed bats, *Tadarida brasiliensis*, on a broad geographic scale and in response to local environmental variables. Significant differences in call structure were observed among populations throughout the species range in the United States, but this variation was not associated with geographic distance or local weather conditions. Observed variability between sites was due primarily to differences between bats, and the flexibility in call structure that can be achieved by individuals. During this study, we observed that bats recorded in the presence of high frequency sounds from chorusing insects used higher call frequencies than bats recorded in silence. This led us to test the hypothesis that bats adjust echolocation call structure in response to local ambient noise. We broadcast experimentally manipulated ultrasonic insect sounds to free-flying Brazilian free-tailed bats and found a positive correlation between the frequency of the insect sound stimulus and the call frequencies used by bats. These results document that bats adjust echolocation call structure to avoid acoustic interference from ambient noise in their local environment.

INTRODUCTION

Variation in the structure of intraspecific communication signals can occur between populations or among individuals within populations. In geographically separated populations, signals may differ as a result of adaptation to local environmental conditions (Wilczynski & Ryan 1999; Slabbekoorn & Smith 2002), learning, or genetic differentiation (Catchpole & Slater 1995). Within populations, plasticity in signal structure allows individuals to respond to varying behavioral and ecological factors, such as changes in predation risk (Endler 1987) or local habitat characteristics (Brumm 2004).

Much of the research on intraspecific variation in communication has focused on signals used in mate choice and species recognition, with fewer studies investigating variability in echolocation signals. Because echolocation involves a single individual that is both signaler and receiver, the incidence and patterns of intraspecific variation may differ from communication signals exchanged between two or more individuals. For example, differences in female preference may drive the maintenance of population dialects in bird song (Baker & Cunningham 1985), but are unlikely to explain patterns of geographic variation in echolocation. In bats, echolocation signals are adapted for foraging, and short-term flexibility in call structure may be especially critical for detecting prey within varying local environments (Arlettaz et al. 2001).

In bats, plasticity in echolocation call structure has been observed in response to a variety of conditions, including proximity to prey (Griffin 1958) and the amount of vegetative clutter in a foraging space (Kalko & Schnitzler 1993; Obrist 1995). Geographic variation in echolocation has also been documented, and variability in call structure has been linked with morphological differences between populations in several species (Heller & von Helversen 1989; Parsons

1997; Francis & Habersetzer 1998; Barclay 1999; Guillen et al. 2000; Law et al. 2002; Aspetsberger et al. 2003). Other species of bats exhibit geographic variation in echolocation that is not associated with morphology or distance between sites (Thomas et al. 1987; O'Farrell et al. 2000; Murray et al. 2001).

Flexibility in call structure may be especially useful in the presence of ambient noise, which can mask weak echoes and decrease the efficiency of echolocation. Potentially masking background noise may arise from both abiotic (e.g. wind, rain, flowing water) and biotic sources (sounds of other animals). Several taxa exhibit changes in signal structure under noisy environmental conditions, including increases in signal amplitude (Lopez et al. 1988; Brumm et al. 2004), duration (Leonard & Horn 2005; Penna et al. 2005), and redundancy (Lengagne et al. 1999), as well as shifts in call frequency (Au et al. 1985; Lopez et al. 1988; Slabbekoorn & Peet 2003). Some species of bats exhibit different call structures when foraging alone or in the presence of conspecifics (Habersetzer 1981; Kalko & Schnitzler 1993), possibly to avoid ‘jamming’ from the calls of neighboring individuals (Ulanovsky et al. 2004). To our knowledge, the calls of other individuals are the only acoustic signals that have been tested in bats for effects on echolocation call structure.

The objective of this research was to investigate the influence of both broad and fine scale effects on the echolocation call structure of Brazilian free-tailed bats, *Tadarida brasiliensis* (F., Molossidae). First, we described geographic variation in this species by documenting call structures from across the United States. Brazilian free-tailed bats within the U.S. are currently placed in two subspecies (Wilkins 1989, Figure 1), although recent molecular studies reveal no discernable genetic structure across the species range in the U. S. and much of Mexico, suggesting panmixia and that subspecific classification is unwarranted (McCracken & Gassel 1997; Russell et al. 2005). These bats also have an especially variable call repertoire, with recordings made in similar habitats and nearby locations yielding very different descriptions of the primary call type (Simmons et al. 1979; Fenton & Bell 1981; Ratcliffe et al. 2004). Given their lack of phylogeographic structure, we hypothesized that any variability observed in the echolocation calls of *T. brasiliensis* would result from factors other than subspecific differences or geographic structuring of populations. The results of our geographic analysis led us to evaluate a second factor that may be responsible for the observed patterns of variability, and to test the hypothesis that bats adjust their call structure to avoid spectral overlap with local ambient noise (e.g. insect choruses) that may interfere with echolocation.

METHODS

Geographic variation

We recorded echolocation calls of 50-60 Brazilian free-tailed bats at each of16 sites, spanning most of the species range in the United States (Figure 1). Data were collected between May and September 2004 and 2005 under an approved protocol from U of Tennessee Animal Care and Use committee, and scientific collection permits from state wildlife agencies. At each site, bats were

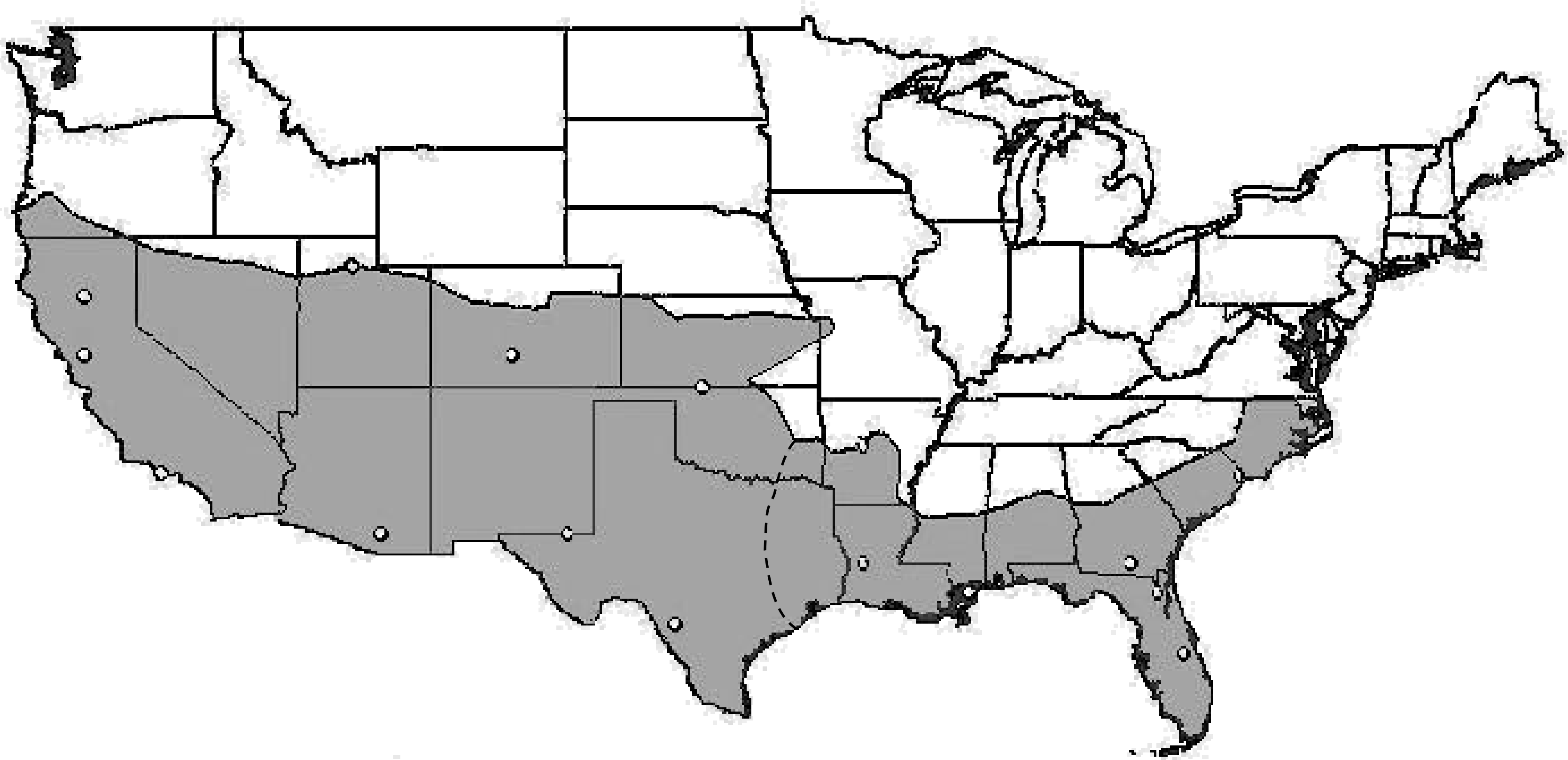


***Figure 1**. Continental US range map of* Tadarida brasiliensis*. Grey area shows the species range. White circles denote the 16 sites at which bats were collected and recorded for the geographic variation study. Dotted line indicates the putative subspecific division between* T.b. cynocephala *in the southeastern US and* T.b. mexicana *in the western US.*

captured at the roost using padded sweep nets, harp traps, or mist nets. After capture, standard measurements were taken from each animal, including mass, forearm length, sex, age, and reproductive condition. To control for possible effects of age (Jones and Ransome 1993; Masters et al. 1995), only the calls of adult animals were recorded.

Within two hours of capture, bats were released individually after a 3.8 cm chemiluminescent tag (Chemical Light, Inc., Vernon Hills, IL) was attached to their dorsal side with multi-purpose glue. The light tag allowed us to track the animal and confirm that recorded calls were emitted by the released bat (Murray et al. 2001). Separate release of marked individuals also ensured that we only recorded a bat once and avoided pseudoreplication, which has been a potentially confounding factor in previous studies that have relied on recordings from free-flying, unmarked bats. Release sites were open areas, such as a baseball field or park, within two miles of the roost. These locations were selected because they provided unobstructed airspace for flying bats, thus standardizing release conditions between sites as much as possible. Prior to the release of bats, we used acoustic monitoring to ensure that other bats were not actively foraging in the area. A real-time, high speed recording system was used to record the bat as it foraged in the area. Signals received by an S-25 ultrasonic detector (Ultrasound Advice, UK, frequency response ± 3 dB between 20 and 120 kHz) attached to a F2000 Control/Filter unit (Pettersson Electronik, Sweden) were captured with a high speed sound card (DAQCard-6062E, National Instruments, USA) and saved on the hard drive of a Dell Inspiron laptop computer. Calls were analyzed with BatSoundPro (Pettersson Electronik, Sweden) using 16-bit resolution and a 200 kHz sampling rate.

From the data-files, we selected high-quality call sequences, according to the following criteria: (i) recordings exhibited a high signal-to-noise ratio, and contained primarily search phase calls, which are emitted before an animal has detected a target insect, (ii) only one bat was present in the recording area, as determined by stable inter-call intervals, and (iii) recordings were at least 15-s in duration. Calls emitted within the first 10-s of release were discarded because they are often shorter in duration and more broadband in frequency than those typically used for foraging. After 10-s, recorded calls generally exhibited the typical call structure used by foraging *T. brasiliensis*, and call sequences often contained feeding buzzes, which indicated successful feeding (EHG, personal observation). We obtained acoustic measurements from spectrograms (frequency x time representation) computed using a 256-point Fast Fourier Transform (50% overlap). From each sequence, we selected the 5-10 highest-quality search calls and measured duration, maximum frequency (at the start of the call), minimum frequency (at the end of a call), and peak frequency (frequency of maximum energy) using Avisoft SasLab Pro (Avisoft Bioacoustics, Germany). Peak frequency was the maximum point in the power spectrum, while minimum and maximum frequency were respectively the lowest and highest frequencies above the background noise, which corresponded well to 20 dB below the maximum peak in the spectrum (Figure 2).

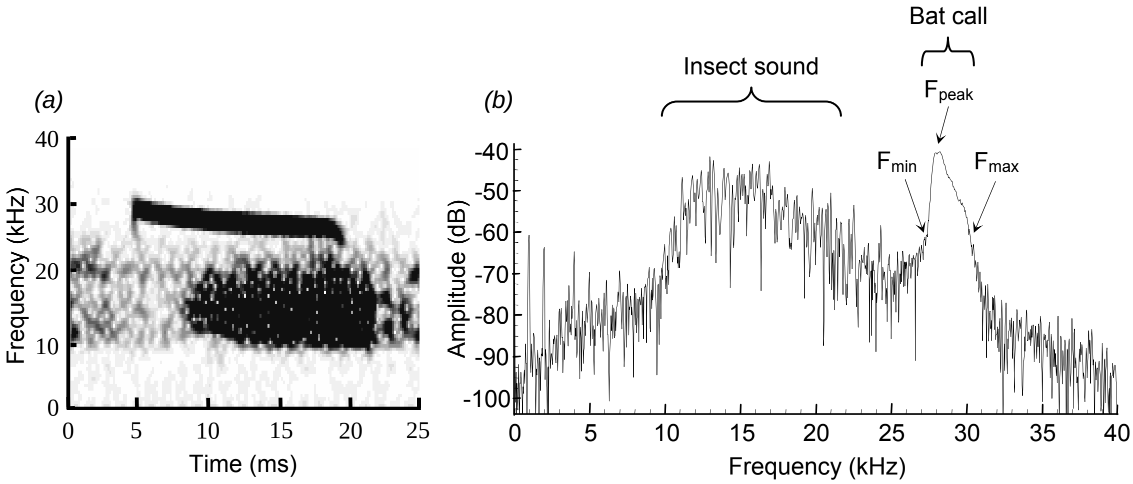


Figure 2. Recording of a search-phase echolocation call and the 21.5 kHz insect sound stimulus. (a) spectrogram (frequency *vs* time) and (b) power spectrum (amplitude *vs* frequency) of both signals. Frequency ranges of the insect sound stimulus and the bat call are marked. The three measured spectral variables of the bat call - maximum frequency (F_{max}), minimum frequency (F_{min}), and peak frequency (F_{peak}) - are indicated on the power spectrum

To determine if differences in call structure existed between sites, we conducted a univariate one-way analysis of variance (ANOVA) for each of the four call parameters, using averaged values for each sequence. We used nested GLM ANOVA to assess which factors were associated most strongly with the observed patterns of variation. In this analysis, the variance components calculated from mean square values indicate the percentage of total variation associated with differences among locations, and among individuals within a location, while the error-variance component refers to the amount of variation due to differences within call sequences of individuals (Sokal & Rohlf 2000). We also performed a linear discriminant function analysis using sequence averages for all call variables to establish if sequences could be correctly assigned to the site of collection.

To test if differences in call structure were associated with geographic distances between populations, we first conducted a principal component analysis on the average sequence values of the four call parameters. We used the first two uncorrelated PC factor scores to calculate a dissimilarity matrix of acoustic Euclidean distances between populations. We then calculated a geographic distance matrix from the latitude and longitude of each location, and compared the acoustic and geographic distance matrices using a non-parametric Mantel test of matrix association (Mantel 1967, MANTEL v. 1.18, Rio de Janeiro, Brazil). The Mantel statistic calculated from these two matrices was then compared to a simulated sampling distribution constructed by randomly reallocating the order of elements in the matrices 1000 times.

Weather conditions also may have influenced echolocation call structure. Body temperature, which is affected by air temperature, is correlated with call frequency in some species (Huffman and Henson Jr. 1991), and humidity can influence call frequency due to it's effect on atmospheric attenuation of acoustic signals (Guillen et al. 2000). Because of these potential influences, we tested for associations between observed call structures and weather conditions at the release site. We gathered weather information for each collection site from NOAA's online database (http://www.ncdc.noaa.gov/oa/climate/stationlocator.html), including temperature, precipitation, wind speed, and relative humidity recorded as close in time as possible to when the bats were released, as well as averages for these variables on the date of study. We conducted a series of linear regressions of each call variable against each weather variable, using a Bonferroni-corrected p-value of 0.0125. To evaluate any cumulative effects of these weather data, we performed a principal component analysis on the eight weather variables, and used the first two PC factor scores to obtain a matrix of Euclidean distances. This weather matrix was then compared to the previously calculated acoustic matrix with a Mantel test, as described above.

Since characteristics of an individual bat may influence echolocation, we tested for associations of sex, body size, and reproductive condition with call structure. Linear regressions for each of the four call variables were performed to test for associations with mass and forearm length. T-tests were used to determine if call structure differed by sex and/or reproductive condition (pregnant vs. lactating females). We excluded non-pregnant and post-lactating females from our analysis of reproductive condition due to low sample sizes for these groups (n<20). We also excluded males from the analysis of reproductive condition because we captured very few males in an active reproductive state.

Broadcast experiments using insect sounds

We experimentally tested the responses of free-flying *T. brasiliensis* to broadcasts of high frequency insect sounds. We performed broadcasts on eight nights between 25 May and 10 June 2005, on a cotton farm in the vicinity of Uvalde, Texas. This site is close to several large Brazilian free-tailed bat colonies, and bats were often observed foraging on insects found in high densities over the crop fields where we conducted our study.

Stimulus signals were constructed from the calls of unidentified orthopterans recorded in the Chihuahan desert of Carlsbad Caverns National Park, Carlsbad, NM (Figure 2). The multiple insect sounds captured in these recordings ranged in frequency from 5-60 kHz, although signal amplitudes were strongest between 8 and 30 kHz. These calls were prominent on one night (11 August 2004) during our study of geographic variation, and appeared to influence the call structure of released bats. We created a series of broadcast stimuli by repeating a 1-s field recording to create a 5-min signal, and shifting the maximum frequency of the insect sound signal to six different frequencies (16.5, 19, 21.5, 24, 26.5, and 29 kHz). These frequencies were selected because they fell within the 8-30 kHz range of high amplitude insect sounds recorded at Carlsbad

Caverns National Park. Further, Brazilian free-tailed bats exhibit maximum sensitivity over a broad hearing range from 10-40 kHz (Henson Jr. 1970), indicating that all broadcast stimuli should have been equally audible to free-flying bats. Shifts in signal frequency were accomplished with the “Frequency Domain Transformation” feature of Avisoft SasLab Pro. A 5-min broadcast of silence was also created as a control. To avoid order effects, we broadcast a mix of these seven stimuli every night and changed the broadcast order on successive nights. We presented each signal on average five times per night and at least ten times over the course of the study.

We began broadcasting stimuli each night between 20:30 and 20:45, when the first bat was sighted in the area, and continued for 2-3 hours during the period of peak bat activity. Broadcast and recording equipment was arranged in a line, with a microphone at 0 and 20 m, and the speaker at 10 m. We broadcast stimuli through an omnidirectional ultrasonic speaker (Avisoft 60401; frequency response ± 5 dB between 15 and 43 kHz) mounted on a tripod 2.5 m from the ground. Two solid dielectric microphones (Avisoft CM16; frequency response ± 3 dB between 10 and 100 kHz) were positioned at a height of 2 m and oriented at 45° above the horizontal and towards the speaker. Stimuli were generated from a Dell Inspiron laptop computer through a high-speed sound card (DAQCard-6062E, National Instruments, Austin, TX) and an amplifier (Avisoft 70101) powered by three 12 V 7.2 A gel cell batteries. High-speed data acquisition was accomplished with Avisoft’s Ultrasound Gate 416, using the same computer that was used for conducting broadcasts. We broadcast stimuli at 83dB, which was the highest intensity possible without overloading the speaker (measured with a B&K ¼ " condensor microphone # 4939 and a B&K measuring amplifier # 2606, Brüel & Kjær, Denmark). Both broadcast and recording were performed using Avisoft RECORDER. Recordings were 5-min long, and made with 16-bit resolution and a 166 kHz sampling rate. Recordings included both the broadcast signal and the calls of free-flying bats in the area.

