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Hearing sensitivity evaluated by the auditory brainstem response in *Miniopterus fuliginosus*

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Abstract: This study evaluated the hearing sensitivity of *Miniopterus fuliginosus*, a frequency-modulating (FM) bat species, by measuring the auditory brainstem responses in the inferior colliculus. The average audiogram was U-shaped. The mean threshold decreased gradually as the frequency increased from 16 to 40 kHz and then decreased rapidly as the frequency reached 46 kHz, with the peak sensitivity occurring at the terminal portion of the echolocation pulse between frequencies of 44 and 56 kHz. The shape of audiogram of *M. fuliginosus* is consistent with other FM bats, and is compared with its vocalization behavior.

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1. Introduction

To perceive their spatial environment in complete darkness, bats emit ultrasound pulses and listen to the returning echoes in a process termed echolocation. Echolocating bats are divided into two groups according to the time–frequency structure of their broadcasts (e.g., Neuweiler, 1984): frequency-modulating (FM) bats and bats with a long constant frequency (CF) component followed by a short FM component. The echolocation sounds are species-specific and are adapted according to the bat's acoustic environment (e.g., foraging habitat); in other words, the auditory system reflects the acoustic environment. To understand the echolocation system of bats, it is therefore important to measure both the hearing sensitivity and the behavioral features.

The auditory brainstem response (ABR) is used to measure hearing sensitivity. It comprises a series of waveforms with short latencies (<10 ms) that collectively represent the neural potential evoked by a sound stimulus. The ABR is recorded either intracranially or extracranially, and is obtained more quickly than behavioral responses. The hearing sensitivities of some echolocating bat species have been quantified by measuring ABRs in the inferior colliculus (Grinnell, 1963; Jen and Suthers, 1982; Boku *et al.*, 2015).

The echolocation calls of eastern bent-winged bats (*Miniopterus fuliginosus*), a frequency-modulating (FM) species, consist of ultrasound signals that exhibit a downward sweep from an initial frequency of approximately 100 kHz to a terminal frequency (end frequency of downward FM sweeps) of approximately 45–50 kHz (Hase *et al.*, 2016). In many bats species, hearing ability are closely associated with their vocalizations. Our recent research using a telemetry microphone system allow us to quantify how *M. fuliginous* changed its vocalizations under acoustically jamming conditions, and showed that the bat tended to increase its pulse frequency by approximately 2 kHz (Hase *et al.*, 2016). We hypothesized that the region of high hearing sensitivity of the bat must cover this increase in frequency to maintain auditory–vocal correspondence. The present study evaluated the hearing sensitivity of *M. fuliginous* by ABR in the inferior colliculus.

2. Materials and methods

2.1 Animal preparation

Three eastern bent-wing bats (*M. fuliginosus*; body weight 12–14 g; one male and two females) were captured from natural caves in Fukui Prefecture in compliance with current Japanese laws. All experimental procedures were approved by the Animal Experiment Committee at Doshisha University.

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The bats were kept in a temperature- and humidity-controlled room ($4 \times 3 \times 2$ m height) under a diurnal light cycle of 12 h dark/12 h light at Doshisha University (Kyoto, Japan). All bats were allowed free access to food (mealworms) and vitamin-enriched water.

The bats were anesthetized with isoflurane during surgery. The scalp fur was shaved, and the skin and muscles over the cranium were retracted. A head post (small metal rod with a flat bottom) was fixed to the top of the exposed cranium with acrylic glue and dental cement.

2.2 Acoustic stimuli

Acoustic stimuli were generated using a custom software program in MATLAB (MathWorks, Inc., Natick, MA, USA). We employed 2 ms tone pips (linear window, 1 ms rise/fall times) at the following frequencies: 4, 8, 16, 32, 36, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 64, 70, 80, 90, and 94 kHz. Acoustic stimuli were presented via a sound card (UA-101, Roland, Shizuoka, Japan; sampling frequency, 192 kHz) and amplifier (SRP-P2400, Sony, Tokyo, Japan) with a programmable attenuator (PA5, Tucker Davis Technology, Alachua, FL, USA). A loudspeaker (ES1, Tucker Davis Technology, Alachua, FL, USA) was positioned 5 cm in front of the bat's head.

