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Presence of single as well as double clicks in the echolocation signals of a fruit bat, *Rousettus leschenaulti* (Chiroptera: Pteropodidae)

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Abstract. We studied the structure of calls emitted by the echolocating fruit bat *Rousettus leschenaulti* (Megachiroptera: Pteropodidae) while flying inside a free-flight room. The echolocation sounds consists of both single as well as double clicks, with single clicks emitted either in the beginning or at the end of click sequences. The duration of clicks was brief, about 1.6 ms and most of the acoustic energy is between 18 to 32 kHz. The use of simple, brief impulsive clicks and the reduction of interpulse interval and duration, when approaching the sides of the walls suggest that they have a good capability of obstacle avoidance as similar to microchiropteran bats. Furthermore, the production of both single as well as double clicks like cave swiftlets in orientation flights has an implication for the evolution of echolocation in bats.

Key words: clicks, fulvous fruit bat, echolocation

Introduction

Among Megachiroptera, bats belonging to the genus of *Rousettus* and *Eonycteris* have retained the prowess of echolocation (Speakman 2001). Generally bats of genus *Rousettus* emit ultrasonic sounds by clicking their tongue (Möhres & Kulzer 1956), however in contrast the bats of *Eonycteris* species have developed a specialized form of echolocation by wing-clapping (Gould 1988). Interestingly, the functions of both forms of echolocation help them in finding their way around in caves where they roost (Altringham 1996). Echolocation in the genus *Rousettus* has been so far well described in *Rousettus aegyptiacus* (Herbert 1985, Waters & Vollrath 2003, Holland et al. 2004, Holland & Waters 2005), *R. amplexicaudatus* (Roberts 1975) and *R. seminudus* (Novick 1958). In most species of *Rousettus*, the echolocation clicks were emitted as double clicks which consists of two sub-clicks separated by a silent interval. Usually the sub-clicks are not distinguishable to human unaided ear (von Herbert 1985), but are heard as only one click. Here we describe the echoclick structure of the Indian fulvous fruit bat *R. leschenaulti*.

Materials and Methods

Three individuals (two ♂♂ and one ♀) of *R. leschenaulti* were captured from their native habitat at Thirupuramkundram temple situated about 16 km from Madurai Kamaraj University campus (9^0 58’ N, 78^o 10’ E) using a mist net (Avinet Inc. USA). Before recording all the bats

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were housed in groups for a maximum of three days in an outdoor enclosure situated in the Botanical Garden of the University. The bats were fed with fruits guava, banana and papaya, and water were provided *ad libitum*. For recording the echolocation clicks we transported the individual bats to a free flight room (3.1 m L x 2.4 m W x 4.0 m H). The flight room was illuminated using a 15-W Philips red darkroom bulb and the illumination had no influence on the bats' echolocation behaviour.

Echolocation signals emitted by *R. leschenaulti* were picked up by a SM2 microphone connected to a S25 bat detector (UltraSound Advice, UK) and transferred into the Portable Ultrasound Signal Processor (PUSP: Ultra Sound Advice, UK) which time-expands 2s of sound by 10X at a sampling rate of 448 kHz. The calls were stored onto Sony *HF*90 cassettes using a Sony WM-D6C Professional Walkman cassette recorder (Sony Corporation, Japan). The bats were released after sunset at the site of capture.

The time-expanded sounds from Walkman were downloaded to a personal computer and analyzed using BatSound v 2.00 (Pettersson Electronik AB, Uppsala, Sweden) with 16 bit A: D converter at a sampling rate of 44.1 kHz. The threshold level was set at 16 in BatSound and a FFT size of 512 points. Interpulse interval (onset of first click to the onset of next click) and the duration of clicks were measured from oscillograms. The peak frequency of the harmonic of all the clicks was noted from the power spectra. We analyzed a total of 18 sequences of echolocation clicks, six sequences per bat. A sequence refers to echolocation clicks that were recorded when a bat makes one or more complete circle flights inside the flight room. We began sound analysis starting from the first click of each sequence for which we could extract all relevant information (i.e., good signal-to-noise ratio). To analyze signal parameters, we selected 10 single and 20 double clicks per sequence and excluded those clicks which were clipped. Initially we analyzed the data to find out whether there were significant differences in the emission of clicks within individual bats using one-way ANOVA. As we found no individual differences within bats, we pooled the data and used non-parametric Wilcoxon Signed-Ranks test to compare each signal parameters.