We located sequences of echolocation calls by searching the oscillogram (amplitude x time representation) for high amplitude signals that indicated the presence of a nearby bat. We only selected sequences for further analysis that: (i) exhibited a high signal-to-noise ratio, (ii) contained primarily search phase calls, and (iii) included calls of only one bat. For multiple sequences recorded within one 5-min recording, we only used sequences separated by > 1 min of silence to minimize the chance of analyzing calls of the same bat. While we cannot eliminate the possibility that we selected multiple sequences from the same bat, the fast flight speed of *T. brasiliensis* (average 6 m/s; Hayward and Davis 1964) and rapid turnover rate of bats foraging in the study area indicate that repeated sampling is unlikely. We selected the 20 highest-quality call sequences for each of the seven broadcast stimuli and measured 10 calls per sequence. For each call, we measured duration, maximum frequency, minimum frequency, and peak frequency from spectrograms generated with a 256-point Fast Fourier Transform (50% overlap). To avoid pseudoreplication, we conducted analyses on the average call measurements of a sequence.

Differences in call structure between stimulus conditions were assessed using univariate one-way ANOVA and Tukey's HSD test for each call variable, as well as a multivariate analysis of variance (MANOVA) that included all four call variables. Pearson's product-moment correlation coefficents were calculated to determine the relationship between each call variable and frequency of the insect sound stimulus.

Call duration and call frequency are negatively correlated in some bat species (Jones 1999), thus a potential issue is that any frequency changes we observed may have been an indirect response to shortened call durations instead of a direct response to frequency of the insect sound stimulus. We used linear regression to test for associations between call duration and each of the spectral variables. To determine if bats were flying closer to the ground in the presence of the insect sound stimulus, we measured signal amplitude from the amplitude envelope for all calls in the dataset, and used a t-test to assess if average call amplitude differed between sequences recorded in silence compared to those recorded in the presence of insect sounds.

Hand-released bats in the absence of insect sounds

As another test of the hypothesis that insect sounds affect echolocation call structure in bats, we recorded additional hand-released bats near Carlsbad Caverns National Park. In contrast to the previous recordings in August 2004, we chose a release site that was distant from nocturnally calling insects, although this was still within the vicinity of the park. Releases were conducted on 16 June

2005 in a large parking lot in which the calls of insects in the surrounding vegetation were not detectable with an S-25 bat detector (Ultrasound Advice, UK). Recording equipment, procedures, and criteria for sequence selection were the same as those described above for our study of geographic variation. We measured calls from 18 individuals, and calculated average values of call duration, maximum frequency, minimum frequency, and peak frequency for each bat. T-tests were used to determine if any of the four call parameters differed between bats recorded in the presence (11 August 2004) or absence (16 June 2005) of insect sounds. To test for multivariate effects, we conducted a MANOVA that included all four call variables in the analysis. Bats were also recorded at Carlsbad Caverns on 10 August 2004, but we chose not to include these data in this analysis because levels of insect activity were not assessed at the time of recording.

RESULTS

Geographic variation

From the 16 sampled sites, we analyzed a total of 3901 calls from 410 individuals. On average, we analyzed 30 call sequences per site, (range: 13-44 sequences/site). Bats exhibited significantly different call structures amongst sites (ANOVA: $F_{15, 394} > 7.2$, $P < 0.0001$ for all four call parameters; Table 1). Our analysis of the nested ANOVA variance components revealed that differences between locations were significant ($F_{14, 3665} > 7.2$, $P < 0.0001$), but explained only

Table 1. Call parameters (average ± standard deviation) at each collection site and results of one-way ANOVA tests.

Location	# Bats	Duration (msec)	Max freq (kHz)	Max freq (kHz)	Peak freq (kHz)
Melbourne, FL	26	11.4±1.5	23.0±1.4	31.7±2.5	26.7±1.7
Gainesville, FL	23	11.2±1.3	22.4±1.5	34.6±4.1	27.7±2.1
Valdosta, GA	25	12.4±1.6	22.8±1.9	29.8±2.7	25.9±2.1
Conway, SC	44	13.5±1.2	20.9±0.9	30.0±2.0	25.2±1.2
Ocean Springs, MI	20	11.2±1.2	22.5±1.2	35.2±3.5	27.8±1.6
Fort Polk, LA	26	12.4±2.1	22.0±1.8	33.2±3.7	26.8±2.0
Hot Springs, AR	33	12.2±1.7	21.3±1.2	31.1±3.0	25.4±1.4
Waynoka, OK	26	11.9±1.5	21.7±1.1	32.8±3.7	26.2±2.1
Uvalde, TX	44	11.0±1.7	22.9±15	34.5±4.0	27.9±2.4
Carlsbad, NM*	27	11.6±1.0	24.5±2.2	35.6±3.6	29.3±2.4
8/10/2004	(13)	11.3±1.0	23.3±2.4	33.2±3.1	28.1±2.8
8/11/2004	(14)	11.8±1.0	25.6±1.4	37.7±2.6	30.5±1.3
Salida, CO	13	12.0±1.2	21.8±1.4	30.8±2.9	25.9±1.7
Tucson, AZ	15	12.3±0.9	22.1±1.2	31.5±2.3	26.5±1.6
Clearfield, UT	21	12.7±1.5	21.6±1.4	30.7±2.5	25.7±1.7
Topanga, CA	16	11.2±1.3	22.2±1.6	37.3±6.0	27.5±2.2
Fairfield, CA	21	11.6±1.6	22.6±1.4	35.2±4.1	27.6±2.0
Los Molinos, CA	30	11.4±1.1	21.4±0.9	34.3±3.6	26.5±1.1
One-way ANOVA	410	$F_{15, 394} = 7.3$ $P < 0.0001$	$F_{15, 394} = 10.8$ $P < 0.0001$	$F_{15, 394} = 10.8$ $P < 0.0001$	$F_{15, 394} = 10.1$ $P < 0.0001$

* Data from Carlsbad, NM are divided by date of collection to illustrate the increased call frequencies of bats recorded on 11 August 2004. We recorded nocturnal insects producing high amplitude, high frequency sounds at the release site on 11 August 2004, but not on 10 August 2004.

15-22% of the variability in call structure (Table 2). We found that differences among individual sequences within a location explained 51-56% of the variation and differences among calls within an individual sequence explained 19-32% of the variation, indicating that there is a high degree of variability in call structure both among and within bats. A linear discriminant function analysis could assign only 21.4% of the sequences to the correct site of collection. This poor classification rate is consistent with the low percentage of variation explained by location in nested ANOVA tests.

Acoustic differences were not associated with geographic distances between sites (Mantel test: $r = -0.06$, $P = 0.29$, Figure 3a). Individual linear regressions and a Mantel test using data from all weather variables revealed no significant associations between weather conditions at the release site and call structure (Mantel test: $r = 0.14$, $P = 0.85$, Figure 3b). Taken together, all of these analyses suggest that geographic patterning in the echolocation calls of Brazilian free-tailed bats is weak to nonexistent, and any differences that might exist are hidden by the large amount of variability among bats within sites.

Mass and forearm length were not associated with changes in call structure ($R^2 < 0.05$ for all parameters), and no differences were found between the echolocation calls of males and females. Lactating females used higher average minimum frequencies than pregnant females (lactating: $n = 41$, 23.1 ± 1.6 kHz, pregnant: $n = 133$, 22.0 ± 1.4 kHz, two-tailed t test: $t_{177} = 4.33$, $P < 0.0001$). Although this finding is statistically significant, the average frequency difference between pregnant and lactating females is small (1.1kHz, or < 5% of

Table 2. Variance components for each call parameter. Explained variance is due to differences: (1) among locations, (2) between individuals at a location, and (3) within individuals at a location.

	% variance due to*		
	locations	individuals	Calls
duration	14.8	53.1	32.1
max frequency	21.4	51.1	27.5
min frequency	24.1	56.8	19.1
peak frequency	21.1	54.0	24.9

* Variance components are calculated from mean square values of nested ANOVA tables.

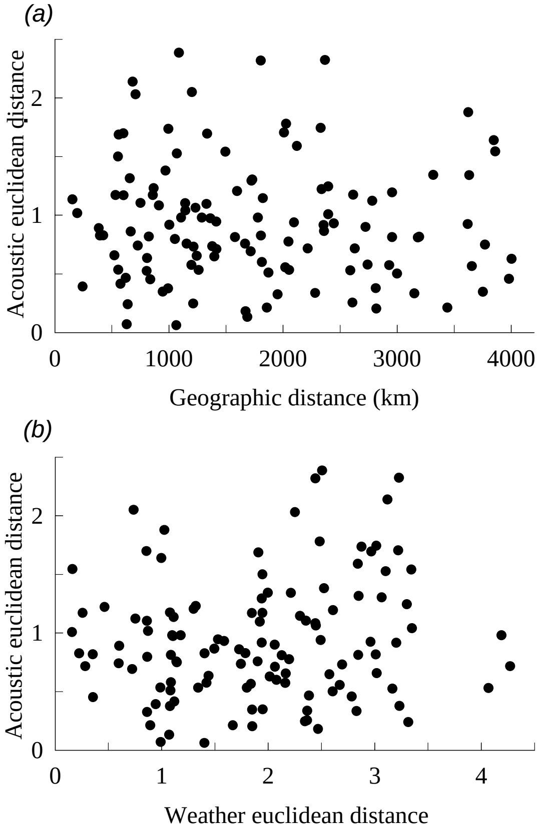


Figure 3. Relationships of acoustic Euclidean distance with (a) geographic distance and (b) weather Euclidean distance. Points represent all possible pairwise comparisons of the sixteen collection sites.

the average), and further sampling is necessary to determine if this relationship is biologically significant.

Calls recorded at Carlsbad Caverns National Park, NM on 11 August 2004 were noticeable outliers, exhibiting significantly higher minimum frequencies compared to other sites and to data collected at the same site on different nights (Table 1). As noted above, recordings from 11 August 2004 contained loud, high frequency sounds produced by nocturnal insects, and such insect calls were not present in recordings from the previous night. This observation motivated us to conduct the experiments described above to test the influence of insect sounds on echolocation call structure.

Effects of insect sounds

Bats exhibited significant differences in call structure in the presence of the seven broadcast stimuli (Figure 4, ANOVA: duration: $F_{6,133} = 8.5$, $P < 0.0001$; maximum, minimum, and peak frequency: $F_{6,133} > 23.5$, $P < 0.0001$, MANOVA (Wilks' Lambda): $F_{24,458} = 9.6$, $P < 0.0001$). Average echolocation call frequency exhibited a strong positive correlation with frequency of the insect sound stimulus (maximum frequency: $R^2 = 0.62$, minimum frequency: $R^2 = 0.46$, peak frequency: $R^2 = 0.55$, $P < 0.0001$ for all three tests). Average call duration and stimulus frequency were negatively correlated, although this was weaker than correlations for the spectral variables ($R^2 = 0.19$, $P < 0.0001$). Control recordings during silence were significantly lower in frequency than calls recorded during experimental broadcasts, with the exception of the two lowest

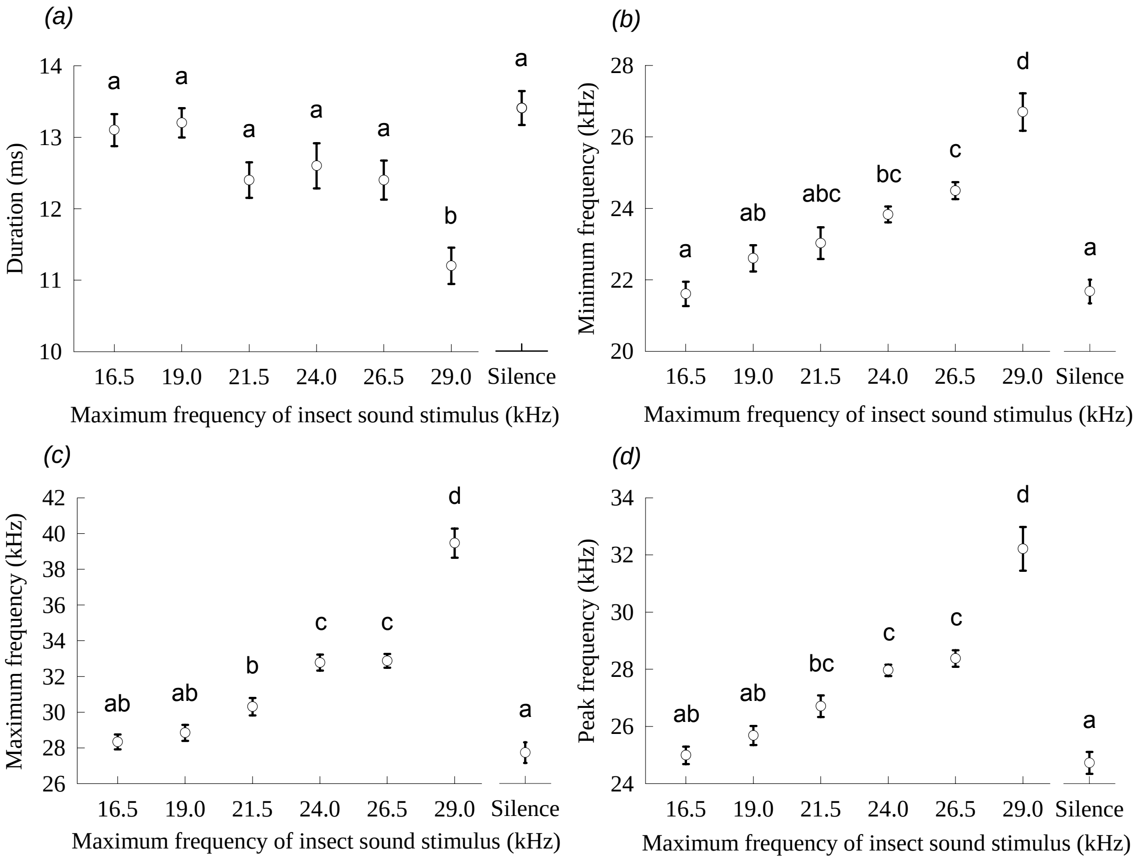


Figure 4. Mean ± standard error for echolocation call parameters recorded during broadcasts of the six insect sound stimuli and silence. (a) duration, (b) minimum frequency, (c) maximum frequency, and (d) peak frequency. Tukey's HSD groupings indicate significant differences across the broadcast stimuli.

frequency stimuli (also marginally for the third lowest frequency stimulus for minimum frequency). Call frequency was negatively correlated with call duration for all three spectral variables (maximum frequency: $R^2 = 0.33$, minimum frequency: $R^2 = 0.19$, peak frequency: $R^2 = 0.27$, $P < 0.0001$ for all three regressions), but we found that average call amplitudes were not different for sequences recorded in silence compared to those recorded in the presence of insect sounds ($t_{138} = -.24$, $P = 0.81$).

Because our study involved unmarked, free-flying bats, it is possible that sex, age, and reproductive condition influenced call structure. However, we believe these factors had little effect on our data because: (1) our geographic variation analysis indicated that sex does not influence call structure in Brazilian free-tailed bats, (2) the study period (25 May – 10 June) spanned only one reproductive period (late pregnancy), eliminating concerns for potential differences between pregnant and lactating females, as well as between subadult and adult bats (Masters et al. 1995), and 3) we broadcast a mixed order of all stimuli each night.

Significant differences existed in the structure of calls recorded from hand-released bats in the presence (11 August 2004) and absence (16 June 2005) of high frequency insect calls (Table 3). Recordings from light-tagged bats where insect sounds were absent were significantly longer in duration (two-tailed t-test:

Table 3. Hand-released calls from Carlsbad Caverns National Park. Call parameters (average ± standard deviation) for recordings at release sites near the park, in which nocturnally calling insects were present (+) or absent (-). Note that bats calling in the presence of high frequency insect sounds used higher average call frequencies than bats recorded in the absence of insect sounds.

Date	# Bats	Duration (msec)	Min freq (kHz)	Max freq (kHz)	Peak freq (kHz)
8/11/2004 (+)	14	11.8±1.0	25.6±1.4	37.7±2.6	30.5±1.3
6/16/2005 (-)	18	13.0±1.2	22.8±1.0	29.3±1.4	26.0±1.0
Two-tailed t-test	32	$t_{30} = 2.8$ P = 0.0009	$t_{30} = -11.7$ P < 0.0001	$t_{30} = -6.6$ P < 0.0001	$t_{30} = -11.0$ P < 0.0001

$t_{30} = 2.78$, $P < 0.0001$), and lower in maximum ($t_{30} = -11.74$, $P < 0.0001$), minimum ($t_{30} = -6.64$, $P < 0.0001$), and peak frequency ($t_{30} = -11.01$, $P < 0.0001$) compared to calls recorded in the presence of insect sounds. MANOVA revealed a similar pattern, with highly significant differences in call structure between nights (Wilks' Lambda: $F_{4,27} = 36.2$, $P < 0.0001$. These results follow the trend observed in the broadcast experiment.

DISCUSSION

Geographic Variation

The substantial variation in call structure that we document here is concordant with other reports of the highly labile echolocation calls of Brazilian free-tailed bats (Simmons et al. 1979; Ratcliffe et al. 2004). While we did observe significant differences in call structure among sites, most of the observed variation occurs between and within individuals (Table 2), and is not associated with geographic distance between locations (Figure 3a) or with putative subspecific distinctions (Figure 1). Observed differences amongst sites may be due to the influence of other local conditions on echolocation that were not addressed in this study, and we have shown that at least one feature of a bats foraging environment (presence of high frequency insect sounds) can strongly influence call structure. Thus, we conclude that the echolocation calls of Brazilian free-tailed bats do not exhibit a discernable geographic pattern, and differences among locations result from the wide range of call structures that can be used by an individual bat, or by different individuals within a population. This observed absence of geographic structure is consistent with studies on gene pool structure that demonstrate panmixia among colonies of Brazilian free-tailed bats throughout North America (McCracken & Gassel 1997; Russell et al. 2005).

While sex and body size have been shown to influence call structure in some bat species (Jones et al. 1992; Barclay et al. 1999; Guillen et al. 2000), we found no evidence for such effects in Brazilian free-tailed bats. We did find that reproductive condition influenced call structure, with pregnant females using lower call frequencies than lactating females. This pattern may be explained by differences in body size and energetic demands of pregnant and lactating females. Use of lower frequencies, which increase a bat's detection range, may be useful for pregnant females that experience reduced maneuverability due to the increased weight of the fetus. Alternatively, lactating females require the highest energetic intake for milk production (Kunz et al. 1995). Use of higher frequency calls may allow lactating females to detect a greater variety of small insects (Pye 1993), although the strength of the relationship between call frequency and detectable target size is not always strong (Waters et al. 1995; Houston et al.2004) and the small differences observed here may not translate to differential detection abilities. Despite this, evidence that Brazilian free-tailed bats feed more heavily on small Hymenoptera during lactation than during pregnancy (Kunz et al. 1995) is consistent with the idea that lactating females eat smaller prey items.

Effects of insect sounds and local environmental conditions

The nocturnal chorus of insect sounds present at Carlsbad Caverns National Park on 11 August 2004 spanned both sonic and ultrasonic frequencies, with the highest amplitude band of calls between 8 and 30 kHz. These insect call frequencies overlap with the echolocation frequencies typically used by Brazilian free-tailed bats (Ratcliffe et al. 2004). While the amplitudes of these insect calls were not measured, the insects clearly provided a loud source of background noise that could potentially influence the echo-reception of bats foraging near the ground. The use of significantly lower frequencies by bats at a nearby site where calling insects were absent suggests that the observed increase in echolocation frequency on 11 August 2004 was due to a temporary adjustment of the bats' calls to avoid overlap with high frequency insect sounds.

