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Research paper

Threshold minima and maxima in the behavioral audiograms of the bats *Artibeus jamaicensis* and *Eptesicus fuscus* are not produced by cochlear mechanics

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Abstract

Behavioral audiograms of *Artibeus jamaicensis* and *Eptesicus fuscus* are characterized by two threshold minima separated by a threshold maximum at 40 kHz, for *A. jamaicensis*, and 45 kHz, for *E. fuscus* [Koay, G., Heffner, H.E., Heffner R.S., 1997. Audiogram of the big brown bat (*Eptesicus fuscus*). Hear. Res. 105, 202–210; Heffner, R.S., Koay, G., Heffner H.E., 2003. Hearing in American leaf-nosed bats. III: *Artibeus jamaicensis*. Hear. Res. 184, 113–122.]. To investigate whether these characteristics are due to cochlear properties, we recorded distortion product otoacooustic emissions (DPOAEs) and calculated DPOAE threshold curves. We found that in both species cochlear sensitivity, assessed by DPOAE recordings, does not show local threshold maxima. The DPOAE threshold curve calculated for *A. jamaicensis* reveals a broadly tuned minimum for frequencies between 20 and 50 kHz and the threshold curve of *E. fuscus* shows a broad sensitive area for frequencies between 15 and 60 kHz. In none of the two species any pronounced threshold irregularities were found. The characteristic pattern of a threshold maximum followed by a minimum observed in behavioral studies seems to be shaped by transfer characteristics of the outer ear and/or neuronal processing in the ascending auditory pathway rather than by cochlear mechanics.

Keywords: Distortion product otoacoustic emission; FM bats; Pinna; Cochlear mechanics

1. Introduction

The Jamaican fruit-eating Bat *Artibeus jamaicensis* and the Big Brown Bat *Eptesicus fuscus* are two of the most widely distributed bat species of the new world. Their diets consist on fruits (*A. jamaicensis*) or insects (*E. fuscus*). The echolocation calls of *A. jamaicensis* contain significant less energy than those of *E. fuscus* and other species of insectivorous bats (Griffin, 1958; Novick, 1963; Surlykke and Moss, 2000). The echolocation signals of *A. jamaicensis* have been described as faint, short, multiharmonic, frequency modulated (FM) calls with energy concentrated mainly between 50 and 104 kHz (Griffin, 1958; Novick, 1963; Schnitzler and Kalko, 1998). On the other hand, echolocation signals of *E. fuscus* are rather long FM calls at a frequency range of 20–50 kHz (Surlykke and Moss, 2000). In contrast to bats that use

Abbreviations: DPOAE, distortios product otoacoustic emissions; FM, frequency modulated; CF, constant frequency

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long constant frequency (CF) echolocation call components and that show highly specialized hearing characteristics, there are no frequency specific cochlear specializations known for *A. jamaicensis* or *E. fuscus*. The hearing abilities of these species assessed by behavioral experiments range from 5 to 120 kHz, with two threshold minimum at 16–56 kHz in *A. jamaicensis*, and 20–64 kHz in *E. fuscus* (Koay et al., 1997; Heffner et al., 2003). The presence of two threshold minima separated by a maxima have been interpreted as an evolutionary adaptation for processing both communication and echolocation signals in separate frequency channels. The threshold maxima has be related to frequency specific pinna gain or to the existence of specific neuronal processing along the ascending auditory pathway that could filter certain frequency ranges and hence determine the resulting behavioral response (Heffner et al., 2003; Koay et al., 2003; Wittekindt et al., 2005).

To investigate whether the characteristics of the behavioral audiogram of *A. jamaicensis* and *E. fuscus* are due to cochlear properties, we recorded distortion product otoacoustic emissions (DPOAEs) to calculate relative DPOAE threshold curves, a method that has proven to be a reliable and objective measure of cochlear sensitivity (Kössl, 1992). DPOAE threshold curves generally are 10–30 dB above most sensitive behavioral or neuronal threshold curves and therefore give the relative course of the threshold of cochlear mechanics but not the absolute hearing sensitivity.

2. Materials and methods

Distortion product otoacoustic emissions were measured from specimens of both sex of *A. jamaicensis* ($n = 8$) and *E. fuscus* ($n = 7$). These bats were captured in Havana city (*A. jamaicensis*) and at Indio Cave (*E. fuscus*), located in the periphery of Havana, Cuba. After the completion of the non-invasive measurements that took place in the Faculty of Biology of Havana University, bats were released at the capture sites.