Results and Discussion

The echolocation sequences of *R. leschenaulti* consist of both impulsive single and double clicks (Fig. 1a), with single clicks occurring either in the beginning (n = 6) or at the end (n = 12) of click sequences. On an average, of six sequences per individual, *R. leschenaulti* emit 10.1 ± 0.09 (n = 3) single clicks and 21.6 ± 0.83 (n = 3) double clicks. There were no inter-individual differences in the emission of both single clicks (One-way ANOVA, $F_{2, 15} = 0.020$, $p > 0.05$ NS) and double clicks (One-way ANOVA, $F_{2, 15} = 0.029$, $p > 0.05$ NS). The pulse repetition rates were 2.50 ± 0.17 (n = 3) single clicks per second and 9.33 ± 1.04 (n = 3) double clicks per second. In both single and double clicks the first peak frequency ranges from 18.08 to 19.70 kHz (Tables 1 and 2), and the second peak in the power spectrum (Figs 1b and c) is -5 dB below the first between 28.52 to 33.64 kHz (Tables 1 and 2). In double clicks, the duration of second clicks is higher than the first clicks (Wilcoxon Signed-Ranks non-parametric test, Z = -5.07, n = 225, p = 0.0001). The interpulse interval between clicks is significantly higher in single clicks than double clicks (Z = -11.23, n = 183, p = 0.0001; Tables 1 and 2). When bats approached the obstacles (sides of wall) the interpulse interval and duration of double clicks decreases (Figs 1d and e). However we did not record any distinct pattern of characteristic "terminal" echolocation sequence as emitted by aerially feeding insectivorous

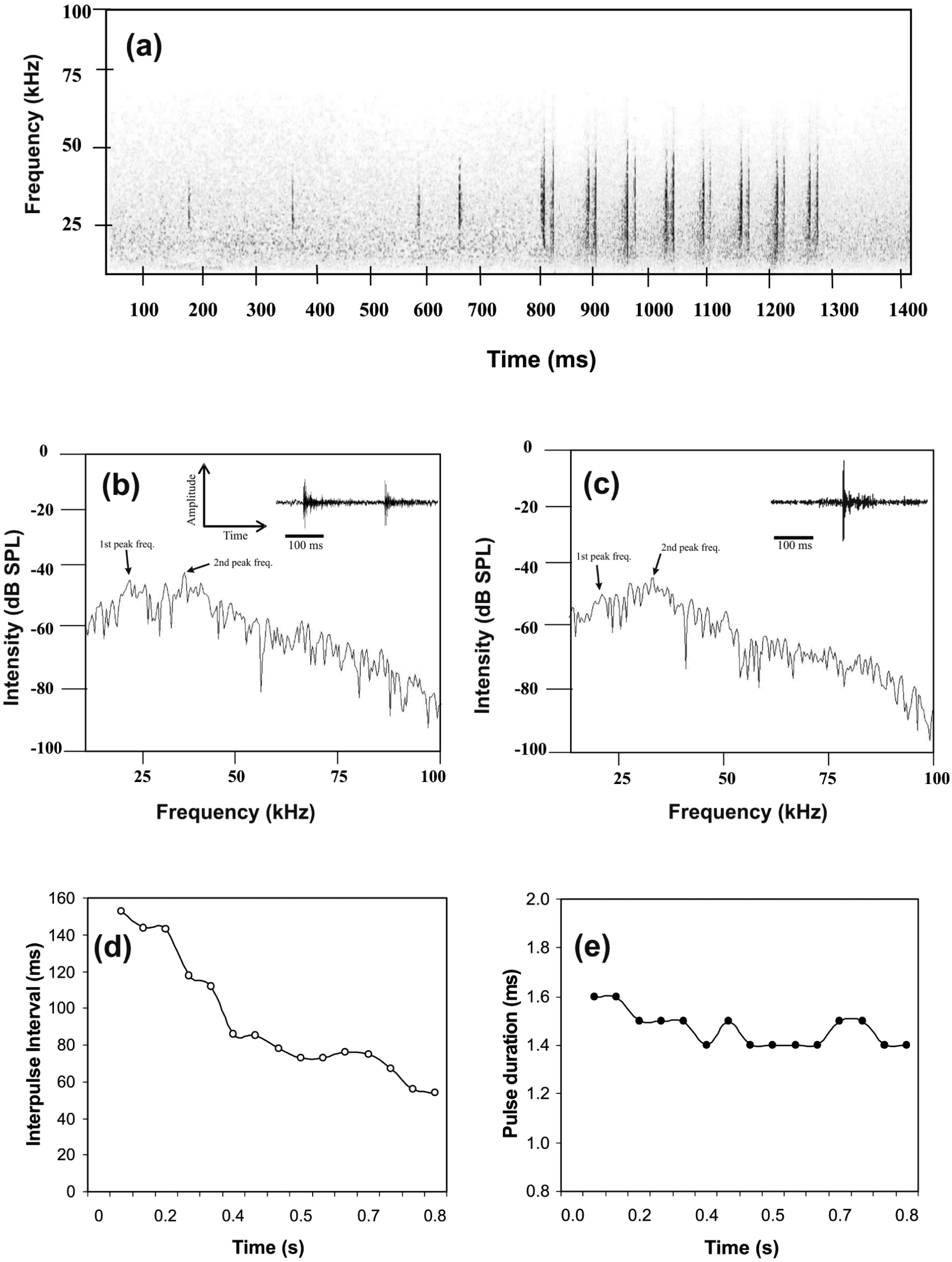

Fig. 1. Echolocation click parameters for a single individual (Bat 1) representing (a) a echolocation click sequence starting with single clicks at the beginning and further continued with double clicks (b) Power spectrum of the first click (from a double–click pair). In-set depicts respective wave form – the scale bar represents the approximate extent of the waveform and amplitude is on an arbitrary scale (c) Power spectrum of a single click and in-set depicts respective wave form (d) echolocation sequence indicating changes in interpulse interval, and changes in duration (e) while approaching the obstacle (sides of the wall)