Results of our experimental broadcast of insect sounds further support the hypothesis that bats adjust their echolocation to avoid spectral overlap with ambient noise from calling insects. This suggests that separating weak echoes of echolocation calls from broadband noise may challenge the signal-processing algorithms of Brazilian free-tailed bats, and that bats adjust call structure to avoid jamming effects from the noise signal. This signal-processing problem is similar to the pyschoacoustic challenge faced by humans in attending to a single speaker under crowded conditions in which several other people are talking simultaneously. Humans are capable of separating one auditory channel (the speaker of interest) from several other auditory channels (other speakers in the room), in what is known as the "cocktail party effect" (Haykin and Chen 2005).

However, this task may be more difficult for echolocating bats, as the amplitudes of echoes are more highly attenuated compared to the amplitudes of original calls, and thus are more easily obscured by high amplitude noise.

It is notable that calls recorded in the presence of the experimental 16.5 kHz and 19 kHz insect sounds were not significantly different from calls recorded in silence (Figure 4), indicating that bats did not respond to the lower frequency stimuli. As the average minimum frequency of Brazilian free-tailed bats is typically above 19 kHz (22.3 kHz for the geographic variation dataset), these frequencies of insect sounds should not interfere with echolocation. Broadcasts at higher frequencies within the spectral range of *T. brasiliensis* echolocation did result in a change in call frequency, with a positive, essentially linear relationship between the spectral parameters of recorded calls and the frequency of the insect sound stimulus (Figures 4b-d). These results demonstrate that Brazilian free-tailed bats are capable of making fine-scale adjustments to their call structure (<1 kHz shifts in call frequency) that lead to decreased spectral overlap with an interfering acoustic signal.

An alternative explanation for the observed changes in call frequency is that bats perceived the insect sound broadcast as a potential foraging “hotspot”, and flew closer to the ground in an attempt to capture prey. Bats flying near the ground may then have shortened call duration to avoid pulse-echo overlap, and shifted to higher call frequencies to allow for shorter target detection distances. We believe this is unlikely because: (1) orthopterans, which were the source of our insect sound broadcast, comprise < 2% of the diet of *T. brasiliensis* (Lee and

McCracken 2005), and (2) call amplitude did not change in response to the presence of the insect sound stimulus, suggesting that bats were flying at similar distances from the ground under all study conditions. Thus, we have no reason to expect that bats directly adjusted call duration across stimuli. Alternatively, bats may have shifted their call frequencies upward to avoid spectral overlap with the insect sound stimulus, resulting in indirect adjustments to call duration. This interpretation explains the association of call duration and call frequency, and conforms to our hypothesis that bats shift their frequencies in a manner that avoids overlap with the insect sound stimulus.

External noise is known to affect flight behavior in some bat species, and presumably has an adverse impact on the efficiency of echolocation. Broadcasts of broadband noise affected the ability of *Plecotus rafinesquii* to navigate between sets of thin wires, although their abilities were not compromised when flying between larger wires (Griffin 1958). Three species of insectivorous bats (*Myotis lucifugus, M. daubentonii, Eptesicus fuscus)* have been shown to avoid foraging over stream riffles compared to calm pools, despite equally or greater insect abundances over riffles (von Frenckell & Barclay 1987; Mackey & Barclay 1989; Rydell et al. 1999). Behavioral avoidance of riffles is likely due to interference from noise produced by fast-flowing water, as broadcasts of high frequency water sounds over calm pools also resulted in reduced foraging activity (Mackey & Barclay 1989). While these previous studies demonstrated behavioral changes in the presence of acoustic interference, they did not investigate whether bats adjust their echolocation structure in response to noise.

M. lucifugus, M. daubentonii, and *E. fuscus* exhibit flexibility in call structure in response to other foraging conditions (Obrist 1995; Schnitzler et al. 2003; Broders et al. 2004), and if experimentally tested, we would expect these species to exhibit a response to high frequency insect sounds similar to that of *T. brasiliensis.*

In bats, call structure in the presence of an external noise source has only been examined in response to the calls of nearby conspecifics. Some bat species have been shown to exhibit a jamming avoidance response, in which individuals alter their echolocation to avoid spectral overlap with the calls of neighboring individuals (Ulanovsky et al. 2004). In this study, we document an analog to jamming avoidance and show that *T. brasiliensis* make fine-scale changes to their echolocation calls in a manner that effectively avoids interference from local sources of environmental noise. It is likely that foraging Brazilian free-tailed bats frequently encounter sources of acoustic interference, such as the sounds of calling insects, and this behavioral flexibility should enhance the efficiency of echolocation for orientation and prey detection.

CHAPTER III

Rapid Jamming Avoidance in Biosonar

This chapter is a version of a paper by the same name that is currently available online in the journal *Proceedings of the Royal Society of London B: Biological Sciences* by Erin H Gillam, Nachum, Ulanovsky, and Gary F McCracken:

Gillam, E.H., Ulanovsky, N., and McCracken, G.F. 2007. Rapid Jamming Avoidance in Biosonar. *Proceedings of the Royal Society of London B: Biological Sciences.* FirstCite early online publication (doi:19.1098/rspb.2006.0047)

My consistent use of "we" throughout this chapter is in reference to my co-authors, Nachum Ulanovsky, Gary McCracken, and myself. I was the primary contributor to this work, which involved the following tasks: (1) development of project design and all data collection, (2) measurement of acoustic signals and most of the statistical analysis of the dataset, (3) most of the gathering and interpretation of the relevant literature, and (4) most of the writing.

ABSTRACT

The sonar systems of bats and dolphins are in many ways superior to man-made sonar and radar systems, and considerable effort has been devoted to understanding the signal-processing strategies underlying these capabilities. A major feature determining the efficiency of sonar systems is sensitivity to noise and jamming signals. Previous studies indicated that echolocating bats may adjust their signal structure to avoid jamming (‘jamming avoidance response’). However, these studies relied on behavioral correlations and not controlled experiments. Here we provide the first experimental evidence for jamming avoidance response in bats. We presented bats (*Tadarida brasiliensis*) with ‘playback stimuli’ consisting of recorded echolocation calls, at one of six frequencies. The bats exhibited a jamming avoidance response by shifting their call frequency away from the presented playback frequency. Approaching bats, challenged by an abrupt change in the playback stimulus, responded by shifting their call frequencies upwards, away from the playback. Interestingly, even bats initially calling below the playback’s frequency shifted their frequencies upwards, ‘jumping’ over the playback frequency. These spectral shifts in the bats’ calls occurred often within less than 200 ms, in the first echolocation call emitted after the stimulus-switch – suggesting that rapid jamming avoidance is an important response for Brazilian free-tailed bats.

INTRODUCTION

Echolocation is a critical sensory system in most bats, and it is used for detecting and assessing prey as well as for orientation and navigation (Griffin 1958; Schnitzler *et al.* 2003). Most echolocating bats use calling patterns consisting of sequences of short calls (pulses) separated by long periods of silence, during which the bat listens to the returning echoes that provide information about the target (Schnitzler *et al.* 2003). Many whale and dolphin species also echolocate, using biosonar pulses that differ in design from those of bats (Cranford & Amundin 2004; Nakamura & Tomonari 2004).

Echolocating animals may experience acoustic interference from ambient sources of noise or from the calls of conspecifics (Dusenbery 1992), which may require a 'jamming avoidance response,' in which the animal adjusts its call structure to minimize interference. It is possible, however, that the signal-processing algorithms of echolocating bats are sufficiently sophisticated that they need not alter their signals. For example, bats may use differences in the direction of arrival of sounds to separate multiple noise and signal sources, similarly to what is done by humans in the 'cocktail party effect' (e.g. Bronkhorst & Plomp 1992). Thus, the study of possible jamming avoidance responses provides a window into the signal-processing capabilities of animals that use biosonar.

Early experiments indicated that long-eared bats (*Plecotus*) are surprisingly resistant to jamming by high-intensity white noise (Griffin *et al.* 1963), but it was unclear whether the bats achieved the reported high performance by changing their calls when the noise was present. In recent years, accumulating indirect evidence has indicated that some bats shift their echolocation call frequencies in the presence of the calls of conspecifics (Habersetzer 1981; Miller & Degn 1981; Obrist 1995; Surlykke & Moss, 2000; Ibanez *et al.* 2004; Ratcliffe *et al.* 2004; Ulanovsky *et al.* 2004). These observations have often been interpreted as a jamming avoidance response. To the best of our knowledge, jamming avoidance has not been studied in echolocating marine mammals.

These previous suggestions for jamming avoidance in bats did not rely on experimental manipulations, but relied rather on analysis of correlations between call frequency and the absence or presence of conspecifics, or on correlations between call frequency and call amplitude. However, because some bats change the frequencies of their echolocation calls under a variety of circumstances unrelated to conspecific calls (Kalko & Schnitzler 1993), correlation-based inferences do not provide conclusive evidence for a jamming avoidance response. Moreover, in previous studies (Habersetzer 1981; Miller & Degn 1981; Obrist 1995; Surlykke & Moss, 2000; Ibanez *et al.* 2004; Ratcliffe *et al.* 2004; Ulanovsky *et al.* 2004) the spatial positions of the bats were unknown – hence it was unclear whether the directional echolocation beams of the bats (Schnitzler & Grinnell 1977; Hartley & Suthers 1989) were aimed towards each other (which may increase the jamming) or away from each other. The correlational approach meant also that no 'time zero' point was available for aligning any observed frequency changes in a bat's calls to the changes in the jamming signals. Thus, to demonstrate a jamming avoidance response that is causally linked to the jamming signals, requires experimental presentation of well-controlled acoustic stimuli, designed to provoke a switch in the bat's call frequency at a known 'time zero'.

Here we report the results of experimental tests of jamming avoidance response in echolocating bats. In the field, we presented free-flying bats (*Tadarida brasiliensis*) with playbacks of pre-recorded echolocation calls at one of six different frequencies. Bats consistently minimized spectral overlap with playback signals by shifting the dominant frequencies of their echolocation calls. In a separate experiment we challenged approaching bats by abruptly switching the frequency of the playback stimulus. Within 200 ms, by the next echolocation call, bats shifted their call frequencies upwards. Our findings provide the first conclusive evidence for a jamming avoidance response in echolocating animals.

METHODS

Recording site and bats

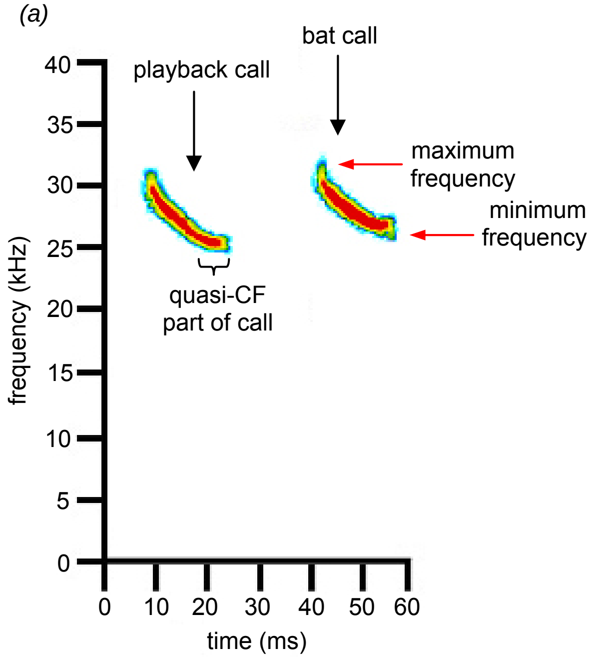
Experiments involving presentation of playbacks of echolocation calls to freely-flying Brazilian free-tailed bats (*Tadarida brasiliensis*), were conducted using methods approved by the University of Tennessee Animal Care and Use Committee. We performed experiments between 23 May and 9 July 2005, on a cotton farm in the vicinity of Uvalde, South Central Texas, within 10 miles of Frio cave, which has been estimated to contain 10 million *T. brasiliensis*. Bats were often observed foraging on insects that were found in high densities over these crop fields.

Acoustic playback stimuli and data acquisition

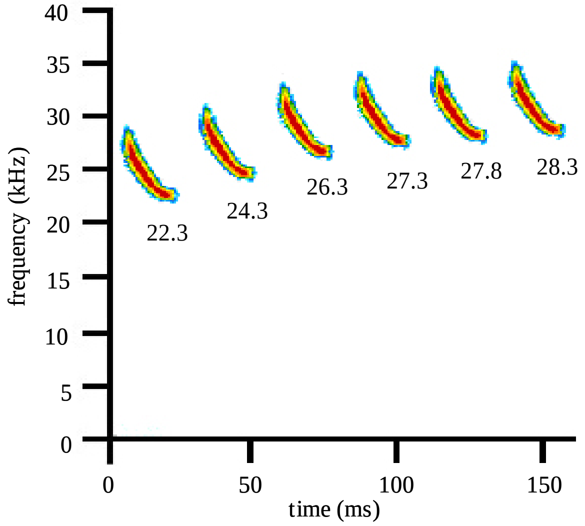
Similar to most insectivorous bats, *T. brasiliensis* utilize short frequency-modulated (FM) sweeps for echolocation (Figure 5a). Call structure in this species may vary between geographic locations (e.g. Ratcliffe *et al.* 2004), so to minimize effects due to this variation, playback stimuli were assembled from recordings of bats foraging at the same study site. We constructed the signal using one prototypical call taken from recordings of the ‘search-phase’ of bat echolocation (Griffin *et al.* 1960; Figure 5a). Although search calls recorded at the study site often exhibited FM structures very similar to this prototypical call, the call structure varies within and between individual bats, so further experiments are needed to investigate possible influences of the detailed FM structure on jamming avoidance responses. To create our stimuli, this prototypical call was repeated at 200-ms intervals for 8.8 s, followed by a 1.45-s sequence of ‘approach’ and terminal ‘feeding buzz’ calls (Griffin et al. 1960). This 10.25-s composite signal was repeated to create a 5-min playback sequence. We then created a series of six playback stimuli by shifting the frequency of this playback signal to one of six different frequency positions (we shifted the frequencies of all search, approach, and buzz calls, together). This resulted in playback stimuli with the following six values for the minimum frequencies of the search calls: 22.3, 24.3, 26.3, 27.3, 27.8, and 28.3 kHz (Figure

Figure 5. Spectrograms (frequency versus time) of search-phase bat calls and playback calls. (*a*) Spectrogram of one search-phase playback call with a minimum frequency of 24.3 kHz (left) and one recorded *Tadarida brasiliensis* search call with a minimum frequency of 25.8 kHz (right). Color scale: Linear, with red corresponding to high intensity values and blue to low intensity values. Red arrows: Minimum and maximum frequencies of the signal. Also shown is the quasi-constant frequency (quasi-CF, or QCF) part of the playback call. Dividing the frequency-range between the minimum and maximum frequency into four frequency quartiles, the lowest-frequency quartile contained 43.9% of the call duration, whereas the highest-frequency quartile contained only 9.5% of the call duration. (*b*) Spectrograms of all the six playback search calls used in this study; numbers below each call represent the minimum frequency, in kHz.

(a)


bat call
playback call
maximum frequency
minimum frequency
quasi-CF part of call
frequency (kHz)
40
35
30
25
20
15
10
5
0
0
10
20
30
40
50
60
time (ms)

(b)


frequency (kHz)
40
35
30
25
20
15
10
5
0
22.3
24.3
26.3
27.3
27.8
28.3
0
50
100
150
time (ms)

5b). A 5-min control broadcast of silence was also created (no sound was presented during those 5 minutes).

Several clarifications are needed regarding the playback stimuli. First, unless stated explicitly otherwise, all references to the ‘frequency’ of a call pertain to its minimum frequency (Figure 5a, lower red arrow). Second, the playback frequencies used in this study (minimum frequencies of search calls between 22.3 and 28.3 kHz) were selected because preliminary experiments indicated that these frequencies span the range of search call frequencies used by these bats when presented with playback stimuli. Third, bats of some species, including *T. brasiliensis*, are attracted to feeding buzzes produced by conspecifics (Balcombe & Fenton 1988; E.H.G. & G.F.M, personal observations), so the purpose of presenting the approach and feeding buzz calls was to attract more bats into the range of our recording equipment. However, for all of our analyses we used only data collected during the time periods when *search*-phase playback calls were presented, and we only measured *search*-phase calls produced by the bats.

Each night, we began playbacks at between *ca.* 20:30 and 20:45, when the first bat was sighted in the area, and continued for 2-3 hours, corresponding to the times of peak bat activity. We presented acoustic stimuli through an omnidirectional ultrasonic speaker (Avisoft Magnat 60401, Avisoft Bioacoustics, Berlin, Germany; frequency response ± 5 dB between 15 and 43 kHz) mounted 2.5 m above the ground on a tripod. Two condenser microphones (Avisoft

CM16; frequency response ± 3 dB between 10 and 100 kHz) were placed in opposite directions 10 m from the speaker. Microphones were positioned at a height of 2 m and oriented at 45° above the horizontal and towards the speaker. Stimuli were generated by a Dell Inspiron laptop through a high-speed sound card (DAQCard-6062E, National Instruments, Austin, TX) and an Avisoft 70101 ultrasonic amplifier. High-speed data acquisition was carried out using Avisoft's Ultrasound Gate 416 and Avisoft RECORDER, using the same laptop that was used for stimulus presentation. Recordings were done with 16-bit resolution and a 166-kHz sampling rate. Recorded files were 5-min long, and included both the playback signals and the calls of free-flying bats in the area.

Static-stimulus experiment

We initially tested for a jamming avoidance response by broadcasting the six playback stimuli and the silence control in a randomized order and recording the calls of free-flying bats in the vicinity. We changed the playback order on successive nights, and presented each 5-min signal five times per night, on average, and at least fifteen times over the course of the entire study. From the data-files, we selected recorded call sequences, according to the following criteria: (*i*) only one bat was present near our recording equipment, as evidenced by the stable inter-pulse intervals of recorded search-phase calls (Speakman & Racey 1991; Ulanovsky *et al.* 2004); (*ii*) we only used sequences separated by > 1 min of silence, in order to minimize the chances of analyzing multiple recordings of the same bat; (*iii*) the recorded call sequences had high signal-to-noise ratio; (*iv*) the sequences consisted primarily of search-phase echolocation calls. Using these criteria, we selected the 30 highest-quality call sequences for each of the 7 playback conditions (6 frequencies + silence), resulting in a total of 210 sequences. We did *not* select sequences based on whether any frequency changes were observed in the bat's behavior. From each sequence, we then selected the highest-quality search calls, 7-10 calls per sequence, and used Avisoft SasLab Pro to measure the call parameters (as described below), for a total of n = 2070 search calls. We then computed the average pulse parameters for each sequence, and used these average values for subsequent analyses of the static-stimulus experiment.

Dynamic-stimulus experiment

To determine whether changes in call frequency were in direct response to the playback signal, we conducted a second experiment in which we abruptly switched the stimulus as an individual bat approached the speaker. We used five of the seven playback stimuli (22.3, 24.3, 26.3, 28.3 kHz, and silence), and performed all of the possible 20 switches between these five conditions. The presence of a single bat was assessed in real time based on the stability of the inter-call intervals, as above, and was later verified offline. The pre-switch playback stimulus was broadcast until an individual bat approached the recording area. We then switched the playback stimulus when the calls of the bat increased in amplitude to a level similar to that of the playback signal, indicating that the bat was approaching our recording system. The switch in playback frequencies resulted in a small temporal gap (< 1.5 sec) between the end of the pre-switch signal and the start of the post-switch signal, and when analyzing the data we used the starting time of the post-switch signal as the alignment point, t = 0. We continued recording until the echolocation calls of the bat were no longer visible on the oscillograms.

