

Echolocation Calls and Neurophysiological Correlations with Auditory Response Properties in the Inferior Colliculus of *Pipistrellus abramus* (Microchiroptera: Vespertilionidae)

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Feng Luo, Jie Ma, An-An Li, Fei-Jian Wu, Qi-Cai Chen, and Shu-Yi Zhang (2007) Echolocation calls and neurophysiological correlations with auditory response properties in the inferior colliculus of *Pipistrellus abramus* (Microchiroptera: Vespertilionidae). *Zoological Studies* 46(5): 622-630. The present study examines the echolocation calls and auditory responses of single neurons in the inferior colliculus (IC) of *Pipistrellus abramus* (Microchiroptera: Vespertilionidae). The data showed that there was a neurophysiological correlation of the auditory response properties with echolocation calls in IC neurons. The echolocation calls of *P. abramus* were broad-band swept from 86.6 to 43.2 kHz. The ending frequencies of the first harmonics which centered around 40 (average, 43.2; range, 37.0-47.0) kHz, were relatively more stable than the initial high frequencies. The average peak frequency was 52.1 (range, 43.3-57.6) kHz of which the majority (81%, 154 of 190 calls) ranged from 50.1 to 60 kHz. We recorded the responses of 75 single IC neurons to pure tones. Most IC neurons had the best frequency (BF) at between 30 and 50 kHz (centered around 40 kHz) (73%, 54 of 75) and between 50.1 and 60 kHz (19%, 14 of 75), respectively corresponding to the ending frequencies of the first harmonics and peak frequencies. The minimum threshold (MT) distribution was wider, and the average MT was significantly higher for neurons with a BF of 30-50 kHz than for neurons with a BF of 50.1-60 kHz (62 ± 11 vs. 49 ± 8 dB SPL, $p < 0.001$, t -test). The latency distribution was also slightly wider for neurons with a BF of 30-50 kHz (71% between 6.1 and 8.0 ms) than for neurons with a BF of 50.1-60 kHz (79% between 4.0 and 6.0 ms). Our study of echolocation calls and auditory response properties of IC neurons suggests that the IC of *P. abramus* can effectively process emitted pulses and echoes during hunting.
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Key words: Echolocation calls, Auditory response properties, Inferior colliculus, *Pipistrellus abramus*.

Echolocating bats navigate and hunt insects by utilizing a biosonar system which includes vocalization and auditory systems. Many parts of the bat's auditory system are specialized for encoding and analyzing echoes from targets (Pollak and Casseday 1989, Covey 2005). By analyzing the temporal and spectral cues of the echoes, bats can extract target information such as the size, shape, and texture of a tiny insect

(Altringham 1996).

During echolocation, the calls emitted by different species of bat vary in frequency and temporal patterns (Kösssl et al. 1999). Different species of echolocating bats can be identified by their echolocation calls. Based on the frequency and temporal patterns of the emitted calls, bats can be broadly divided into 3 categories, the frequency-modulated (FM), constant-frequency (CF)/FM, and

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click bats (Neuweiler 2003).

In the ascending auditory pathways, the inferior colliculus (IC) occupies a strategic position in that it receives and integrates excitatory and inhibitory inputs from many lower-level auditory nuclei as well as descending inputs from the auditory cortex (Covey and Casseday 1995, Casseday et al. 2001, Jen et al. 2001). Previous studies showed that large numbers of IC neurons in bats are tuned to the frequency range of their echolocation calls. Those studies included certain FM bats, such as *Eptesicus fuscus* (Jen and Schlegel 1982, Casseday and Covey 1992) and *Molossus molossus* (Mora et al. 2004), and CF-FM bats, such as *Rhinolophus ferrumequinum* (Möller et al., 1978, Schuller and Pollak 1979) and *Pteronotus parnellii* (Pollak and Bodenhamer 1981, Jen and Kamada 1982). However, most of those studies mainly dealt with neurophysiological aspects of hearing without information on correlations with the behavioral ecology of the bats.