Table 1. Duration, Interpulse interval and peak frequencies of single clicks produced by three *R. leschenaulti*. Data were given as mean ± SD. For each bat 60 single clicks were pooled from six echolocation sequences.

	Duration (ms)	Interpulse interval (ms)	1st Peak frequency	2nd Peak frequency
Bat 1 (♂)	1.62 ± 1.08	174.30 ± 32.91	18.08 ± 1.28	30.02 ± 5.39
Bat 2 (♂)	1.59 ± 0.30	187.76 ± 30.41	18.87 ± 1.34	31.93 ± 5.65
Bat 3 (♀)	1.60 ± 0.36	178.77 ± 48.64	19.30 ± 1.03	30.51 ± 3.46
Mean	1.61	180.28	18.76	30.82
SD	0.02	6.86	0.62	0.99

bats, in which the duration and IPI of echolocations calls become much shorter when they reach the targets.

On an average, *R. leschenaulti* emitted 21% of sound sequences as single clicks. Thus producing single clicks may represent an inherent flexibility in the vocalization mechanism of *R. leschenaulti* allowing these bats to avoid pulse-echo overlap, since they live in closed environment like caves, wells or man-made structures. A similar pattern of echo-click design is also reported in cave swiftlets. For example *Aerodramus fuciphagus, A. maximus, A. salanganus,* and *A. vulcanorum* are known to emit single as well as double clicks (Thomassen et al. 2004), whereas *A. vanikorensis*, *A. ocistus* and *A. sawtelli* typically emits single clicks (Fullard et al. 1993, Thomassen et al. 2004). Thus the possession of both good visual system (Thangadurai 2001) and echolocation with a characteristic echo-click pattern similar to cave swiftlets in *R. leschenaulti* has implications for the evolution of echolocation in bats (Springer et al. 2001). In the present study we could not get sufficiently long sequences because the structure of signals was strongly influenced by obstacles (walls) close to flying bats. Furthermore, short distances of walls caused often turning of bats. However recording in natural conditions in their roosting caves may presumably provide precise sound characteristics to confirm our objectives. The long duration of second click as compared to the first click, is may be apparently due to multiple overlapping echoes reflected from the flight room.

Our analysis showed that the echolocation clicks of *R. leschenaulti* were close to the range of frequencies reported for *R. aegyptiacus* and *R. amplexicaudatus* with most of the

Table 2. Duration, Interpulse interval and peak frequencies of double click produced by three *R. leschenaulti*. For each bat 75 double clicks were pooled from six echolocation sequences.

Call parameters	Bat 1	Bat 2	Bat 3	Mean	SD
1st click					
Duration (ms)	1.51	1.48	1.49	1.49	0.01
1st peak frequency (kHz)	19.25	19.23	19.23	19.24	0.01
2nd peak frequency (kHz)	27.35	34.95	33.97	32.09	4.13
2nd click					
Duration (ms)	1.63	1.60	1.57	1.60	0.03
1st peak frequency (kHz)	19.70	19.35	19.40	19.48	0.19
2nd peak frequency (kHz)	28.52	33.03	33.64	31.73	2.80
IPI (ms)					
Within clicks	24.68	23.65	28.04	25.46	2.30
Between clicks	96.44	88.26	102.29	95.66	7.05

acoustic energy ranging between 18–32 kHz (Roberts 1975, Waters & Vollrath 2003). Also the range of maximum and minimum frequency of clicks matches with their best hearing sensitivity at 30–50 kHz (Rajakumari 1994). The brief impulsive clicks of *R. leschenaulti* may be helpful for good obstacle avoidance (Waters & Vollrath 2003, Holland et al. 2005). The reduction of interpulse interval and duration when approaching the obstacles further reiterates that the function of echolocation in orientation is similar to microchiropteran bats (Griffin 1958, Griffin et al. 1958)

Generally, in megachiropteran bats, vision and olfaction are the only available sensory system for general orientation and food acquisition (Altringham & Fenton 2003). But in microchiropteran bats this function is predominantly taken over by echolocation (Griffin 1958) that helps both to detect prey and to find their way in dark environs. Although *R. leschenaulti* relatively use vision and olfaction for foraging (Thangadurai 2001), the exact function of echolocation clicks is not yet studied in detail. Perhaps, our study provides the first report on this species describing its echolocation features and further study is extensively needed to reveal whether this bat uses echolocation for food acquisition.

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