For analysis, we selected the 10 highest-quality call sequences for each of the 20 switches, using the same selection criteria as above, and the additional criterion that the sequence contained at least ten calls pre-switch and ten post-switch. This resulted in a total of 200 sequences. We then extracted two subsets out of those 200 sequences: (*i*) the 'main dataset', defined as a subset of sequences where before the switch (t < 0) the pre-switch playback frequency differed by > 3 kHz from the bat's frequency (= the *average pre-switch bat frequency*), and where at the switch (t = 0), the new playback frequency differed by < 1.75 kHz from the bat's average *pre*-switch frequency. These criteria resulted in 39 sequences in the main dataset (1078 total calls), for which we expected a jamming avoidance response to occur after the switch (t > 0) because of the small frequency separation between the playback and the bat calls at t = 0. (*ii*) The 'control dataset', defined as a subset of sequences where both before (t < 0) and at the switch moment (t = 0), the playback frequency differed by > 3 kHz from the average pre-switch bat frequency. These criteria resulted in 24 sequences in the control dataset (673 total calls), for which we did not expect a jamming avoidance response to occur after the switch (t > 0), because of the larger frequency separation between the playback and the bat calls at t = 0.

Selection of a value of > 3 kHz for delineating these subsets of the data was informed by the results of the static-stimulus and dynamic-stimulus experiments, as described below.

For the population analyses of the frequency shifts, we computed for each individual sequence the differences between the frequency of each bat call and the corresponding average pre-switch bat frequency. These differences are, by definition, 0 kHz before the switch ($t < 0$), so that any post-switch frequency shift will be expressed as a deviation from 0 kHz. We then pooled all the 39 sequences of the main dataset, or 24 sequences of the control dataset, and grouped these data into 1-s time bins. For each time-bin we then computed the following three average frequency values: (1) average for all the 39 sequences of the main dataset or 24 sequences of the control dataset; (2) average only for the sequences in which at $t = 0$ the bat was calling at a frequency above the post-switch playback signal ($f_{playback} < f_{bat}$), and (3) average only for the sequences in which at $t = 0$ the bat was calling at a frequency below the post-switch playback signal ($f_{playback} > f_{bat}$). We plotted the data only for time-bins that included ≥ 25 calls per bin in *all* these 3 averages.

The inter-call interval of search-phase calls in the dynamic-stimulus experiment had an average of 227 ± 55 ms (mean ± s.d.). Averages were calculated over intervals < 350 ms, to remove potential bias due to missed calls. Averaging over intervals < 500 ms resulted in an average inter-call interval of 266 ± 89 ms.

Measurement of pulse parameters

After the conclusion of the experiments, and following selection of all sequences for analysis, we extracted data from each selected file by digitally high-pass filtering the recording using a finite impulse response filter with 5-kHz cutoff, and computed the spectrogram (frequency × time representation) using a 1024-point Fast Fourier Transform (93.75% overlap). For the 166-kHz sampling rate we used, this gave a 162-Hz frequency resolution.

We excluded all the playback calls, which were easily identified based on their inter-call interval and spectro-temporal shape, both of which were highly reproducible due to our usage of a single replicated call with a fixed interval. From the spectrogram of the search-phase calls of the bats, we measured (*i*) minimum frequency and (*ii*) maximum frequency, defined as the lowest and highest frequencies above the background noise, respectively (Figure 5a) (both of these measurements also corresponded well with –20 dB points below the maximal peak of the power spectrum, data not shown), (*iii*) call bandwidth, defined as the maximum frequency – minimum frequency, and (*iv*) inter-call interval, defined as the time between the onsets of consecutive calls. Unless otherwise stated, we used the minimum frequency in our analyses because *a*) calls at lower frequencies are less subject to atmospheric attenuation and signal degradation than are calls at higher frequencies (Lawrence & Simmons 1982), and *b*), the quasi-constant-frequency region near the lowest frequency of the call allows for more precise measurement of minimum frequency than is possible for the higher frequency portions of the call. From the oscillogram, we measured the call amplitudes for the 39 sequences in our main dataset. Finally, we returned to the spectrograms of the original recordings and measured the numerical values of the minimum frequencies of the playback calls (22.3, 24.3, 26.3, 27.3, 27.8, and 28.3 kHz; Figure 5b), using the same methods and same settings that were used for measuring the bat calls (1024-point Fast Fourier Transform, 93.75% overlap).

Doppler shift estimation (dynamic-stimulus experiment)

To estimate the effect of the Doppler shift caused by the bat approaching or flying away from our microphones, we used the following values: average flight speed during foraging, $v = 6$ m/s (Hayward & Davis 1964) (minimal reported flight speeds are 5 m/s, Vaughan 1966); average frequency of all bat calls in the main dataset of the dynamic-stimulus experiments, $f = 25.22$ kHz; speed of sound, $c = 331.4$ m/s. These values were substituted into the formula of the relative Doppler shift between an approaching bat ($t << 0$) and a bat flying away ($t >> 0$): $2 \times v \times f / c$, yielding a difference value of 0.91 kHz for an approaching bat versus a bat flying away from the microphone.

Statistical tests

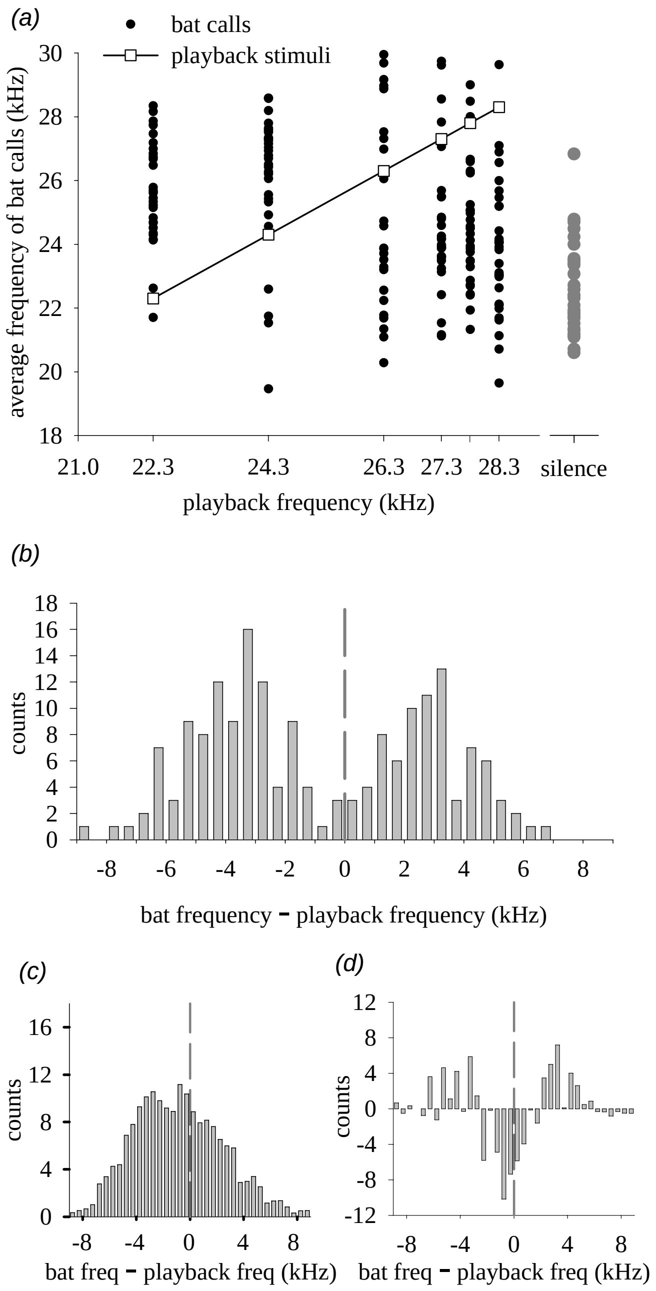
For the Monte-Carlo simulations of the static-stimulus experiment, we randomly reshuffled the playback frequency associated with each bat-call frequency, and calculated a new set of frequency differences. This random reshuffling was repeated 1000 times. We constructed histograms of the real and the simulated data using 0.5-kHz bins between –9 and 9 kHz. For the simulated data, we divided the counts by the number of permutations used (n = 1000) in order to create an identical sample size for both distributions (n = 180). We then performed a χ^2 test to compare the real and the simulated distributions (the test's results were similar with other bin-sizes). The simulations were done using Matlab (Mathworks, Natick, MA, USA). For this and all other statistical tests, we used a $p < 0.05$ significance level.

RESULTS

Static-stimulus experiment

A scatter plot of the average frequency in each sequence of bat calls, versus the corresponding playback frequency, indicated that the bat calls were usually displaced above or below the frequency of the playback stimuli (Figure 6a). To quantify this observation, we performed three analyses. First, we pooled data from the two lowest-frequency playbacks (22.3 and 24.3 kHz) into a 'low' group, and data from the two highest-frequency playbacks (27.8 and 28.3 kHz) into a 'high' group (Figure 6a, two left-most versus two right-most columns of black dots). Average call frequency differed between the low and high groups, with bats exhibiting higher-frequency calls in the presence of lower-frequency playbacks (two-tailed t-test: $t = 4.37$, $df = 118$, $p < 0.0005$).

Figure 6. Static-stimulus experiment. (*a*) Frequency of bat calls versus frequency of playback stimuli. Each dot represents the average frequency of one sequence of search calls, recorded from one bat; black dots: recorded bat calls, n = 180. Gray dots: ‘Silence’ control (n = 30). Open squares: The six frequency values used for the search-phase playback stimuli. (*b*) Distribution of frequency-difference values (average bat frequency – playback frequency), pooled over all the 6 playback frequencies (n = 180); bin size, 0.5 kHz; vertical dashed line indicates zero. (*c*) Distribution of reshuffled frequency-difference data from the Monte-Carlo simulation. (*d*) Difference between the original histogram in *b* and the Monte-Carlo simulation in *c*.


(a)
bat calls
playback stimuli
average frequency of bat calls (kHz)
18
20
22
24
26
28
30
21.0
22.3
24.3
26.3
27.3
28.3
silence
playback frequency (kHz)
(b)
counts
0
2
4
6
8
10
12
14
16
18
-8
-6
-4
-2
0
2
4
6
8
bat frequency − playback frequency (kHz)
(c)
counts
0
4
8
12
16
-8
-4
0
4
8
bat freq − playback freq (kHz)
(d)
counts
-12
-8
-4
0
4
8
12
-8
-4
0
4
8
bat freq − playback freq (kHz)

Second, we subtracted the frequency of the playback from the frequency of the bat's calls, and constructed a histogram of these differences (Figure 6b). This histogram showed a bimodal distribution of the frequency differences, with a trough near zero and peaks on either side of zero. This pattern indicates that most bats did not call at or near the frequency of the playback, suggesting a jamming avoidance response. Monte Carlo simulations of randomly reshuffled frequency differences (Figure 6c; see Methods) showed a unimodal distribution that was significantly different from the bimodal distribution of our data (χ^2 test: $X^2 = 69.57$, $df = 35$, $p < 0.0005$). This suggests that the trough near zero (Figure 6b) is real, and provides evidence for a jamming avoidance response in the presence of conspecific calls.

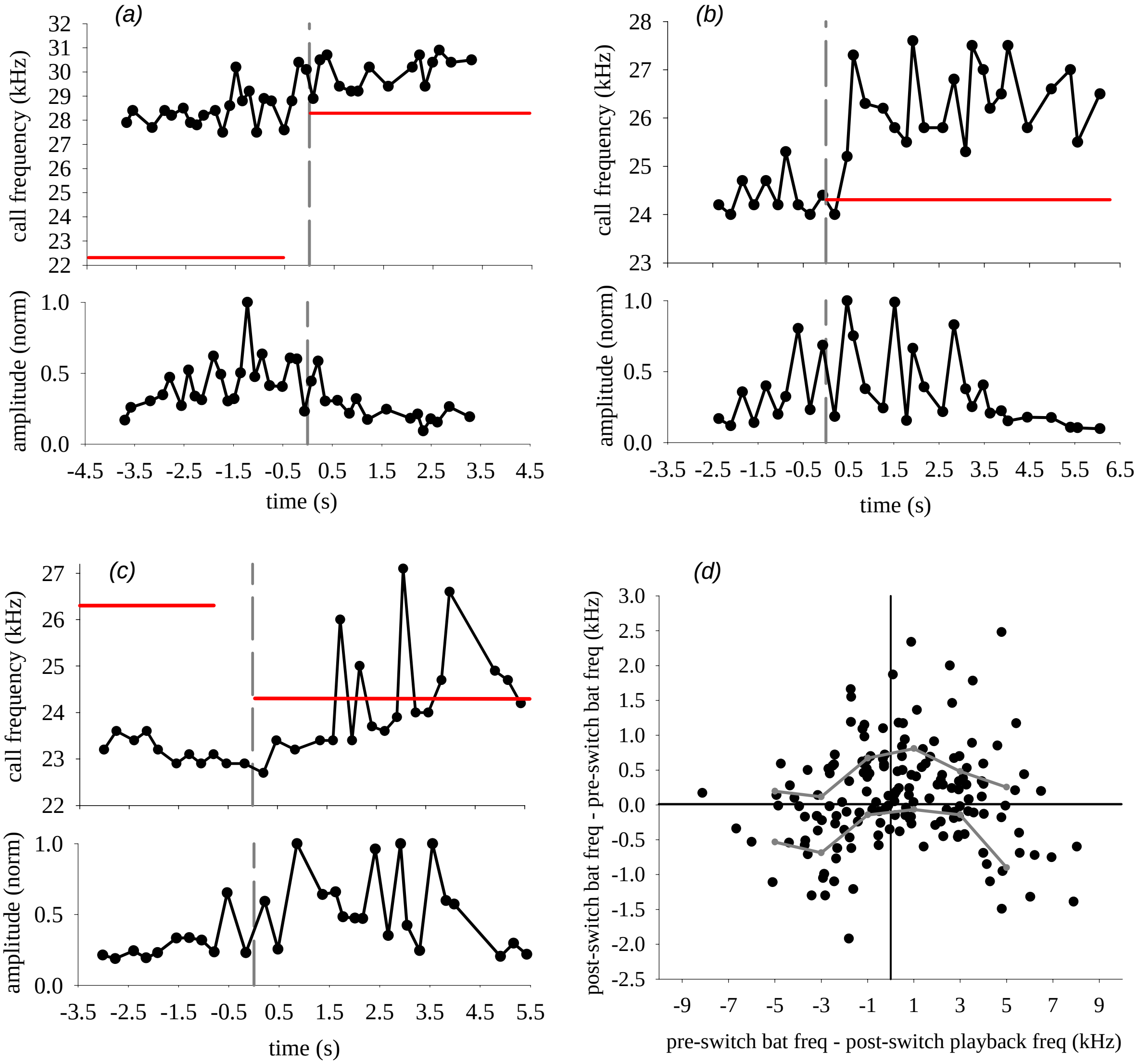
Finally, the call frequencies used by bats in the presences of the 'silence' control (Figure 6a, gray dots) were significantly lower than the frequencies used by bats in the presence of any of the six playback stimuli (black dots) (one-sided t-test: $t > 4.27$, $df = 58$, $p < 0.0001$, individually for five of the six comparisons, with the 28.3-kHz playback yielding $t = 2.61$, $p = 0.0057$; all six t-tests remained significant after application of a Bonferroni correction for multiple comparisons, which yields a significance threshold of 0.0083). This suggests that in the presence of playback calls, the bats tended to shift their call frequencies upwards rather than downwards. Another asymmetry in the bats' behavior is seen in Figure 6d, which shows the difference between the real and the Monte-Carlo-simulated data: Although bats employed both positive and negative frequency shifts, the bats seemed to avoid particularly the frequencies below the playback stimulus, i.e., a larger portion of the frequency differences forming the trough was to the left from 0 than to the right from 0 (Figure 6d, sign test for the number of sequences between –3 and 0 kHz *vs.* their number between 0 and +3 kHz: $p < 0.02$). We will return to these asymmetries later.

<u>Dynamic-stimulus experiment</u>

Sequences of bat call frequencies collected in the dynamic stimulus experiment (Figure 7a-b, top panels), illustrate that the bats shifted their call frequencies upwards in response to the stimulus-switch at t = 0. In Figure 7b, the initial rapid shift upwards was larger than 3 kHz. Note, also, the gradual increase in the amplitude of the calls as the bat approached the microphone, and then the gradual decrease as it flew away (Figure 7a-b, bottom panels).

The average frequency difference between the post-switch and pre-switch bat calls plotted *vs* the frequency difference between the pre-switch bat call and the post-switch playback stimulus (Figure 7d suggested the following: If at t = 0 there is a small frequency-difference between the playback and the bat frequency (x-axis < ±1.75 kHz), the bats shifted their call frequencies, and these shifts are mostly upwards; however, if at t = 0 there was a larger frequency-difference between the playback and the bat frequency (x-axis > ±3.0 kHz), the bats did not shift frequencies. This was the motivation for dividing our sequences into a 'main dataset,' with x-axis between ±1.75 kHz, and a 'control dataset,' with x-axis larger than ±3.0 kHz, as described above (see Methods).

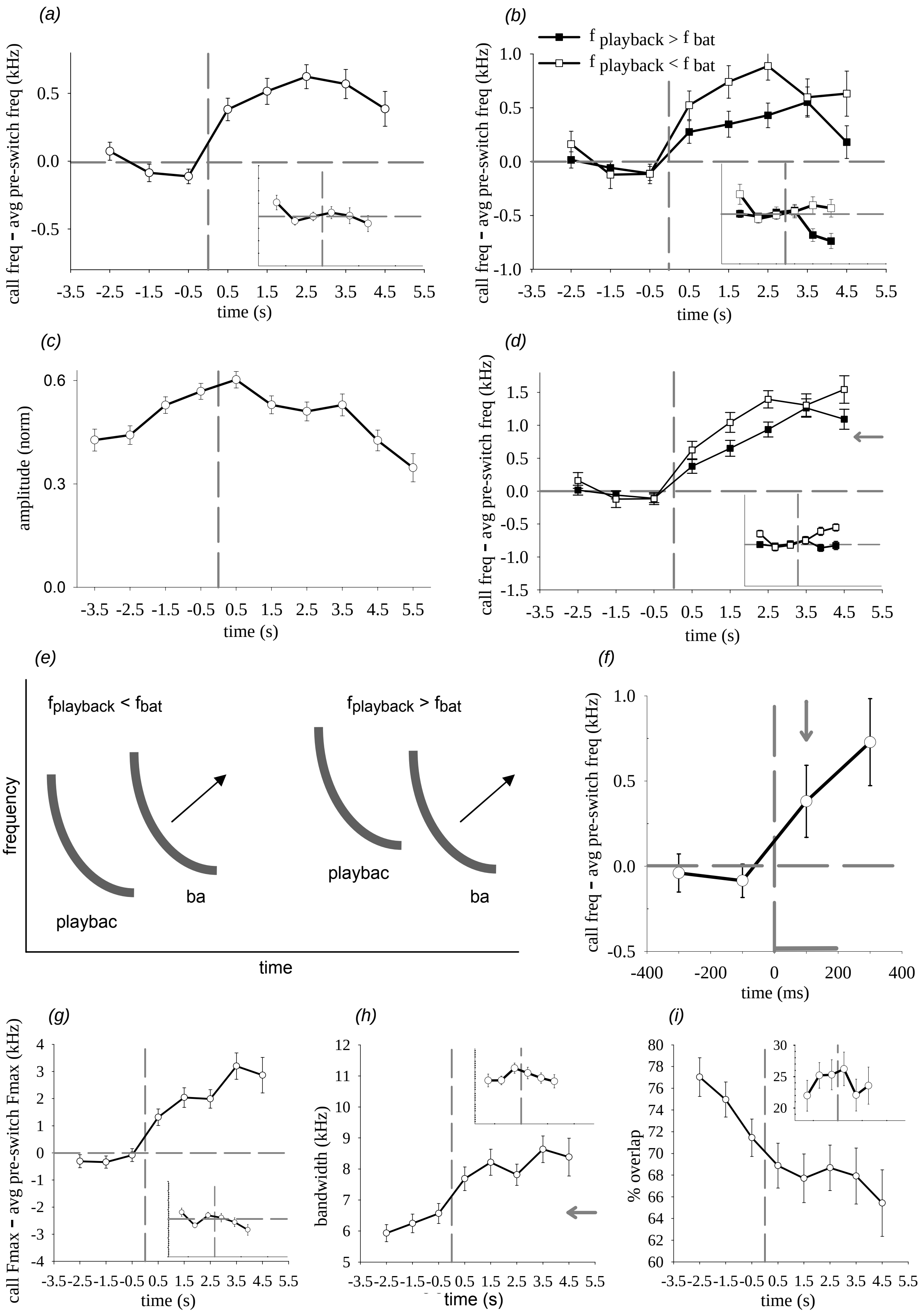
Figure 7. Dynamic-stimulus experiment: Examples. (*a*-*b*) Examples of recorded sequences of bat search calls, where the bats adjusted their call frequency in response to the playback. Top panels: Call frequency versus time; red lines indicate the playback frequency. Bottom panels: Normalized call amplitudes versus time, showing the gradual increase in recorded amplitude as the bat approached the microphone, and then the gradual decrease as the bat flew away. (*a*) The playback stimulus switched at t = 0 from a frequency of 22.3 kHz to 28.3 kHz (red lines); t = 0 (vertical dashed line) corresponds to the start of the post-switch stimulus. (*b*) The playback stimulus switched at t = 0 from 'silence' to a frequency of 24.3 kHz. (*c*) The playback frequency switched at t = 0 from 26.3 to 24.3 kHz. Here the bat slowly shifted its frequency upward, eventually 'jumping' over the playback frequency. (d) Population graph showing for each sequence (dots) the bat's frequency shift at t > 0 compared to t < 0 (y-axis) versus the frequency difference between the bat calls and the playback stimulus (x-axis). We included in this plot all sequences in which no 'silence' stimuli occurred before or after the switch (n = 164/200 sequences). Gray lines, 25th and 75th percentiles of the y-values of the dots, computed in 2-kHz bins along the x-axis.