To record DPOAEs, the bat's head was fixed with a mouth holder made of dental acrylic. A closed coupler system, incorporating two adjacent conical tubes for stimulation and recording, was placed in the meatus under visual control at a distance 0.3–1 mm from the tympanum. One coupler channel was connected to a 1/4″ Brüel and Kjaer 4135 microphone, and two 1″ Microtech Gefell MK 103.1 microphone capsules served as speakers connected to the other coupler channel. To determine DPOAE threshold curves that reflect the relative sensitivity of non-linear cochlear mechanics, we presented two pure tones of the primary frequencies f1 and f2 and measured the most prominent cubic distortion product 2f1–f2. For the experiments, a Pentium PC with a Microstar 3000a/212 DSP board (A/D and D/A sampling rate of 250 kHz) was used for generation of the two pure tone stimuli and for the FFT analysis of the microphone signal. To improve the signal to noise ratio, a total of 180 responses to the two stimuli that had a constant phase relation were averaged before FFT analysis. The recording system was calibrated in situ for constant SPL at the microphone membrane using white noise. The white noise was generated by addition of sinusoids of constant phase relation. For a detailed description of the measuring procedures see Kössl et al. (1999).

In both species, distortion product otoacoustic emissions were measured for frequencies from 10 to 115 kHz. Fig. 1(a) shows an example measured in *A. jamaicensis* giving the frequency spectrum of the two stimuli and the distortion product otoacoustic emissions. The most prominent DPOAE is at the frequency 2f1–f2. The DPOAE level was optimized by choosing a f1 frequency that, at low stimulus levels, produced maximum DPOAE levels for a given f2 frequency.

For the threshold measurements, f2/f1 was set to the best ratio and growth functions of the 2f1–f2 DPOAE were recorded by increasing the level of f1 and f2 in 2 dB increments (Fig. 1(b)). The levels of the two stimuli always were adjusted in such a way that the f1 stimulus was 10 dB louder than the f2 stimulus. From growth functions measured for the range of chosen f2 frequencies, the f2 stimulus level that was sufficient to generate a small distortion of −10 dB SPL (threshold criterion) was interpolated and defined as the threshold value for a given f2 frequency. We generally measured growth functions only once for each f2 frequency. One growth function consists of 200 averages in the time domain. In some cases, we repeated the measurement of a growth function to check for repeatability. In these cases, the growth functions as well as the threshold values did not change within 3 dB.

3. Results

In both species, there was no obvious dependence of the best ratio on the frequency of f2. The corresponding best ratios f2/f1 (f1 < f2) ranged from 1.13 at f2 frequency of 10 kHz to 1.21 at 115 kHz f2 frequency for *A. jamaicensis*, and from 1.17 to 1.09, for *E. fuscus*.

The data for the average threshold curves were sampled from six specimens of *A. jamaicensis* and four of *E. fuscus* (Fig. 2). In two other individuals from *A. jamaicensis* and three other from *E. fuscus* the threshold curves were more than 20 dB less sensitive. These data were excluded from the average dataset. In none of the individual threshold curves of both species we found any irregularities in form of threshold maxima (Fig. 2).

The DPOAE threshold curve of *A. jamaicensis* is characterized by a broadly tuned minimum in the frequencies range of 20–50 kHz where thresholds are below 25 dB SPL (Fig. 3). The DPOAE threshold increases with lower and higher test frequencies. In the average threshold curve a small relative threshold maximum of <5 dB at 35 kHz is not significantly different from the threshold values at 30–40 kHz (ANOVA: $F = 0.8837$; $p = 0.4280$). The course of the average threshold curve parallels the course of the minimal threshold values obtained from all animals (Fig. 3).

In comparison with *A. jamaicensis*, the DPOAE threshold curve of *E. fuscus* shows a broader tuned minimum, in the frequency range between 15 and 60 kHz (Fig. 3). Minimum average values below 20 dB SPL are found for frequencies between 35 and 45 kHz. The DPOAE threshold increases pronouncedly for frequencies lower than 15 kHz and higher than 70 kHz. The shape of the average curve is also similar to the curve of minimal thresholds over all animals (Fig. 3).

4. Discussion

In bats and other mammals, DPOAE threshold curves give a good indication of the mechanical sensitivity of the cochlea. A correspondence of maximum 2f1–f2 levels and of minimum 2f1–f2 thresholds with the range of best hearing has been established for other mammals (Faulstich et al., 1996; Mills and Sheperd, 2001) including bats