Communication calls of little brown bats display individual-specific characteristics

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(Received 2 December 2009; revised 23 May 2010; accepted 26 May 2010)

Bats' echolocation signals have been shown to be situation-, colony-, and individual-specific, but whether or not these findings apply to bats' communication signals is not fully understood. The primary goal of this study was to test the hypothesis that the communication calls of adult little brown bats (*Myotis lucifugus*) are individual specific. Bats were paired to form focal pairs from June 2007 to August 2008. Each bat's vocalizations were recorded on a PC-based digital recorder with a custom made ultrasonic microphone. The vocal signals were first classified using a previously established classification scheme. Three acoustic parameters (the minimum and maximum frequencies, and the call duration) of two of the dominant call-types, the steep-FM and broadband noise bursts, of individual bats were further analyzed. Discriminant function analysis, and multi- and univariate analyses of variance of these parameters revealed that these vocal signals were individually distinct and likely contain individual signatures to allow bats to identify individuals acoustically. © 2010 Acoustical Society of America. [DOI: 10.1121/1.3455835]

PACS number(s): 43.80.Ka [JAS]

Pages: 919–923

I. INTRODUCTION

The echolocation system of bats has been studied extensively, and research over the last several decades has shed light on the spectrotemporal characteristics of echolocation signals, how they are used to acquire the static and dynamic information of a target, and how they are processed in the brain. Studies of bats' echolocation signals have revealed that these signals are situation- (Siemers and Schnitzler, 2004), colony- (Pearl and Fenton, 1996), and individual-specific (Masters *et al.*, 1995; Kazial *et al.*, 2001; Fenton *et al.*, 1999; Yovel *et al.*, 2009a, 2009b). At this time, it is unclear the extent to which these findings apply to bats' communication signals.

Echolocating bats are highly social animals, and sound communication plays an important role in their social interactions (Fenton, 1984). Recent studies showed that communication calls produced by infants during mother-young interactions carry individual signatures (Bohn *et al.*, 2007; Knornschild and von Helverson, 2008). Another recent study in Brazilian free-tailed bats (Bohn *et al.*, 2009) showed that their songs (i.e., stereotypical combinations of acoustic elements) also differ individually; whether or not the acoustic elements themselves are individually distinct in the adults was not determined, however. Nonetheless, individual signatures have been found in communication signals of adult marine mammals that echolocate, such as dolphins (Watwood *et al.*, 2005; Janik *et al.*, 2006) and whales (Weiß *et al.*, 2006). Therefore, it is plausible that communication calls of

adult echolocating bats are also individual specific (i.e., have acoustic characteristics which differ among individuals).

To test the above hypothesis, we investigated the sound communication calls of adult male little brown bats (*Myotis lucifugus*). We analyzed in detail three acoustic parameters (the minimum and maximum frequencies, and the call duration) of two of the dominant call-types that these bats produce. Discriminant function analysis, and multi- and univariate ANOVA of these parameters revealed that these signals were individually distinct.

II. METHODS

A. Subjects

Adult male little brown bats (*M. lucifugus*) were collected in Spring 2007 and Spring 2008 from Starved Rock State Park in Utica, IL and maintained in an environmental room at 27 °C and 60% relative humidity. The bats were kept in two separate cages (46×61×46 cm), divided by collection year. Therefore, there was one home cage for the Spring 2007 collection and one home cage for the Spring 2008 collection. Typically, permit restrictions allow collection of male bats only; however a female bat was accidentally collected in 2008 and therefore included in the study. Food (meal worms) and water were changed daily and made available *ad libitum*. Following 2 weeks of acclimation to the laboratory environment, microchips were implanted into the neck of individual bats by the staff members of the Department of Animal Resources at the University of Illinois, as a means of individual bat identification.

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FIG. 1. Schematic diagram of the experimental setup showing the placements of the audiovisual recording devices and playback loudspeaker.