(a)
(b)
(c)
(d)
call frequency (kHz)
amplitude (norm)
time (s)
32
31
30
29
28
27
26
25
24
23
22
1.0
0.5
0.0
-4.5
-3.5
-2.5
-1.5
-0.5
0.5
1.5
2.5
3.5
4.5
5.5
6.5
post-switch bat freq - pre-switch bat freq (kHz)
pre-switch bat freq - post-switch playback freq (kHz)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-9
-7
-5
-3
-1
1
3
5
7
9

Population analysis of the main dataset (Figure 8a) demonstrated that bats made rapid changes to the frequencies of their calls when the playback stimulus was switched at $t = 0$ to within a small frequency-difference (< 1.75 kHz) from the bat's frequency. Such changes were not observed in the control dataset, where the frequency shift of the playback stimulus was to within > 3.0 kHz from the bat's frequency (Figure 8a, Inset). Very similar results were obtained in the subset of sequences in which the stimulus was switched from 'silence' to a playback frequency that was close to the bat's frequency (data not shown). In other words, the response of the bats was frequency-specific, occurring only when the post-switch playback frequency was close to the bat's frequency – suggesting a jamming avoidance response.

To examine the effect of having a positive versus negative initial frequency difference between the playback and the bat calls, we decomposed the dataset into two groups of sequences (Figure 8b), based on whether the average pre-switch bat frequency was above the post-switch playback frequency (open squares) or below it (closed squares). The bats that used frequencies above the playback (open squares) shifted their call frequencies upwards, away from the playback, as expected from a jamming avoidance response. However, many bats that used frequencies below the playback (closed squares) also shifted their frequency upwards – *towards* the playback frequency. Comparison of the average frequency at $t > 0$ versus $t < 0$ showed that 100% of the sequences in the $f_{playback} < f_{bat}$ group exhibited an upward frequency shift (14/14 sequences, sign test: $p < 0.0005$), and 72% of the sequences in the $f_{playback} > f_{bat}$ group also

Figure 8. Dynamic-stimulus experiment: Population analysis. (*a*) Average frequency-difference values versus time. Main dataset is shown in the main plot, while the control dataset is shown in the inset. Averages were obtained by aligning the sequences at the start of the post-switch stimulus (t = 0), pooling all sequences and grouping them into 1-s time bins. Errorbars denote mean ± s.e.m. Vertical dashed line, t = 0; horizontal dashed line, frequency-difference = 0. (*b*) Average frequency-difference versus time, using the same data as in figure 8a, decomposed based on whether the post-switch playback frequency was above the average pre-switch bat frequency (closed symbols) or below it (open symbols). (*c*) Average normalized amplitude of recorded bat calls, computed for the main dataset. (*d*) Data from figure 8b corrected for Doppler shift. Gray arrow: Average frequency difference between the playback frequency and the bat frequency, for the group of sequences where $f_{playback} > f_{bat}$. (*e*) Schematic of a spectrogram summarizing the bats' responses in the dynamic-stimulus experiment. When the playback frequency is below the bat's frequency ($f_{playback} < f_{bat}$, left), the bat shifts its frequency upwards, away from the playback; when the playback frequency is above the bat frequency ($f_{playback} > f_{bat}$, right), the bat also shifts its call frequency upward, towards and beyond the playback frequency. (*f*) Data from Figure 8a replotted on a finer time-scale (bin size = 200 ms). Arrow: First time-bin that showed a significant upwards frequency shift by the bats. (*g*) Average frequency-difference versus time, using the maximal frequency, Fmax. (*h*) Average call bandwidth versus time. Arrow shows the bandwidth of the playback call, 6.6 kHz.. (*i*) Average frequency overlap between the bat calls and the playback, defined as the percent of the bat call bandwidth overlapped by that of the playback. Inset, control dataset.


(a)
call freq – avg pre-switch freq (kHz)
0.5
0.0
-0.5
-3.5 -2.5 -1.5 -0.5 0.5 1.5 2.5 3.5 4.5 5.5
time (s)
(b)
f playback > f bat
f playback < f bat
1.0
0.5
0.0
-0.5
-1.0
call freq – avg pre-switch freq (kHz)
-3.5 -2.5 -1.5 -0.5 0.5 1.5 2.5 3.5 4.5 5.5
time (s)
(c)
amplitude (norm)
0.6
0.3
0.0
-3.5 -2.5 -1.5 -0.5 0.5 1.5 2.5 3.5 4.5 5.5
time (s)
(d)
call freq – avg pre-switch freq (kHz)
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-3.5 -2.5 -1.5 -0.5 0.5 1.5 2.5 3.5 4.5 5.5
time (s)
(e)
$f_{playback} < f_{bat}$
$f_{playback} > f_{bat}$
frequency
playbac
ba
playbac
ba
time
(f)
call freq – avg pre-switch freq (kHz)
1.0
0.5
0.0
-0.5
-400 -200 0 200 400
time (ms)
(g)
call Fmax – avg pre-switch Fmax (kHz)
4
3
2
1
0
-1
-2
-3
-4
-3.5 -2.5 -1.5 -0.5 0.5 1.5 2.5 3.5 4.5 5.5
time (s)
(h)
bandwidth (kHz)
12
11
10
9
8
7
6
5
-3.5 -2.5 -1.5 -0.5 0.5 1.5 2.5 3.5 4.5 5.5
time (s)
(i)
% overlap
80
78
76
74
72
70
68
66
64
62
60
30
25
20
-3.5 -2.5 -1.5 -0.5 0.5 1.5 2.5 3.5 4.5 5.5
time (s)

exhibited an upward frequency shift (18/25, sign test: $p < 0.05$). These upward frequency shifts were maintained almost as long as we could reliably record the bats as they flew away from the speaker (on average, up to $t = 3.5$ s). No upward frequency shifts were observed in the control dataset, and a downward shift was observed for the control group with $f_{playback} > f_{bat}$ (Figure 8b, Inset).

Two possible explanations can be invoked for the counter-intuitive frequency shift of bat calls towards the playback frequency. First, this may be an artifact caused by the Doppler shift due to the bats' motion. Second, the bats may have been shifting their frequencies towards and *beyond* the playback frequencies, perhaps in order to 'jump' over the playback frequency: Figure 7c shows an example of a recorded bat sequence where this seems to be the case, with the bat slowly shifting its call frequencies upwards, eventually reaching frequencies higher than the playback.

To determine the magnitude of the Doppler shift due to the bats' motion, we first plotted the average amplitudes of recorded bat calls (Figure 8c): These amplitudes increased as the bat approached our recording system ($t \sim 0$), remained high as the bat flew near our system, and then decreased as the bat flew away ($t \geq 3.5$ s). Because we performed the frequency-switch of the playback as the bat was approaching the microphones (Figure 8c, $t = 0$ is on the rising phase of the amplitude curve), this meant that at times $t << 0$ there was a positive Doppler shift from the approaching bat – so the actual pre-switch frequencies were *lower* than what we recorded. Conversely, because of the negative Doppler shift for a bat flying away, the post-switch frequencies were *higher* than those recorded. Using the estimate of a Doppler shift of 0.91 kHz for a bat approaching the microphone (t << 0) versus a bat flying away (t >>0; see Methods), we re-plotted the data from Figure 8b with a linear rise in the Doppler shift from a 0-kHz shift at t = 0 to a 0.91-kHz shift at t = 4.5 s (Figure 8d). A linear change in the Doppler shift was used because we did not know the direction of the bat's flight immediately after t = 0. Therefore, this estimate may be inaccurate at t ~ 0, but at t >> 0 it provides a reasonable approximation of the Doppler shift. The main point conveyed by Figure 8d is that the Doppler-corrected frequency shift (Figure 8d) was even *larger* than our initial measurements (Figure 8b).

Next, to determine whether the bats indeed shifted their frequency *beyond* the playback frequency for sequences with $f_{playback} > f_{bat}$ (Figure 8d main plot, closed squares), we computed the average value of $f_{playback} – f_{bat}$ for these sequences, using pre-switch f_{bat} and post-switch $f_{playback}$. This frequency difference was 0.82 kHz. We then plotted this difference value in Figure 8d (gray arrow). Since the bat frequencies after the switch were *above* the gray arrow (Figure 8d closed squares, t = 3.5 and 4.5-s bins), this demonstrated that the bats shifted their frequency not only towards, but also beyond the playback frequency. For the $f_{playback} > f_{bat}$ group, we also directly examined individual call sequences for evidence of upward shifts, calculating the percentage of sequences in which the bat's call frequency was above the playback frequency presented to the bat. For time-bins 0.5, 1.5, 2.5, 3.5 and 4.5 s, these percentages were 29%, 42%, 48%, 65% and 63%, respectively. This demonstrates that after 5 seconds there was an increase of > two-fold in the number of bats calling above the playback signal, with the majority of sequences surpassing the playback frequency by the last two time bins.

Figure 8e shows a schematic summarizing this behavior of the bats. When the bats used call frequencies above the playback (Figure 8e, left), they shifted their call frequencies upwards, away from the playback. When the bats used call frequencies below the playback (Figure 8e, right), they also tended to shift their call frequencies upwards, towards and beyond the playback – ‘jumping’ over the playback frequency.

Finally, to address how quickly the bats reacted to the stimulus-switch, we reexamined the dataset from Figure 8a using smaller, 200-ms, time bins rather than 1-s bins (Figure 8f). This higher temporal resolution demonstrates that a significant upward frequency shift was apparent already in the first time-bin after the switch (Figure 8*f*, arrow; one-sided t-test for this bin: $t = 1.80$, $df = 30$, $p < 0.05$). This bin was centered at t = 100 ms, and spanned the times from t = 0 to 200 ms (gray horizontal bar). Therefore, on average, the bats shifted their call frequencies upwards within less than 200 ms. Because the bats’ average inter-call interval during search phase was 227 ± 55 ms (mean ± s.d.), this means that many bats shifted their call frequencies upwards already in their first call after the stimulus-switch.

Other changes in call structure

Components of calls other than the minimum frequency also changed in response to the playback stimuli. A plot of the changes in the *maximum frequency* of the bat calls, Fmax, shows that at t > 0 the bats rapidly shifted their Fmax upwards, with an average shift of +3 kHz at t = 3.5 s (Figure 8g). The *bandwidth* of the bat calls also increased at t > 0, as seen in Figure 8h (t-test of –0.5 s time-bin versus 3.5 s time-bin in Figure 8h: $t = 3.92$, $p < 0.0002$). In the static experiment, the bandwidth also increased in the presence of playbacks compared to the 'silence' condition (bandwidth = 6.74 ± 2.30 kHz, mean ± s.d., compared to 4.15 ± 1.93 kHz for 'silence'; t-test: $t = 5.86$, $p < 0.0001$). The increase in bandwidth, combined with the upward frequency shift, suggests that the bats were decreasing the *frequency overlap* between their calls and the playback stimuli. A slight, but significant decrease in the frequency overlap, from 71% to 65% overlap, was indeed observed between the t = –0.5 s time-bin and the t = 4.5 s time-bin (Figure 8i, one-sided t-test: $t = 1.35$, $p < 0.05$).

Measures of non-frequency call parameters showed that the *amplitude* of the calls did not increase after the stimulus-switch in the dynamic experiment (no stepwise increase in amplitude at t = 0 in Figure 8c), indicating that the bats did not increase their call loudness in response to the playback. However, the *inter-call interval* was slightly and significantly shorter in the static-stimulus experiment under the playback *versus* the 'silence' conditions (mean ± s.d. = 247 ± 34 *versus* 262 ± 17 ms, respectively; two-sided t-test: $t = 2.12$, $df = 197$, $p < 0.05$), suggesting that the bats increased their call-rate in the presence of conspecifics. The *duration* of the calls also was shorter under the playback *versus* the ‘silence’ conditions (11.8 ± 1.6 *versus* 13.7 ± 0.9 ms; two-sided t-test: $t = 5.81$, $df = 197$, $p < 0.0001$). As a result the *duty cycle*, defined as the percentage of time when a bat is calling, was not significantly different between the playback and the ‘silence’ conditions (duty cycle: 4.98 ± 0.97 and 5.33 ± 0.74 percent, respectively; 2-sided t-test: $t = 1.72$, $df = 197$, n.s.). This suggests that the bats did *not* increase the redundancy of their signals, an increase that has been previously reported as a response to noise in other taxa (e.g. Lengagne *et al.* 1999).

In summary, the jamming avoidance response in *T. brasiliensis* consisted of several changes to the bats’ calls, including an upward frequency shift, increase in bandwidth, decrease in duration, slight decrease in spectral overlap between the bat call and the jamming call, and an increase in call-rate.

DISCUSSION

Jamming avoidance response and its role in electrolocation have been well documented in a number of weakly electric fish, particularly the knife fish *Eigenmannia* (Watanabe & Takeda 1963; for reviews see Heiligenberg 1991; Metzner 1999). Here, we provide the first direct experimental evidence for jamming avoidance in echolocating animals. Our ‘static stimulus experiment,’ where we presented playbacks of pre-recorded calls shifted to one of six frequencies, demonstrated that free-flying bats (*Tadarida brasiliensis*) avoided using frequencies that were close to the presented stimulus frequency, creating a notch in the distribution of used frequencies (Figure 6b). A causal link between stimulus and response was demonstrated in the 'dynamic stimulus experiment,' which involved abruptly switching the playback stimulus as a bat approached our recording equipment. Here, bats clearly exhibited a jamming avoidance response, by shifting their call frequencies upwards (Figures 3, 4). Surprisingly, the bats that originally used frequencies *below* the playback frequency also shifted upwards, 'jumping' over the frequency of the playback stimulus (Figure 8d). Finally, we showed that the jamming avoidance response was very rapid, with the bats shifting their frequencies within < 200 ms of the stimulus-switch (Figure 8f).

Comparisons to previous studies of jamming avoidance

Several previous studies in echolocating bats have provided evidence that some bat species which produce frequency-modulated (FM) signals ('FM bats') shift their call frequency in response to conspecifics (Habersetzer 1981; Miller & Degn 1981; Obrist 1995; Surlykke & Moss, 2000; Ibanez *et al.* 2004; Ratcliffe *et al.* 2004; Ulanovsky *et al.* 2004). This includes the species studied here (Ratcliffe *et al.* 2004) and the related species *Tadarida teniotis* (Ulanovsky *et al.* 2004). In species that produce constant-frequency (CF) signals, so-called 'CF bats,' no robust frequency shifts have been found (Jones *et al.* 1994) – but this may be expected, since auditory neurons in CF bats have an extremely narrow-band tuning to the bat's call frequency (Suga *et al.* 1987), making spectral jamming less likely. Similarly, no shifts were found in the bat *Taphozous perforatus,* which is an FM bat that uses unusually narrowband calls (Ulanovsky *et al.* 2004).

In some of the previous studies of FM bats, the evidence for frequency shifts consisted of examples of recordings in which two or three bats were flying together and maintained particularly large frequency differences between their calls (Habersetzer 1981; Miller & Degn 1981; Surlykke & Moss 2000). Other studies have shown that groups of bats flying in the same area exhibit a larger variation in frequencies compared to ‘virtual groups’ constructed from calls of bats flying alone (Obrist 1995; Ibanez *et al.* 2004; Ratcliffe *et al.* 2004; Ulanovsky *et al.* 2004). The most extensive evidence for frequency shifts involved a recent study of *Tadarida teniotis* (Ulanovsky *et al.* 2004), which suggested long-term ‘static’ frequency shifts as well as more rapid dynamic shifts within a ~1-s timescale, when two bats were flying together. Interestingly, several of these previous studies have indicated a bias for upward frequency shifts (Obrist 1995; Ibanez *et al.* 2004; Ulanovsky *et al.* 2004), similar to the current study.

Although the frequency differences observed in previous studies can be interpreted as a jamming avoidance response, other interpretations are likely, particularly because echolocating bats are known to shift their call frequencies under a variety of circumstances, such as when approaching a cluttered environment (Kalko & Schnitzler 1993). For example, bats flying in groups may fly at different speeds compared to solitary bats, or at different heights, or at different distances from vegetation – all of which may aid in collision avoidance. Therefore, changes in call design reported in previous studies (Habersetzer

1981; Miller & Degn 1981; Obrist 1995; Surlykke & Moss, 2000; Ibanez *et al.* 2004; Ratcliffe *et al.* 2004; Ulanovsky *et al.* 2004) may have been due to these or other behavioral factors, rather than to a jamming avoidance response to the conspecific calls. Because of the lack of experimental manipulations, these studies do not provide information about the behavioral significance of any observed frequency shifts. Moreover, several methodological difficulties were inherent to all previous studies, which relied on recording the calls of free-flying bats and then using a post-hoc correlation analysis of call parameters. First, the locations of the recorded bats relative to each other and to the recording microphone were unknown, so it was unclear whether the bats were approaching or departing from each other, which may influence whether jamming avoidance was to be expected at all. Second, because experimental manipulations were not used, there was no 'time zero' around which to measure any presumed frequency changes, confounding the analysis of any dynamic frequency shifts.

In the current study, explicit experimental manipulations allowed us to overcome these methodological limitations. By switching the playback frequency as the bat approached our speaker, and by then aligning the analysis to the switch-time (time zero), we provide the first demonstration that frequency shifts are causally linked to experimental playback stimuli. These frequency shifts were very rapid, occurring in some bats within less than 200 ms, suggesting that these frequency shifts are not caused by factors such as changes in the bat's height or the level of ultrasonic clutter, which are unlikely to change appreciably within 200 ms – but were, in fact, induced by the playback calls themselves.

The jamming avoidance described in this study differs from the jamming avoidance response in the electrolocation system of weakly electric fishes in that jamming avoidance in fishes typically develops slowly, sometimes over a few tens of seconds (e.g. Kawasaki, 1997), in contrast to the very rapid frequency shifts that occurred in the bats. In other respects, the asymmetric response that we report for the bat *T. brasiliensis*, which shifted its frequencies mostly upwards, is similar to some species of fish (Apteronotidae) that also exhibit an asymmetric response, always shifting their discharge frequency upwards (Heiligenberg *et al.* 1996). However, in weakly electric fishes, the picture is known to be more complex, as other species (Eigenmannidae) exhibit a symmetric jamming avoidance response, shifting their frequency upwards when encountering a lower-frequency conspecific signal and shifting downwards when encountering a higher-frequency signal (Heiligenberg 1991). Other species of echolocating bats may also exhibit a symmetric jamming avoidance response, similar to that of the weakly electric fish *Eigenmannia*.