The amplitudes of the stimuli were calibrated using a microphone (type 7016, Aco Co., Tokyo, Japan) placed on the bat's head. The sound level of each stimulus was expressed as the peak-to-peak sound pressure level (SPL) (p–p, re. 20 μPa). Sound stimuli were initially presented at 80 dB SPL p–p and then decreased in 5 dB steps until reaching 20 dB SPL p–p. The repetition rate was 20 times/s (inter-stimulus interval, 50 ms), and the ABR measurement at each frequency was repeated 100 times at each intensity.

2.3 Recording procedure

ABR measurements were conducted in a sound-attenuated room. We fixed each bat in a custom-made polystyrene foam holder to prevent any movement of the shoulders or feet. Using a fine needle, a small hole was made in the dorsal skull, directly over the inferior colliculus (approximately 0.5 mm posterior to the lambda and 2 mm lateral to the midline). A tungsten microelectrode (impedance, 30–50 kΩ) was inserted into the inferior colliculus using a hydraulic micromanipulator (MO-8, Narishige, Tokyo, Japan). Recordings were obtained at an intracollicular depth of 100 μm. The ABR was amplified using a differential amplifier (DAM 80, World Precision Instruments, Sarasota, FL, USA) and filtered using a dual variable filter (0.1 Hz to 5.0 kHz; VBF 8, Kemo Ltd, Dartford, Kent, UK). The recordings were stored in a personal computer via an analogue-to-digital converter (Micro 1401, Cambridge Electronic Design, Milton, Cambridge, UK).

2.4 Data analysis

In off-line analysis, background noise was reduced using a digital band-pass filter (300 Hz to 3 kHz). The recorded waveform responses were measured using a custom program in MATLAB and averaged over the 100 repetitions of each frequency at each intensity. We analyzed the waveforms that occurred within the first 7 ms of the sound reaching the bat's ear. Three or four positive peaks were observed in this time window, and the latency and intensity of each peak were quantified. The peaks were labeled according to Boku *et al.* (2015); because the second and third peaks were difficult to distinguish from one another, they were grouped together as P2/P3 in this study. The latency of the fourth waveform peak corresponds to the travel time from the cochlea to the inferior colliculus (Haplea *et al.*, 1994). In the previous study (Boku *et al.*, 2015), the fourth peak was simply the largest peak, and perhaps the only distinguishable peak at the lower levels tested. It is also an acceptable to choose the largest peak because it provides the best estimate of the threshold under these experimental conditions. Therefore, in the present study, we used the largest peak (it appeared as the fourth peak) as an indicator of hearing sensitivity to measure the amplitude as the ABR threshold in this study. The threshold at each frequency was defined as the point at which the largest peak exceeded the noise level by at least 0.25 μV.

3. Results

Figure 1 presents the ABR waveforms of bat A, obtained at 46 kHz and 20–80 dB SPL p–p. At 40–80 dB SPL p–p, the first positive peak (P1) appeared at 1.9–2.5 ms, the second and third positive peaks (P2/P3) at 3.0–3.3 ms. At 25–80 dB SPL p–p, the fourth positive peak (P4) appeared at 3.8–4.7 ms and exhibited the highest ABR amplitude. No clear peaks were observed at 20 dB SPL p–p. As sound intensity increased, the