B. Experimental procedures for analysis of communication calls

Between June 2007 and August 2008, pairs of identified bats ($n=5$ pairs) were placed in a plastic mesh cage ($7 \times 21 \times 25$ cm with Plexiglass frame) in a sound attenuated booth once a week for 30 min (Fig. 1). Recordings were conducted at peak times of daily activity (~ 5 pm, midnight, and 2 a.m.; Melendez *et al.*, 2006).

Under infrared lighting (Kodak safelight filter #10 affixed to a 60-Watt incandescent lamp), the behaviors of paired bats were recorded with a Sony camcorder (DCR-DVD-301 equipped with *nightshot* feature). Bats were recorded as pairs, to induce the exchange of communication calls. The video signal was synched to the bats' vocal signals recorded on a PC-based digital recorder (PC-Tape), with a custom made ultrasonic microphone having a flat frequency response from 15–120 kHz, with a roll-off of 10 dB per octave and 6 dB per octave at 15 kHz and 120 kHz, respectively (Siemers and Schnitzler, 2004; Feng *et al.*, 2006). The microphone was placed 15 cm from the cage.

Bats' vocalizations were analyzed by two independent observers. At the beginning of each recording, an independent observer was instructed to recite the identification number of an individual bat and to visually indicate which bat corresponded to the particular identification number. Calls were analyzed for one bat per pair. Acoustic communication signals were digitized (16-bit A/D conversion) at a sampling rate of 192 kHz, saved as WAV files, and displayed for analysis (fast Fourier transformed with 1024 points) using Selena, a custom-designed sound analysis software (Feng *et al.*, 2006).

Individual vocalizations were classified using an established classification scheme (Melendez *et al.*, 2006). We found that pairs consisting of bats from the same home cage displayed very low levels of interaction. For this reason, experimental pairs consisted of bats from different home cages. Additionally, each bat was only paired once. Three of the pairs recorded contained one male and one female (recorded

FIG. 2. (Color online) Spectrograms of the two dominant communication call types (FFT size 1024, Hamming window): (A) Broadband noise burst. (B) Downward FM call. (C) Steep-FM call. (D) Broadband click train

prior to her death in April 2008). The female bat rarely called, and therefore data collection and analysis were limited to males' calls.

C. Data analysis

Discriminant function analysis (DFA) and multivariate analysis of variance (MANOVA) were used to determine whether or not communication calls were individual-specific. DFA provides an assessment of whether or not bats' sound communication calls can be used to discriminate between individuals. MANOVA assesses the likelihood that the means of two or more groups have the same sampling distribution; in this case, whether or not individual bat's calls are the same. Posthoc univariate ANOVA was conducted subsequently to confirm results from the MANOVA (Duntelman, 1984; Naguib *et al.*, 2001).

III. RESULTS

A total of 15,103 calls were recorded from 5 focal pairs. As reported previously (Melendez *et al.*, 2006), there were four dominant call-types (Fig. 2(A)–2(D)): broadband noise-burst (BNB), downward-FM (DFM), steep-FM (StFM) and broadband click trains (BCT). StFM was the most frequently emitted (94.4%; Table I), and BNB was the second most common communication calls (4.24%; Table I). DFM and BCT were the least common calls (1.3% and 0.03%, respectively; Table I).

TABLE I. Number of calls emitted for each call type.

Call type	Number of calls
Steep-FM	14 262
Broadband noise burst	641
Downward FM	196
Broadband click train	4

TABLE II. Analysis of the minimum and maximum frequency and the duration of the steep-FM calls ($n=30$) and broadband noise bursts ($n=30$) of 5 individual bats. Shown are mean and standard error of these acoustic parameters.