Hypotheses accounting for jamming avoidance in the bat

Several explanations may account for the upward shifts in the bats' call frequencies. First, the bats may be exhibiting a vocal startle response to the playback stimuli (a 'surprise response'), rather than be avoiding jamming, and this startle response may be expressed as an upward frequency shift. However, the long duration of the response, lasting several seconds – as long as we could record the calls (Figure 7a,b) – suggests that these frequency shifts do not reflect an instinctive, transient, startle response. Second, if the bats are avoiding jamming, they may prefer to shift their frequencies upwards rather than downwards if they have more sensitive hearing at above-average frequencies than at below-average frequencies. However, this explanation is unlikely because published audiograms of *T. brasiliensis* suggest that the hearing of this bat is most sensitive over a wide frequency range between 10-40 kHz (Henson Jr. 1970), covering frequencies both above and below the bat's dominant frequency. Third, the observed increase in Fmax (Figure 8g) and bandwidth (Figure 8h) after the stimulus switch could reflect an attempt by the bats to specifically avoid jamming of their highest call frequencies. Yet, similar increases in these call parameters are often noted when bats are attempting to gain more detailed information about their environment, such as when foraging in the presence of vegetative clutter (Obrist 1995). Brazilian free-tailed bats often forage in the presence of multiple conspecifics (Ratcliffe *et al.* 2004), so increasing the Fmax and bandwidth would provide a foraging bat with more precise information about the location of nearby conspecifics, which may be helpful for reducing mid-air collisions. Fourth, the bats may be changing their calls in order to minimize the frequency overlap with the playback stimuli, as reflected by the significant decrease in overlap that was observed following the stimulus switch (Figure 8*i*). However, the decrease in overlap was small in size, from 71% overlap just before the switch (at t = –0.5 s) to 65% overlap long after the switch (at t = 4.5 s). This small decrease was most likely caused by the increased bandwidth of post-switch calls rather than by frequency shifts, and it suggests that the bats were not attempting to substantially reduce the frequency overlap.

A fifth hypothesis, that can account for the results of both the static-stimulus and dynamic-stimulus experiments, is that the jamming power of playback calls is not uniform across frequencies – but that the narrowband, so called ‘quasi-constant-frequency’ (QCF) component that occurs near the end of the playback call (Figure 5a), produces the most effective jamming. Two factors may add to the jamming potency of the QCF part of the playback call. First, this part of the playback call is relatively long in duration (see Figure 5a). Second, it contains lower frequencies, which are least subject to atmospheric attenuation (Lawrence & Simmons 1982). Therefore, we propose that the bat’s sonar is most jammed if the lowest (QCF) frequency of the *playback* call is anywhere within the bandwidth of the bat’s own call. This hypothesis explains why in the dynamic-stimulus experiment the bats tended to shift their frequencies upwards, above the playback frequency (Figure 8d) – because an upward shift puts the QCF part of the playback call below the bandwidth of the bat’s own call.

This hypothesis also accounts for several results of the static-stimulus experiment. First, the hypothesis explains why, compared to the ‘silence’ condition, the bats preferentially shifted their frequency upwards when the playback frequencies were presented (Figure 6a). Second, the hypothesis suggests that the bats’ frequencies should form an asymmetric ‘hole’ mostly *below* the playback frequency, because the bats calling below the playback would shift their frequencies upwards, above the playback frequency. This asymmetry in the ‘hole’ was observed in the static-stimulus experiment (Figure 6d). The hypothesis does not explain however the finding that bats used lower call frequencies when presented with higher playback frequencies, which is the opposite of what we might expect (Figure 6a) – although this could reflect a physical limit of the bats’ ability to shift their frequency upwards when presented with the highest playback frequencies. Thus, our hypothesis explains the results of the dynamic-stimulus experiment (Figures 3, 4), as well as most of the results of the static-stimulus experiment (Figure 6).

In conclusion, several intriguing questions remain. For example, what happens when two bats approach one another: do they both shift their frequencies upwards? What, if any, are the rules that govern their ‘group behavior’ under such conditions? One way to address these questions experimentally is to use sequences of playback calls that do not have a fixed frequency as in this study, but rather change their frequencies across successive calls, according to the time-course reported here for the real bats. It would also be informative to digitally manipulate the lowest-frequency (QCF) and highest-frequency parts of the playback calls, in order to test the hypothesis that there are differential effects of various parts of the playback call on the bats’ behavior. These and other experiments could help elucidate the ability of echolocating bats to forage and avoid collisions when flying in high-density groups that often consist of tens or hundreds of bats (Adams & Simmons 2002) – an ability that is yet to be matched by man-made airborne radars.

CHAPTER IV

Flight in a Column Formation: Echolocation Calls of Brazilian Free-Tailed Bats, *Tadarida brasiliens*is, During Mass Emergence from Cave Roosts

My consistent use of “we” throughout this chapter is in reference to my co-authors, Gary McCracken, Nickolay Hristov, and myself. I was the primary contributor to this work, which involved the following tasks: (1) development of project design and collection of all acoustic data, (2) measurement of acoustic signals and all statistical analysis, (3) all gathering and interpretation of the relevant literature, and (4) all of the writing.

ABSTRACT

Brazilian free-tailed bats, *Tadarida brasiliensis*, emerging from cave roosts in tight serpentine columns must monitor the surrounding environment and avoid collisions with nearby conspecifics. The objectives of this research were to describe and quantify the structure of calls produced by emerging *T. brasiliensis* to assess how bats are able to effectively orient in a column formation. We recorded emergence calls from two roosts with approximate colony sizes of 350,000 and 35,000 bats. Brazilian free-tailed bats emit two distinct call types during emergence that we categorize as sweep and hook calls, both of which are significantly different from echolocation calls emitted by foraging bats. We propose that hook calls are used to localize the positions of nearby bats within the column, which is important for collision avoidance, and that sweep calls are used to gain information about predators and other objects in the relatively uncluttered environment outside of the column. Both call types exhibited significant structural differences between sites, although a detailed assessment of sweep calls found no relationship between call structure and the number of bats emerging from a roost, as quantified using thermal imaging technology. Site differences in calls may be associated with the spacing of bats during emergence.

INTRODUCTION

Numerous studies demonstrate that bats modify the structure of their echolocation signals under different behavioral and ecological conditions. As examples, bats alter call structure in response to the proximity of insect targets (Griffin 1958), the amount of vegetative clutter in an environment (Rydell 1990, Kalko and Schnitzler 1993, Obrist 1995), and the presence of conspecifics (Obrist 1995, Gillam et al. 2007). Since no call structure is ideal for all situations, flexibility will be critical for bats to adapt calls to fit differing demands for obtaining information (Obrist 1995).

A situation that has not been well-explored concerns if and how bats alter their echolocation calls during emergence flights from roosts. Bats exiting roosts in high densities face the dual challenge of avoiding collisions with other bats and evading predators (Lee and Kuo 2001). While short, broadband calls are ideal for precise localization of nearby conspecifics, long, narrowband signals will be best for long distance detection of avian predators. Characterization of emergence calls may provide insight into how bats avoid collisions and identify predators, thus accommodating the apparent need for two conflicting call structures.

Emergences of Brazilian free-tailed bats, *Tadarida brasiliensis*, from day roosts can range from a few individuals leaving a tree-hole or bat house to millions of bats exiting in huge densities from large limestone caves (Davis et al. 1962, Cockrum 1969). At larger colonies *T. brasiliensis* emerge in a tight serpentine column (Wilkins 1989), which appears to be a tactic for avoiding predation by raptors that commonly hunt near cave entrances (Lee and Kuo 2001). However, bats emerging in columns are separated from each other by very short distances and are at high risk of collision with other bats.

The foraging calls of Brazilian free-tailed bats consist of long, narrowband signals (typically 10-14 ms duration with a 2-15 kHz bandwidth) that are well suited for detecting prey at long distances in open environments (Simmons et al. 1979, Ratcliffe et al. 2004, Gillam et al. in press). Calls emitted by *T. brasiliensis* emerging from a building roost were substantially more broadband (35 kHz) and shorter in duration (7.1 ms) than foraging calls, and contained a constant-frequency (CF) component at the start of the call not previously reported in this species (Simmons et al. 1979). These calls were recorded under emergence conditions (i.e. bats exiting a small opening) that appear to be very different from those experienced at a large cave colony in which hundreds of bats emerge every second.

In this study, we document call structures used by Brazilian free-tailed bats during mass emergences from one large cave roost and one smaller cave roost in South Central Texas. Audio recordings were made simultaneously with thermal imaging video recordings of bats exiting each roost to investigate relationships between call structure and the number of emerging bats.

METHODS

We recorded emerging *T. brasiliensis* at two roosts in South Central Texas; 1) Ney Cave near Bandera, TX, which contains a large maternity colony estimated to contain 300,000 –400,000 bats (Betke et al. in prep, TH Kunz and N Hristov personal communication), and 2) the Bamberger Chiroptorium near Johnson City, TX, an artificial cave built in 1998 that currently houses approximately 30,000 – 40,000 Brazilian free-tailed bats during the spring and summer months (TH Kunz and N Hristov, personal communication).

Field recordings

Audio and thermal imaging recordings of emerging bats were taken at Ney Cave on 26 and 27 June 2006 and at the Chiroptorium on 2 July 2006. Recordings began at the time bats first exited the roost and continued until bats stopped exiting (Chiroptorium), or until an approximately equal number of bats were observed entering and exiting the roost (Ney Cave).

We obtained real-time audio recordings using Avisoft CM16 solid dielectric microphones (Avisoft Bioacoustics, Germany, frequency response of ± 3 dB between 10 and 100 kHz) mounted on light stands. We placed two microphones at Ney Cave, with one oriented perpendicular to the emerging column at a height of 1.5 m, and another placed directly below the column at a height of 1 m and oriented upwards. Due to the smaller colony size at the Chiroptorium, we only used one microphone, which we pointed perpendicular to the emerging column at a height of 1.5 m. At both sites, high-speed acoustic data acquisition was accomplished with Avisoft's Ultrasound Gate 416 connected to a Dell Inspiron laptop running Avisoft RECORDER. Recordings were 5-min long, but sampling was continuous as there was no time-gap between consecutive recording files. Recordings were made with 16-bit resolution and a 166 kHz sampling rate.

We used a FLIR/Indigo Systems Merlin Mid infrared thermal camera to image bats as they emerged from the roost. The camera acquired 12-bit intensity values in 320 x 240 digital video format at a rate of 60 frames per second, which was recorded directly to a computer hard drive (Kunz et al. in press). At both sites, the camera was oriented perpendicular to the emerging column. While we were able to obtain video of the entire column at Ney Cave, the landscape at the Chiroptorium prevented a complete census, and a small, unknown percentage of bats were not filmed. This counting error should not have affected our analyses, as video data was used only for relative comparisons.

Data measurement and analysis

After recordings were complete, we removed extraneous noise by digitally high-pass filtering all sound files using a finite impulse response filter with a 15-kHz cutoff. For analysis we selected only calls with a high signal-to-noise ratio in which signal structure could be accurately assessed. All measured calls were separated by a minimum of 5-s, although this interval was often greater. Due to the large number of individuals exiting the cave simultaneously and the fast flight speed of emerging bats (8.6 ± 1.6 m/s, J Reichard, personal communication), it is highly unlikely that calls recorded at >5-s apart were emitted by the same bat. We obtained acoustic measurements from spectrograms (frequency x time representation) computed using a 1024-point Fast Fourier Transform (93.75% overlap). For each selected call, we measured five variables; 1) duration, 2) maximum frequency (at the start of the call), 3) minimum frequency (at the end of a call), and 4) peak frequency (frequency of maximum energy) using Avisoft SasLab Pro (Avisoft Bioacoustics, Germany). We calculated 5) bandwidth by subtracting minimum frequency from maximum frequency for each measured call.

Methods for analyzing video data are described in Kunz et al. (in press) and Betke et al. (in review). Pixels from video recordings were identified as a bat with an adaptive filtering method that assessed changes in pixel values over time, such that significant deviations over short time periods indicated the presence of a bat. Bats were then tracked using a recursive Bayesian filtering method and a data-association algorithm that assigned new observations to previously established tracks. These methods allowed hundreds of bats to be simultaneously tracked and for accurate censuses of the numbers of bats exiting a roost. Data was outputted at 600-frame intervals, which yielded a count of the number of bats emerging every 10-s. Due to differences in the camera's field of view, we were unable to calculate the density of emerging bats, and instead report flow rates as the number of bats emerging in 10-s intervals.

We aligned our video and audio data so that each 10-s emergence count was associated with measurements of one 'sweep call' that was randomly selected from the same 10-s period; sweep calls were short, frequency-modulated (FM) signals that exhibited a sweeping structure common to the echolocation calls of most aerial-hawking bats (Figure 9a, Griffin 1958). We performed a series of linear regressions to determine if emergence count was associated with any of the call variables. Since call data were not normally distributed and common transforms did not fix this problem, we conducted a non-parametric bootstrap analysis. This analysis, which was conducted with NCSS (Statistical Systems, Kaysville, UT), used 3000 bootstrap samples and yielded bias-corrected R^2 estimates.

In addition to calls recorded during emergence, we obtained measurements from echolocation calls of free-flying *T. brasiliensis* foraging over a cotton field in the same region of South Central Texas (Gillam et al. in press). These calls were recorded and measured using the same equipment and methods described in this study. We selected one call from each of 30 sequences, which were most likely from different individuals (see Gillam et al. in press, details). Due to unequal sample size, unequal variances, and lack of normality, we used non-parametric bootstrap analyses for all comparisons between call types. We also used bootstrap comparisons to assess effects of location and date on call structure. Each analysis involved resampling the dataset 3000 times and computing a bootstrap p-value. Bootstrap comparisons

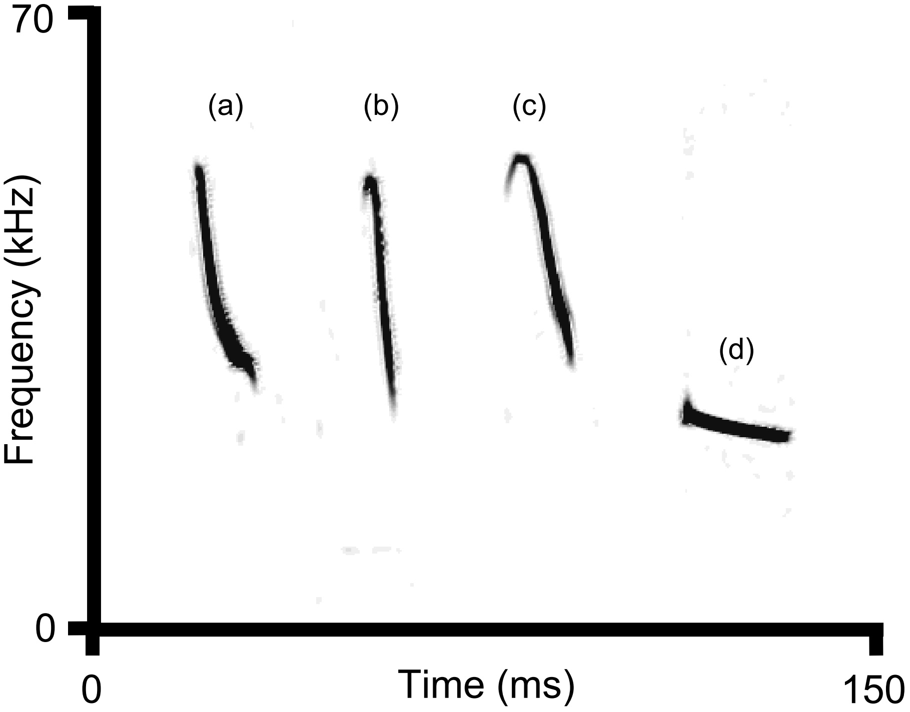


Figure 9. Spectrogram depicting an assemblage of recorded call types. (a) sweep call recorded during 26 June emergence at Ney Cave. (b) hook call with a small hook section recorded during 26 June emergence at Ney Cave. (c) hook call with a larger hook section recorded during 2 July 2006 emergence from Bamberger Chiroptorium. (d) echolocation call recorded from *T. brasiliensis* foraging over a cotton field in South Central Texas in May 2005.

were performed in SPSS 15.0 (SPSS Inc., Chicago, IL) using the BOOTDIFF macro (Hayes 2005).

RESULTS

On 26 June, the emergence at Ney Cave began at 6:37pm and ended at 9:44pm. During this time, we observed three distinct emergence periods separated by periods of no emergence activity (11-min emergence, 88-min break, 6-min emergence, 26-min break, 56-min emergence). A census analysis from thermal imaging data estimated that a total of 370,556 bats exited the roost. On 27 June, bats began exiting the cave at 6:11pm and stopped at 9:43pm, with three separate emergences (9-min emergence, 69-min break, 8-min emergence, 66-min break, 60-min emergence) and a total census estimate of 355,846 bats. The substantially shorter emergence at the Chiroptorium on 2 July started at 7:28pm and stopped at 8:42pm, with two distinct periods of activity (6-min emergence, 63-min break, 5-min emergence) and a total estimate of 31,417 bats (although some bats were missed). Average and peak flow rates (bats/10-s) were respectively 816 and 5,948 at Ney Cave, and 582 and 3,714 at the Chiroptorium. To provide a visual representation of the recorded sounds, a short section of an emergence recording from the Chiroptorium is shown in Figure 10. From the emergence recordings at both caves, we identified two distinctive call types, which we describe as (1) sweep calls, and (2) hook calls.

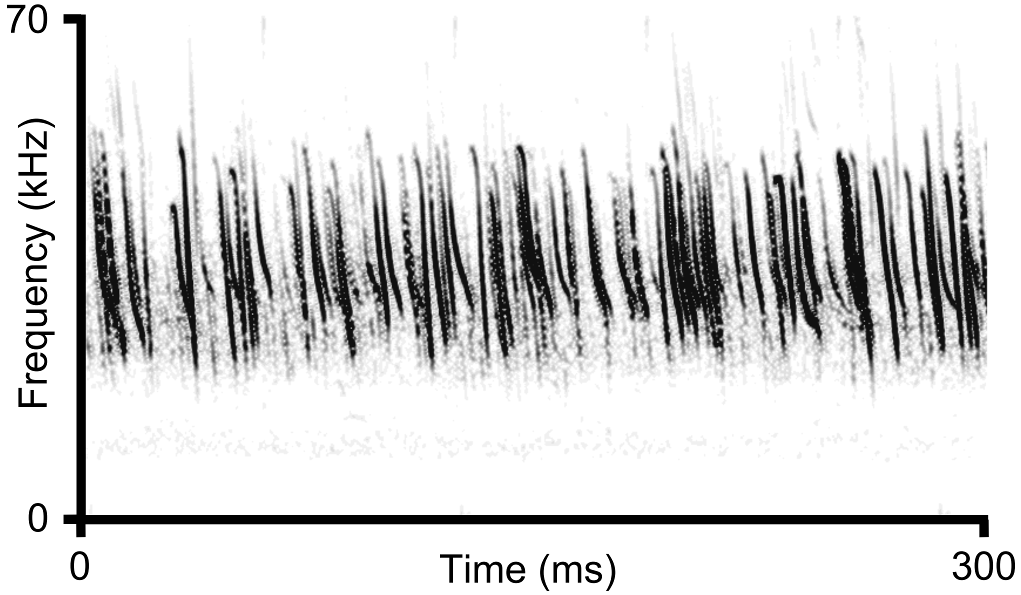


Figure 10. Spectrogram (frequency x time representation) of an emergence recording from Bamberger Chiroptorium on 2 July, 2006. The signal has been high-pass filtered using a cut-off of 15 kHz, which was below the minimum frequency of recorded calls.