Pipistrellus abramus, commonly called the house bat, belongs to the suborder Microchiroptera of the Vespertilionidae. This species is widely distributed from Russia to China, Japan, Korea, Taiwan, Vietnam, Myanmar, and India (Simmons 2005) and roosts in natural habitats such as caves, tree cavities, and crevices, as well as in old buildings from which they typically emerge to hunt insects at dusk (Funakoshi and Uchida 1978). Some aspects of the ecology and physiology of this bat species have been studied in Japan (Uchida et al. 1988), Taiwan (Lee 1995, Lee and Lee 2005, Chao 2001), and China (Huang and Huang 1982, Gu et al. 2003, Feng et al. 2003). However, in spite of its wide distribution, correlations between its behavioral ecology and auditory response properties have not been studied. The purpose of this study was to examine correlations of auditory response properties of neurons in the IC of this bat species with its echolocation calls. Specifically, we studied correlations of echolocation calls with the body size and auditory response properties of IC neurons of this species. We report herein that IC neurons of this species can effectively process the emitted pulses and echoes.

MATERIALS AND METHODS

Measurement of body size and recording of echolocation calls

Seven female and 5 male bats (*Pipistrellus*

abramus) were captured by mist netting above the lawn from July to Sept. on the campus of Central China Normal University (114°33'N, 30°35'E), Wuhan, Hubei, China. We measured the length of the right forearm to the nearest 0.1 mm with a vernier caliper. The body weight was measured using a spring balance to an accuracy of 0.5 g. These bats were housed in a temperature- and humidity-controlled cage and were given unlimited access to larvae of *Tenebrio molitor* and water. They were used to record echolocation calls as well as for recording the auditory responses of their IC neurons to sound stimulation.

To record echolocation calls of these bats, we released each bat in an empty room (12 x 6 x 4 m), and the bat's emitted echolocation calls were recorded using a Pettersson D980 bat detector (Pettersson Elektronik, Uppsala, Sweden) before being fed into a notebook computer (time-expansion 10x). The echolocation calls were analyzed using the software BatSound pro 3.31 (Pettersson Elektronik), with a fast Fourier transformation (FFT) size of 512 points and a Hamming window. We only analyzed the call sequences recorded from a bat that flew straight toward the bat detector and within 2 m of it.

Surgical procedures

Prior to the electrophysiological experiments, the bat was anesthetized with pentobarbital sodium (45-50 mg/kg body weight (bw)). The hair overlying the skull was cropped, and the skin was incised with ophthalmologic surgical scissors. A local anesthetic (procaine hydrochloride) was applied to the open wound area. The muscles overlying the skull were reflected, and the skull was scraped clean of tissue and then swabbed with 100% ethanol. A 1.5 cm long nail was glued onto the exposed skull with acrylic glue and dental cement. The bat was then allowed to recover for 1-4 d prior to the first electrophysiological recording.

On the day of the recording session, the bat was put inside a holder, which was fastened in a bracket, and its head was oriented with its eyesnout line pointed to 0° azimuth and 0° elevation of the frontal auditory space. The head was immobilized by fixing the shank of the nail into a metal rod with a set screw. A small hole of 200-500 μm in diameter was then made in the semitransparent skull above the IC for insertion of a glass pipette electrode filled with 2 M NaCl (with an impedance of 5-10 MΩ) to record the sound-activated neural

responses. An indifferent electrode (of Ag-AgCl silver wire) was placed beneath the nearby temporal muscles. Recordings were conducted inside a double-walled anechoic room (at a temperature of 28-30°C) and the ceiling and inside walls were covered with convoluted polyurethane foam to reduce echoes. Each recording electrode was visually placed at the surface of the IC and advanced with a stepping hydraulic micropositioner (Model 640, David Kopf Instruments, Tujunga, California, USA) from which the recording depth was read. Procedures were in accordance with NIH guidelines for experiments involving vertebrate animals.

Acoustic stimuli

To generate acoustic stimuli, continuous sine waves from a function generator (Agilent 33220A, Kuala Lumpur, Malaysia) were formed into pure tones by a homemade tone burst generator driven by a stimulator (Sen-7203, Nihon Kohden, Tokyo, Japan). The tones were amplified by an ultrasonic power amplifier (homemade) after passing through decade attenuators (LAT-45, Leader, Yokohama, Japan) before being fed into a small condenser loudspeaker (AKG model CK 50, 1.5 cm diameter, 1.2 g). The loudspeaker was placed 38.5 cm away from the bat's ear and 30° contralateral to the recording site. Calibration of the loudspeaker was conducted with a 1/4" microphone (4939, B&K, Narum, Denmark) placed at the bat's ear using a measuring amplifier (2610, B&K). The output of the loudspeaker was expressed in dB SPL referred to 20 μ Pa root mean square.