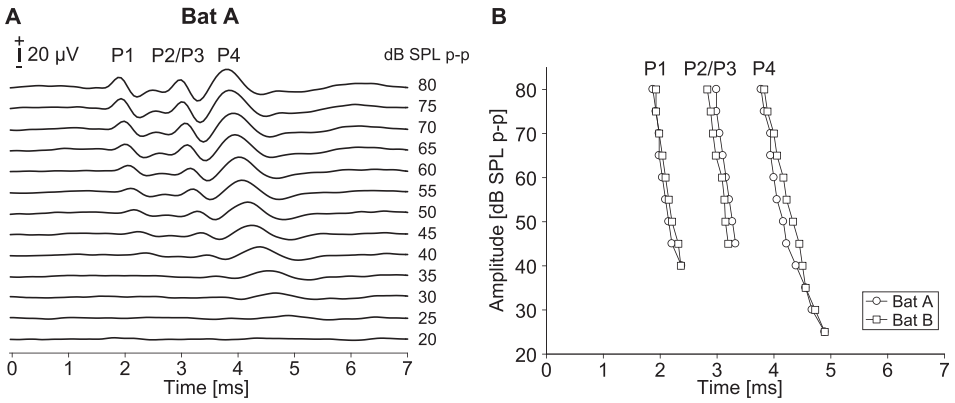

Fig. 1. (A) Waveforms of the auditory brainstem response (ABR) in bat A, recorded in the inferior colliculus at 46 kHz and 20–90 dB peak-to-peak sound pressure level (SPL p–p). Three distinct peaks were identified and labeled P1, P2/P3, and P4. The waveform values are the averages of 100 measurements, and the vertical scale bar is 20 μV. (B) Sound intensity–latency trading for the three peaks at 46 kHz in bat A and B.

ABR amplitude increased and the ABR latency decreased [Fig. 1(B)]. In a linear approximation, the latency of P1 decreased by 9.6–11.2 μs/dB, that of P2/P3 by 9.6–10.5 μs/dB, and that of P4 by 12.8–17.6 μs/dB, as the sound intensity increased from 45 to 80 dB SPL p–p.

Figure 2(A) shows the mean hearing sensitivities of the three bats according to the amplitude of P4. The average audiogram was U-shaped for all bats. The mean threshold decreased gradually as the frequency increased from 16 to 40 kHz, and then decreased rapidly as the frequency reached 46 kHz. The lowest threshold was approximately 25 dB SPL p–p at frequencies of 46–54 kHz. The mean threshold then increased gradually from 56 to 94 kHz. The highest threshold was 68 dB SPL p–p at 16 kHz.

4. Discussion

The lowest threshold was observed between 46 and 54 kHz at approximately 25 dB SPL p–p, and this range corresponded to the dominant frequency [i.e., terminal frequency, Fig. 2(B)] of echolocation pulses in *M. fuliginosus* reported by Hase *et al.* (2016). A relationship between hearing sensitivity and the dominant frequency of the echolocation pulse has been observed in other FM bat species (Grinnell, 1963; Obrist and Wenstrup, 1998). In addition, the profile of the ABR audiogram of *M. fuliginosus* was very similar to that of another *Miniopterus* species, *M. schreibersii* (Jen and Suthers, 1982), suggesting similar hearing sensitivity profiles among the species of this genus.

In general, the intensity-latency (IL) functions of the ABR are measured to study several aspects of auditory temporal processing (such as development and nerve injury) in many species of animals, including cat (Huang and Buchwald, 1978), dolphin (Ridgway *et al.*, 1981), and gerbil (Burkard and Voigt, 1989). Several previous studies have investigated the IL trading of various FM bat species to understand the temporal

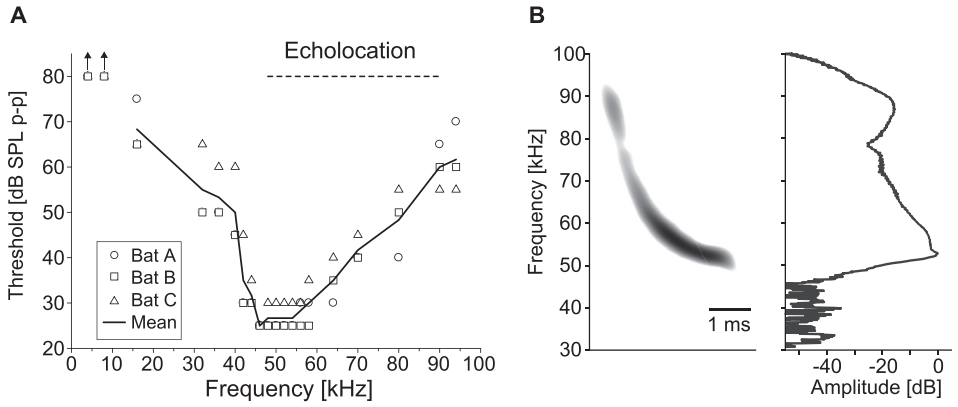

Fig. 2. (A) Hearing sensitivity of *M. fuliginosus*. The solid line represents the mean auditory brainstem response (ABR) threshold of the three bats. The arrows indicate that no ABR was observed in any bat at 4 or 8 kHz at an intensity of 80 dB peak-to-peak sound pressure level (SPL p–p). (B) Spectrograms of the echolocation sounds (left panel) and power spectrum (right panel) of *M. fuliginosus*. This call was produced by one bat during flight in the recording room.