Call type	Bat	N	Min freq	Max freq	Duration
Steep-FM	A2	30	36.04 ± 0.36	96 ± 0	8.2 ± 0.40
	A8	30	44.71 ± 0.45	92.7 ± 0.89	7.93 ± 0.34
	A9	30	36.84 ± 0.52	96 ± 0	10.17 ± 0.31
	A10	30	46.84 ± 0.24	91.96 ± 0.25	7.6 ± 0.32
	A12	30	39.38 ± 0.59	96 ± 0	10.37 ± 0.26
Broadband noise burst	A2	30	4.26 ± 0.04	85.58 ± 1.54	38.43 ± 7.58
	A8	30	4.2 ± 0	95.82 ± 0.13	38.6 ± 1.39
	A9	30	4.2 ± 0	95.9 ± 0.1	25.8 ± 3.14
	A10	30	4.23 ± 0.01	95.8 ± 0.14	20.9 ± 1.65
	A12	30	4.2 ± 0	95.9 ± 0.1	129.3 ± 5.73

To investigate whether or not bat's communication signals were individual specific, we analyzed the StFM and BNB calls quantitatively. Specifically, we analyzed the minimum and maximum frequencies, and the signal duration of 30 StFM and 30 BNB calls per bat (Table II)—these were features used for identifying different call-types in an earlier study of vocal communication in little brown bats (Barclay *et al.*, 1979). A total of 30 calls were randomly chosen from different recording sessions for only one bat per bat pair. Discriminant function analysis revealed that 89% of the StFM calls and 66% of the BNB calls could be correctly assigned to individual bats.

Multivariate analysis of variance (MANOVA) revealed that both the StFMs (Wilks' $\lambda=0.130$; $F=25.83$; $p<0.001$) and BNBs (Wilks' $\lambda=0.113$; $F=40.40$; $p<0.001$) were individually distinct. Subsequent univariate ANOVA showed that (i) all three acoustic parameters chosen could be used to differentiate between StFM calls of individual bats, and (ii) only the maximum frequency and duration could be used for differentiating BNB calls of individuals (Table III).

IV. DISCUSSION

Results of the present study showed that two communication calls (the StFM and BNB calls) of adult *M. lucifugus* were individual specific, displaying distinct spectral and temporal characteristics. These calls can potentially be differentiated by three acoustic parameters: the minimum frequency, maximum frequency, and duration of the call. However, it remains to be determined whether or not the individual-specificity represents individual signatures, allowing bats to

identify individuals acoustically. Previous in-depth analysis of the vocal signature of bats has focused on their echolocation calls (Masters *et al.*, 1995; Obrist, 1995; Kazial *et al.*, 2001; Fenton *et al.*, 1999; Siemers and Kerth, 2006; Kazial *et al.*, 2008a, 2008b; Yovel *et al.*, 2009a, 2009b). The echolocation calls of the big brown bat (*Eptesicus fuscus*) have been shown to differ among individuals within a family and between adults and juveniles (Masters *et al.*, 1995; Kazial *et al.*, 2001). Comparative studies in several other bat species, including *Euderma maculatum*, *Eptesicus fuscus*, *Lasiurus borealis*, and *Lasiurus cinereus*, showed intra- and inter-individual variability in echolocation calls. Unlike the communication calls for which individual differences occur in both the spectral and time domains, the differences in echolocation calls were predominantly in their call frequency and FM pattern (Obrist, 1995). The temporal characteristics of echolocation calls, such as the call duration, are actively controlled during hunting and optimized to avoid overlap between the emitted pulse and returning echo (Jones, 1999), and therefore the variability between individuals is difficult to assess.

Previously, Balcombe showed that mother bats can identify their pups based on the pups' vocal signals (Balcombe, 1990). Therefore, it would not be too surprising if adult bats can also discriminate their own vocal communication signals, as it would be evolutionarily advantageous for bats to have the ability to recognize a non-threatening familiar individual over a potentially threatening stranger using acoustic cues (Osborne, 2005; Tripovich *et al.*, 2008). This ability is particularly handy because bats live in dark caves, form large social colonies, and have poor vision (Fenton, 1984).