Recording of neuronal responses

A pure tone of 4 ms duration (with 0.5 ms rise-decay times) was delivered at 2 pulses per second (pps) through the loudspeaker to isolate sound-activated IC neurons. Upon isolation of a neuron, its action potentials were amplified (ISO-DAM, WPI, Sarasota, USA) and synchronously sent to an oscilloscope (TDS210, Tek, Beaverton, USA), an audio monitor (Grass AM9, Kernersville, USA), and a computer for acquisition of peri-stimulus-time histograms (PSTHs) (with a bin width of 0.5 ms). By systematically changing the frequency and amplitude of the pure tone, we determined each neuron's best frequency (BF) and minimum threshold (MT). The BF was defined as the frequency requiring the lowest amplitude to elicit a neural response. At the MT, the neuron responded

to the presented BF sound stimuli with 50% probability. Using a custom-written program, PSTHs were obtained with responses of IC neurons to 32 BF sound stimuli delivered at 20 dB above the MT from which the first spike latency was obtained. A neuron's frequency tuning curve (FTC) was plotted with the combinations of frequencies and amplitudes that elicited the minimal response of the recorded neuron.

RESULTS

Body size and echolocation calls

The forearm length and body weight of *P. abramus* were 32.8 ± 1.7 (range, 30.2-40.0) mm and 4.4 ± 0.9 (range, 2.5-5.5) g, respectively. We recorded a total of 190 calls from 24 call sequences of 12 *P. abramus* in free flight. The echolocation calls of this bat species were broadband FM signals which typically contain 1 to 2 harmonics (Fig. 1A), with the predominant first harmonic sweeping from 86.6 to 43.2 kHz. Call characteristics of this bat species determined in the present and previous studies are shown in table 1. Because we could only obtain the mean value of call data from previous studies, we only present descriptive comparisons here to show differences in calls recorded in different places. It is clear that the first harmonic of the echolocation calls obtained in the present study had a broader frequency range and change rate but a shorter duration than data obtained in previous studies. The frequency with the most energy (i.e., the peak frequency, measured from the power spectrum, with a FFT size of 512 points) was 52.1 ± 2.4 kHz in the present study (Fig. 1B), which was slightly lower than 58.1 ± 3.8 kHz from calls of bats obtained in Guizhou Province, China. However, there was little difference among the ending frequencies of the first harmonics of echolocation calls of bats caught in 3 different places (i.e., 43.2 ± 1.8 kHz from calls of bats in Wuhan, China, 45.9 ± 1.6 kHz from calls of bats in Guizhou, China, and 46.7 ± 1.9 kHz from calls of bats in Taiwan).

Auditory response properties of IC neurons

The auditory response properties of 75 IC neurons were examined. When stimulated with single pulses, 55 (73%) neurons discharged 1 or 2 impulses (phasic responders) (Fig. 2A), 11 (15%) neurons discharged 3-5 impulses (phasic bursters)

(Fig. 2B), and the remaining 9 (12%) neurons were tonic responders which discharged impulses throughout the entire pulse duration (Fig. 2C, D).

Figure 3 shows the distribution of BFs and their relationship with recording depths. BFs ranged from 18.9 to 79.3 kHz, which span the entire range of the peak frequency and ending frequency of the first harmonics of the bat's calls. Most BFs were either between 30 and 50 kHz (54 of 75, 73%) or between 50.1 and 60 kHz (14 of 75, 19%) (Fig. 3A, B). A linear regression analysis showed that these neurons were tonotopically organized within the IC such that the BF of these neurons significantly and progressively increased with recording depth ($p < 0.001$).

The MT of these neurons ranged from 29 to 80 (average, 58 ± 12) dB SPL with most (46 of 75, 61%) between 40 and 60 dB SPL (Fig. 4A). The first spike latency ranged from 3.2 to 10.4 (average, 6.3 ± 1.5) ms in which the majority (68 of 75,

91%) were between 4 and 8 ms (Fig. 4B).