Sweep Calls

Sweep calls were the most common call type recorded during emergence (Figure 9a). These calls began with a steep downward slope and transitioned to a quasi-constant frequency (QCF) section that often ended with a short FM tail (Table 4, Figure 9a). We measured one sweep call from every 10-s increment of emergence recordings, for a total of 944 sweep calls (885 calls from Ney Cave and 59 calls from the Chiroptorium). On average, calls were 8 ms long, ranged from 53 to 29 kHz, and had a peak frequency of 33 kHz. Linear regressions revealed that the flow rate of emerging bats was not a strong predictor of call structure and explained only a small amount (< 15 %) of variation in any call variable (bootstrap parameter estimates ± standard error: duration $R^2 = 0.09 \pm 0.02$; min frequency $R^2 = 0.09 \pm 0.02$; max frequency $R^2 = 0.002 \pm 0.003$; peak frequency $R^2 = 0.13 \pm 0.03$; bandwidth $R^2 = 0.01 \pm 0.007$).

Hook Calls

Hook calls were distinguished from sweep calls as highly broadband signals that began with a distinctive convex hook of variable size, followed by a steep linear downward slope (Figure 9b,c). While these calls were prevalent in our recordings, they were substantially less common during times when relatively few bats were emerging from the roost (<100 bats in a 10-s period), which prevented us from conducting a comparison to bat counts extracted from thermal video data. Instead, we measured 50 hook calls from each of the 3 recording

Table 4. Mean ± standard deviation of the five measured call variables. Data has been divided by call type (sweep, hook, foraging) and the location of collection (Ney Cave, Bamberger Chiroptorium).

	Emergence Sweep		**Emergence Hook**		**Foraging**
	NC	BC	NC	BC	
N	884	59	100	50	30
Dur (ms)	7.9 (1.0)	9.8 (1.6)	6.7 (0.8)	7.8 (1.4)	12.8 (1.1)
Fmin (kHZ)	28.8 (2.1)	27.5 (2.0)	25.0 (2.4)	24.4 (3.2)	22.7 (1.5)
Fmax (kHz)	53.6 (3.5)	47.8 (7.0)	52.2 (2.2)	50.0 (2.9)	27.0 (3.0)
Fpeak (kHZ	32.9 (2.8)	30.8 (1.7)	50.6 (2.2)	33.0 (3.0)	24.5 (1.6)
Bandwidth	24.8 (3.2)	20.3 (5.8)	27.2 (2.2)	25.7 (3.2)	4.3 (2.4)

nights. On average, the 150 measured calls were 7 ms long and spanned a frequency range of 25 to 52 kHz, with a peak frequency of 45 kHz.

Comparisons Between Call Types

Comparison of emergence calls to the echolocation signals of foraging *T. brasiliensis* (Figure 9d) revealed that both sweep and hook calls were shorter, more broadband, and higher in minimum, maximum, and peak frequencies than foraging calls ($p < 0.0001$ for all analyses).

Visual comparison of sweep and hook calls revealed two major structural differences: (1) a low-frequency QCF section was present in sweep calls and absent in hook calls, and (2) a high-frequency hook section was present in hook calls and absent in sweep calls. Comparison of hook and sweep calls revealed significant differences ($p < 0.0001$) for all call variables. On average, sweep calls were longer, less broadband and higher in minimum and maximum frequency, although these differences were small (Table 4). The largest discrepancy was the substantially lower peak frequency of sweep calls compared to hook calls. In general, peak frequency was found in the lower frequency QCF section of sweep calls and in the upper half or hook section of hook calls.

While we found no effect of date on call structure, there was a significant effect of location for both call types. Sweep calls from Ney Cave were shorter, more broadband, and higher in frequency than sweep calls from the Chiroptorium ($p < 0.0001$ for all call variables). The same pattern was observed for hook calls, although no differences were observed in minimum frequency ($p < 0.0001$ for the other four variables). The most striking difference between sites was a 17 kHz higher average peak frequency of hook calls from Ney Cave (Table 4). Due to this site effect, all reported comparisons between call types were performed separately for each location.

DISCUSSION

Simmons et al. (1979) stated that calls of emerging Brazilian free-tailed bats began “with a short constant-frequency (CF) component with a duration of 1.5 ms at 55 kHz”. We believe that the described CF region corresponds to the hook structure reported in this study. While both studies used the same method of sound analysis (Fast Fourier Transform), we suspect that signal resolution was limited in 1979 by the relatively slow processing speed of computers. Alternatively, current processors allow us to quickly perform FFT analyses using a very large FFT length (1024), which results in much greater frequency resolution. Spectrograms created using a lower FFT length (64) show the hook section as a constant frequency, similar to Simmons et al. (1979). Further, the duration and frequency of Simmon’s CF region are very similar to the hook structures observed in this study.

Emergence vs. Foraging Calls

The differences observed between the emergence and foraging calls of *T. brasiliensis* are analogous to changes in other species related to the amount of vegetative clutter at a feeding site. Bats foraging amongst thick vegetation use short, broadband signals to accurately localize objects in the environment and assist in collision avoidance (Rydell 1990, Kalko and Schnitzler 1993, Obrist 1995). Similarly, emerging Brazilian free-tailed bats use short, broadband signals, evidently in response to the highly cluttered conditions created by the presence of many bats. The high frequency of emergence calls also results in short detection distances, which will be useful when bats need information about nearby targets. Alternatively, the long, narrowband calls of foraging bats are ideal for flying in relatively uncluttered conditions and searching for distant insects. Interestingly, foraging *T. brasiliensis* also increase the bandwidth and frequency of their calls in the presence of echolocation playbacks (Gillam et al. 2007), indicating that foraging bats also adjust call structure to gather more detailed information in the presence of conspecifics.

Sweep vs. Hook Calls

While sweep and hook calls are both emitted during emergence, they exhibit substantive differences in call structure. It is implausible that these calls were produced by different species, as the vast majority of bats at both sites are *T. brasiliensis* (Davis et al. 1962, G McCracken personal communication) and the echolocation calls of other species in the area exhibit very different call structures. Therefore, sweep and hook calls are evidently distinctive call types produced by emerging Brazilian free-tailed bats that likely allow the bats to obtain different types of information about their surroundings.

Because flying bats can direct their calls by moving their head from side-to-side (Simmons 1973), it is likely that individuals near the edge of the emergence column direct some calls towards nearby bats and other calls towards the uncluttered environment outside of the column. We propose that *T. brasiliensis* use hook calls when oriented towards the column in order to accurately localize the position of adjacent bats. The distinctive hook region provides a precise starting point to a call for accurate target ranging (Bradbury and Vehrencamp 1998) and a high peak frequency will increase detail about a bat's immediate surroundings. We further propose that sweep calls are emitted when bats direct signals outside of the column and are primarily used for assessing characteristics of the surrounding environment. The low frequency, high amplitude QCF region of sweep calls should lead to longer detection distances, which will be important for identifying raptors that are in the vicinity but are further away than adjacent bats. This hypothesis also explains our observation that hook calls are less common during periods of sparse emergence when bats do not form a column. Greater spatial separation will decrease the risk of collisions, and target-ranging information obtained from hook calls should be less important. Further, if column formation is a tactic for predator avoidance (Lee and Kuo 2001), bats emerging at low densities may be more susceptible to predation and information from sweep calls about distant targets will be important for detection of raptors. While more detailed research is necessary to confirm the conditions under which bats emit sweep and hook calls, it seems plausible that a mix of the two call types will allow bats to obtain the information needed for collision avoidance and predator detection.

Changes in the Structure of Sweep Calls

We did not find that bats altered call structure in reference to the number of individuals exiting the roost, although we only assessed this relationship with sweep calls. If the proposed functions of sweep and hook calls are correct, we would not necessarily expect a relationship between sweep call structure and emergence count, as the surrounding area will generally be open and uncluttered. Alternatively, it is possible that bats adjust their spatial distribution in response to changes in the flow rate of emerging bats. Further assessment of hook calls and flow rate of emerging bats will be necessary to determine if this is the case.

Despite the lack of a relationship between emergence count and sweep calls, the significant differences observed between locations suggest that bats may adjust their call structure under different emergence conditions. The colony at Ney Cave was much larger than the Chiroptorium colony, and qualitative visual assessments indicate that bats are spaced closer together as they leave Ney Cave than eaving the Chiroptorium. The shorter, higher frequency calls recorded at Ney Cave would be consistent with a tighter spatial distribution, as such calls attenuate faster and provide more accurate target range estimates. The much higher (17 kHz average) peak frequency of bats emerging from Ney Cave may also be related to spatial distribution. The general use of a higher peak frequency during a dense emergence may serve as a form of collective jamming avoidance, as greater signal attenuation may decrease the interference produced by conspecific calls and allow bats to be spaced closer together. Further testing is necessary to determine if the peak frequency used by bats at different caves is associated with colony size.

While emergence in a tight column likely provides a predator dilution effect (Wilson 2000), bats face the task of avoiding collisions with conspecifics present at very short distances while still monitoring the area outside of the column. Brazilian free-tailed bats appear to solve this problem by emitting two call types, which are used for extracting different information from the surrounding environment. These results further substantiate the high flexibility of echolocation in this species (Simmons et al. 1979, Ratcliffe et al. 2004, Gillam et al. 2007, Gillam et al. in press), and demonstrate that Brazilian free-tailed bats orient and echolocate effectively even when flight conditions are very different from those experienced during foraging.

CHAPTER V

Eavesdropping by Bats on the Feeding Buzzes of Conspecifics

This chapter is a version of a paper by the same name currently under review in the *Canadian Journal of Zoology* by Erin H Gillam.

ABSTRACT

Echolocation calls of most bats are loud in amplitude and subject to eavesdropping by nearby conspecifics. Bats may be especially attentive to 'feeding buzz' calls, which are emitted immediately before prey capture and indicate successful hunting. While previous work has shown that some species are attracted to feeding buzzes, these studies did not provide a well-controlled test of eavesdropping since comparisons were made between responses to natural and altered signals (eg. forward vs backward broadcasts of calls). In this study, I assessed the importance of feeding buzzes by conducting playbacks of controlled echolocation stimuli. I presented free-flying Brazilian free-tailed bats, *Tadarida brasiliensis,* with echolocation call sequences in which feeding buzz calls were either present or absent, as well as a silence control. I determined levels of bat activity by counting the number of echolocation calls recorded in the presence of each stimulus, and found significantly greater bat activity in response to broadcasts that contained feeding buzzes compared to broadcasts without feeding buzzes. These results indicate that bats are especially attentive to conspecific feeding buzz calls, and that eavesdropping should allow a bat to more readily locate rich patches of insect prey.

INTRODUCTION

Food resources often occur in small, ephemeral patches that are separated by larger areas of poor quality. While such an uneven distribution may increase the time an animal must dedicate to foraging (Stephens and Krebs 1986), efforts to locate areas of high resource density may be enhanced by monitoring conspecific cues that indicate successful feeding (McGregor 2005). For example, the conspicuous sounds of an agouti, *Dasyprocta punctata*, chewing a nut attract other agoutis to a feeding site (Smythe 1970). Such passive information transfer via eavesdropping on conspecific foraging cues can occur at the feeding site (McQuoid and Galef 1993, 1992; Nieh et al. 2004), or at a colony or roost, which may serve as an 'information center' (Chauvin and Thierry 2005; Ratcliffe and ter Hofstede 2005; Wright et al. 2003).

Several species of bats gain knowledge of food resources by attending to the cues of conspecifics. Short-tailed fruit bats, *Carollia perpiscillata*, alter their food preferences based on olfactory cues obtained in the roost from conspecifics that have recently fed (Ratcliffe and ter Hofstede 2005). Evening bats, *Nycticieus humeralis*, follow successful foragers from roosts to rich feeding areas, with bats alternating the roles of leader and follower on subsequent trips (Wilkinson 1992). Group departures from a roost have been observed in several other species (Fenton et al. 2004; Racey and Swift 1985), although it is often unclear if such behavior is due to passive information transfer (Wilkinson 1992), active recruitment of roostmates via communication calls (Wilkinson and Boughman 1998), or bottlenecks at the roost exit (Speakman et al. 1992).

High amplitude echolocation calls of bats reveal information about the foraging success of an individual and could be especially susceptible to eavesdropping. Attention to the calls of nearby conspecifics potentially can lead to opportunistic aggregations of bats at insect-rich locations (Bell 1980; Fenton et al. 1976; Vaughan 1980). Specifically, bats may be attracted to terminal-phase, ‘feeding buzz’ calls, which are emitted as a bat captures an insect. These calls indicate successful foraging and exhibit a substantially different structure from calls used when a bat is searching for (search-phase), or approaching (approach-phase) a prey target (Griffin 1958).

Three previous studies have investigated the response of bats to echolocation call sequences containing feeding buzzes. Barclay (1982) broadcast signals of feeding *Myotis lucifugus* (which contained search, approach, and feeding buzz calls) to free-flying conspecifics, and found that bat activity was significantly higher when the foraging signal was played forwards compared to trials in which the signal was played backwards. Leonard and Fenton (1984) found similar results when performing a forward/backward playback experiment with the foraging calls of *Euderma maculatum*, and suggested that this species may use echolocation to regulate individual spacing within a feeding area. Balcombe and Fenton (1988) performed the most direct test of eavesdropping on feeding buzz calls, showing that foraging activity of *Lasiurus borealis* greatly increased in the presence of repeated conspecific feeding buzzes compared to presentations of an unedited foraging sequence that contained all three phases of bat echolocation.

The objective of this study was to further test the hypothesis that bats eavesdrop on the echolocation calls of nearby conspecifics and are especially attracted to feeding buzzes. Although this question has been investigated in the past, previous playback experiments did not specifically address the response of bats to the presence of feeding buzzes in realistic echolocation sequences. The backward broadcasts conducted by Barclay (1982) and Leonard and Fenton (1984) altered important information about the echolocation signal, including the direction of a call's frequency sweep and the temporal pattern of the call sequence (Barclay 1982). It is possible that the altered signal was not recognized by bats as a sequence of foraging calls, resulting in lower responses compared to the forward broadcast, independent of the presence or absence of feeding buzzes. Balcombe and Fenton (1988) used a 'super-stimulus' of 51 repeated feeding buzzes that lacked the search- and approach-phase signals that almost always occur between consecutive buzzes (Schnitzler and Kalko 2001). Thus, none of these studies investigated responses of bats to realistic playbacks in which terminal-phase signals were either present or absent. In this study, I performed a controlled experiment in which I compared bat activity in response to two echolocation playback stimuli: (1) a call sequence that contained only search-phase calls, and (2) the same search-phase sequence with a typical series of approach-phase and feeding buzz calls added at regular intervals. This design allowed us to control for other stimulus characteristics, such as call frequency and duration.

I chose to investigate the effects of conspecific feeding buzzes with Brazilian free-tailed bats, *Tadarida brasiliensis*. This species is highly gregarious, forming colonies that reach into the millions in South Central Texas (Davis 1962). Despite their ability to disperse 25 or more kilometers from the roost (Davis 1962; Williams et al. 1973), these bats experience a high interaction rate with conspecifics while foraging (Ratcliffe et al. 2004). The echolocation calls of *T. brasiliensis* are generally narrowband and relatively low in frequency (average minimum frequency of 22.3 kHz; Gillam and McCracken in press), and as a result calls will propagate substantial distances in the environment and be audible to nearby bats. This foraging behavior makes *T. brasiliensis* an optimal species for studying the response of bats to the feeding buzzes of conspecifics.

METHODS

Field Experiments with Echolocation Playbacks

I performed playback experiments between 21:20 and 00:20 on eight nights from 3 June to 12 June 2006. All experiments were performed on a cotton farm in the vicinity of Uvalde, Texas, which is close to several large Brazilian free-tailed bat colonies (*Tadarida brasiliensis)*, and bats were often observed foraging on insects found in high densities over the crop fields where I conducted the study.

Playback stimuli were constructed from previously obtained recordings of bats foraging at the study site. The first playback signal, referred to as “Feeding Buzzes Present”, contained calls from the search, approach, and terminal phases of bat echolocation (Figure 11a*).* I assembled this signal by repeating one typical search-phase call at 200-ms intervals for 8.8-s, and appending a 1.45-s sequence of approach-phase and feeding buzz calls. This 10.25-s composite sequence was repeated to create a 10-min playback. The second playback signal, referred to as “Feeding Buzzes Absent”, contained only search-phase calls (Figure 11b), and was constructed by repeating the same search-phase call from the first playback at 200-ms intervals to create a 10-min signal. I also used a 10-min control broadcast containing no sound, referred to as “Silence”.

Each night, I broadcast six replicates of each stimulus in a mixed order, and changed the playback order on successive nights to control for temporal effects. This design ensured an even distribution of the stimulus presentations throughout the evenings over the study period. I broadcast stimuli through an omnidirectional ultrasonic speaker (Avisoft 60401, Avisoft Bioacoustics, Germany; frequency response ± 5 dB between 15 and 43 kHz) mounted on a tripod 3 m from the ground. Broadcast amplitude was 74 dB at 10 cm from the speaker, as measured with a B&K ¼ " condensor microphone # 4939 and a B&K measuring amplifier # 2606 (Brüel & Kjær, Denmark). This is lower than the

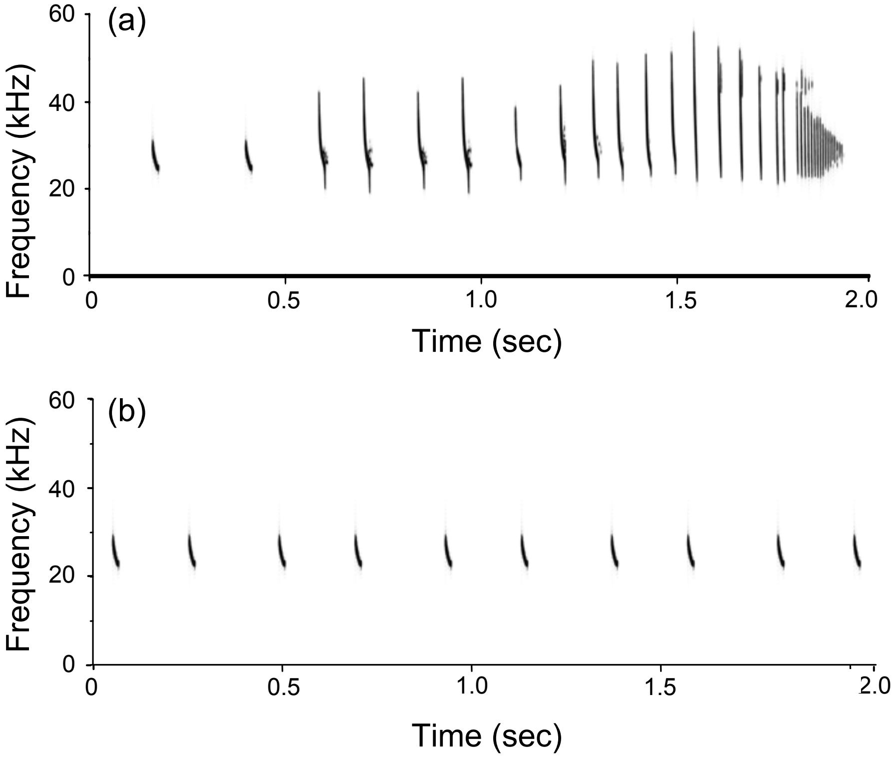


Figure 11. Spectrograms of the final 2-second portion of the echolocation playback stimuli. (a) “Feeding Buzzes Present” stimulus containing search, approach, and terminal-phase calls. The preceding 8.25-s of the call sequence that is not shown is composed of search-phase calls identical to the first four signals in this shortened sequence. (b) “Feeding Buzzes Absent” stimulus containing search-phase calls only. The preceding 8.25-s of the call sequence that is not shown is identical to the depicted calls.

typical call amplitude of many insectivorous bats (>100 dB; Lawrence and Simmons 1982; Waters and Jones 1995), but was the highest intensity possible without overloading the speaker. A solid dielectric microphone (Avisoft CM16; frequency response ± 3 dB between 10 and 100 kHz) was positioned 2 m to the left of the speaker at a height of 3 m and oriented directly upward. Stimuli were generated from a Dell Inspiron Laptop through a high-speed sound card (DAQCard-6062E, National Instruments, Austin, TX) and an amplifier (Avisoft 70101) powered by three 12 V 7.2 A gel cell batteries. High-speed data acquisition was accomplished with Avisoft's Ultrasound Gate 416 through the same laptop that was used for broadcasts. Both playback and recording were conducted with Avisoft RECORDER. Recordings were 5-min long, but sampling was continuous as there was no time-gap between consecutive recording files. Recordings were made with 16-bit resolution and a 166 kHz sampling rate, and included both the playback signal and the calls of free-flying bats in the area.