processing of their echolocation sounds (Burkard and Moss, 1994; Boku *et al.*, 2015). Special attention has been paid to IL trading because the latency shift causes ranging error, and the echolocation system must build on physiological constrains. Simmons and his colleagues have shown that the amplitude-induced latency shift of ABR in the big brown bat, *Eptesicus fuscus*, is consistent with its target ranging error (Simmons *et al.*, 1990). In a recent study, the latency of P4 was shortened by 12–15 μs/dB at 44–104 dB peak-equivalent SPL in awake *P. abramus* (Boku *et al.*, 2015). Our results showed that the latency of the P4 peak decreased by 12.8–17.6 μs/dB as the sound intensity increased from 45 to 80 dB SPL p–p, and these data are comparable with those obtained from *P. abramus*. This relationship demonstrates that the IL trading of the ABR is relatively similar across species that share similar echolocation pulses, even among different families. Our data suggest that the temporal processing of echolocation sounds up to the brainstem level are similar among these FM bats.

Our previous research demonstrated that *P. abramus* and *M. fuliginosus* emit echolocation pulses in the same frequency range and increase their pulse frequency by a maximum of approximately 5 kHz to avoid acoustic jamming (Takahashi *et al.*, 2014; Hase *et al.*, 2016). On the other hand, the ABR audiograms of both species were most sensitive near the terminal frequency of their FM pulse, and the sensitive region extended to a higher frequency by approximately 6 kHz. The hearing sensitivity profile may be related to the degree of upward shift that the bat exhibits under jamming. Therefore, we suggest that the acoustic fovea of the FM bat, although probably not as highly specialized as that of the CF–FM bat, has broadened to accommodate their jamming avoidance behavior. Future research is required to confirm this hypothesis.

In many FM bats, the best hearing sensitivity occurs at the dominant frequency (i.e., terminal frequency) of their echolocation pulse, and their high-sensitivity region often extends toward lower frequencies. For example, the sensitivity drops by a maximum of ~5 dB within 10 kHz region lower than the dominant frequency in several FM bats, including little brown bats (Grinnell, 1963), red bats (Obrist and Wenstrup, 1998), and Japanese house bats (Boku *et al.*, 2015). Therefore, *M. fuliginous*, which shows a 28 dB decline in 10 kHz, may have poor low-frequency hearing. On the other hand, *M. fuliginous* has better high-frequency sensitivity than *P. abramus*. The sensitivity at 10 kHz higher than the dominant frequency is worse than that of the dominant frequency by 8 dB (46–56 kHz) in *M. fuliginous* and by 20 dB (42–52 kHz) in *P. abramus*. The highest frequency produced detectable ABR was 96 kHz in *M. fuliginous* and 80 kHz in *P. abramus*, and from the dominant pulse frequency, hearing sensitivity declined by 0.7 dB/kHz (46–96 kHz) in *M. fuliginous* and by 1.1 dB/kHz (42–80 kHz) in *P. abramus*. This greater hearing sensitivity in high-frequency range confers several advantages on *M. fuliginous*. It has been shown that a wider pulse band-width correlates with better echolocation performance, including better target accuracy and target resolution in perceiving prey (Simmons, 1973; Siemers and Schnitzler, 2004). The better high-frequency sensitivity of *M. fuliginous* might allow it to utilize wider frequency information in its FM echo, and possibly advantages of a wider pulse band-width.