The MT distribution was wider and the average MT was significantly higher for neurons with BFs of between 30 and 50 kHz than for neurons with BFs of between 50.1 and 60 kHz (62 ± 11 vs. 49 ± 8 dB SPL, t -test, $p < 0.001$) (Fig. 4C). In parallel with this finding, the latency distribution was also slightly wider for neurons with BFs of between 30 and 50 kHz than for neurons with BFs of between 50.1 and 60 kHz (Fig. 4D). Most (39 of 55, 71%) latencies of the former were between 6.1 and 8.0 (average, 7.2 ± 0.6) ms while most (11 of 14, 79%) latencies of the latter were between 4.0 and 6.0 (average, 5.0 ± 0.6) ms (t -test, $p < 0.001$).

The FTCs of all 75 neurons were V-shaped, and the bandwidths progressively increased with the sound amplitude (Fig. 5A-D). These FTCs could be classified as asymmetrical (60%, 45 of 75) (Fig. 5A, B) and symmetrical (40%, 30 of 75) (Fig. 5C, D) based on the slopes of the 2 limbs.

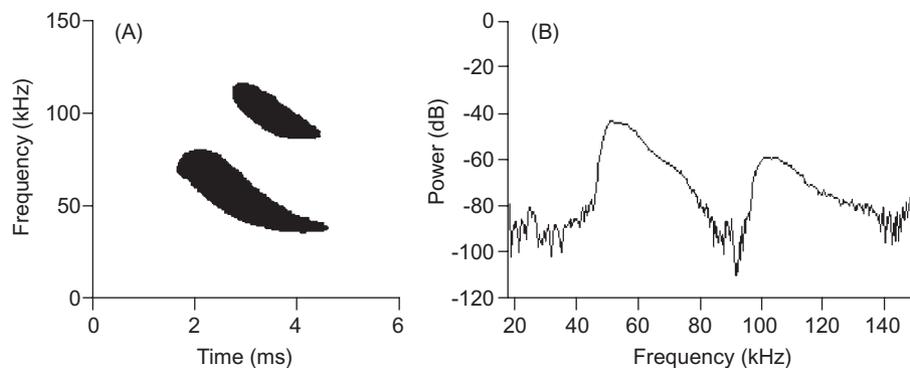


Fig. 1. Sonogram (A) and power spectrum (B) of an echolocation call from *Pipistrellus abramus* in free flight.

Table 1. Call characteristics of *Pipistrellus abramus* determined in the present and previous studies

Parameter	Echolocation calls recorded in different places		
	Wuhan, China (present study)	Guizhou, China (Feng et al. 2003)	Taiwan (Chao 2001)
Duration (ms)	2.6 ± 0.8 (1.3-4.7)	3.5 ± 0.4	6.0 ± 1.5 (1.2-9.7)
Peak frequency (kHz)	52.1 ± 2.4 (43.3-57.6)	58.1 ± 3.8	-
Pulse interval (ms)	71.0 ± 36.5 (15.3-172.4)	46.8 ± 17.5	82.7 ± 9.5 (54.0-113.0)
First harmonic			
Starting frequency (kHz)	86.6 ± 10.2 (62.0-105.0)	78.0 ± 5.7	53.3 ± 5.3 (42.9-71.8)
Ending frequency (kHz)	43.2 ± 1.8 (37.0-47.0)	45.9 ± 1.6	46.7 ± 1.9 (41.3-51.8)
Frequency range (kHz)	43.4 ± 10.6 (17.0-61.0)	-	6.6 ± 4.3 (1.0-24.1)
Change rate (kHz/ms)	18.5 ± 6.5 (5.8-30.7)	-	2.7 ± 2.2 (0.2-12.9)
Second harmonic			
Starting frequency (kHz)	119.5 ± 11.7 (88.0-144.0)	100.0 ± 2.8	-
Ending frequency (kHz)	90.5 ± 5.2 (72.0-100.0)	92.0 ± 2.8	-

Data displayed in table are expressed as the mean \pm standard deviation (maximum-minimum).

The FTC was defined as asymmetrical if the frequency range difference between the high- and low-frequency limbs at the same amplitude above the MT exceeded 20%. Otherwise, the FTC was considered to be symmetrical. The asymmetric FTC curves had a steep slope either at the low-frequency limb (18%, 8 of 45) (Fig. 5A) or at the high-

frequency limb (82%, 37 of 45) (Fig. 5B). Conversely, the slopes of both limbs of symmetrical FTCs were similar (Fig. 5C, D).