Pulse Count Analyses

All acoustic measurements and analyses were conducted with Avisoft SasLab Pro. I digitally high-pass filtered all recordings to remove background noise, using a finite impulse response filter with a 5-kHz cutoff. I excluded from analysis files that contained high levels of wind noise. To assess levels of bat activity in the presence of the three playback signals, I performed a pulse train analysis that automatically detected and counted echolocation calls. This analysis provided information about relative bat activity in the presence of each playback stimulus, but could not be used to estimate the number of bats in the recording area since the calls of individual bats could not be distinguished. I used a hysteresis searching method to detect calls, in which an amplitude peak was counted only if it exceeded the pre-peak amplitude by a pre-defined threshold (Specht 2004). The value of this hysteresis threshold influences the pulse counts produced by the program. To ensure that I chose an appropriate value, I counted several recordings by hand and compared my counts to those produced by the pulse train analysis at different hysteresis settings. A 20dB hysteresis threshold yielded the most accurate pulse counts, and thus was used for all analyses.

The pulse count analysis also was influenced by the amplitude threshold setting, with lower thresholds resulting in increased detection of weak signals and a higher final pulse count. I counted the calls in each recording file using three amplitude thresholds: 100 mV, 300 mV, and 500 mV (Figure 12). This allowed me to assess the relative amplitude of detected calls and gain insight into how close bats were flying to the recording system. Use of multiple thresholds was preferred to directly assessing the amplitude of each call, as the latter approach required the logistically difficult task of individually excluding each recorded playback call from analysis instead of simply subtracting the total number of playback calls from the pulse count (see below). While the 100 mV analysis detected the greatest number of calls, the 300 mV and 500 mV analyses counted a decreasing number of pulses, only detecting higher amplitude signals (Figure 12).

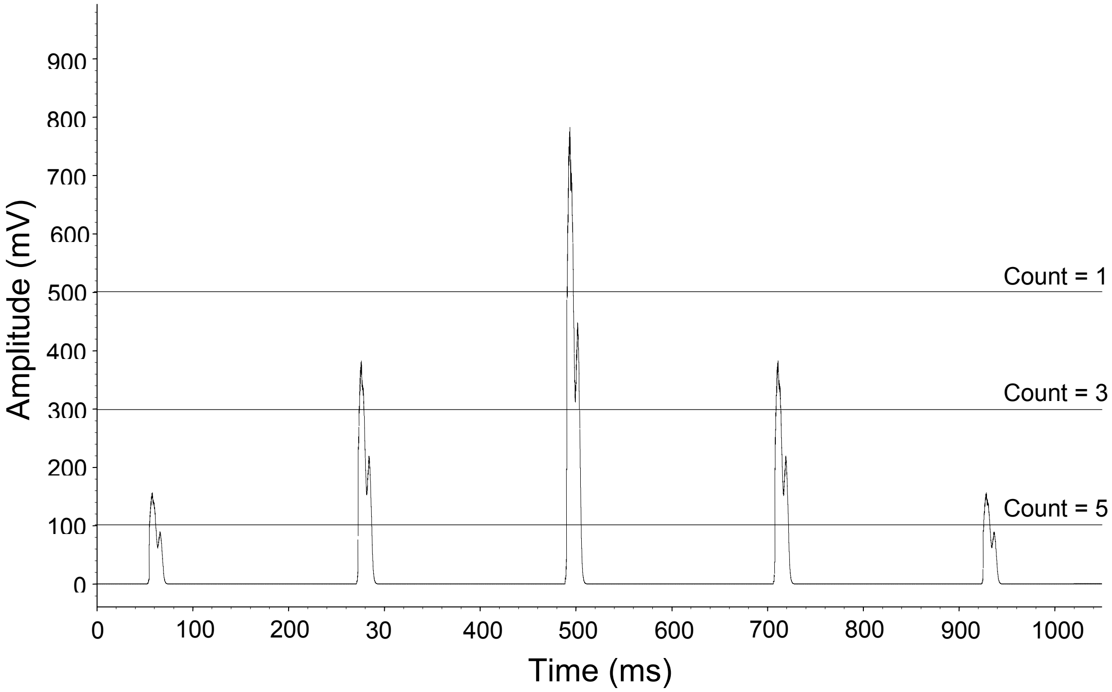


Figure 12. Amplitude detection thresholds. Amplitude envelope depicting the detection of five calls with inter-call intervals of approximate 210 ms. In this example, the 100 mV amplitude threshold detects all five calls, the 300 mV detects three calls, and the 500 mV threshold only detects the loudest call.

Playback calls were present in recordings, but were only detectable by the 100 mV analysis. To obtain a pulse count that excluded the playback calls, I broadcast each stimulus before bats arrived at the study site and counted the number of detected pulses in these “bat-free” recordings. I then subtracted the appropriate playback pulse count (“Feeding Buzzes Present” or “Feeding Buzzes Absent”) from the counts produced by the 100 mV analyses. Another issue was that some bat calls overlapped with playback calls and were not counted. Although this led to lower pulse counts, I chose not to include a correction for this overlap error in the final analysis, as corrections resulted in only small changes to the final counts and the unadjusted value was more conservative

Statistical Analyses

I tested if the number of detected bat calls differed between the three playback stimuli (Feeding Buzzes Present, Feeding Buzzes Absent, Silence) by conducting a one-way ANOVA for each of the amplitude thresholds, and conducting post-hoc Tukey-Kramer multiple comparison tests. A significance level of 0.05 was used for all tests.

RESULTS

I analyzed 40 ten-minute recordings for each of the three playback signals (n=120 total). Bat activity was significantly different between the three stimuli at 100 mV ($F_{2,117} = 3.14$, $P = 0.047$), 300 mV ($F_{2,117} = 13.36$, $P < 0.0001$), and 500 mV ($F_{2,117} = 14.66$, $P < 0.0001$). For the 300 mV and 500 mV analyses, Tukey-Kramer tests revealed that bat activity was significantly greater in response to “Feeding Buzzes Present” compared to “Feeding Buzzes Absent” or “Silence” (see Tukey-Kramer groupings and legend, Figure 13). Despite a significant p-value for the ANOVA test, no differential response between broadcast stimuli was observed for the 100 mV analysis, although there is an obvious trend for the same pattern of increased activity in response to the “Feeding Buzzes Present” stimulus (Figure 13).

DISCUSSION

The results of this study support the hypothesis that bats eavesdrop on the echolocation calls of conspecifics and are attracted to terminal-phase feeding buzzes that indicate successful foraging by other bats. While significant differences existed between stimuli for all three analyses, it is interesting that the largest differences were observed in the 300 and 500 mV analyses (Figure 13). This suggests that the playback stimulus containing feeding buzzes not only attracted more bats, but that these bats more closely approached our speaker system, as revealed by the high amplitude of the detected echolocation calls. Overall, these results provide evidence that bats approach conspecifics emitting terminal-phase calls, likely in an attempt to enhance feeding success or to gain more detailed information about the signaling animal and its foraging area.

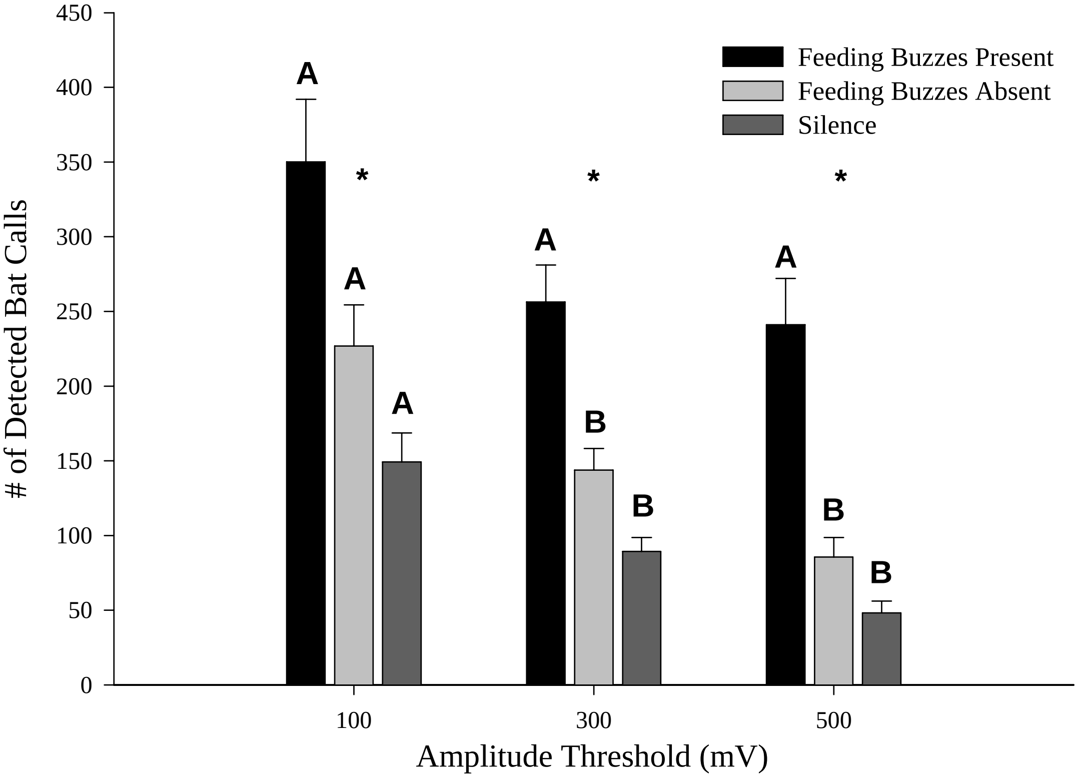


Figure 13. Levels of bat activity in the presence of each playback stimuli. Bat activity was assessed by pulse count analyses using three different amplitude thresholds (100, 300, 500 mV). * indicates ANOVA was significant at the 0.05 level. Letters indicate results of Tukey-Kramer multiple comparison tests from one-way ANOVA; counts from stimuli labeled “A” are not significantly different from each other, but are significantly different from stimuli labeled “B”. For the 300 mV and 500 mV threshold analyses, significantly more bat activity was detected during broadcast of the “Feeding Buzzes Present” stimulus compared to the “Feeding Buzzes Absent” or “Silence” stimuli. While Tukey-Kramer comparisons did not assign these differences in the 100 mV analysis (all are grouped into “A”), ANOVA revealed significant differences between stimuli ($p = 0.047$) and a trend similar to the other analyses is observed.

Our findings agree with those of previous studies demonstrating that bats are attracted to terminal-phase feeding buzzes (Barclay 1982, Leonard and Fenton 1984, and Balcombe and Fenton 1988). While Barclay (1982) reported that more bats responded to foraging calls played forward compared to backward broadcasts, he also indicated feeding buzzes may not be critical to eavesdropping, as there were no differences in the response of *M. lucifugus* to conspecific ‘foraging’ and ‘non-foraging’ sequences. However, because the non-foraging playback used in the study was recorded during swarming, when bats aggregate for mating (McCracken and Wilkinson 2000), the signal may have contained social communication calls that could have attracted bats to the playback despite the absence of feeding buzzes. Here we demonstrate that when exposed to two realistic and otherwise identical echolocation stimuli, the signal containing approach-phase and feeding buzz calls was more attractive, suggesting that bats pay particular attention to the portion of a call sequence that is associated with insect capture.

The question remains as to whether eavesdropping on conspecific feeding buzzes represents information parasitism or information transfer. Information parasitism occurs if foraging success decreases when conspecifics are attracted to a bat’s foraging area, while information transfer occurs if sharing information about foraging areas either does not affect or increases an individual’s foraging success (Wilkinson 1992). Information transfer can be further divided into: (1) passive transfer, which occurs when animals mimic the behavior of conspecifics (Wilkinson 1992), and (2) active transfer, in which information is actively relayed to other individuals, often through a developed system of communication, such as the dance language of honey bees, *Apis mellifera* (von Frisch 1967).

The conditions in which *T. brasiliensis* forage suggest that eavesdropping on the echolocation calls of conspecifics is best described as passive information transfer. Brazilian free-tailed bats aggregate in large numbers and ultimately encounter many conspecifics while foraging (Ratcliffe et al. 2004). Despite a high interaction rate, agonistic interactions are rarely observed (GF McCracken, personal observation) and large numbers of bats are commonly seen foraging over crop fields in close proximity to one another. If information parasitism were occurring, agonistic encounters would be expected when bats forage at high densities (Racey and Swift 1985).

The rich food sources exploited by Brazilian free-tailed bats also compliment the hypothesis of passive information transfer. Moths are a major food source for *T. brasiliensis*, sometimes comprising over 80% of their diet (Whitaker et al. 1996; Lee and McCracken 2002). Noctuid moths, such as *Helicoverpa zea*, are very abundant in South Central Texas, and the distribution of these insects is highly variable in space and time (Fitt 1989). Mass emergences of billions of moths occur asynchronously over crop fields within brief time windows (Raulston 1990), resulting in strong spatial and temporal heterogeneity in resource availability (JK Westbrook and EH Gillam, unpublished data). These extremely high densities of moths suggests that the presence of multiple bats feeding in close proximity is unlikely to affect the foraging success of any individual bat. Such rich, ephemeral patches of insects have previously been suggested as ideal conditions for information transfer, as patches contain sufficient prey to support successful foraging by multiple bats but do not persist long enough to warrant territoriality and defense (Wilkinson 1992). Eavesdropping on conspecifics by Brazilian free-tailed bats should allow individuals to enhance foraging success by decreasing the amount of time spent in a poor area and gaining information about the presence of new, ephemeral patches that are rich in insect prey (Galef and Giraldeau 2001).

While echolocation signals in bats are primarily used for orientation and prey detection, it has been suggested that echolocation evolved from social communication calls (Fenton 1984). Most bats emit a wide range of social calls that are associated with several behaviors, including mating (Bradbury 1977), mother-young interactions (Balcombe and McCracken 1992), and alarm signaling (Russ et al. 1998). The results of this study enhance the link between echolocation and social calls by further demonstrating that echolocation calls can have a communicative function.

CHAPTER VI

Conclusions and Directions for Future Research

CONCLUSIONS

Chapter II

- Brazilian free-tailed bats exhibit significant variation in call structure among populations with regard to call frequency and duration. However, the observed variation is not associated with geographic distance or local weather conditions. I conclude that little or no geographic patterning exists in the echolocation calls of *T. brasiliensis*, and that the observed variability in call structure is due primarily to the variability within and between individual bats.
- There is a positive correlation between the echolocation call frequencies used by bats and the experimental broadcast frequencies of ambient insect sounds. This finding indicates that bats adjust their echolocation call structure to avoid acoustic interference from ambient noise in the local environment.
- Brazilian free-tailed bats exhibit substantial flexibility in call structure, and variability in call parameters are as expected for maximizing the efficiency of echolocation under different behavioral and ecological conditions.

Chapter III

- The results of this chapter provide the first experimental evidence for jamming avoidance in bats

- Using an experimental playback design, I found that free-flying Brazilian free-tailed bats shifted their call frequencies away from the frequencies of playback calls that simulated the presence of other bats foraging in the same airspace. When I challenged approaching bats with a playback near their call frequency, a jamming avoidance response also was observed, although there was a bias for shifting upwards to higher frequencies.
- As expected, bats challenged with a playback frequency below their initial calling frequency exhibited positive shifts. An unexpected finding was that bats exposed to a playback higher in frequency than their own calls also shifted upwards.
- Spectral shifts in call frequency often occurred within less than 200 ms, in the first echolocation call emitted after the stimulus-switch. Such a rapid reaction suggests that jamming avoidance responses are important for ensuring effective echo reception and signal processing.
- A model proposed to explain the observed jamming avoidance response hypothesizes that bats are most sensitive to interference from the high amplitude, lower frequency portion of echolocation calls known as the “quasi-constant frequency” region.

Chapter IV

- Brazilian free-tailed bats emerging from roosts emit two distinct call types, that I describe as sweep and hook calls. Both sweep and hook calls are significantly different from the echolocation calls typically emitted by free-flying *T. brasiliensis* foraging over a cotton field.

- I hypothesize that hook calls provide bats with information about the distance to nearby bats within the emergence column, which is important for regulating individual spacing. I hypothesize that bats use sweep calls for gaining information about targets in the relatively uncluttered environment outside of the column, such as avian predators.
- The structure of sweep calls does not change in relation to the number of bats emerging from the roost. However, both hook and sweep calls differed significantly between sites, perhaps due to associations with the spacing of bats during emergence.

Chapter V

- I assessed bat activity in response to echolocation playbacks by counting the number of recorded echolocation calls that exceeded a pre-set amplitude threshold. I found that bat activity was substantially greater in response to playbacks that contained search, approach, and feeding buzz calls compared to playbacks that contained only search calls, or silence.
- Brazilian free-tailed bats are especially attentive to feeding buzz calls of conspecifics. Eavesdropping should be an effective strategy for increasing individual foraging success, as Brazilian free-tailed bats forage on insects that are often found in rich, ephemeral patches that can support simultaneous foraging by multiple bats.

DIRECTIONS FOR FUTURE RESEARCH

Several potential areas of research arise from the findings of this dissertation. I believe one of the most important areas for further investigation concerns jamming avoidance in bats. While our experiments in Chapter 4 tested the reaction of bats to signals from a stationary speaker, in reality bats use jamming avoidance during encounters with fast-flying conspecifics in a shared airspace. Use of microphone arrays to estimate 3D positions of multiple bats, will allow us to answer questions about the fine details of jamming avoidance, such as: “Is the onset of JAR related to the position and flight direction of other bats in the area?”, and “Are spectral shifts maintained for long periods of time (several minutes) or do bats dynamically adjust their call frequencies in reference to the proximity of conspecifics?”. Answers to these questions will not only provide a better understanding of JAR under realistic flight conditions, but will allow us to further quantify the flexibility of echolocation in Brazilian free-tailed bats.

In Chapter 4, I propose that the “quasi-constant frequency”, or QCF, section of echolocation calls causes the greatest amount of interference, and that bats shift to higher call frequencies in an attempt to minimize overlap with the QCF region of other bats. While this hypothesis fits most of the observed responses, it has not been experimentally tested. Laboratory range discrimination tests, in which bats are exposed to broadcasts of calls varying in the intensity and structure of the QCF region, would be useful for testing the proposed hypothesis. Further field playbacks using a range of echolocation call types would determine if the extent of frequency shifts is related to the structure of the overlapping signal. Overall, these studies will provide insight into how the characteristics of interfering signals influence the efficiency of echolocation.

Further examination of calls produced by bats during emergence would also be a worthwhile area of research, as the behavioral and ecological conditions experienced by emerging bats are substantially different from foraging situations. Predation risk is often higher at roosts, especially by owls and raptors, and call structure will influence how efficiently avian predators can be detected and avoided. Substantial inter- and intra-specific variation exists in patterns of emergence (column, clustered, single bats), which will influence the risk of collision and potentially the call structure used by bats. Finally, previous studies of intraspecific variability in echolocation have strictly examined foraging calls, and adding emergence calls to the description of a species’ echolocation repertoire will better characterize natural variability in call structure.

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