A previous study showed that the hearing sensitivity at low frequencies corresponded to the isolation calls of conspecific pups of various species of echolocating bats (Bohn *et al.*, 2006); thus, the hearing sensitivity at low frequencies correlated with the frequency range of their communication sounds. In the present study, the threshold at 16 kHz was approximately 40 dB higher in *M. fuliginosus* than in *P. abramus*, suggesting a greater intraspecies difference in the hearing sensitivity at low frequencies (<40 kHz) than at high frequencies. This could imply *M. fuliginosus* does not use a low-frequency range for the communication as does *P. abramus*. Currently, there is limited knowledge regarding their vocal communications (i.e., the frequency range used for non-echolocation vocalization) in *M. fuliginosus.* This issue can be investigated in future research by thoroughly recording and quantifying the vocalizations of these two species, including their echolocation and communication sounds.

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References and links

Bohn, K. M., Moss, C. F., and Wilkinson, G. S. (**2006**). "Correlated evolution between hearing sensitivity and social calls in bats," Biol. Lett. **2**, 561–564.

Boku, S., Riquimaroux, H., Simmons, A. M., and Simmons, J. A. (**2015**). "Auditory brainstem response of the Japanese house bat (*Pipistrellus abramus*)," J. Acoust. Soc. Am. **137**, 1063–1068.

Burkard, R., and Moss, C. F. (**1994**). "The brain-stem auditory-evoked response in the big brown bat (*Eptesicus fuscus*) to clicks and frequency-modulated sweeps," J. Acoust. Soc. Am. **96**, 801–810.

Burkard, R., and Voigt, H. F. (**1989**). "Stimulus dependencies of the gerbil brain-stem auditory-evoked response (BAER). I: Effects of click level, rate, and polarity," J. Acoust. Soc. Am. **85**, 2514–2525.

Grinnell, A. (**1963**). "The neurophysiology of audition in bats: Intensity and frequency parameters," J. Physiol. **167**, 38–66.

Haplea, S., Covey, E., and Casseday, J. (**1994**). "Frequency tuning and response latencies at three levels in the brainstem of the echolocating bat, *Eptesicus fuscus*," J. Comp. Physiol. A **174**, 671–683.

Hase, K., Miyamoto, T., Kobayasi, K. I., and Hiryu, S. (**2016**). "Rapid frequency control of sonar sounds by the FM bat, *Miniopterus fuliginosus*, in response to spectral overlap," Behav. Processes **128**, 126–133.

Huang, C.-M., and Buchwald, J. (**1978**). "Factors that affect the amplitudes and latencies of the vertex short latency acoustic responses in the cat," Electroencephalogr. Clin. Neurophysiol. **44**, 179–186.

Jen, P. H.-S., and Suthers, R. A. (**1982**). "Responses of inferior collicular neurones to acoustic stimuli in certain FM and CF-FM paleotropical bats," J. Comp. Physiol. A **146**, 423–434.

Neuweiler, G. (**1984**). "Foraging, echolocation and audition in bats," Naturwissenschaften **71**, 446–455.

Obrist, M. K., and Wenstrup, J. J. (**1998**). "Hearing and hunting in red bats (*Lasiurus borealis, Vespertilionidae*): Audiogram and ear properties," J. Exp. Biol. **201**, 143–154.

Ridgway, S., Bullock, T., Carder, D., Seeley, R., Woods, D., and Galambos, R. (**1981**). "Auditory brainstem response in dolphins," Proc. Natl. Acad. Sci. U.S.A. **78**, 1943–1947.

Siemers, B. M., and Schnitzler, H.-U. (**2004**). "Echolocation signals reflect niche differentiation in five sympatric congeneric bat species," Nature **429**, 657–661.

Simmons, J. A. (**1973**). "The resolution of target range by echolocating bats," J. Acoust. Soc. Am. **54**, 157–173.

Simmons, J. A., Moss, C. F., and Ferragamo, M. (**1990**). "Convergence of temporal and spectral information into acoustic images of complex sonar targets perceived by the echolocating bat, *Eptesicus fuscus*," J. Comp. Physiol. A **166**, 449–470.

Takahashi, E., Hyomoto, K., Riquimaroux, H., Watanabe, Y., Ohta, T., and Hiryu, S. (**2014**). "Adaptive changes in echolocation sounds by *Pipistrellus abramus* in response to artificial jamming sounds," J. Exp. Biol. **217**, 2885–2891.