The sharpness of the FTC was expressed by a $Q_{10\text{-dB}}$ value which was obtained by dividing the BF by the bandwidth of the FTC at 10 dB above the MT. The $Q_{10\text{-dB}}$ value of these FTCs ranged

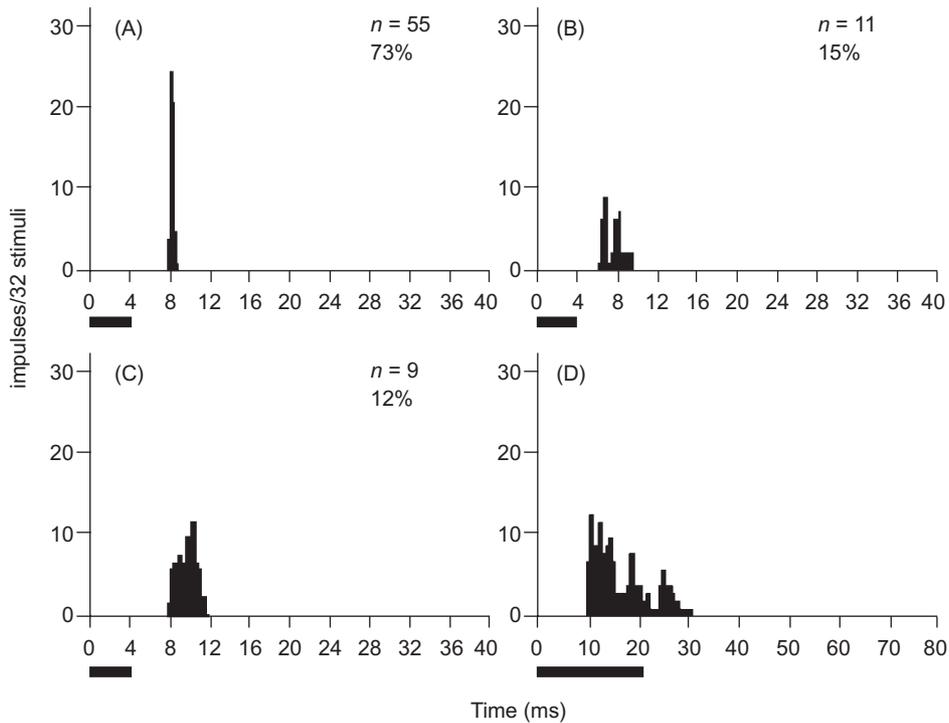


Fig. 2. Peri-stimulus-time histograms (PSTHs) showing the discharge patterns of 3 representative inferior collicular neurons: a phasic responder (A), a phasic burster (B), and a tonic responder (C, D). *n*, number of neurons. The best frequency (BF) (kHz), minimum threshold (MT) (dB SPL), and depth (μm) of these 3 neurons were 35.4, 56, 672 (A); 30.5, 54, 419 (B); 41.2, 48, 922 (C, D).

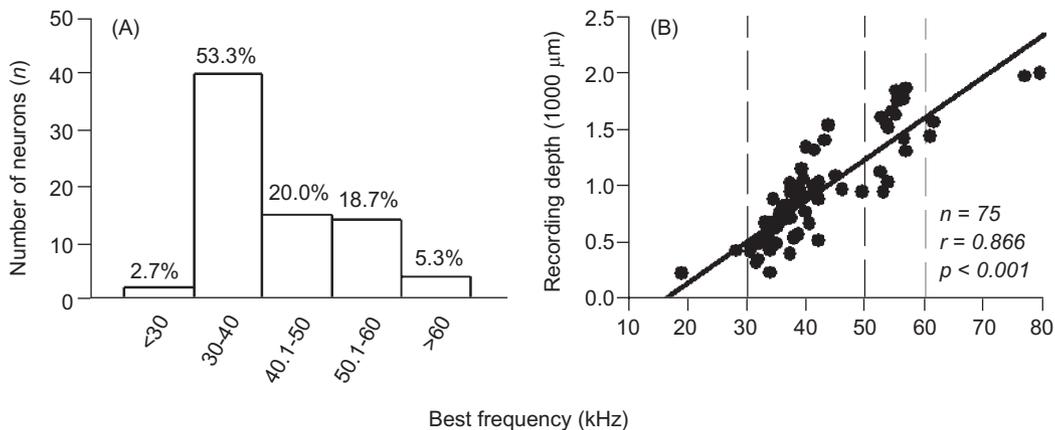


Fig. 3. Distributions of the best frequencies (BFs) in inferior collicular (IC) neurons of *Pipistrellus abramus* (A) and the relationship between BFs and recording depths (B). The linear regression and correlation coefficient of plot B are shown by a diagonal line and *r*. *n*, number of neurons; *p*, significance level; 2 frequency ranges of 30-50 and 50.1-60 kHz are divided by 3 vertical dashed lines (see text for details).