Results of our study are compatible with those of Carter and colleagues in adult white-winged vampire bats, *Diaemus youngi* (Carter *et al.*, 2008). These investigators showed that the antiphonal call of adult white-winged vampire bats carried individual signatures. In addition to individual calls, Bohn and colleagues recently studied the songs of adult Brazilian free-tailed bats, *Tadarida brasiliensis* (Bohn *et al.*, 2009); they defined bat "songs" using the classification scheme in birds (Marler and Slabbekoorn, 2004; Catchpole and Slater, 2008), i.e., songs are longer and contain multiple syllables (or notes and/or phrases) that are often combined in

TABLE III. Univariate analysis of variance (ANOVA) on individual acoustic features.

Call	Acoustic parameter	F	P
Steep-FM	Min frequency	140.1	<0.0001
	Max frequency	23.82	<0.0001
	Duration	15.9	<0.0001
Broadband noise burst	Min frequency	1.85	0.122
	Max frequency	44.46	<0.0001
	Duration	95.26	<0.0001

a stereotypical manner. Bohn *et al.*, (2009) reported significant variations between individuals; however, the researchers cautioned that these results could have been affected by the more pronounced level of variation found within individuals. That is, there was a high level (72%–92%) of variation from one individual's song rendition to the next rendition, which they suggested could have had an effect on individual signature analysis. Therefore, analysis of the acoustic syllables themselves i.e., calls (versus songs) as in this study, is necessary for gaining further insight into individual signatures. Putting the caveat aside, the various studies in echolocating bats collectively show the presence of individual-specific characteristics in not only their echolocation signals but also their sound communication signals.

Results from echolocating bats are compatible with those from studies in echolocating marine mammals: dolphins (Watwood *et al.*, 2005; Janik *et al.*, 2006) and whales (Weiss *et al.*, 2006). These studies in marine mammals show that features such as duration and frequency spectrum can be used to distinguish the sound communication signals of individuals. Furthermore, these individual call signatures appear to be dependent on the behavioral context. For example, Watwood *et al.* (2005) showed that signature whistles, defined as the most common whistle recorded from an individual, are more likely to be used when a dolphin is separated from their partner than when the dolphins are together. Although the present study did not explore the dependence of individual signatures on the nature of social interactions, it is possible that a similar dependence exists, since little brown bats are also highly social animals.

Individual signatures of communication calls have been well documented in frogs and birds. Studies in the American bullfrog revealed that these animals can discriminate individuals on the basis of their vocal signals; the critical difference being the call's fundamental frequency (Davis, 1987; Bee and Gerhardt, 2001a, 2001b, 2001c). A recent study in the concave-eared torrent frogs (*Odorrana tormota*), a species producing variable and highly complex calls, revealed that these animals also have individual-specific vocal signatures, despite its unlimited call repertoire (Feng *et al.*, 2009b). Similar to the little brown bats, the signatures are contained in the spectral and temporal domains of the vocal signals, and are behaviorally important for neighbor-stranger discrimination (Feng *et al.*, 2009a). Numerous studies in birds revealed that they can discriminate among individuals based on song signatures (Krebs *et al.*, 1981; Brooks and Falls, 1975; Falls, 1982); birdsongs can differ according to distinct song types, song sequences, or vocal signatures (Weary *et al.*, 1990; Weary and Krebs, 1992; Pincemy and Guyomarc'h, 2005; Sharp *et al.*, 2005; Ranjard and Ross, 2008).

In conclusion, the present study shows that the communication calls of adult little brown bats carry individual-specific differences. Thus, for bats that echolocate, it may be possible to discriminate individuals on the basis of the animals' echolocation and/or communication calls. Further studies are necessary to determine: (i) whether or not these individual-specific differences also serve as their individual call signatures which can be used to distinguish individuals

acoustically (ii) whether or not these phenomena are common to all species of echolocating bats, (iii) whether the call differences are dependent on the animal's behavioral context.

ACKNOWLEDGMENTS

This research was supported by a Grant No. R01DC04998 and a Fellowship No. F31DC008759 from the National Institute for Deafness and Communication Disorders. We thank the Illinois Department of Natural Resources for permitting collection of bats, and the UIUC Department of Animal Resources for assistance in care of the bats.

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