from 1.8 to 12.4. About 1/5 (18%, 10 of 55) of the neurons in the range of 30-50 kHz and most (71%, 10 of 14) of the neurons in the range of 50-60 kHz had Q_{10-dB} values exceeding 5 (Fig. 5E).

DISCUSSION

Body size and echolocation calls

In this study, we show that *P. abramus* uses high-frequency calls for echolocation (Fig. 1). It has been reported that the vocal apparatus of small animals can emit calls of high frequencies with high pressure (Bradbury and Vehrencamp 1998). Because the Rayleigh principle dictates that most sound energy is diffracted by a target if the sound wavelength is larger than the target, bats use high-frequency calls with very short wavelengths for echolocation, which enables them to detect small insects (Pye 1993).

Compared with other bats of the family

Vespertilionidae, *P. abramus* is a small-sized bat (with an average bw of 4.4 g). The peak frequency of this bat species was 52.1 kHz which is higher than the 40.6 kHz of larger-sized FM bats such as *Myotis ricketti* (with an average bw of 22.5 g) but lower than the 76.5 kHz of smaller-sized bats such as *Tylonycteris pachypus* (with an average bw of 3.4 g) (Ma et al. 2003, Zhang et al. 2005). These data suggest that the peak frequency of echolocation calls used by the bats is correlated with the body weight. These observations are in agreement with previous studies which showed that the frequency of echolocation calls used by bats increases inversely with body size (Fenton et al. 1998, Bogdanowicz et al. 1999, Jones 1999).

On the other hand, it has been reported that animals with a small head and body size typically have small tympanic membranes which resonate with higher-frequency calls than animals with large tympanic membranes (Nummela 1995). This report tempted us to speculate that the difference in the peak frequency of the calls emitted by these

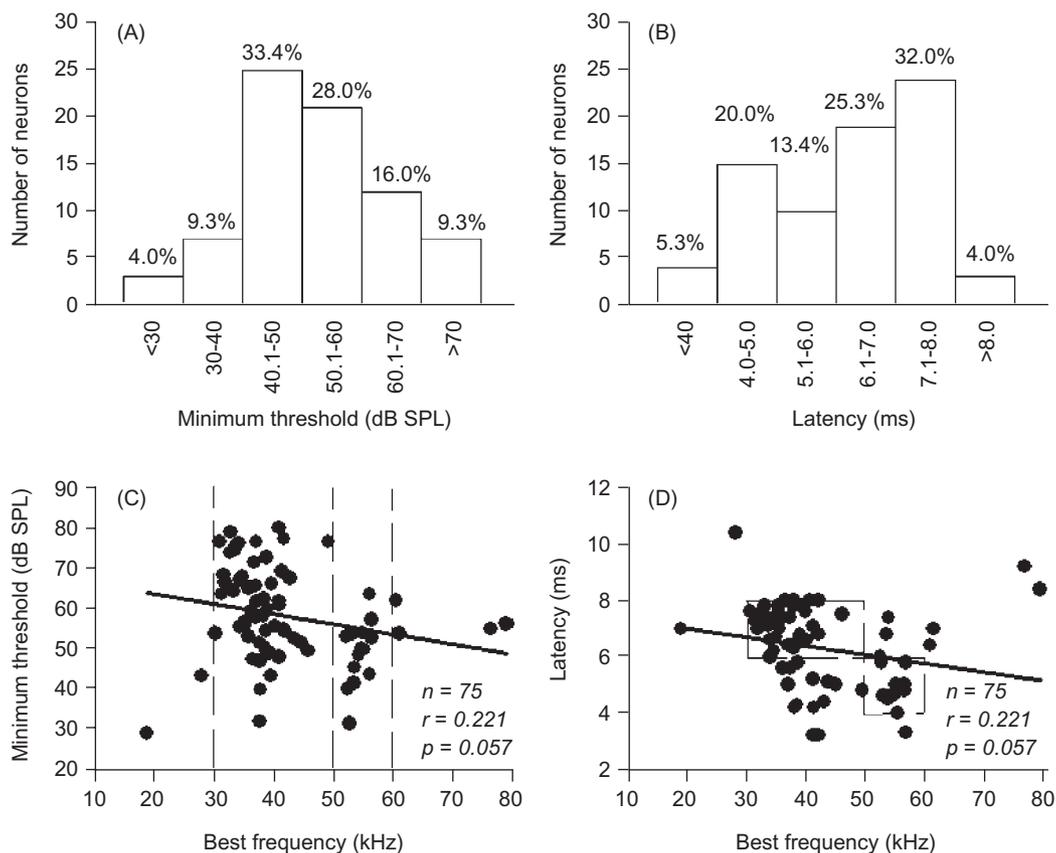


Fig. 4. Distributions of minimum thresholds (MTs) (A) and latencies (B) of neurons in the inferior colliculus (IC) of *Pipistrellus abramus*. Correlation of MTs (C) and latencies (D) of these neurons with their best frequencies (BFs). The dashed boxes in D represent 2 latency ranges of 4-6 and 6.1-8 ms.

3 bat species might also be related to the size of their tympanic membrane. Further studies are needed to confirm this speculation.

Echolocating bats use different information-gathering strategies for flying and hunting in confined and open environments (Griffin et al. 1960, Schnitzler and Kalko 2001, Surlykke and Moss 2000). Because sound absorption severely constrains echolocation calls over long distances and calls of higher frequencies are more strongly attenuated than lower ones (Bazley 1976), *Pipistrellus* bats have very flexible echolocation calls and are able to change the duration and bandwidth of the shallow CF-like tail of the FM signals (or delete it altogether) (Altringham 1996). In agreement with those studies, our observations showed that *P. abramus* prefers to search and pursue insects in open spaces above the vegetation and shrubs where there are low insect densities, but no obstacles to using shallow FM sweeps of long durations (~9.7 ms). However, when detecting potential prey or flying into a cluttered environment, the bat shortens the duration (1.3-4.7 ms) of the FM signals swept downward from 86.6 to 43.2 kHz (Table 1). This bat species also forages among trees, around buildings and street lamps, and in air space rich in insects over ponds. A long, shallow sweep would increase the range of echolocation by integrating auditory input over the full duration of the echo,

while a short steep sweep could allow the bat to obtain more information within the same time and avoid overlaps between emitted calls and echoes (Neuweiler 1989).

We showed that the echolocation call parameters of *P. abramus* obtained in the present study differed from those of previous reports (Table 1). What might be the reasons for these different observations? Conceivably, these different observations might be simply due to geographical variations in echolocation calls of the same species as reported previously (Neuweiler 1989). However, it is more likely due to the different ways that the echolocation calls were recorded in these 3 studies. For example, Chao (2001) recorded the calls of bats flying in the open, while Feng et al. (2003) and we recorded echolocation calls in a closed space. As such, the duration, pulse intervals, and ending frequency of the first harmonics recorded by Chao (2001) are higher but the frequency range and change rate of the first harmonics were lower than our data (Table 1). We also noted that Feng et al. (2003) only recorded the echolocation calls of nursing female *P. abramus* in Guizhou while we recorded the echolocation calls from both sexes of this bat species in Wuhan. However, whether there are sexual differences in the echolocation calls of this bat species needs further studies.

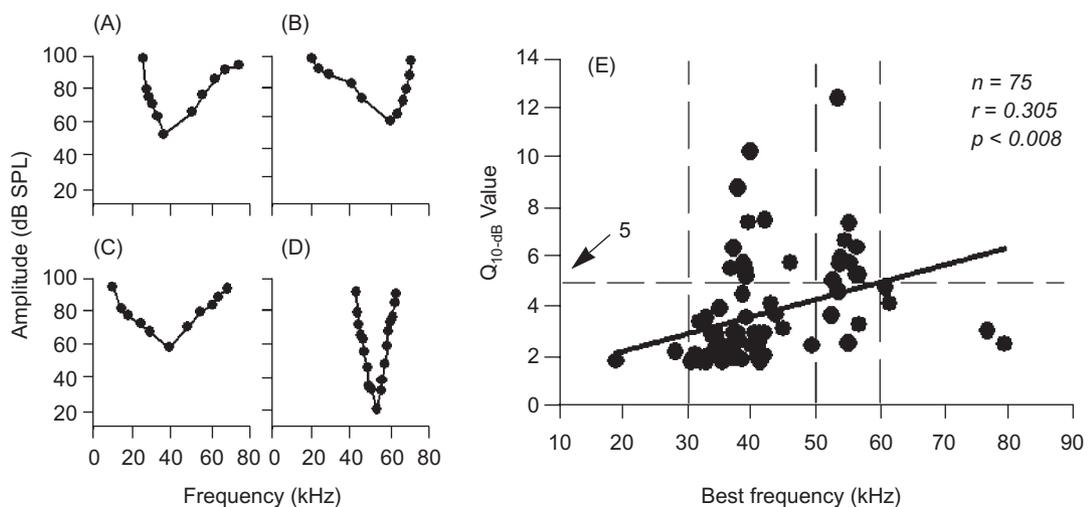


Fig. 5. Different types of frequency tuning curves (FTCs) of neurons in the inferior colliculus (IC) of *Pipistrellus abramus*. A, B, Asymmetrical FTCs with a steep slope at the low- (A) or high- (B) frequency limb; C, D, symmetrical FTCs that were broadly (C) or narrowly (D) tuned to the stimulus frequency; E, correlation between Q_{10-dB} (BF divided by the bandwidth at 10 dB above the minimum threshold (MT)) values and the best frequencies (BFs). The horizontal dashed line at $Q_{10-dB} = 5$ is our arbitrary criterion for classifying a neuron as broadly (< 5) or narrowly (≥ 5) tuned to the stimulus frequency.

Auditory responses of neurons in the IC of *P. abramus*

All neurons recorded in the present study discharged to an acoustic stimulus in 3 ways: phasic response (73%), phasic bursts (15%), and tonic response (12%). In the IC of *E. fuscus*, the percentages of neurons with corresponding discharge patterns were 48.3%, 19.1%, and 25.7%, while the remaining neurons discharged with other patterns such as on-off response, off response, and inhibitory units (Jen and Schlegel, 1982). Whether there are neurons with such discharge patterns in the IC of *P. abramus* needs to be investigated in further studies.

Most BFs of the IC neurons obtained from this study were between 30 and 60 kHz which covers the main frequency component of the echolocation calls of this bat species (Figs. 1, 3). In particular, the BFs of a large group of neurons cover the peak frequency and ending frequency of the first harmonic calls. These findings suggest that the IC of this bat species can effectively process the emitted calls and returning echoes. These neurons are tonotopically organized along the dorsal ventral axis of the IC, similar to previous findings in the IC of *E. fuscus* (Casseday and Covey 1992), *P. parnellii* (Pollak and Bodenhamer 1981), *R. pusillus* (Luo et al. 2006), and other mammalian species including cats (Semple and Aitkin 1979) and chinchillas (Nuding et al. 1999).

All FTCs obtained in the present study were V-shaped (Fig. 5A-D). This observation differs from the FTCs obtained from the IC neurons of other FM bats such as *Myotis* (Suga 1969), *E. fuscus* (Casseday and Covey 1992), and *M. molossus* (Mora et al. 2004). In addition to the V-shaped FTCs, closed and double-peak FTCs were also obtained in these studies. Whether these different observations are simply due to a sampling bias or specific differences between species remains to be further investigated.

We observed that neurons with BFs of between 30 and 60 kHz varied widely in their MT, ranging from 30 to 75 dB SPL (Fig. 4C). This finding suggests that they are able to respond to emitted calls and returning echoes across different phases of hunting. These neurons also had a wide range of $Q_{10\text{-dB}}$ values, but some of them were sharply tuned to large $Q_{10\text{-dB}}$ values (> 5) (Fig. 5E). Conceivably, these sharply tuned neurons enable the bat to effectively process the ending and peak frequencies of its echolocation calls. Similarly sharply tuned neurons have also been

reported in another FM bat (Casseday and Covey 1992). These neurons are comparable to the filter neurons found in CF-FM bats (Moller et al. 1978, Pollak and Bodenhamer 1981, Pollak and Casseday 1989). It has been proposed that sharply tuned filter neurons enable CF-FM bats to detect the wing-beating of insects in cluttered environments (Neuweiler 1989).

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